IUFRO

WP 2.09.01 Seed Orchards

PROCEEDINGS

IUFRO Seed Orchard Conference 2017

SEPTEMBER 4-6, 2017 BÅLSTA, SWEDEN

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IUFRO SEED ORCHARD CONFERENCE 2017

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WELCOME TO
IUFRO Seed Orchard Conference 2017

Dear Delegates, Colleagues, Ladies and Gentlemen,

It is my great pleasure to welcome you at the IUFRO Seed Orchard Conference 2017 in Bålsta, Sweden, on behalf of the organizing institutions – the International Union of Forest Research Organizations (IUFRO), the Swedish University of Agricultural Sciences (SLU), the Forestry Research Institute of Sweden (Skogforsk) and Svenska Skogsplantor.

This conference is being held as part of activities initiated in 2007 with the aim to facilitate research related to seed orchards worldwide and make access to research outputs more efficient. In 2007, the first international conference focused on seed orchards was held and, the following year, a working party devoted to seed orchards was formed under IUFRO Division 2.

Seed orchards are the most commonly used forest tree production populations in the world and, at the same time, the most cost-efficient way to produce genetically improved seed for reforestation. Seed orchards represent one of the keys to sustainable forest management because they produce healthier and more productive forests. It is of vital importance to continue a focused research on seed orchards and to seek methods of improving both quality and quantity of their seeds. In Sweden, for instance, seed orchards are currently supplying 62% of Norway spruce and 94% of Scots pine seedlings and these figures are still growing, while the seeds are progressively becoming more improved.

The objective of this conference is to exchange the most recent scientific knowledge related to forest tree seed orchards and their integration in the forestry practice. We hope to create a pleasant environment for sharing expertise and ideas for future research and to encourage collaboration among research teams involved in different aspects of seed orchards. We will cover topics such as linking seed orchards with long-term tree breeding, seed orchard design and management, cone and seed health and development, fertility variation and genetic diversity of seed crops, seed pathology, seed testing and storage, gene conservation and impact of seed orchards on forestry and society. I am certain that all oral and poster presentations will be highly valuable to all participants and will lead to constructive discussions throughout and after the Conference.

I would like to express our sincere gratitude to our sponsors, namely the Swedish Forest Tree Breeding Association (Föreningen Skogsträdsförädling), Södra's Foundation for Research, Development and Education (Södras Stiftelse för Forskning, Utveckling och Utbildning), Holmen Skog, SCA (Svenska Cellulosa Aktiebolaget) and its affiliated Rydéns fund, Sveaskog, Bergvik skog and SLU, for their generous support. I extend my thanks to all members of the Organizing Committee and Scientific Committee for their hard work and genuine interest to make the Conference successful and I warmly thank you all – distinguished delegates, students, sponsors' representatives and guests – for your kind participation.

I wish all of you a very pleasant and memorable stay at the Conference and hope that you will find it greatly beneficial for your future work. I also wish the IUFRO Working Unit 2.09.01 "Seed Orchards" many enthusiastic followers and a successful and fruitful continuation of collaborative efforts.

Yours sincerely,

Tomáš Funda

Tomáš Funda
Conference Chair
Swedish University of Agricultural Sciences
Deputy Coordinator of IUFRO Working Party 2.09.01 "Seed Orchards"

Photo: Ulfstand Wennström, Skogforsk

OUR SPECIAL THANKS TO

FÖRENINGEN SKOGSTRÄDSFÖRÄDLING
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Photo: Curt Almqvist, Skogforsk

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The number of participants: 106

Photo: Lars Klingström, Holmen Skog

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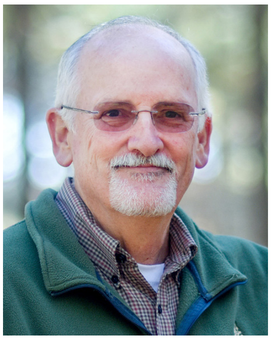
Steve McKeand, North Carolina State University, USA



Dr. Steve McKeand
Professor
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Steve McKeand has been a Professor of Forestry and Environmental Resources for over 30 years and is Director of the Cooperative Tree Improvement Program (http://treeimprovement.org//) at North Carolina State University. In addition to directing the program, Steve conducts research in support of the Tree Improvement Cooperative, teaches graduate and undergraduate courses in forest genetics, directs graduate students, and conducts outreach and extension programs for the 35 company and government agency members of the Cooperative as well as for foresters and forest landowners throughout the southern US.

Steve received his BS in Forestry in 1976 and MS in Forest Genetics in 1978, both from Purdue University. In 1983, he completed his PhD in Forest Genetics at NC State University.

Title: Management strategies to increase genetic gain and to maintain healthy trees in loblolly pine seed orchards and forests in the southern US

Alain Roques, French National Institute for Agricultural Research INRA, France

Dr. Alain Roques
Research Director of Forest Zoology
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Alain Roques (D.Sc., Ph.D.) is the leader of the Forest Zoology unit with INRA Orléans, France. He is a forest entomologist with 31 years of experience on the biology, ecology and behavior of forest insects. He got his PhD in 1975 and DSc in 1988. Until the late 2000s, his activity mostly focused on the mechanisms underlying the colonization of conifer cones by insects, developing EU research programs in seed orchards of Europe and China. During the recent years, he turned towards biological invasions and the effect of global warming on the populations of terrestrial invertebrates. He especially coordinated the inventory of alien terrestrial invertebrates in Europe realized within the DAISIE project. He was also the editor of a recent book devoted to the relationships between climate change and insects: “Processionary Moths and Climate Change: An update”. He published more than 150 peer-reviewed papers, of which 65 dealing with cone and seed insects, 25 books and book chapters, and presented 93 communications at international conferences.

Title: Why are insect pests more impacting in seed orchards than in surrounding forests? The experience of long-term surveys of pest damage in France

KEYNOTE SPEAKERS

Christer Löfstedt, Lund University, Sweden

Dr. Christer Löfstedt
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Christer Löfstedt's research interests include all aspects of insect chemical communication with focus on the evolutionary and applied aspects of pheromone communication in moths. Ongoing research ranges from molecular biology of pheromone reception and pheromone biosynthesis to studies of insect behaviour in the field. Christer is involved in several projects aimed at biorational control of pest insects by means of insect pheromones, one of the foremost alternatives to the use of conventional insecticides. He teaches various aspects of chemical communication on undergraduate courses in chemical ecology, evolutionary ecology, sensory biology, molecular ecology and biotechnology.

Christer is head of Department of Biology at Lund University since 2010, President of The International Society of Chemical Ecology and fellow of the Royal Swedish Academy of Sciences. He was appointed professor in Ecology at Lund University in 1998.

Title: "Three out of the Big Five": Challenges in pheromone-based control of moths in spruce seed orchards

Juha Kaitera, Natural Resources Institute Finland (Luke), Finland

Dr. Juha Kaitera
Natural Resources Institute Finland

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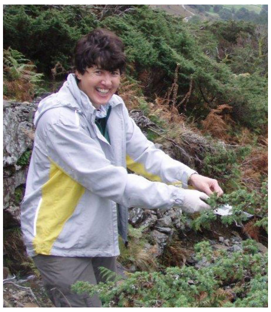
Juha Kaitera is a forest pathologist and a Senior Scientist at the Natural Resources Institute Finland in Oulu, Finland. He is a Docent at the University of Oulu with an expertise in epidemiology of rust diseases. His research has concentrated especially on scleroderris canker of pine and spruce, pine stem rusts, spruce cone rusts and root and butt rot diseases (especially *Heterobasidion*), of which he has published over 60 peer-reviewed scientific papers. His recent work has focused on pine stem rusts (*Cronartium, Peridermium*) and cone rusts of spruce (*Thekopsora, Chrysomyxa*), of which he has published over 40 peer-reviewed articles. The research of cone rusts has concentrated on their fruiting and sporulation, life cycle, host and alternate host range, and effect on seeds.

Title: Biology of cone pathogens of *Picea* – *Thekopsora* and *Chrysomyxa* cone rusts

KEYNOTE SPEAKERS

Shelagh McCartan, Forestry Commission, United Kingdom



Dr. Shelagh McCartan
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Shelagh McCartan is a Seed Scientist and Work Area Leader for Plant Production at Forest Research in Great Britain. She co-ordinates a programme of applied research with two focus areas:

- upgrading, testing, stratifying and storing tree seeds to improve the cost-effectiveness and efficiency of plant production; and
- modelling thermal time to predict the impact of climate change on dormancy-germination cycles of tree seeds.

She is the Coordinator of IUFRO WG 2.09.03 – Seed Physiology and Technology.

Title: Bad seed is robbery of a worse kind …

Xiao-Ru Wang, Umeå University, Sweden

Dr. Xiao-Ru Wang
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Umeå University, Sweden

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Xiao-Ru Wang is a Professor in plant genetics at the Department of Ecology and Environmental Science, Umeå Plant Science Centre, Umeå University, and an Adjunct Professor at the Beijing Forestry University, China. Her research covers population genetics and phylogenetic history of Eurasian conifer species. She teaches graduate and undergraduate courses in genetics and evolution and molecular ecology. Recently she leads a few research projects in Sweden on genetic function of pine and spruce seed orchards and the related consequences for forest production and long-term adaptation to changing climate.

Xiao-Ru received her PhD in Forest Genetics from the Swedish University of Agricultural Sciences (1992) and MSc (1987) and BSc (1984) in agronomy from Beijing Forestry University, China.

Title: Seed orchards – linking basic research and forest production

KEYNOTE SPEAKERS

Jaroslaw Burczyk, Kazimierz Wielki University, Poland

Dr. Jaroslaw Burczyk
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Jaroslaw Burczyk is a Professor in Biology (PhD in biology, 1992). He has long experience in population genetics of forest trees, including gene flow, mating systems and management of forest genetic resources. His particular interests are management of forest genetic resources in natural and artificial populations (seed orchards). He works on statistical mating models (e.g., neighborhood model) to investigate the determinants of reproductive success. Currently he is head of Department of Genetics, and Vice Rector for Science of Kazimierz Wielki University in Bydgoszcz, Poland. He gained scientific experience through extended sabbaticals in Oregon State University, Corvallis, USA (Fulbright Fellowship) and ENGREF-INRA, Nancy, France.

Title: **Monitoring mating patterns in seed orchards: adjusting expectations to reality**

Yousry A. El-Kassaby, University of British Columbia, Canada

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Yousry A. El-Kassaby is a Professor and Associate Dean, Graduate and Postdoctoral Studies, Faculty of Forestry, University of British Columbia, Vancouver, Canada. Prior to his current post, Yousry worked in the industry as Director, Genetics and Product Development, CellFor Inc., and as Director, Applied Forest Research, Pacific Forest Products Ltd, both based in Victoria, British Columbia. He has received numerous research grants in the area of forest genetics and tree improvement and has authored and co-authored more than 300 refereed publications, book chapters and invited papers. His research interests are diverse and cover tree domestication, tree improvement delivery system, seed and seedling biology and production, gene conservation, biotechnology and genomics. Yousry is a member of the IUFRO Extended Board and Coordinator of Division 2 (Physiology and Genetics) and recipient of the IUFRO Scientific Achievement Award (2010). He is an Adjunct Professor at Fujian University and Nanjing University (China), Honorary Professor at Beijing University (China) and Professor Honoris causa at the Czech University of Life Sciences Prague (Czech Republic). He serves as Associate Editor to the Canadian Journal of Forest Research.

Yousry is Ph.D. in population and quantitative genetics (1980: University of British Columbia, Canada), M.Sc. in quantitative genetics (1977: University of Tanta, Egypt) and B.Sc. in Genetics (1970: University of Alexandria, Egypt).

Title: **Tree breeding in the genomics era – opportunities and challenges**

KEYNOTE SPEAKERS

Milan Lstibůrek,Czech University of Life Sceinces Prague, Czech Republic

Dr. Milan Lstibůrek
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Milan Lstibůrek is a Professor and Departmental Head at the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague, Czech Republic. He is a graduate of the Czech University of Life Sciences (Ing. in Forestry), Swedish University of Agricultural Sciences (MSc. in Forest Biology), and North Carolina State University (Ph.D. in Forestry). He is currently an Adjunct Professor at the North Carolina State University. His area of interest covers quantitative forest genetics. Milan developed and evaluated numerous breeding strategies and seed orchard layouts.

Title: **Seed orchard designs**

Photo: Curt Almqvist, Skogforsk

NEW CHALLENGES FOR SEED ORCHARD MANAGEMENT OF LOBLOLLY PINE IN THE SOUTHERN US

Steve McKeand, North Carolina State University Cooperative Tree Improvement Program, USA
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J.B. Jett, Serenia O'Berry, and Austin Heine, North Carolina State University Cooperative Tree Improvement Program, USA

Seed orchards of loblolly pine (*Pinus taeda* L.) continue to be the dominant delivery system for genetic gain to landowners in the southern United States. Over 400,000 hectares of genetically improved loblolly pine are planted each year, and about 98% of these seedlings come from seeds produced in seed orchards. Plantation establishment with individual open-pollinated (OP) and full-sib (FS) families is standard practice in the South, and landowners have more options for what to plant than ever before. With the shift of landownership from large vertically integrated forest products companies to more forest investment and land management companies, there has been a fundamental shift in the availability of genetically improved seedlings to landowners. When the large forest products companies controlled the production of improved seedlings, the best genotypes typically went to their own lands, and lesser genotypes were sold to other landowners. Today, essentially all genetically improved pine families are available to all southern landowners, and over the last decade, a true seedling market has evolved.

As is typical in open markets, a significant price differential for the best seedlings has also emerged. Prices per thousand seedlings range from about $50 USD for average or "run of the mill" open-pollinated families to $229 USD for the best full-sib families.

Given this four-fold difference in price, nursery and seed orchard vendors have a strong incentive to produce the best genetic quality seedlings possible for landowners. Mass production of full-sib families has become a more routine practice in the southern US. This past growing season, there were 116 million seedlings of full-sib families established in plantations, representing about 15% of the loblolly pine seedlings produced in the South. Over the last 10 years, annual production of full-sib families has increased from 26.5 million to 116.3 million seedlings.

This increase in demand for full-sib seedlings has led to studies such as the pollination bag study that has been ongoing with members of the NC State University Cooperative Tree Improvement Program. This study, which is aimed at finding a pollination bag prototype that can increase cone and seed yield, was initiated due to this rapid rise in demand for the best seedlings. We found that we can double the odds of female strobili survival from time of bagging to cone harvest by using better pollination bags for operational production of full-sib seed.

The down side of the mass production of full-sib seedlings is that heavy aerial lift equipment is required in seed orchards to monitor strobili, install pollination bags, collect pollen, make 2-3 pollinations, remove pollination bags, monitor crops for pests, and collect cones 18 months after pollination. This tremendous increase in traffic has resulted in significant increases in soil compaction and is negatively affecting tree health and cone production. In a recent study, we found the percentage of "usable soil" (defined as the volume of soil having a soil strength < 2750 kPa using a soil penetrometer) around seed orchard trees ranged from an average of only 7% to 18% in the traffic rows for the five orchards sampled but was 18% to 45% in the sample positions near the tree where traffic was minimal. This increase in soil compaction was negatively correlated with cone yield.

The benefit from increased genetic gains from mass production of full-sib seedlings is having a significant impact on productivity and profit for landowners, but the increased demand for the best seedlings has come at a cost to seed orchards, a critical production factory of the entire plantation system of the southern US. Orchard managers have become more sensitive to the need to maintain healthy trees with improved fertilization and soil management regimes and pest management practices. We will discuss mitigation factors and seed orchard design options to enhance tree health and vigor as well as increase the efficiency of OP and FS seed production.

Keywords: Genetic gain, *Pinus taeda*, soil compaction, tree health

WHAT IS CAUSING CONELET ABORTION IN A LODGEPOLE PINE SEED ORCHARD IN ALBERTA, CANADA?

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Seed orchard production is fundamental to the success of any tree breeding program. This is particularly true for seed orchards designed for producing planting stock in the heavily invested mountain pine beetle regions of west central Alberta, Canada. Despite efforts to bring a seed orchard, designed to produce seed for an area of 1,106,686 ha and a target yield of approximately 6.6 million seeds/year, into full production through application of gibberellic acid ($GA_{4/7}$), yields have never materialized above 20% of its target in the last decade of production. In the summer of 2015, 34 of 110 lodgepole pine parent clones were selected for experimental treatment for a total of 284 trees. Three injection dates were selected (June 30th, July 20th and August 6/7th), and three ramets per clone treated at each date, plus three control trees per clone. The rate of $GA_{4/7}$ application was not tested and all grafted ramets were a minimum of 12 years old prior to treatment.

Three key results were obtained: 1) Six of the 34 clones showed sensitivity to $GA_{4/7}$ regardless of application dose based on stem diameter; 2) female conelet production increased significantly in the spring of 2016 for the first two treatment dates; and 3) between the spring of 2016 and the fall of 2016, all increases in conelet production diminished through high abortion rates.

In the fall of 2015 and 2016 all cones were counted on the treatment and control trees for the 34 clones and five cones per tree were randomly selected to further investigate the seed yield per cone. All cones collected to date represent 'untreated' yields and the '$GA_{4/7}$ treatment' harvest cones will be collected September 2017. Results will be presented on clonal sensitivity to $GA_{4/7}$, seed and cone yields per clone over two years, conelet development after $GA_{4/7}$ treatments and conelet abortion rates measured on three branches per ramet in the spring *versus* fall of 2016 and 2017.

Further Information:
This work is supported through an NSERC /Industrial Research Chair in Tree Improvement and Alberta Agriculture and Forestry. Industrial partners include: West Fraser, (Blue Ridge Lumber, Hinton Wood Products, Sundre Forest Products, Alberta Plywood), Weyerhaeuser Timberlands (Grande Prairie and Pembina divisions), Canfor, Millar Western, Alberta Newsprint, Alberta-Pacific Forest Industries, and the Huallen Seed Orchard Company (HASOC).

Keywords: Lodgepole pine, conelet abortion, low yielding, *Pinus contorta*

ADDING PINECONES IN CLUSTERS ON LODGEPOLE PINE BY STEM-INJECTION AND PASTE APPLICATION OF PLANT GROWTH REGULATORS

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Exogenously applied gibberellins, especially in combination with cytokinins, dramatically boosted pinecone production. When the treatments using plant growth regulators (PGRs) were optimized, female cones appeared on long-shoot buds of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) in positions normally occupied by male cones. Large clusters of pinecones – sometimes with 30 or more cones each – developed on branches. Such cones were morphologically normal in every way and produced normal seed. Exogenous application of PGRs affected the profiles of phytohormones and some of their metabolites in long-shoot buds during cone bud initiation and differentiation. We used high performance liquid chromatography-electrospray ionization tandem mass spectrometry with multiple reaction monitoring mode to analyze changes in gibberellins, abscisic acid and cytokinins, as well as some of their metabolites. We also studied differences in the type of treatment, i.e. stem-injection versus branch-paste application of PGRs. To minimize damage to trees, we created a method that successfully identified sensitive genotypes. Genotype-dependent effects were noted as well. We developed an operational method suitable for large-scale application in seed orchards.

Keywords: Long-shoot bud, lodgepole pine, gibberellin, cytokinin, pinecone yield

EXTREMELY HIGH SEED YIELDS OF A NEW SEED ORCHARD CONCEPT (NSOC) OF CHINESE FIR *(Cunninghamia lanceolata)*: INNOVATION, TECHNIQUES AND MANAGEMENT

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In China, plantation is not only adopted for commercial purpose but also for regenerating or rehabilitating other types of forests, which turns out to be a great drive for seed orchard development. The large-scale establishment of seed orchards started in 1980s and was completed in 1997, with a total area of 13,523 ha reached at the end of the year nationwide. However, the total seed yield from those seed orchards was only 226427 kg in 1999, with an average of 16.73 kg/ha. Chinese fir, an ever-green wind-pollinated conifer species, is one of the most important tree species in southern China, covering one quarter of the overall plantation area. It is of great importance to commercial wood production and it accounts for roughly half of the domestic plantation timber production. The breeding program for Chinese fir was initiated in 1964-1965 and the first clonal seed orchard (the first generation) was established in 1966 at Yangkou forest farm of Fujian Province. In 1983, the first clonal seed orchard of Chinese fir with the second generation was established at Guangzhuang forest farm, Fujian Province, with a total number of 345 clones. In 2003, the first clonal seed orchard of Chinese fir with the third generation was established at Yangkou forest farm of Fujian Province. Over the past 50 years, Chinese fir has received a considerable breeding effort. Seed yields of Chinese fir seed orchards in Fujian and Guangdong province were an epitome of the lower seed yields of forest seed orchards for many years in China (Fig 1) before the NSOC. Although the sale price of the Chinese fir seed is 10 times higher than that of ordinary commodity seed, it is still impossible to balance the investment into the seed orchards.

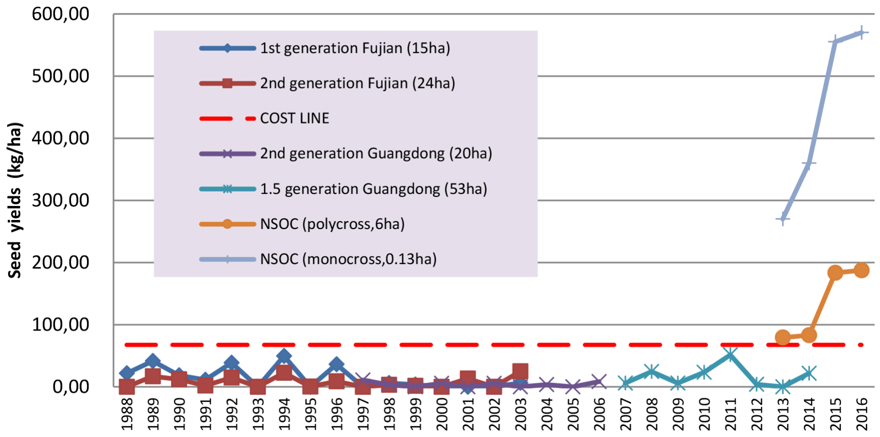

Figure 1. Seed yields of Chinese fir seed orchards in China,1988–2016.

Comparison of the harvest of Chinese fir seed orchard in the past (A) and NSOC (B,C)

Can seed yields of seed orchards be raised effectively? We put forward a novel design in 2002, called "New seed orchard concept". The main idea of the new concept is composed of the following four key points:

Small: The area of a seed orchard will be relatively small with a suggested area of 6–10 ha. This size of seed orchard will be well managed in practice, especially in the mountain area of southern China.

Dwarfing: The height of the mother tree will be controlled manually at a suggested height of 2.5–3.0 m. The operation of dwarfing will be illustrated in details below.

Parents: All clones that become the parents of a seed orchard must be those cross with "free love" after many years and recurrent selection, based on the observation and determination of combining ability among candidate parents, according to the seed yields and growth performance of their progenies as selection index.

Fertilization: The optimal fertilization protocol was put forward to increase seed yields of Chinese fir seed orchards based on the nutritive element content of the mother tree and soil, site conditions, and characteristics of flowering and fruiting of the mother tree. Key techniques included nutrition diagnosis, types and ratio of fertilization, time, frequency and amount of fertilization.

The seed yields per unit area of NSOC Chinese fir with polycross reached 187.5 kg/ha in 2016, which was 10 times the average yields per unit area of other Chinese fir seed orchards and 5times the highest yields per unit area recorded in other Chinese fir seed orchards in China.

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Keywords: Seed orchard, genetic improvement, seed yield, crown management, Chinese fir

EFFECT OF GENETIC THINNING OF SCOTS PINE SEED ORCHARDS

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More than 3000 hectares of Scots pine seed orchards have been established in Finland since 1954. The initial planting density of the orchards has varied between 250 to 400 grafts per hectare. When the grafts grow bigger, their crowns need to be released from competition to improve flowering and seed production. When the need for thinnings became topical in the 1980's, a computer programme was developed to incorporate also genetic values of the orchard clones into the thinning decisions (Nikkanen & Pukkala, 1987). The original programme was made for VAX mainframe computer, but it was transformed in 2002 into a MS Excel based (Visual Basic) application for PC (Kinnunen & Karvinen, 2010).

The purpose of thinning of seed orchards is to regulate the growing space of the grafts and at the same time to improve the genetic value of the orchard by using the newest clone and progeny data in selection of the grafts to be removed.

The computer programme used in thinning can utilise clone-wise standardized (average 50, range approximately 0 to 100) performance levels for the following traits: quality, height growth, survival, female and male flowering. The user sets weights for each trait, and a weighted mean index value is calculated for each clone. The relative importance of the traits can be adjusted in the programme. Furthermore, every individual graft is monitored for its phenotypical condition (size, health). The final index value for each graft is the product of clonal and phenotypic graft values.

The thinning programme iteratively compares every graft to its neighbours and removes the worst graft by index until a pre-defined target density (grafts/ha) is achieved. Density can be adjusted in row direction (maximum number of unthinned grafts), or in two dimensions by comparing grafts within a circle of a desired diameter. The smaller the diameter of the circle, the more even the density will be. On the other hand this will diminish improvement in the genetic value of the seed orchard.

The output of the programme includes in addition to a map which indicates the grafts to be removed in thinning also a summary giving averages for the traits used in thinning and status numbers for the orchard both before and after thinning. The summary information helps in choosing among several thinning alternatives produced with different weighting among the traits and other initial settings.

Since 1986 altogether 107 first-generation Scots pine seed orchards with a total area of 1742 ha have been thinned in Finland. Twenty-four seed orchards (312 ha) have been thinned twice and one orchard (30 ha) three times. Also two 1.5-generation elite seed orchards have already been thinned in recent years.

The original density of first-generation seed orchards was 400 grafts/ha when established. After first thinning about 150-170 grafts/ha remain, and after second thinning ca. 90 grafts/ha. 1.5-generation seed orchards have about 250 grafts /ha when established, and after 1st thinning the density will drop down to 135 grafts/ha.

We compared the genetic effect of thinning in three seed orchards. Two of the seed orchards were first-generation orchards and one of them had been thinned twice. The third studied orchard was a 1.5-generation seed orchard. In the first thinning 50 to 61% of the grafts remained in the seed orchard and in the second thinning 27% of the original number of grafts were left.

The performance level for both height and quality typically increased 5 to 7 units, which corresponds 10 to 14% improvement compared to the theoretical population mean (Fig. 1). The improvement in height from thinning of the elite seed orchard No. 404 was only 3 units due to a greater weight given to quality. The second thinning yielded approximately an equal improvement as the first thinning. In the seed orchard No. 282, this resulted in a total of about 25% increase in performance level values compared to the population mean.

Genetic thinning had a negligible effect on female flowering and cone production. In the orchard No. 404, it slightly reduced pollen production.

The realized selection intensities for height in thinning of the seed orchards were relatively modest varying from 0.27 in first thinning to 0.62 in second thinning. These values were only 40 to 50% relative to the theoretically obtainable values with the used thinning percentage. There are two explanations for this lower than expected level of obtained selection intensity. First, selection for other goals (mainly quality) reduced the selection intensity for height. Second, the need to fulfill also the spatial requirements of the operation reduced the selection intensities.

The genetic thinning of a seed orchard changes the graft numbers per clone from approximately even distribution to follow more that of linear deployment (Fig. 2).

The presented examples show that it is feasible to combine in seed orchard thinning both the need for increasing the growing space for the grafts to promote seed production and improving the genetic value of the seed orchard by incorporation the clonal genetic information into decision making.

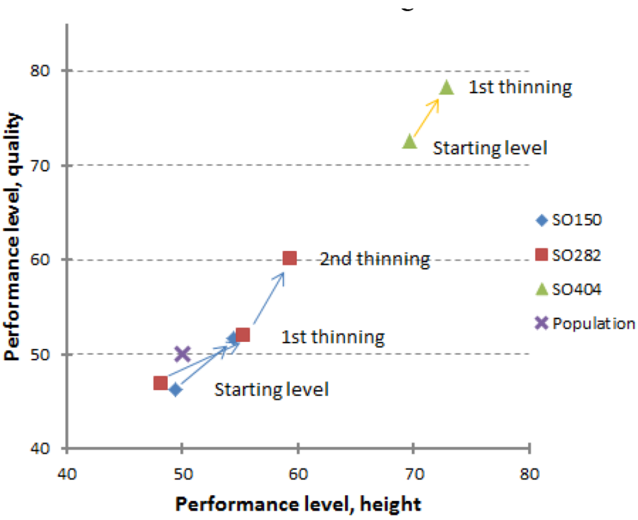

Figure 1. The effect of genetic thinning on the average genetic level in height and quality in three different seed orchards (Nos. 150, 282, 404). Seed orchard No. 282 has been thinned twice and No. 404 is a 1.5 generation seed orchard. Theoretical population average is also indicated.

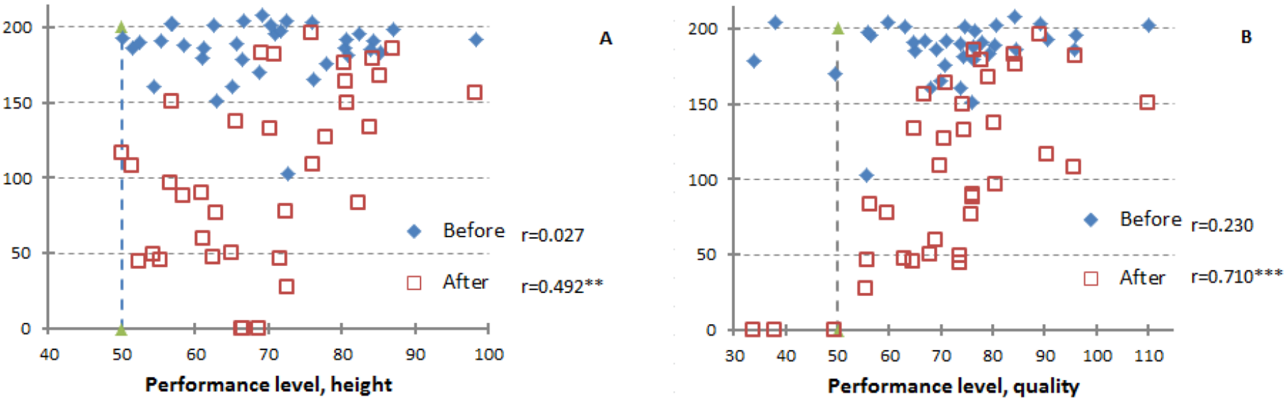

Figure 2. The number of grafts per clone before and after thinning in seed orchard No. 404 as a function of performance level for height (A) and quality (B). The correlation between the number of grafts and the trait in question is also given. The vertical dashed line gives the theoretical population average.

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Keywords: Seed orchard, genetic gain, selection intensity

COMPARISON OF POLLINATION BAGS FOR MASS PRODUCTION OF CONTROL CROSS SEEDS IN LOBLOLLY PINE

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Over the past 10 years, operational deployment of fullsib families of loblolly pine (*Pinus taeda* L.) has gained prominence relative to open-pollinated seedling stock or seed orchard mixes in the southern United States. To produce control-cross seed, a pollination bag must be used to isolate female strobili from outside pollen contamination, and a single, known pollen is applied at time of maximum female strobili receptivity. In the spring of 2014, the members and staff of the NCSU Cooperative Tree Improvement Program designed and installed a study to compare four pollination bag prototypes. Bags from PBS International were compared to the industry-standard Lawson pollination bag with and without a support wire. Open-pollinated flowers were also monitored for comparisons. Based on preliminary results from the 2014 installation, another round of prototype testing began in the spring of 2015. The main objective of this study was to compare cone survival, seed yields, and seed efficiencies of cones produced from these bags types.

For the 2014 version of the study, cone survival and seed yield per cone were calculated following cone harvest in October 2015. Significant differences in cone survival were found between the industry standard Lawson pollination bag (L) and PBS-A and PBS-B bag prototypes. Lawson with a support wire (Lw) was also superior over the control for cone survival. The odds of female strobili survival from time of bagging to cone harvest in these three bag types was approximately twice as high as that of the Lawson pollination bag. Bag type did not significantly affect seed yield per cone with the least squares mean among bag types only ranging from 73 to 78 seed per cone. Open-pollinated (OP) cones however, were significantly different than all bag types with a least squares mean of approximately 111 seed per cone.

Cone analysis was also performed to determine seed efficiency per cone, the proportion of filled seed out of the cone's seed potential. Number of first year aborted ovules, second year aborted ovules, filled seed, empty seed, fertile scales, and infertile scales were recorded for each cone. For seed efficiency per cone, bags performed similarly, but open-pollinated strobili were significantly different than all bag types for seed efficiency. The open-pollinated cones had a much lower proportion of first year aborted ovules and a higher proportion of filled seed than cones from all pollination bags. This finding was not unexpected and suggests that problems with pollen availability or viability exist in pollination bags that do not occur in open-pollinated cones.

The most interesting finding from the 2014 installation was the increase in cone survival for PBS-A, PBS-B, and the Lawson bag with a support wire. Stiffer/stronger bag material used in PBS-A and PBS-B or the addition of a support wire to Lawson pollination bags is believed to be the reason for better survival. With over 1.4 million pollination bags put up in the southeastern United States in both 2016 and 2017, this increase in cone survival will substantially increase seed production. The 2015 analysis is underway with preliminary results of cone survival per bag again suggesting significant differences between the Lawson bags and other PBS prototypes.

Keywords: *Pinus taeda*, seed orchard, cone analysis

WHY ARE INSECT PESTS MORE IMPACTING IN SEED ORCHARDS THAN IN SURROUNDING FORESTS? THE EXPERIENCE OF LONG-TERM SURVEYS OF PEST DAMAGE IN FRANCE

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Though having received little attention until large tree-improvement programs were initiated, insects exploiting seed cones during the pre-dispersal phase of seed development are nowadays considered as a critical factor susceptible to decrease the expected magnitude of seed crops. An updated review of the entomofauna associated with seed cones of conifers in Eurasia reveals approximately 170 native species of phytophagous insects, which belong to 7 different orders. In addition, a few species of exotic insects, essentially seed chalcids in the genus *Megastigmus*, have been introduced through unregulated seed trade.

About 70% of these insect species are highly hostspecific, being only capable to exploit cones and/or seeds of congeneric conifer species. The length of the reproductive cycles in conifers, i.e. from 1 to 3 years from flower bud initiation to seed maturation depending on species, offers large opportunities of development to different guilds of specialized insects, which may entirely exploit the seed cone niche and thus affect natural regeneration in forest stands as well as seed yield in seed orchards. Insect damage to seed crops in Europe, Russia, and China has been reviewed for the native species of genera *Abies, Cedrus, Cupressus, Juniperus, Larix, Picea, Pinus*, as well as for introduced conifers, e.g. *Pseudotsuga*. In most cases, insects appeared to limit seed crops at a large extent but damage importance, although largely fluctuating in space and time, was usually larger in seed orchards than in the surrounding stands of the same conifer species. Thus, insects frequently destroyed more than 50% of the viable seeds in the French seed orchards of Douglas-fir as well as in the Siberian larch seed orchards of Northern China.

Despite tree longevity, the production of viable seeds is concentrated upon a limited number of crops (key crops) in most conifer species because of both masting process and variation in seed viability with tree age. The effective impact of seed cone insects on natural regeneration thus depends on the temporal adjustment of insect population dynamics to these key crops, that could appear in an asynchronous pattern between seed orchards and their surrounding stands due to the management of seed orchards for flowering improvement (girdling, gibberelline injection, …). To cope with masting and space-time discontinuity in tree flowering, specialized insects have evolved adaptive patterns such as prolonged diapause (up to 7 years in some species). Such strategies however result in a lot of mortality, being only effective in preserving species but not populations when null crops occurs during several successive years. In seed orchards, with a provoked more or less regular flowering, such strategies could be not needed. Moreover, in case of null crops in stands surrounding a flowering seed orchard, the adult insects emerging there can be expected to migrate to the orchard.

To address these questions, the invasion of several French Douglas-fir seed orchards by host-specific seed chalcids migrating from surrounding conspecific plantations has been monitored for a 32-year period from initial flowering (1985) on. The orchard cone crops were entirely harvested every year to prevent the establishment of any resident chalcid population. Models based on passive diffusion of females emerging in the surrounding plantations with a downwind drift allowed to estimate the relative contribution of each plantation to orchard re-infestation. Results suggested that only a few plantations located within a radius of 5 km from the orchards contributed significantly. However, the relative contribution of each plantation strongly varied among years, depending on both the relative availability of seeds with regard to the numbers of adult insect emerging in the plantation and the wind patterns. Insects thus appeared to be fixed in the plantation when the crop is large there but the seed orchard could be completely infested when it presents a medium crop whilst the cone crop in the surrounding plantations dropped from large the year before to low/null the present year. Finally, a predictor for orchard infestation was proposed based on the mean ratio of cone crop size from one year to the next in the surrounding plantations. Several practices aimed at assisting the orchard managers were also suggested: orchard establishment in regions with a low density of Douglas-fir plantations, removal of plantations within a radius of 5 km of the orchard, annual harvesting of the orchard cone crop, notwithstanding its size.

However, these results concern a couple where both partners, tree and insects, are exotic. These highly specialized insects only face a few native competitors and natural enemies in the invaded area, and thus can occupy the total available niche space. It would be important to carry out a similar comparative monitoring seed orchard- surrounding stands using native tree species, e.g. *Picea* or *Pinus*, and their native cone and seed pests.

Keywords: Conifers, seed, cone, insects, damage, regeneration

"THREE OUT OF THE BIG FIVE": CHALLENGES IN PHEROMONE-BASED CONTROL OF MOTHS IN SPRUCE SEED ORCHARDS

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Three moth species, viz. *Cydia strobilella* (Tortricidae), *Dioryctria abietella* (Pyralidae), and *Eupithecia abietaria* (Geometridae), are among the five major pests in European spruce seed orchards and cause dramatic reduction in the seed yield. Current methods to suppress these pest insects, including chemical insecticides and application of *Bacillus thuringiensis*, are not reliable or efficient enough to prevent significant losses. In agriculture, in vineyards and in fruit orchards, sex pheromones constitute an economically efficient and environmentally friendly alternative to pest control by conventional insecticides. Similarly, sex pheromones have a great potential for monitoring and control of moth pests in spruce seed orchards.

We identified the female-produced sex pheromones of *C. strobiella, D. abietella*, and *E. abietaria*. Flight phenology of the three species was monitored using sticky traps baited with the synthetic sex pheromones. We furthermore attempted to correlate trap catch with cone damage in Swedish spruce seed orchards. Spruce flowering, potential seed crop, and cone damage, however, vary enormously between years. This makes it hard to establish a clear relationship between trap catches and variation in these variables, which limits the value of pheromone-based monitoring of the pests for practical purposes.

Sex pheromones may also be used for the direct control of pest insects. The mating behaviour of moths can potentially be disrupted by releasing high doses of synthetic sex pheromones. We performed mating disruption trials with *C. strobilella* and *D. abietella* and found that pheromone treatment almost completely shut down catches of males in pheromone traps. Larval presence and abundance in cones were, however, not always reduced by pheromone treatment. Trials to optimize the application of sex pheromones for mating disruption in spruce seed orchards are in progress.

The identification of sex pheromones for three of the major pests in spruce seed orchards and subsequent demonstration of their potential use for monitoring and mating disruption has set the scene for pheromone-based control of these moths. However, the successful implementation of such IPM-strategies involves several challenges and an element of *"Catch 22"*: The sex pheromones of *C. strobilella* and *E. abietaria*, albeit identified, are not commercially available. The lack of commercially available pheromone products prevents orchard managers from using the pheromones in control programs. If there is no market there will be no supply and with no supply there is no market! This obstacle has to be overcome in cooperation between pheromone researchers and orchard managers.

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Keywords: Sex pheromone, *Cydia strobiella, Dioryctria abietella, Eupithecia abietaria*, IPM

CONSEQUENCES AND MITIGATION OF PEST DAMAGE IN SWEDISH NORWAY SPRUCE SEED ORCHARDS

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Seeds from genetically improved Norway spruce (NS) orchards are in high demand for forest regeneration in Sweden. Annually, around 200 million NS seedlings are produced for planting. Although NS is quite easy to propagate by rooted cuttings, and perhaps soon by somatic embryogenesis, the vast majority of regeneration material will most probably come from seed orchards. In Sweden, the establishment of a third cycle of seed orchards is almost complete, with about 370 ha of NS orchard area. Seed from third-cycle orchards have an estimated genetic superiority in production at full rotation of 25%, with additional gains arising from improved stem quality. While these orchards are expected to produce 5 kg of seed per ha annually, resulting in 220 million seedlings, typically extreme annual variations in cone production can create a deficit of improved material, even though older orchards (first- and second-cycle with less genetic gain) remain in production and can contribute to seed crops. When good seed years occur, cones and seed are often heavily damaged by pests. Most devastating are attacks from four insect species, *Dioryctria abietella* (Spruce cone worm), *Cydia strobilella* (Spruce seed moth), *Eupithecia abietaria* (Spruce cone looper), and *Strobilomyia anthracina* (Spruce cone maggot), as well as infection by cone-rust fungus *Thekopsora areolata* (Cherry-spruce rust). A rough estimate over several years reveals that about 40% of the potential crop is destroyed by insects. During recent years, up to 70% of the cones have been infested by *T. areolata*. Lack of improved seed and seedlings results in large economic losses from future production. Stumpage value of improved seedlings in Sweden is estimated at 1,7 billion SEK per year during the second half of this century. A deficit of seed from a major species may also shift spatial species distribution negatively.

To mitigate the deficit of improved NS seed, a Research and Application Program was initiated in 2016 by The Swedish Tree Breeding Association, in collaboration with the Seed Orchard Owners. The goal is early and abundant flowering and seed production in orchards, and pest management to minimize crop losses. This should be achieved by:

1. implementation of existing know-ledge and methods;
2. development of routines for adaptive management; and
3. new and co-organized research for both flower stimulation and pest control.

Researchers and orchard managers will collaborate closely to achieve the objectives. Experience and knowledge from similar orchard conditions in neighboring countries will be reviewed and utilized. The program is also used as a platform for proposals and co-funding of additional supportive research projects. Initially, a web-based “Best practice manual” is being made available from current knowledge and experience, then continuously updated through the 4- to 6-year program period.

Keywords: *Picea abies*, seed demand, pest control, flower stimulation, research program, adaptive management

PHEROMONE-BASED MATING DISRUPTION OF PEST INSECTS IN EUROPEAN SPRUCE SEED ORCHARDS

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Picea abies seed orchards have been established in Europe with the purpose of producing high and stable seed yields to meet market requirements. The yields from such orchards, however, are often low and irregular, and an important reason for this insufficient seed supply is damage caused by cone-feeding insects. The moths *Cydia strobilella* (Tortricidae) and *Dioryctria abietella* (Pyralidae) are among the worst pests in spruce seed orchards because their larvae consume the seeds or other parts of the spruce cone. Infestation rates of these species can reach 80-90% and there are currently no efficient control methods available. Many moths communicate with long-range sex pheromones for mate finding and high doses of synthetic pheromones can potentially disrupt the mating behaviour of pest species, providing a green alternative to traditional chemical pesticides for population control. We have previously identified the female-produced sex pheromone of C. *strobilella* (Löfstedt et al. 2012) and D. *abietella* (Wang et al. 2010)and used these compounds in small-scale mating disruption experiments, were we have altered the type of dispenser, the density of dispensers, the amount of pheromone per dispenser, and the position of the dispenser in the spruce trees. Although captures of males in pheromone-baited monitoring traps have been drastically reduced in treated areas versus control areas, a subsequent significant reduction of cone infestation has rarely been observed. In recent years, we have used a wax formulation (SPLAT) for release of the active compounds to control both pest species. Wax droplets have been applied as 2 g point sources at the upper part of spruce trees. To evaluate the efficacy of the treatment, we have analysed catches of males in pheromone traps and larval presence and abundance in sampled cones. The results from these experiments will be presented.

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Keywords: Pest management, spruce seed orchard, *Cydia strobiella, Dioryctria abietella*, pheromone

IS SEED YIELD IN SEED ORCHARDS AFFECTED DIFFERENTLY IN THE PRESENCE OF ONE INSECT SPECIES OR A SUBSET OF SPECIES? PRELIMINARY RESULTS FROM FRANCE AND SWEDEN 2015-2016

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Seed orchards of coniferous tree species have been established in many countries to produce high quality seeds for forestation. However, the seed production in seed orchards is far below the demand, and one reason for the low and unpredictable seed production is damage caused by insects. Currently, only one insecticide is registered for use in seed orchards in both Sweden and France, and this is not active against all occurring pest species. The project in focus here aims to explore if it is a risk that pesticide targeted against certain pest species can affect other pest species through relieved intraspecific competition. This is an important issue in pest control in general when using more specific insecticides or pheromones (as opposed to broad-spectrum insecticides), when several species frequently inhabit the same resource. The risk is that species not or less affected by the insecticide can increase its feeding, resulting in a low effect of the treatment on seed yield and seed quality. To study an effect on yield and quality of seeds, strobili (female spruce flowers that will develop to cones) were protected by fine mesh bags from early season. We then inoculated the strobili with eggs of *Cydia strobilella* in two different densities and later the cones with larvae of *Dioryctria abietella* also in two densities. We also inoculated them with a combination of the two species in order to see if that had any effect on seeds. The preliminary results mainly focus on seed yield measured as easily extractable seeds, since seeds damaged by insects often get stuck to the cone by resin or due to damaged scales.

Keywords: *Picea abies*, spruce seed orchard, insecticide, pest management, cone and seed insects

BIOLOGY OF CONE PATHOGENS OF *Picea – Thekopsora* AND *Chrysomyxa* CONE RUSTS

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Cone rusts reduce significantly seed production especially in seed orchards of *Picea* (Southerland et al. 1987; Singh & Carew 1990). We investigated the seasonal sporulation of cone rusts in *Picea abies* cones after an excellent cone crop in seed orchards and sample areas. The effects of cone rusts on various *Picea* hosts and alternate host plants *(Prunus, Pyrola)* were also investigated for a decade. *Thekopsora areolata* was the most significant cone rust that destroyed most of the cones of *P. abies* in several seed orchards in 2006 (Kaitera, 2013). The rust colonized systemically infected cones and formed fruiting stages in cone scales in the first year in mid- and late summer (Kaitera, et al. 2009). These aecia sporulated 1-4 years later (Kaitera & Tillman-Sutela, 2014). The number of seeds and germination of seeds were significantly reduced in infected cones. The rust sporulated on a wide range of susceptible *Picea spp*. (Kaitera, et al. 2014, 2017). Another pathogenic rust, *C. pirolata*, destroyed ccasional cones of P. abies, where it colonized systemically most of the infected cones. The rust fruited and sporulated in the first year in cone scales (Kaitera, et al. 2009). A previously unknown *Chrysomyxa* rust fruited and sporulated in cone scales of *P. abies*, where it was identified as a member of the *C. ledi*/*C. rhododendri* complex (Kaitera, et al. 2010). The rust infected mainly single cone scales in infected cones of many *Picea species*, but occasionally the number of infected scales was high on *P. abies* (Kaitera, et al. 2017). The rust did not colonize entirely the infected cones or reduce the number of seeds, and therefore, had little effect on seeds. Therefore, rust control should be focused on *Thekopsora areolata* especially in seed orchards. Such control might be possible by removing infected cones from live trees along routine cone collection. The other pathogenic rust, *C. pirolata*, should be controlled only in seed orchards with high *C. pirolata* incidence, while C. ledi does not have to be controlled at all.

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Keywords: Cone rusts, *Chrysomyxa, Picea*, seed orchards, *Thekopsora*

INTEGRATED PEST MANAGEMENT EXPERIENCE AND FUNCTIONALITY IN CONE CROP PROTECTION IN FINLAND

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The investigation of several years of cones revealed the most damaging agents of cone crops in Finnish seed orchards. The most important and common insects were cone moth *Dioryctria abietella* and spruce seed moth *Cydia strobilella*. Their infestation rates varied from 10 or 80 percent from year to year. *Eupithecia* cone loopers can be partly responsible for the damage caused by *D. abietella*. Spruce cone maggot, *Strobilomyia antracina* was also frequently encountered but it was relatively local. In general, of the known seed parasitoids, *Megastigmus strobilobius* was more common than *Plemmeliella abietina*. The cherry spruce rust *Thekopsora areolata* was also common in the cones and reached epidemic levels in 2006.

There is a challenge to find suitable insecticides for such a minor use as forest tree seed production. In Sweden a biological product Turex WP 50 was found to be efficient against *D. abietella* and *Eupithecia abietaria* (Glynn & Weslien, 2004; Rosenberg & Weslien, 2005). In 2015 Turex WP 50 was approved in Finland for use in spruce seed orchards against lepidopteran pests. Nevertheless, it was not a solution against specialist seed predator *C. strobilella*. In 2014 an effort was made to test for two insecticides that targeted *C. strobilella* eggs. They are laid to the female flowers before the scales close and cone starts to develop. There were, however, practical difficulties in the application of the insecticides largely due to varying weather in the spring of 2014. Calypso SC 480 (a.i. thiacloprid) showed a minor, statistically not significant, tendency for lower infestation rates of *C. strobilella* than Steward WG 300 (a.i. indoxacarb) or control. Therefore, these insecticides were tested again in 2017 and the flowering phases were simultaneously recorded. Preliminary results will be presented.

Today plant protection calls for an integrated pest management (IPM) approach. Therefore, in the Nordic countries a collaborative effort was made to monitor the cone and seed insects to be able to determine threshold levels and timing of the potential insecticide applications. Species specific pheromones for *D. abietella* and *C. strobilella* had been recognized and synthetically produced in Sweden (Wang, et al. 2010; Löfstedt, et al. 2012). Initially, white delta traps baited with synthetic female pheromones were set at height of 1.5 m in spruce seed orchards. In Finland the trap captures were low despite the high damage rate observed in the cones. Majority of spruce seed orchards monitored were close to 40-50 years in age except for one that was 15-20 years .

As the flowers and cones grow in the upper part of the tree crowns, different trap heights (1.5 m, 3 m, 6 m, 9 m and 14 m) for both *D. abietella* and *C. strobilella* were tested in an old and a young spruce seed orchard where the tree tops reached 16 - 19 m and 7 - 9 m in height, respectively. In the old orchard *D. abietella* males were caught mostly at 14 m, only some at 9 m and none at 1.5 m. *C. strobilella* males were equally attracted to traps at 1.5 m and 14 m but *C. strobilella* catch at 9 m was more than double the catch at 1.5 m and at 14 m. In the new orchard the greatest catch of *C. strobilella* was at 9 m and almost zero at 1.5m. *C. strobilella* was not caught above the canopies and, in addition, only few *D. abietella* males were found at 14 m. The *D. abietella* catch at 9 m was small and the catch at 1.5 m was four times greater than the one at 9 m. Finally, comparisons among trap heights of 1.5 m, 3 m, 6 m and 9 m revealed that greatest trap captures for both species were at 6 m. *D. abietella* tend to fly and be attracted to pheromones higher from the ground than *C. strobilella*.

More knowledge is needed on the correlation of the damage rates and trap captures in space and time. Currently the knowledge of IPM potential is being transferred to seed orchard managers.

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Keywords: *Picea abies*, insecticide, seed predator, moth, integrated pest management

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GENOTYPIC DIVERSITY AND REPRODUCTIVE BIOLOGY OF *Thekopsora areolata*, THE CAUSAL AGENT OF CHERRY SPRUCE RUST IN NORWAY SPRUCE SEED ORCHARDS

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Swedish forestry is sustained by yearly planting of about 200 million seedlings, produced in seeds orchards planted with superior trees. The seed orchards are intensively managed to produce large quantities of high quality seeds. However, today there are a deficit of plants origin from Swedish seed orchards mainly because irregular flowering as well as pest and pathogen infections.

The fungal pathogen *Thekopsora areolata* causal agent of cherry spruce rust heavily affects Norway spruce seed production. Using newly developed microsatellite markers and a hierarchical sampling strategy, population structure and reproductive mode of *T. areolata* were investigated. From one location in Norway, two locations in Finland and five locations in Sweden one aecium per infected cone were analysed. While in two locations in Sweden multiple aecium were sampled per scales, cones and locations. The results show an over-all high genetic diversity in *T. areolata* at all hierarchical levels with no genetic structure indication high gene flow and random mating. At the cone/scale level non-random mating were observed for which several distinct hypothesis will be presented. Future research directions and management implication will be discussed.

Keywords: Epidemiology, population genetics, *Thekopsora areolata*, Norway spruce, cone, rust

BAD SEED IS ROBBERY OF A WORST KIND...

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Trees often produce large quantities of unproductive seeds, that is, dead, empty or insect-infested seeds. In some species, these unproductive seeds are sometimes difficult to separate out from productive seeds during processing, resulting in poor quality seed lots. In addition, tree seeds are often dormant, requiring stratification to germinate rapidly and uniformly over a wider temperature range. These traits present specific challenges to seed traders and nurserymen, influencing the cost-effectiveness and efficiency of plant production in nurseries. This presentation deals with three case studies:

1. European silver fir (*Abies alba* Mill.) produces seed crops that often have a high proportion of empty seeds. These empty seeds sometimes have thickened seed coats or contain brown-black material that makes separation of filled and empty seeds very difficult. A three-stage process known as imbibition-drying-separation (IDS), however, can be used to improve tree seed lot quality. This process exploits differential drying, and thereby, creates a window of opportunity where these categories of seeds can be separated using conventional means. In this particular case, a poor-quality seed lot had an initial germination capacity of 64%, which increased to 81% after upgrading by IDS. This process, therefore, enabled seeds to be sown in plug trays, resulting in lower labour costs in the nursery. Thus, IDS can be used to improve poor-quality seed lots, which would otherwise not be commercially viable for seedling production in container nurseries.
2. European ash *(Fraxinus excelsior)* is under severe threat from an emerging fungal disease (ash die back), which is caused by *Hymenoscyphus fraxineus*. This pathogen has been detected in seeds, indicating a potential pathway for the introduction of the disease into new areas. Hot-water treatments, however, have been used successfully to eradicate diseases based on the principle that pathogens are killed at temperatures that are not harmful to seeds. In this case, seed quality tests including tetrazolium, excised embryo, and germination tests showed that ash fruits can survive 5 hours at 44ºC with minimal damage to the seeds. This temperature-time exceeds that previously shown to eradicate *Hymenoscyphus fraxineus* in ash seedlings. Thus, hot-water treatments can provide a simple, cost-effective management tool, potentially enabling disease-free plants to be raised from infected ash fruits.
3. Douglas fir *(Pseudotsuga menziesii)* seeds are often relatively or conditionally dormant. Relatively dormant seeds do not require a pre-chill but often germinate slowly or erratically over a narrow temperature range. After a short pre-chill (3-5°C), these seeds germinate quicker and more uniformly over a wider temperature range. Usually, Douglas fir seeds are pre-chilled for a few weeks, often 3 weeks, before sowing in nurseries, but the optimal pre-chill duration is much longer, ranging from 25-48 weeks. In this particular case, a 12-week pre-chill promoted earlier and more uniform germination of Douglas fir seeds in the poly-tunnel. Thus, longer pre-chills can extend the growing season by several weeks, potentially enabling the production of 1.0 planting stock.

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Keywords: Seed processing, seed testing, nursery production

GERMINATION OF SCOTS PINE SEEDS AFTER FREEZING OF HARVESTED CONES *IN VITRO*

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Fundamental physiological change in life span of orthodox seeds is their dehydration at the final stage of development (Kermode, et al. 1986). In a dry state, seeds can tolerate a number of diverse environmental conditions, including extremes in temperature. For Scots pine in Fennoscandia, where temperatures below –30°C are common during winter months, this is critical as seeds must simultaneously mature and become freezing resistant in order to survive the harsh winter conditions.

During seed ripening and over-wintering phase, Scots pine seed development can be disturbed or interrupted by negative temperatures, leading to seed damage and low germinability. In material collected from northern Sweden, Kardell (1974) demonstrated that in natural environments the proportion of Scots pine seed with damaged embryos – as determined with tetrazolium test – increased from zero in September to 53% at maximum in February. In a later study, however, he found that during the period from the beginning of September to the middle of October, freezing of collected cones at temperatures of –5°C, –10°C and –15°C for two, four and eight hours gave an increased germination. In the following year, same combinations of time and temperature caused both embryo damage and decreased germination (Kardell, 1976). With these contradictory results on the role of sub-zero temperatures on Scots pine seed maturation, optimizing the timing of cone collections is problematic.

The objectives of the current study were to evaluate if cone water content could be used to predict the water content of the seeds inside them and to test cold hardiness of harvested Scots pine seeds while still in cones to determine how their subsequent germination and possible freezing injury depend on cone water content.

For the study, Scots pine (*Pinus silvestris* L.) cone and seed water content were analyzed in two consecutive seasons during maturation stage in the autumn and during two winter months before seed dispersal. Cones with different water contents were subjected to 2 h freezing at –30°C and seed viability (as based on seed X-raying and esterase activity measurements) and laboratory germination after treatment were analyzed.

On each collection, seed water content followed closely to that of cones and the general relationship could be described with a logistic function. On average, cones had 5–10%-units higher water content than the seeds inside them. The higher the cone water content, the higher was the probability that at least one seed per cone was damaged and the lower was the germination after freezing treatment. Critical cone water content for zero germination after freezing at –30°C was approximately 35% (fresh weight basis). This corresponds with 22.5% water content in seeds.

These results stress the importance of maturation drying in order that the seeds could attain hardiness for winter months. It is suggested that monitoring cone water content should become a routine analysis in seed orchards and used as an indicator of both seed ripening and the development of frost tolerance of seeds.

Keywords: Freezing tolerance, maturation drying, seed moisture, x-ray, esterase activity

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TRANSCRIPT PROFILING DURING EARLY CONE DEVELOPMENT IN NORWAY SPRUCE

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Conifers, ginkgo, cycads and gnetophytes comprise the four groups of extant gymnosperms holding a unique position of sharing common ancestry with the angiosperms. Comparative studies of gymnosperms and angiosperms are the key to a better understanding of ancient seed plant morphologies, how they have shifted over evolution to shape modern day species, and how the genes governing these morphologies have evolved. In the current project we utilize recent developments in nucleic acids sequencing technologies to unravel the transcript profiles underlying early cone development in the conifer Norway spruce *(Picea abies)*. Cone development in Norway spruce spans almost one calendar year. In order to follow the transcriptional changes that occur in the different bud-types we have collected male- female and vegetative buds throughout the growth season and analysed the mRNA and smallRNA transcriptomes using Illumina Next generation sequencing. The data generated within this project will be deposited in ConGenIE database and made available for further comparative studies. Growth and development of male-, female- and vegetative lateral organs are sequentially initiated from distinct bud meristems during the summer months. Hence, by collecting buds at different time points it is possible to discriminate between male- female- and vegetative buds before lateral organ development commence in late summer or early autumn. By comparing transcriptomes between these early stages of bud development we aim to identify genes of importance for male- female- and vegetative meristem identity, as well as the genes responsible for lateral organ development in the different bud-types. Of specific interest in this context are sets of proteins that belong to the MADS-box gene family of transcription factors. MADS box genes have been implicated in regulating reproductive organ development in both angiosperms and gymnosperms. In a preliminary study we have identified MADS-box genes and their alternatively spliced isoforms in Norway spruce. We constructed a phylogenetic tree from sequence alignments of *Picea abies, Pinus radiata, Arabidopsis thaliana and Solanum lycopersicum*. The phylogenetic analysis showed that, four PaMADSX splice variants such as *PaMADSX_AC, PaMADSX_BC, PaMADSX_AD, PaMADSX_BD* together with other gymnosperms MADS-box genes form an orthologous sister clade to the floral integrator *SOC1* in angiosperms. qRT-PCR experiments indicate that the different *PaMADSX* splice variants are differentially expressed in different bud types and that all *PaMADSX* splice variants are induced by Gibberelic Acid (GA) suggesting that *PaMADSX* can be used as a molecular marker for successful cone-inducing GA-treatments.

Keywords: Norway spruce, cone development, transcript profiling, MADS-box genes, gibberelic acid (GA), cone inducing

DIFFERENCES IN SEED MATURATION TIME OF NORWAY SPRUCE CLONES AFFECT GERMINATION ENERGY OF SEED LOTS

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Cone collections of Norway spruce *(Picea abies)* start typically in September in Finnish seed orchards. Early start is favored by cone collection entrepreneurs due to easier collection conditions. Cone samples are occasionally collected and inspected beforehand, but the intention is often to check the extent of possible pest damages, and maturity evaluation is limited to anatomical maturity assessment by radiography. Therefore, there is a risk that seeds may not yet have attained full, physiological maturity in the earliest collections and this may affect seed quality.

In the oldest Norway spruce seed orchards clone numbers may exceed 100, but in the newest clone numbers are closer to 20. As the clone numbers decrease, it is increasingly important that a large – and known – proportion of them contribute to the final seed and seedling lots to ensure sufficient genetic diversity for forest regene-ration. We hypothesize that poorly timed cone collections may have a detrimental effect for seed quality (i.e. germinability) and that clones may mature at different times. This may cause unintentional and uncontrolled effects for genetic diversity as seedlings of slowly germinating clones are at risk of becoming poor quality seedlings (Himanen & Nygren, 2014). To create a practical tool to assess clone maturity, cone water content was studied as an indicator for the development of germinability.

Cones were collected from a first generation seed orchard (Sv 403, Suhola) in central Finland on seven occasions from mid-September 2012 to March 2013. Five clones were selected on the basis of approximately the same latitudinal location of plus trees and sufficient flowering in spring 2012 to produce enough healthy cones to be collected. From each clone, five ramets were selected. Cones that were severely damaged by insects or diseases (mainly by *Thekopsora areolata*) were excluded from the collections. In the laboratory cone water content was measured from one eight of each cone according to International Seed Testing Association (2007) guidelines. Seeds from the remaining cone were extracted and approximately 50 seeds from each cone were germinated on Petri dishes on moist blotter paper in a germination cabinet at 20°C for 21 days. The remaining seeds from each cone were stored in airtight containers at +4°C. In May 2013 germination test was repeated with the stored seeds. By that time, the seeds from the first cone collection had been stored at +4°C for eight months and from the last collection 2.5 months.

Cone water content of the clones decreased dramatically, but not uniformly between the first (September 17) and the fourth (October 29) collection time. On October 1, there was a 15%-unit difference in water content of the cones between the fastest and slowest maturing clone. By the fourth collection time, the decrease leveled off and the differences in cone water content between clones disappeared excluding one clone. In winter between January and March there was, however, an additional slight decrease in cone water content of all clones.

Germination percentage energy (day 7) and capacity (day 21) increased steadily as cone water content decreased in all clones. However, variation between clones was considerable, i.e. a certain level of cone water content did not indicate the same level of germinability in all clones. For example, clone E456D reached 80% germination energy already on September 25 at the cone water content of 39%, whereas clone E246 did not reach this until November 26 at the cone water content of 27%. Short storage at +4°C adversely affected the germination energy of all clones and the differences in germination between fast and slow maturing clones were exacerbated (Figure 1).

According to the study material, cone water content predicts the development of germination energy and can be used as a tool in operational seed orchards management to help to assess the correct collection timing. However, the same water content does not indicate the same germinability in all clones and therefore several clones must be sampled.

The large differences observed between the maturation timing between the clones raise the question should clones be collected at different times to ensure the highest possible germinability for seed lots. The current practice of collecting cones mainly early in the autumn may cause unintentional and uncontrolled reduction in the presence of some of clones in the seed crop and seedling lots as slow germination is linked to an increased chance of the seedling to be of poor quality and being culled from the marketable seedlings.

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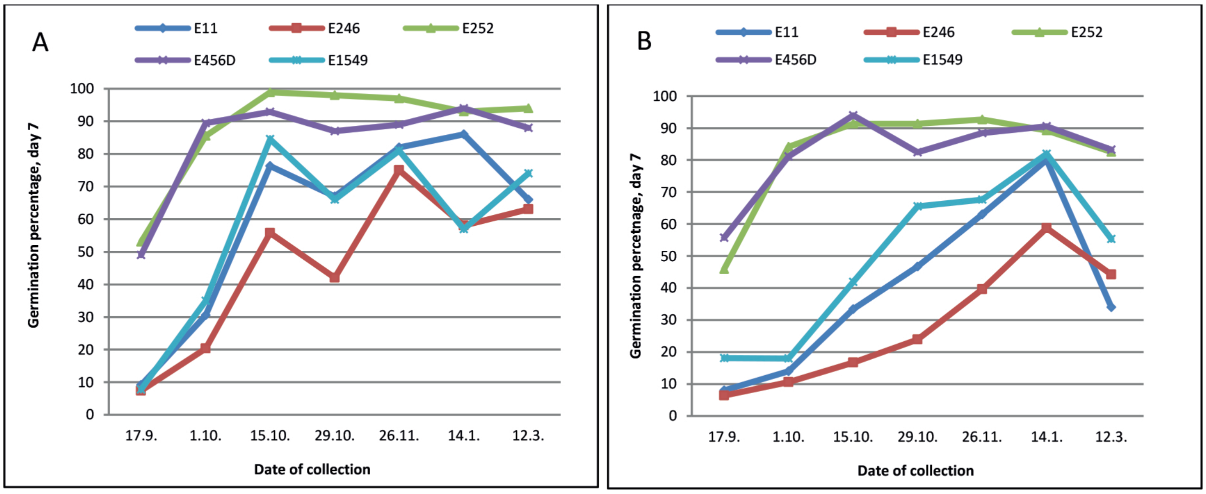

Figure 1. Laboratory germination percentage on day 7 of five Norway spruce clones (E11, E246, E252, E456D and E1549) collected at seven different occasions from a 1. generation seed orchard in Finland. Figure 1A presents germination right after collection and 1B the germination after a short term seed dry storage at +4 °C

Keywords: Genetic diversity, germinability, seed collection, seed maturity

SEED ORCHARD – LINKING BASIC RESEARCH AND FOREST PRODUCTION

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A seed orchard is an intensively managed population of selected plus trees that are used to produce genetically improved seeds at large operational scale. More than a half century has passed since the first seed orchards were established for forest production, and presently it still represents the most cost-effective strategy for increasing forest harvest potential of conifer species. In Sweden the advanced pine and spruce seed orchards are predicted to produce seeds with about 25% greater volume production than unimproved seeds. In seed orchard management, the major concern has been on balancing genetic gain with genetic diversity, e.g. by varying the number and kinship of parents, the frequency of each parents, and the spatial planting of the orchard. The effects of these designs on the genetic quality of seed crops are often assessed by marker-based mating system analyses that evaluate reproductive success of the parents, and pedigree structure and diversity in seed crops. If gametic contribution and mating success among parents deviate significantly from the theoretical expectation, a considerable part of the expected production gain and diversity will be lost. Mating system in seed orchards thus has been a subject of active genetic research; results from this field have not only provided valuable reference to orchard establishment and management operations, but also improved our understanding of the reproductive biology and genetic control of embryo abortion in many tree species.

The deployment of seed orchard crops in large–scale reforestation has generated public concerns for long-term ecological consequences to reginal forests. This becomes particularly important under climate change scenarios to forecasting the response of orchard crops to local conditions. Forest trees show strong evidence of local adap-tation to discrete habitats and continuous environmental gradients. Seed orchard operations on one hand can assist gene flow from a different climate regime, by translocation crops across climate zones, to speed up the adaptation of local forests to changing climate. On the other hand, high levels of gene flow between populations from different environments can counteract divergent selection, resulting in the dilution of locallyadapted alleles. Understanding and predicting these different evolutionary outcomes requires extensive empirical testing, and seed orchard materials with known pedigree information offer a powerful opportunity for this examination. Seed orchard crops can be tested in manipulative conditions in the lab and transplanted into multiple sites across environmental gradients to determine the growth and fitness response of seedlings of different genetic background (mating types and pedigree). Such projects require close collaboration between researchers and breeders to establish experimental systems that allow long-term follow-up studies. Genomic resources that are available for major forest species (e.g. whole exome data) will greatly assist with the understanding of geneticenvironmental interactions that shape adaptive divergence between populations, and the breeding and deployment strategies to meet future challenges.

Keywords: Climate adaptation, genetic diversity, gene flow, mating system, seed crops

DIVERSE FECUNDITY IN WILD CHERRY SEED ORCHARDS AS A CONSEQUENCE OF SPATIAL-GENETIC VARIANCE

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Wild cherry (*Prunus avium* L.), belonging among the top rated hardwood timbers, exhibits gametophytic self-incompatibility controlled by the multi-allelic S-locus. This mechanism promotes out-crossing and prevents self-fertilization (Vaughan, et al. 2007). Besides sexual reproduction though insect-mediated pollination, wild cherry often propagates vegetatively via suckering (Jolivet, et al. 2013). Therefore, the genetic structure analysis and consequent optimization among candidates for tree breeding activities (e.g. seed orchard establishment) or an appropriate treatment of already established seed orchards is needed.

Two wild cherry seed orchards with the contrasting difference in fecundity (35 kg versus 364 kg of annual crop production) but resembling in other characteristics such as age, occurrence of pollen contamination source, flowering and availability of pollinators were subjects of the study. The seed orchard with the low level of seed production (SO 1) comprises from app. 300 trees formally belonging among 59 clones with an effective number of clones (Kang, et al. 2001) equal to 31.9. The seed orchard with the satisfactory level of fecundity (SO 2) composed from app. 200 trees divided into 28 clonal groups and an effective number of clones being 25.8.

Genetic analysis of seed orchards utilizing 16 SSRs loci and S-locus polymorphism revealed an unexpected distortion of the genetic architecture, which resulted in significant reduction in the effective number of clones being approximately double in SO 2 compare to SO 1. Additional reduction in fertility of SO 1 is attributable to the seed orchard structure (clumps of identical genetic units) due to forage preferences of bees (Cartar and Real, 1997).

In conclusion, diverse fecundity of seed orchards was explained by intrinsic spatial genetic pattern and the study proved the importance of *apriori* evaluation of genetic composition among candidates for tree breeding activities.

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Keywords: *Prunus avium* L., seed orchard structure, fecundity, S-locus, SSRs

GENETIC GAIN AND GENE DIVERSITY OF SEED ORCHARD CROPS

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Seed orchards are the major tool for deploying the improvement generated by breeding programs and assuring the consistent supply of genetically improved seed. Attainment of genetic gain and monitoring of gene diversity through selection and breeding were studied considering the factors: selection intensity; genetic value; coancestry; fertility variation; and pollen contamination. The optimum goal of a seed orchard is achieved when the orchard population is under an idealized situation, i.e., panmixis, equal gamete contributions from all parental genotypes, non-relatedness and no pollen contamination. In practice, however, due to relatedness among parents, variation in clonal fertility and ramet number, and gene migration from outside, the realized genetic gain and gene diversity deviate from the expectation. In the present study, the genetic value of seed orchard crops (genetic gain, *G*) could be increased by selective harvest, genetic thinning and/or both. Status number *(Ns)* was used to monitor the loss of gene diversity in the process of forest tree domestication, and calculated to be reasonably high in most seed orchards. Fertility of parents was estimated based on the assessment of flowering or seed production, which was shown to be under strong genetic control. Variation in fertility among orchard parents was a general feature and reduced the predicted gene diversity of the orchard crop. Fertility variation among parents could be described by the sibling coefficient (Ψ). Ψ was estimated to be 2 (CV= 100% for fertility). In calculating Ψ, it was possible to consider, besides fertility variation, the phenotypic correlation between maternal and paternal fertilities, and pollen contamination. Status number was increased by controlling parental fertility, e.g., equal seed harvest, mixing seed in equal proportions and balancing parental contribution. By equalizing female fertility among over-represented parents, it was possible to effect a favorable tradeoff between gene diversity and seed production. If the status number of orchard crop is not large enough, loss of gene diversity, random drift in gene frequency and potential inbreeding problems could occur in subsequent generations. Genetic loss or erosion did not seem to be alarming during the domestication of forest trees, because a large number of parents are commonly used in first-generation seed orchards. An understanding of reproductive processes and monitoring of the impacts of the management practices are essential to maximize genetic gain and to maintain sustainable gene diversity in seed orchard programs

Keywords: Status number, effective population size, group coancestry, inbreeding, fertility variation, ramet variation, gene migration, sibling coefficient

MATING SYSTEM TEMPORAL VARIATION IN A COASTAL DOUGLAS-FIR SEED ORCHARD

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As a key component of the tree improvement delivery system, seed orchards play a significant role in packaging the genetic gain and diversity captured through breeding. To reach this goal, seed orchards are expected to function as a closed, perfectly random mating population, which, in reality, is rarely fulfilled due to parental fertility variation, reproductive phenology asynchrony, and gene flow (pollen contamination). The actual performance of the mating system within the orchard is thus of primary interest. We studied the mating system dynamic of a second generation, wind-pollinated, coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seed orchard over a four-year period to obtain information under various combinations of seed crop management practices such as supplemental mass pollination and/or bloom delay (overhead cooling) under different cone-crop sizes (high vs. low). This study utilized microsatellite markers to generate DNA fingerprints of the seed orchard's parental and offspring (4 years seed crop) populations, respectively. The DNA fingerprinting data were used to assign the seed crop parentage through pedigree reconstruction. The parentage assignment permitted the determination of parental gametic contribution, selfing rate, and the extent of external gene flow. Among the studied 4 years, parental contribution for 80% of the gametes ranged from 37 to 64%, selfing rate ranged between 12 and 17%, and pollen contamination ranged between 10 and 28%. These results provided evidences for persistent fertility variation, greater than usual selfing rates, and various levels of pollen intrusion and these estimates were affected by the various crop management practices implemented.

Keywords: Douglas-fir; seed orchard; pollen contamination; pedigree reconstruction; microsatellites

CLONAL VARIATION IN STROBILUS AND CONELET PRODUCTION AND ITS EFFECT ON GENETIC DIVERSITY OF SEEDS IN THE SECOND GENERATION SEED ORCHARD OF KOREAN BLACK PINE (*Pinus thunbergii* Parl.) IN SOUTH KOREA

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The tree population means not only the concept of a group of trees but also the genetic group for study of gene diversity between clones or its generations. The genetic variation in population is effected mainly by population size, fertility variation and mating system. Seed orchard consists of selected trees from the base or breeding population with the aim of producing improved seed. Most seed orchards show the large variation in flowering ability between species, age and clones. Unequal parental contribution could be a serious problem to genetic diversity in seed production and may influence the genetic gain. So continuous research of fertility variation, effective population size, heritability and parental balance is important to estimate genetic diversity of seed in the seed orchards.

This study was performed to survey the genetic diversity of seeds in the second generation seed orchard of *P. thunbergii* established at An-myeon Island, South Korea in 2006. The size of orchard was 1 ha with 5 m × 5 m spacing. There were 26 combinations and the entire number of ramets was 308 (at least 3 to 16 ramets per combination). The objectives of this study were to survey the variation of female, male strobili and seed conelet production among combinations, and to estimate effective population sizes (gene diversity) and genetic parameters (variance component, heritability and correlation) based on the annual variation of strobilus and conlelet production among clones.

The numbers of female, male strobili and conelet were surveyed for two consecutive years (2015 and 2016). The number of female and male strobili were counted on the entire ramets from 26 combinations. The averages of female strobili were 164.6 and 187.1 and those of male strobili were 2,257 and 5,113 each year. The multiple range analysis of Tukey HSD showed that there was a larger difference in male strobilus production than female strobilus among combinations. The conelet production was classified as nine groups in 2015 and seven groups in 2016. Especially, the average of conelet production was not much difference among combinations in 2016, showing 17 combinations were grouped as the same one.

The effective population size (i.e., status number) was larger than 20 for strobilus and conelet in both studied years except only for male strobilus in 2015. The broad-sense heritabilities of male strobilus and conelet were 0.236 and 0.238 in 2015, and 0.140 and 0.170 in 2016, respectively. The broad-sense heritability of female strobilus was higher in 2016 (0.194) than 2015 (0.068).

The correlation between female, male strobilus and conelet production was positive and statistically significant among clones. The correlations between volume growth data from KFRI in 2009 and strobilus production, and volume growth and conelet production did not show significant differences. These results implied that volume growth and conelet production are independent traits each other. A consist monitoring of reproductive characteristics could be needed for the selection breeding of advanced generation.

The results would be useful for efficient management of seed orchard such as stable, mass production of seed and for basic information of the establishment of advanced generation seed orchards.

Keywords: Korean black pine, second generation seed orchard, flowering, conelet, gene diversity, status number

MONITORING MATING PATTERNS IN SEED ORCHARDS: ADJUSTING EXPECTATIONS TO REALITY

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Open pollinated seed orchards are established with the expectations of limited background pollination and random mating among genotypes present in an orchard. Any departures from these assumptions likely reduce genetic gains predicted from seed orchards. Therefore the knowledge on mating patterns in seed orchards can be useful in assessing the utility of seed lots from a particular orchard, but also it may be useful in designing new guidelines for seed orchard establishment. The interest in monitoring mating patterns in seed orchards with the aid of genetic markers started more than three decades ago and it stimulated a number of studies building our current knowledge in this field. However, various statistical approaches and different genetic markers often provided contrasting and unreliable estimates of background pollination or other mating parameters, and the determinants of mating patterns within seed orchards remain still poorly understood. Nevertheless, with the aid of refined statistical mating models that account for genotyping problems and already available genetic markers, new insights into the factors underlying background pollination, as well as male and female fertility variation in seed orchards are readily possible, hopefully extending our knowledge on seed orchards' functioning.

Keywords: Mating system, gene flow, fertility variation, genetic markers, seed orchards

THE USE OF NEW METHODS IN EVALUATING THE GENETIC VALUE OF THE PROGENY OF SEED ORCHARDS

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In Poland, seed orchards, as an essential part of forest seed base, were initially established in early 1970s. Their importance gradually increased over time, and the principles of establishing and manging these facilities were simultaneously improved. In the late 1980s, a detailed methodology for establishing clonal and seedling seed orchards was developed and implemented into practice. Those principles are still being applied nowadays. An original method of distributing ramets and seedlings was developed. In order to preserve the genetic variability of a progeny, a minimum number of clones was adopted - 40 for pine and spruce and 30 for other species. Depending on the category of seeds orchards, three or four schematic fellings are planned, with a target spacing of 12 x 12 m. The distribution of ramets in clones and of seedlings in progenies is random, with minimum distances retained in clones and seedling in families. Initially, the dispersion of plants was randomly drawn by hand and since the 1990s by a designed computer program (J. Zajączkowski, unpublished data), taking into account the randomness of distribution and minimum distances between specimens. From the theoretical point of view, the adopted principles of establishing and managing seed orchards seem to fully meet the adopted assumptions concerning the genetic diversity of consecutive forest generations. However, in practice, it emerged that the genetic diversity and the share of undesirable genotypes among the progeny are modified to a varying degree by genotypes being alien for the plantation, errors in plantation layout and influx of alien pollen. The currently available research methods have made it possible to verify the correctness of establishing seed orchards and information about their breeding value. In Poland, the assessment of the distribution of pine grafts in pine seed orchards is conducted based on the variability of molecular markers (cpDNA: Pt 26081, Pt. 45002, Pt. 36480, Pt. 30204, Pt. 15169, Pt. 71936, PCP 1289, PCP 41131, PCP 87314. The laboratory that carries out the analyses is the Forest Gene Bank in Kostrzyca, and the obtained data are used by the Forest Research Institute to plan future felling. To date, all strains on 4 plantations have been verified, obtaining a variable level of errors, which affect basic parameters of genetic diversity. For example, the seed plantation No. MP/3/41163/05 was verified for 67 mother trees represented by 1770 ramets (6 to 35 per mother tree). Before verification, the relative effective number of clones was 0.93. Laboratory analyses modified the set of ramets in the plantation, which reduced the relative effective number of clones to 0.92. The occurrence of 66 alien genotypes has been proven, which is 3.72% of all ramets. We believe that despite the negligible number of errors they could have been an effective source of alien pollen for the plantation; therefore, defective strains were removed from it. In summary, the development of laboratory techniques has opened up new possibilities of improvements in seed production. The obtained results may be used for verifying existing plantations and for planning the arrangement and number of mother trees on newly established plantations in order to obtain theoretically assumed genetic variability.

Keywords: Seed orchard, genetic variability, molecular marker

CONE PRODUCTION VARIATION IN A CLONAL SEED ORCHARD OF SCOTS PINE (*Pinus sylvestris* L.)

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The seed orchard is one of the most important links between present and future generations to transmit gene diversity to plantation forestry. However, clonal and annual variation of reproductive characters has a great impact on this transmission. The cone production was assessed in a clonal seed orchard of Scots pine (*Pinus sylvestris* L.) for three consecutive years (2014, 2015, 2016). The variation of clonal and annual, including cone fertility variation, and correlation among years for cone production were estimated from six grafts selected random of each 25 clones.

Averages of cone number were 127, 112 and 117 for the years. Large differences in annual cone production were found between the three years at both individual graft and clone levels. The most productive 30 grafts (representing 20% of total observed grafts) produced about 26% of total cone production in the orchard for the years, while it was less than 26% for the most productive five clones. The differences were also supported by results of Analysis of Variance (MANOVA). According to results of MANOVA, statistically significant ($p < 0.05$) differences were found for the cone production among years, while cone productions of clones were similar. Besides, the result showed that clone x year interaction was statistically significant for cone production.

Cone fertility variation, defined the contribution of zygotic parents (i.e., total fertility), estimated based on numbers of cone was similar across the years and close to 1. There were generally positive and significant ($p \le 0.05$) correlations between the years (2014 & 2016, $r=0.181$; *2015 & 2016*, $r=0.306$) for cone production at the graft level, while the correlations were not significant ($p>0.05$) at the clone level.

Keywords: Mating system, gene flow, fertility variation, genetic markers, seed orchards

SEED PRODUCTION POTENTIAL OF SCOTS PINE SEED ORCHARD IN POLAND

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Seed orchard production potential is one important factor for forest management and affects the long-term tree breeding strategy. The mean value of seed production levels is required for planning of the seed orchard area needed in each breeding region or in a whole country. In Poland, forty years ago, when seed orchards were established, it was assumed, as was reported in literature, that mean seed production per hectare of Scots pine seed orchard will be about 5 kg, but it was never studied for different age of orchards and management regime for particular conditions in Poland. Additionally, the management system for seed orchards has changed considerably in Poland in the last 10 years, now including crown pruning and tree topping (Kowalczyk, et al. 2013). Therefore, there is need to recognize the real potential productivity of seed orchard in current environmental and management conditions in Poland.

Seed production obtained in this study is based on the declared data concerning cone collections in database of Forest Reproductive Material Office (bnl.gov.pl). The data comes from orchard with ages between 6 and 40 years. In many cases not all cones were collected from seed orchard, because it was not economically profitable, or because there was no need for seeds from seed orchard in the particular provenance regions, so data are biased in some extend. To estimate the real seed production potential, a case study was done in the one of the most productive young seed orchard located in Gałowo (no. in national register MP/3/41175/05, age 15), planted in good soil conditions in spacing 6 x 6 m (277 stems/ha) and tree stem pruned up to 3,5 – 4.0 m from ground. In selected orchard the control cone harvesting was done in three years (2013, 2014 and 2016).

Results obtained were higher than those previously reported in the literature and in the Polish database. The production per-hectare in the seed orchard examined in this work, based on declared data, varied between 0.3 and 29.6 kg/ha/yr. Prescher et al. (2005), reported seed production levels between 4.3 to 19.1 kg/ha/yr were achieved in six periodically pruned Swedish Pinus sylvestris seed orchards with orchard ages of 20 to 44 years and spacing of 130 to 350 stems per ha. Compilation of seed production data for most of the harvested Polish Scots pine seed orchards gave an average seed production of 7.15 kg/ha/yr. in reporting period. In analyzes of future seed supply of Scots pine from seed orchards in Poland we suggest using production levels of 10 kg/ha/yr. Data indicates that it is possible to collect cones each year, but every 3 - 4 years we observe very good crop.

In the case study conducted in Gałowo seed orchard in agreement with the other studies, we found big difference between clone productivity. The average cone production per clone is 10 kg/year, but the most productive clone reached 19.9 kg/year, fig. 1. There are also clones producing very little cones. The most productive 27 clones (35% of clone composition in seed orchard) produce 50% of total seed orchard's crop. There is significant correlation on average productivity per clone in observation period. Analyzing spatial structure of clone production, we found important spatial effect up to 10 spacing (60 m), caused probably by the microenvironmental and genotypic variability. Single tree heritability of cone production is rather high: 0.37 +/- 0.04.

The high production reported in this study is caused by favorable environmental condition in Gałowo were the seed orchard is located. It indicates that intensive management of operational seed orchards will probably easily reach 20 kg cone production per hectare. This influences on seed orchard programme in Poland (Chałupka, et al. 2011) and implies the proper estimation of number and area of next generation seed orchards in breeding region.

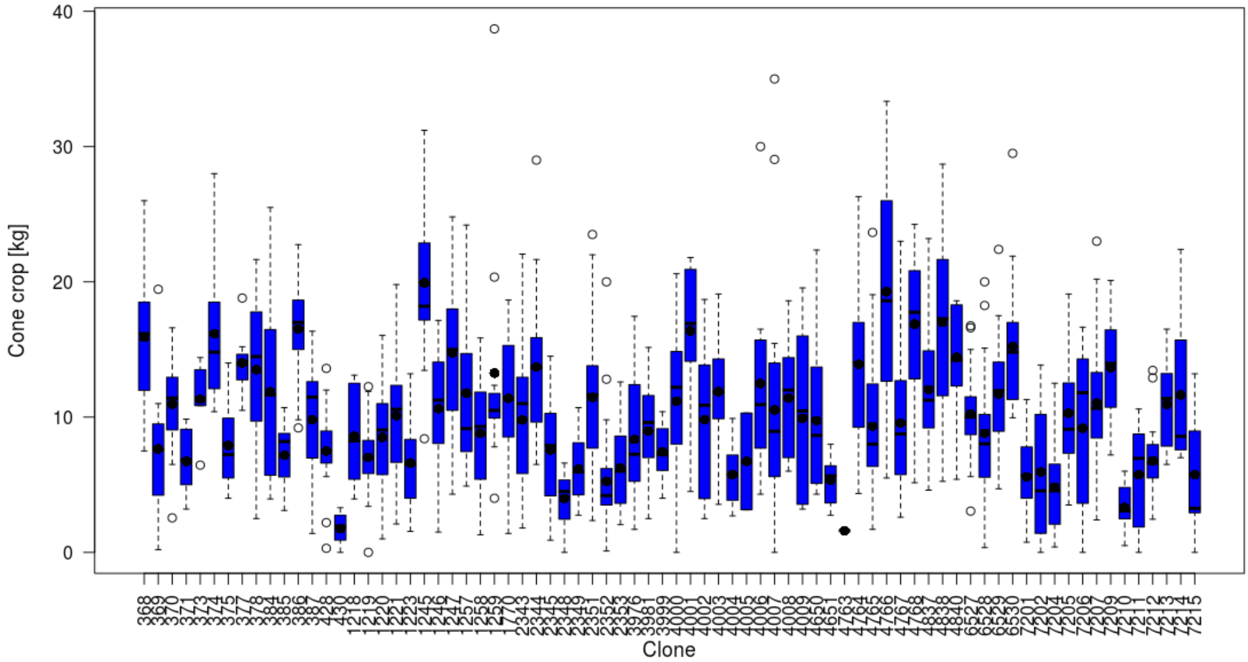

Fig. 1. Cones production in a Polish Scots pine seed orchard located in Gałowo, in the years 2013, 14 and 16. The box plot demonstrate variability of production potential (black dots indicate means).

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Keywords: Seed orchard, Scots pine, cone production, heritability, potential seed production

USING NEXT GENERATION SEQUENCING TO DISTINGUISH GENOTYPES AND ESTABLISH MARKER SET FOR POLLEN CONTAMINATION AND ADAPTIVE DIFFERENCES BETWEEN UNCONTAMINATED, OUTCROSSED AND NATURAL STAND SEEDLINGS; PROCEDURES, PROBLEMS AND PROSPECTS

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Seed orchards are powerful tools that facilitate deployment of improved trees during reforestation. Seed orchard management has always been concerned with pollen contamination from unimproved material, especially when orchards have been moved south relative the parental origin to improve seed yield. Not only would this affect genetic gain but also hardiness for the deployment area. Earlier studies have mainly used multi-allelic markers such as SSRs to establish contamination rates in orchards, and results have been diverse. To better establish the amount of contamination and pollen contribution, among seed-orchard parents and outside pollen to the seed crop, we are using next generation sequencing techniques. We have also tested the seed crop and representative stand seeds for hardiness and growth, to compare the three categories of seedlings (stand, outcrossed and where both parents are orchard clones). In addition we aim to establish a reduced set of high quality markers that can make clear distinction between genotypes.

We are studying four Scots pine and four Norway spruce seed orchards in Sweden with a total of 177 and 361 unique parental genotypes, respectively. Investigating individual parents' contribution and pollen contamination to the seed crop over several years (three crops for each orchard in this study) would give a good estimation of the genetic composition of the crop. In addition, comparing different management plans and deployment among these will also provide insight into how management shapes genetic composition of orchards' seed crop.

The main difficulty from using high throughput sequencing is the massive amount of information gained and which of this data is trustworthy. We have evaluated two major workflows and found that the reference guided mapping of RAD (Restriction site associated DNA) sequences is advantageous compared to de novo assembly of overlapping RAD-tags as a sequence reference for SNP-calling. Both Scots pine and Norway spruce can otherwise suffer from paralogue scoring which results in excess of heterozygotes. However, the non-reference guided SNP-calling also results in software algorithms that wrongly separate variable RAD tags as two different sequences and thus return excess of homozygotes. By using trees with known pedigree and haplotype material; segregation patterns and heterozygosity patterns can be used as evaluating workflow methods and establish additional filtering steps to build a reliable marker panel for parental analysis.

The project includes a massive workload to sequence around 8 000 offspring from the eight orchards, where all parents have been sequenced and four crops of around 1200 offspring have been sent in for sequencing. Preliminary results so far are more than 10 000 high quality SNPs that correctly distinguish individuals and quantify relationship between trees and the genetic structure among orchards. In general, the two species show different genetic structure across the country, where Scots pine in our studied orchards belongs to a single uniform genetic group, while Norway spruce is an admixture of at least two distinct genetic clusters, one northern and one southern cluster. All four spruce seed orchards show a different genetic composition of these two clusters.

Keywords: Parentage assignment, adaptation, GBS, pollen contamination, genetic clustering

CLONAL VARIATION IN FLOWERING IN THE 1.5 GENERATION SEED ORCHARD OF *Pinus koraiensis*

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A forest seed orchard is defined as a plantation of genetically superior trees that ideally produce seeds free from genetically inferior pollen sources, and intensively managed to produce regular and abundant crop of source and easily harvested seed. A 1.5 generation seed orchard (recruit from many first-generation open-pollinated testing programs with lots of parents from which to choose) using parents would give more gain than all-progeny orchards and is essentially equal to the gain from selecting both progeny and parents. The cumulative genetic gains of a 1.5 generation seed orchards are about 20~30%. Depending on the size of the breeding program, the selection intensity, and the weighting of traits, the superiority generally varies. The main purposes of the present study were to survey the variation in flowering among clones, to investigate the abundance of female, male strobili and conelet on each ramet, and to estimate genetic parameters (heritability and correlation) of flowering in a 1.5 generation seed orchard of *Pinus koraiensis*.

Pinus koraiensis is a species of pine known commonly as the Korean pine. Its seeds are extensively harvested and sold as pine nuts, particularly in northeastern China and south Korea; it is the most widely traded pine nut in international commerce. This species is native to northeastern Korea and Japan. Its native habitat is in subalpine forests at elevations of 4,200 to 8,000 feet (1,300~2,500m) above sea level.

Clonal variation in flowering among 53 clones, 713 trees of *Pinus koraiensis* in a seed orchard (1.7 ha) established in 1995 was investigated for 4 years (2014~2017) at Chuncheon branch office of National Forest Seed and Variety Center. The data showed differences in production of both female and male strobili among clones, and were very large and statistically significant for all years examined. Based on the flowering frequency, the expected gamete contribution of the different clones to the offspring was calculated. According to the calculation, 5 clones among 53 clones contributed to 50% of total conelet in 2016. Differences in female strobilus production among clones were more variable than in male.

Gene diversity of seeds was estimated from the flowering frequency on the basis of the concept of the effective number of parents (i.e., status number). The genetic parameters of flowering assessment are going to be presented and discussed for the management of seed orchards.

Keywords: 1.5 generation seed orchard, clonal variation, *Pinus koraiensis*, flowering assessment

TREE IMPROVEMENT IN THE GENOMICS ERA – OPPORTUNITIES AND CHALLENGES

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Traditional tree breeding programs have captured impressive gains for many species all over the world. These long-term endeavours are resource-dependent, require organizational commitment and cover vast geographic territories. Until now, the classic recurrent selection scheme, with its repetitive cycles of selection, breeding, and testing, has been the scheme of choice. Selection of parental candidates is often conducted in natural stands and/or plantations. Breeding involves the development of a structured pedigree produced from crosses among the selected parents following established mating designs. The breeding cycle is completed by testing and evaluation. This process identifies the elite individuals which are then introduced to seed orchards for mass production of genetically improved seed for reforestation. While successful, this current scheme is static and not responsive to market or climate changes. The availability of relatively affordable genomic information for "non-model" species such as trees, with their known large and complex genomes, allows the creation of innovative developments that eliminate most of the traditional recurrent selection steps, thus making breeding more responsive and nimble. Such novel developments include the use of genomicbased assembled pedigree evaluation, the blended single-step evaluation, and the development of revolutionary genomic selection methods that predict mature phenotypes performance at an early age through the use of their genotypes. This genomic revolution has significant ramification as the model unit of quantitative genetics has shifted from being the line of descent to the allele, thus creating a paradigm shift requiring drastic reevaluation of past and current practices. These exciting developments have a carryover effect affecting future tree improvement delivery systems.

Keywords: Next generation sequencing, genetic variance decomposition, genomic relationship, linkage disequilibrium, Mendelian error

THE PROVISION OF FOREST TREE SEED IN AUSTRIA: CURRENT STATUS AND HISTORIC DEVELOPMENT

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Austria is forested to almost 50% of its area. Regeneration of the forest is done mainly with natural regeneration, but still annually about 40 million seedlings are planted. There is the strong political will to use mainly national forest reproductive material, for gene conservation purposes but mainly to preserve local adaption in this mountainous country. At the same time climate change is a serious concern, as conifer forests at low altitudes are affected by numerous stress factors. There is a strong variation among tree species in the proportion of seeds that are coming from national sources. While for the main tree species, e.g. Norway spruce and European larch, a large part of seeds is coming from Austrian forests and seed orchards, for other less planted species a large part of the tree seeds is coming from sources abroad. The smaller part of the seeds used is coming from national seed orchards albeit there is a strong variation among tree species and among years, e.g. almost 100% of wild cherry seeds are coming from seed orchards, while this number amounts to only about 24% in Silver fir. Compared to seed collection from stands, seed production in orchards is more frequent; the orchards thus are a very important source of seeds for nurseries.

In Austria, forest tree seed orchards are managed by private enterprises and governmental agencies; efforts to establish forest tree seed orchards started in the late 1950s by the Austrian Federal Forests. The orchards installed during the 1990s by the Austrian government were designed as gene conservation orchards. Currently there are 53 active seed orchards from 18 tree species, which are managed by BFW on behalf of the Austrian government. Additional 24 seed orchards from 8 tree species are managed by forest enterprises (most of these are run by the Austrian Federal Forests); these latter seed orchards were mainly installed as plus-tree orchards for breeding purposes.

There is a high demand for good quality and improved forest reproductive material in Austria. During the last 10 years research activities have been undertaken to identify and develop superior material in Norway spruce, European larch and Douglas fir, particularly in relation to climate change. This research also includes the identification of molecular markers for specific traits (e.g. drought resistance, wood density, form) to assist selection. Results show that provenances of Norway spruce and European larch exhibit a high genetic variation in their reaction to drought. These data can also be used to identify genes involved in drought resistance. For European larch, a “Breeding-without-Breeding” approach (El-Kassaby & Lstibůrek, 2009) has been successfully implemented to identify clones for a second-generation seed orchard.

Historically Austria has been rather a source of tree seeds; especially conifer seeds have been exported to other areas of Europe. Again Norway spruce and European larch have been among the species traded most. In the frame of a recently completed EU-project (FORGER), the historic trade routes for the latter two species have been scrutinized. We were able to show that there has been tremendous transfer of these species’ reproductive material in the temperate part of Europe during the late 19th century. Centres for trading of seeds were located in Germany and Austria. Especially Alpine plant material of European larch was transferred outside the native range across Europe, with the result that the native gene pool in the Sudetes and Carpathians was strongly altered by Alpine plant material (Jansen & Geburek, 2016). A similar pattern was also obtained for Norway spruce (Jansen, et al., in press), although differences in provenance performance have become less evident compared to European larch.

Keywords: Forest reproductive material, seed orchards, translocation, gene conservation, forest genetic resources

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WHOLE-ROTATION GAIN FROM USE OF IMPROVED NORWAY SPRUCE: CASE STUDY IN LATVIA

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Norway spruce *Picea abies* (L.) Karst. covers large areas in Northern Europe, being the most economically important conifer tree species. It is the third most common trees species in Latvia's forest after Scots pine and birch. Around 80 % of Norway spruce stands are regenerated with planting, mostly using genetically improved stock (in 2015 around 52 % of all Norway spruce nursery stock in Latvia was grown from genetically improved seed orchard material), which highlights the practical importance of Norway spruce breeding. Fennoscandian and Baltic countries, including Latvia, have active breeding programmes for Norway spruce. Breeding achievements together with climate change and legislation may work as driving factors for intensified management in Latvia – highly productive spruce plantations on former agricultural land can be grown focusing purely on wood production, meanwhile diversifying ecosystem services in other stands.

However, there is a lack of studies related to possible changes in growth dynamics when using genetically improved – seed orchard – material in Latvia. Globally there is a focus on development of growth models for genetically improved material of economically important forest tree species.

In this study full rotation projections were simulated using a tree growth model developed in Latvia. The aim of the study was to examine whether stand level projections with initial height for different sets of genotypes at young age as input values reflect definite differences in growth at the end of rotation. The potential gain in height, mean annual increment (MAI), and net present value (NPV) using 3 % discount rate was estimated.

The evaluation was done using data from four genetic trials containing open-pollinated families and clonal material. Material used consisted of ~ 600 genetic entries and ~ 20000 trees, and was divided in two groups: firstly, 10% of highest families/clones reflecting selection intensity used in Latvian forest tree breeding programme to obtain set of clones for seed orchard establishment, and, secondly, remaining 90% of genotypes as a reference group. Final felling in simulations was projected when mean diameter at breast height (DBH) had reached target diameter (31 cm) according to current legislation in Latvia.

Selection of 10% of best genetic entries for use in seed orchards showed considerable gains in the present simulations. Estimated predicted genetic effect for height at young age for 10% of highest families/clones was 11% comparing to the reference group. At the moment of projected final felling age, the best families had on average, 5% higher mean height, 43 % higher MAI and 52% higher NPV. Meanwhile the time required to reach the target DBH was by 17% shorter in comparison to reference group, reaching it on average at age of 42 years. Despite earlier projected cutting age, selection of 10% of best genetic entries resulted in by 52 m^3/ha higher yield. All in all, accelerated growth and reduced rotation length were important factors improving economic gain and thus affirming the importance of use of seed orchard crop in forest regeneration and afforestation.

Acknowledgements: study has been supported by research program "Forest tree breeding for establishment of high genetic quality stands".

Keywords: Tree growth modelling, genetic gain, Norway spruce breeding, Norway spruce plantations, open-pollinated families, clones

THE GERMAN BREEDING CONCEPT – THE BASIS FOR NEW SEED ORCHARDS

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From the 1980th up to now the proportion of natural regeneration and planting has reversed from 20 and 80% to 85 and 15%, respectively. The number of forest tree breeding institutions has decreased continuously in Germany. Therefore, a breeding workshop was held in 2011 to receive an overview on the present status. Four invited speakers gave an overview on the forest tree breeding activities in their countries followed by the analysis of the situation in Germany from different points of view (summarised in Liesebach, 2011). As a result of this workshop a concept on forest tree breeding in Germany was prepared (Liesebach, et al. 2013). The concept is focusing on six species (Douglas-fir, Scots pine, Norway spruce, sycamore maple, larches, and oaks. Due to the limited number of remaining breeding stations and reduced staff the ongoing work needs to be concentrated. In the concept it is laid down that breeding is a continuous process which costs money. With substitutes of the 'Waldklimafonds', a joined grant program of the Federal Ministries of Agriculture (BMEL) and Environments (BMUB), the realisation of the concept could be started in 2014 (Meißner, et al. 2015; Janßen, et al. 2017).

Depending on the breeding progress, the strategy for the 6 species varies from selection of stands over the establishment of seed orchards, the creating of parents of families and the selection of clones. In addition to existing regions of provenance we start to delineate breeding/deployment zones taking climate change into consideration (Liepe & Liesebach, 2017). The approach for Douglas-fir will be presented in details.

The breeding activities will be completed by evaluation of the genetic diversity and pollination structure (see presentation by J Wojacki & H Liesebach).

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Keywords: Forest tree breeding, seed orchard, climate change, deployment zone, *Pseudotsuga menziesii*

SEED ORCHARD DESIGNS

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The particular mating pattern within a seed orchard is a complex function of many factors, including spatial arrangement (seed orchard design), clonal genealogy and size, reproductive phenology, gametic contribution, propensity to selfing, and the extent of extraneous gene flow. In my presentation, I will provide an overview of traditional designs developed during the 1950s to early 1970s (pure rows, chessboard, completely random, randomized complete block, fixed block, rotating block, reversed block, unbalanced incomplete block, balanced incomplete block, cyclic incomplete block, balanced lattice, permutated neighborhood, and systematic designs). I will discuss some theoretical background related to formulating mathematically optimum solutions to the spatial allocation. I will then highlight the development of recent schemes (R2SCR, MI, and ONA), suitable to advanced generation seed orchards, followed by their anticipated theoretical efficiency.

Keywords: Spatial allocation, panmixia, selfing and mating among relatives, genetic gain and diversity

SELECTION FOR SOLID WOOD PROPERTIES TO ESTABLISH SEED ORCHARDS FOR *Eucalyptus nitens* IN NEW ZEALAND

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Eucalyptus nitens is the most important commercial eucalypt species in New Zealand with an advanced breeding programme, moving towards its fourth generation. Breeding is based on open-pollination, with implementation of genomic tools underway. *E. nitens* has been grown predominantly for pulp wood production with a rotation age of 15 years in Southland, the southernmost region of New Zealand and the major *E. nitens* plantation area. This species boasts a mean annual increment in excess of 20 m^3/ha/year. *E. nitens* is recognised not only for its fast growth but also for good form and cold-hardiness. Wood quality traits have not been the focus of breeding until recently, with a greater interest in the use of *E. nitens* for higher value solid wood products. Selection criteria for solid wood production were proposed in the latest breeding plan and are regarded as an essential focus for next generation selections. Two progeny trials were assessed for growth and form, and wood density at age six. Measurements for solid wood properties at age seven were undertaken at one site. Selections were carried out to establish two new production seed orchards: one with a focus on solid-wood production, and one with a focus on high-yield pulp wood production. These two new production lines consisted of material determined to have superior BLUP estimated breeding values for larger growth, higher wood density, lower growth strain, lower wood shrinkage and lower internal checking. Genetic parameters for solid wood traits and implications of genomics as proof-of-concept will be discussed.

Keywords: *Eucalyptus nitens*, breeding, wood quality, seed orchard, genomic selection

FEMALE FERTILITY VARIATION IN A CLONAL SEED ORCHARD OF SPECIAL VARIETY OF SCOTS PINE

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Female fertility variation is of major interest for the economics and biology of seed orchards, especially for the efficiency and diversity of seed orchard crops. A clonal seed orchard of *Pinus sylvestris* L. var. *compacta* (TOSUN) established in Karabuk province of Turkey in 2004 aiming *ex situ* conservation. The orchard includes 823 ramets of 26 clones changing from 3 to 37 grafts. Five ramets per clone were chosen randomly and all numbers of female strobili in entire crown of 130 grafts were counted in 2015 and 2016. Average numbers of female strobili per clone were ranged from 4.2 to 75.0 in 2015 and from 0.0 to 39.2 in 2016. Clonal rank correlation of female production between 2015 and 2016 was 0.42 and statistically significant. The top 30% of clones produced 51% of total female strobili in 2015 and 82% of total female strobili in 2016. Most of female strobili were single while a few of them formed clusters. Number of strobili per cluster ranged from 2 to 15. The occurrence of clusters in 2015 and 2016 was very similar. The cluster including average 15 strobili was in fourth and sixth order in years 2015 and 2016, respectively. Year variation was statistically significant but other factors (clone, clone year interaction) were not statistically significant. Female fertility variation (Ψ_f), effective status number (N_s) and relative status number (N_r) were 2.14, 12.15 and 0.48 in 2015 and 3.56, 7.29 and 0.28 in 2016, respectively. The effective status number was half of census number in 2015 and one third of census number in 2016. So, the genetic diversity based on female fertility variation of young seed orchard was low both in 2015 and 2016. On the other hand, female fertility variation itself between two consecutive years was highly different. The differences for two consecutive years supported general argument that the fertility variation is not stable in young seed orchards.

Figure 1. Clonal seed orchard in winter.

Keywords: *Pinus sylvestris*, status number, relative status number, cluster of strobili

Figure 2. A graft in the clonal seed orchard.

Figure 3. Samples of female strobili.

Figure 4. A cluster of female strobili.

EFFECT OF BIOTIC STRESS ON STROBILI AND CONE PRODUCTIONS IN A NATURAL POPULATION OF BRUTIAN PINE (*Pinus brutia* TEN.)

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Productivity of reproductive traits such as number of strobili and cone is influenced by many genetic and environmental factors or called biotic and a-biotic factors such as stress, temperature, soil properties, hormone, practices and pests. Pine processionary moth *(Thaumetopoea wilkinsoni)* is one of the most important biotic factors because of its stress or damage on Brutian pine (*Pinus brutia* Ten.) forests.

Effect of the pine processionary moth on strobili and cone production in a natural population of Brutian pine was examined based on the number of female and male strobili and juvenile (two years) and mature (three years) cones. Fertility variation was also investigated as the proportion of the number of strobili and cones counted from individual trees in the population. Besides, number of strobili and cones were also related to growth characteristics (height, diameter at breast height and diameter at base) and the insect stress. For this purpose, the reproductive and growth data were collected from 100 individual trees sampled randomly, of which 55 were under insect-induced stress in April-May of 2017.

The individuals under stress had about 50% higher strobili and cone productions than that of control. The growth characters were positively correlated ($p \leq 0.05$) with strobili and cone production for both stressed and control individuals. Fertility variation, estimated based on the number of strobili and cones, ranged from 1.20 to 1.48 and were similar in both stressed and control individuals. However, variation was higher in cone production than in strobili production.

Keywords: Effective number of parents, fertility, growth, pest, reproductive

INTERACTION BETWEEN CONE PRODUCTION AND GROWTH TRAITS IN A MEDITERRANEAN CYPRESS (*Cupressus sempervirens* L.) PLANTATION

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Estimation of the interaction between cone production and growth traits had important roles in selection and management of seed sources, and also an important stage of breeding programme. This study was carried out on an eleven year plantation of Mediterranean Cypress (*Cupressus sempervirens* L.) to estimate relation between mature cone production and growth traits (height, diameter at breast height, and diameter at base), and also cone fertility variation based on collected data from 90 individual trees sampled randomly in April of 2017. While many studies were conducted on the interaction between reproductive and growth traits in different forest tree species, the present study was the first investigation in Mediterranean Cypress.

Average of number of cones per tree was 72, while it ranged from 20 to 150 in individuals. However, the coefficient of variation *(CV)* of cone production showed that there was a small difference among individuals for cone production. Estimated fertility variation (1.21, 83% of total trees) in the present study could have been acceptable level for a typical population.

Averages of height, diameter at breast height, and diameter at base were 5.1 m, 11.1 cm and 8 cm, respectively.

Height and diameters had positive and significant ($p \leq 0.05$, $r > 0.29$) effects on cone production. Results of the study were discussed for forestry practice and genetic-breeding of the species.

Keywords: Fertility, growth, relation, seed

POLLEN CONTAMINATION AND MATING STRUCTURE IN MARITIME PINE CLONAL SEED ORCHARDS

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Maritime pine (*Pinus pinaster* Ait.) is a major forest tree species in south-western Europe. In France, a breeding program based on a recurrent selection strategy was initiated in the 1960s with the objective to develop improved varieties for growth and stem straightness. Open-pollinated seed orchards currently supply more than 90% of maritime pine improved stocks for plantation forestry in France (41 million seedlings in 2015-2016). However pollen contamination and mating structure in such seed orchards are still largely unknown.

In this study, we investigated the genetic composition of seed collections from clonal seed orchards through SNP genotyping considering three factors:

1) The seed orchard location (3 sites with contrasted pedo-climatic conditions)
2) The pollination year (three seed collections: 2011, 2013 and 2014).
3) The maternal genotype on which seeds were collected.

Three data sets were analyzed:

1) 1,524 offsprings from four maternal parents over three seed orchards and two seed collections (2011, 2013);
2) 590 offsprings from 20 maternal parents in one seed orchard (2013);
3) 450 offsprings sampled from commercial seedlots from three seed orchards (2014).

Paternity and/or parental analyses were carried out on these 2,564 offsprings with the Cervus software (likelihood inference methodology with 99% confidence level) based on 60 SNPs developed by Vidal *et al.* (2015). Moreover, each parental genotype was evaluated over two pollination years (2015, 2016) for reproductive phenology traits (precocity of female and male flowering as well as female number of flowers) in the three seed orchards.

Pollen contamination rates were globally high (from 20 to 96%) but highly dependent on seed orchard location. An effect was also detected for both the maternal parent and the pollination year. Even if all parental genotypes contributed as father in the offspring, differential paternal reproductive success was highlighted. This is only partly explained by the variable number of ramets deployed per parental genotype in each orchard. Site and annual variation in pollen contamination can result from a number of geographical (vicinity of plantations), orchard design (zones, ramets/genotype), ontogenetic (orchard age) and environmental factors (pedo-climatic conditions) affecting flowering and/or mating success. Reproductive phenology of parental genotypes and climatic data in each orchard will be presented to tentatively find correlations with pollen contamination rates.

Keywords: *Pinus pinaster* Ait., clonal seed orchard, SNP genotyping, pollen contamination, mating structure

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DETECTING HERITABLE VARIATION IN CHLOROPHYLL FLUORESCENCE AND NEEDLE SPECTRAL REFLECTANCE IN SCOTS PINE (*Pinus sylvestris* L.) STRUCTURED POPULATION

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Both processes enumerated by chlorophyll fluorescence (ChlF) parameters and foliage properties reflected in spectral reflectance are considered to be polygenic, so tools of quantitative genetics can be used to reveal their heritable variance.

The study was conducted within test plantings of half-sib progenies originated from two pine seed orchards of Western Bohemian pine provenance. These test plantings were designed in incomplete blocks in two geographically different localities. Preselected phenotypes within half-sib progenies of seed orchards were subjected to the paternal assignment using SSRs markers. The sum of 523 individual trees from the both sites was sampled in the summer (July and August) of 2014.

Collected foliage samples were analyzed for fast chlorophyll fluorescence (ChlF), water content, spectral reflectance measured between 350 and 2500 nm with step of 1 nm and for content of photosynthetic pigments (chlorophylls a and b, total carotenoids).

Parameters derived from the OJIP part of ChlF kinetics curve characterize individual processes associated with the exciton trapping by light-harvesting antennae, energy utilization in Photosystem II reaction centers and its transfer further down the photosynthetic electron-transport chain. Spectral reflectance may be dissected into several regions, were the region of visible light corresponds to photosynthetic pigments contents, the near-infrared region and shortwave infrared region comprises information about various needle components like, water content, phenolics or cellulose.

The statistical analysis was based on mixed linear models with reconstructed pedigree based on single sequence repeat (SSR) marker analysis forming a relationship matrix that is an integral part of this analysis.

In most studied traits, significant narrow sense heritability was found, ranging from 0.14 to 0.33. A novel approach in detecting heritable variation along the reflectance curve suggested the existence of selection pressure on reflectance properties in the visible region (arguably due to crucial importance of photosynthesis) and relatively weaker selection on spectral properties at longer wavelengths.

Knowledge of pigment- or spectral reflectance- based stress markers is already established for recognizing abiotic stress resistant genotypes. The spectral signals such as single wavelength reflectance or spectral indices serve as indirect markers often correlated to production or stress adaptive traits in breeding. The heritability pattern along the fluorescence kinetics curve suggests the possibility of further analyses revealing quantitative trait loci (QTL) involved in primary photosynthetic processes. The observed pattern enables the dissection of heritable structure of primary photosynthetic processes on a fine scale.

Keywords: Chlorophyll fluorescence, foliar spectral reflectance, pedigree reconstruction, heritability, *Pinus sylvestris*

IMPLEMENTATION OF THE OPTIMUM NEIGHBORHOOD ALGORITHM SEED ORCHARD DESIGN

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Optimum Neighborhood Algorithm (ONA) is a new efficient seed orchard layout. The ONA utilizes local heuristics based on the assumption that the most common genetic exchange in seed orchards occurs among adjacent neighbours. Its aim is primarily uniform spatial distribution among adjacent genetic entries, thus it should promote panmixia. Achieving this goal also ensures effective separation of ramets of individual clones, thus minimizing inbreeding (Chaloupková et al. 2016). Here we present operational benefits of using ONA design in forest tree breeding. The first is the possibility of using ONA under variable clonal sizes. Secondly, we present some examples of combining ONA with additional designs. This method is suitable for establishing orchards of advanced generations. Third, some examples of using clonal rows and explaining the principle of minimizing the neighborhood effect, which is achieved by the original structure of the algorithm, are presented. Fourth, we suggest interesting possibility of using the ONA to refill empty positions, which arose in the existing orchards by the mortality within some of the clones. If these particular ramets are no longer available, the ONA, depending on the current layout of the orchard, creates the best possible scheme with remaining clones available in the stock. Finally, ONA is available for different shapes of orchards (non-rectangular). Some other examples of expanding ONA are currently under development. We consider the inclusion of positive and negative assortative mating. There is also the possibility of tweaking the size of the optimized neighbourhood of each position. In addition, we also mention other parameters that affect the scheme and the possibility of their implementations to the existing algorithm. A software tool for users interested in using ONA is currently available online and is continuously updated.

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Keywords: Seed orchards design, random mating, clonal rows, advanced generation seed orchards

UTILIZATION OF RESISTANT LOCAL NORWAY SPRUCE VEGETATIVE VARIANTS IN FOREST REGENERATION IN THE ORE MOUNTAINS, CZECH REPUBLIC

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The aim of this research project, undertaken during the period 2015–2018, is to utilize specially selected vegetative variants of resistant (tolerant against emissions) Ore Mountains population of Norway spruce for forest regeneration in forest management in the region. These areas are administrated by a number of participants of the project, including the Municipal Forests (MF) Chomutov, Forests of the Town Jirkov, Forests Jáchymov and MF Klášterec (the last named organization will participate in the project as user of the project results). The project includes other activities, such as DNA analyses, which will contribute to returning (repatriation) of the original gene pool material of resistant Ore Mountains Norway spruce to its natural habitat. As part of the planned activities, locations in the areas administrated by the above mentioned organizations were selected for the establishment of four research plots ((0.5 ha each), with vegetatively reproduced variants (rooted cuttings) of Norway spruce resistant forms. On these research plots, Norway spruce planted variants will be periodically evaluated in the future by assessing their qualitative and quantitative characteristics. Additionally, on the MF Chomutov area, an *in-situ* seed orchard (1 ha) will be established to produce seed of the resistant forms, which should be used in the future for forest regeneration on similar areas of other participants and users of the results. The reserve seed orchard of the same character and size will also be established on the area under the administration of PEXÍDR Ltd. (other participant in the project), while planting material for these objects will also be propagated and planted by this company. The establishment of two *in situ* and *ex situ* hedge orchards (1 ha) for vegetative reproduction of Norway spruce resistant forms is also part of the planned activities of this project. Both hedge orchards will be established on the area under the MF Klášterec and PEXÍDR Ltd. administration. The capacity of this vegetatively produced material from these hedge orchards is calculated not only for fellow participants of the project and users of this project results, but also for other interested forestry enterprises in the Ore Mountains. In the project, the plans are to use certified clones of resistant Ore Mountain Norway spruce as the source of vegetative propagation material for planting rooted cuttings and grafting, and these are also intended for DNA analyses. These clones are concentrated in the already aging clone *ex-situ* collections which were established by the Department of Biology and Breeding of Forest Tree Species (Forestry and Game Management Research Institute /FGMRI/ Jíloviště – Strnady) over three periods (1970, 1972 and 1991) in the area of the former FGMRI Administration of Experimental Forest Objects, which is currently under the administration of Forest Jíloviště Ltd. For clones from the above mentioned certified sources of qualified reproductive material, their molecular-genetic characteristics will be investigated using DNA analysis in order to determine the genetic variability of these clones. The main output of the research project will be the establishment of guidelines for the use of vegetatively propagated reproductive material of Ore Mountains Norway spruce resistant forms in the forest management units of the Ore Mountains area, to be used by local forest owners and managers. Results of the project will be provided to users based on contractual arrangements (Certified Methodology - Nmet, Functional Sample - Gfunk) and through the publication of results in special reviewed periodicals (Results Category Jsc), in accordance with the project outline.

Keywords: Hedge orchards, seed orchards, resistant Norway spruce, experimental plantings, Ore Mountains, Czech Republic

EVALUATION OF LARGE ISOLATION TENTS FOR HIGH-QUALITY SEED PRODUCTION IN A SCOTS PINE SEED ORCHARD

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Context and aim of the study

Seed orchards are the most widely used forest tree production populations in the world, supplying the forest industry with consistent and abundant seed crops of superior genetic quality. The genetic quality can however be severely affected by non-random mating among parents and by the occurrence of background pollination.

We analyzed mating structure and background pollination in large isolation tents that were established in a clonal Scots pine seed orchard "Västerhus" in northern Sweden (13.7 ha, 28 replicated parents, 136.3 ± 88.8 ramets per parent, 63°18′ N & 18°32′ E, planted in 1991) with the objective 1) to form a physical barrier against background pollen and 2) to create reproductive isolation from surrounding trees by inducing earlier flowering in the tents.

Materials and Methods:

We built six isolation tents, each covering 10-13 ramets, which represented three different treatments (T: tents only, F: tents with a fan to promote air circulation, and P: tents with supplemental pollination from five pollen donors) and two levels of unprotected controls (CI: near the tents and CO: far from the tents). We scored flowering phenology inside and outside tents, and further installed four pollen traps to quantify the abundance of ambient pollen (two within the seed orchard in a close vicinity of the tents and the other two ca 400 m from the seed orchard's boundary in opposite directions).

We analyzed 5,683 offspring (3,692 from tents and 1,991 from open controls) collected from four maternal parents over three consecutive pollination seasons 2010-2012. We genotyped them at 9 nuclear microsatellite loci and assigned their paternities using the simple exclusion method. We used the genetic data to estimate male reproductive success, effective number of fathers and rates of pollen contamination and self-fertilization in the analyzed seed crops and thereby evaluated the usability of the tent method in seed orchard management and forestry practice.

Results:

Tent trees shed pollen and exhibited maximum female receptivity approximately one week earlier than control trees. The majority of matings in tents occurred at short distances, on average 78.3% within two trees apart (ca 5 m) and only 5.3% further than six trees apart (> 16 m). Self-fertilization was relatively high in tents due to overrepresentation of some parents, with individual-tree rates exceeding 50% (average 21.8%), but it was substantially reduced by the supplemental pollination treatment (average 7.7%). Supplemental pollination was successful and consistent across years, fertilizing on average 61.6% of seeds in P tents (59.8%, 48.2% and 76.3% in pollination seasons 2010, 2011 and 2012, respectively). Pollen contamination was low in controls (5.3%, 7.1%, and 4.8% in the three years) but all tents remained entirely free of foreign pollen. Moreover, we detected only five tent seeds that had been sired by seed orchard fathers that did not occur in the tents. The low contamination is in line with assessment of pollen traps that captured ca 10 fold more pollen within the seed orchard than 400 m apart from it during (maximum) female receptivity of seed orchard parents. Effective numbers of fathers were lower in seed crops produced in tents as compared with those produced in open controls (and the air circulation by portable fans did not bring any notable improvement in this regard), but the application of supplemental pollen substantially improved the crops' genetic status, including inbreeding.

Conclusions and recommendations

The tested isolation tents have proven to be an effective method in preventing trees within them from background pollination by both creating a physical barrier against background pollen and by shifting reproductive phenology of trees within them.

In order to optimize the genetic gain and diversity of seed crops, more pollen donors can be involved in the supplemental pollination treatment and/or seed crops from several smaller tents with different parental compositions can be mixed. The tent system is flexible in length and width of the tents and thus offers the opportunity to cover larger areas of seed orchards, theoretically comprising of entire plantations. Seed orchards can also be designed to accommodate such tents later by covering a specific number or composition of ramets.

When combined with supplemental pollination, the tent isolation could become a useful management practice for producing high genetic quality crops in forest tree seed orchard.

Keywords: Mating structure, paternity assignment, genetic diversity, isolation tent, seed orchard, Scots pine

ASSESMENT OF FEASIBILITY OF OPEN POLLINATED LANDES X CORSICA *Pinus pinaster* SEED ORCHARD

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Maritime pine *(Pinus pinaster)* is a very plastic pine species subjected in France to one of the most advanced conifer breeding program (4th generation varieties just released after marker-enabled forward selection; Vidal et al., 2015, 2017). The selection per se is performed by INRA and FCBA while the testing involves additional partners (the National Forest Service and CPFA) into the French maritime pine breeding cooperative (further designed as FMPcoop, official French name is "GIS pin maritime du futur").

Since the pioneer work by AFOCEL (now operating as FCBA) focusing on an elite pan-Corsica selection (Devinas synthetic provenance, Alazard, 1998), products of Inter provenances crosses between Landes and Corsica (LC varieties) are used beside the pure Landes provenances. This combination allows to get a different compromise between growth and form while obtaining better pest, cold and drought tolerance than any of each provenance (Harfouche, et al., 2000).

In order to enable a fully informed assessment of the performance of preexisting LC varieties to be taken into account for the design of the new ones, we performed a first quick molecular study in order to characterize the genetic contribution involved in the seedlots from (1) the LC2 seed orchards (seeds are harvested on Landese mother clones which are control-pollinated with Corsican fathers) and (2) the free pollination of LC1 hybrids (mother trees produced by control cross). We thus preselected 18 nuclear SSR chosen from their performance in Maritime pine gene-flow and landscape genetic studies (Ribeiro et al., 2002; AFOCEL, 2005; Santo-DelBlanco, et al., 2015). From the results of the screening of parental population and selected LC hybrids, we defined and optimized a set of 11 markers amplified in two multiplexes which revealed highly powerful to discriminate between Corsican and Landese genetic contributions. These SSR panels allowed us to establishing that the open pollinated seedlots from LC2 orchards are bona fide interprovenance hybrids and that products of free-pollination of F1 hybrids retains a highly variable (but significant) proportion of Corsican genetic contribution (5 to 60% estimated with STRUCTURE) averaging ca 20%.

The signs of occurrence of two gene pools discriminated by STRUCTURE within the Landese provenance, and the increase in their relative importance from the F1 hybrids to their open-pollinated sibs, could correspond to a wide contribution of pollen from outside the seed orchard (thus mainly genetically unimproved – or improved less than expected – fathers). Together with the correct provenance assignation, this would be further validated through SNP genotyping using the marker panels setup for pedigree reconstruction (Vidal et al 2015) and for high power provenance diagnostic (Jaramillo-Correa, et al., 2015). Outside application for total pedigree reconstruction (in order to enable forward selection), the SSR panel described here would be more performing than SNP regarding economic cost of the genotyping, especially for small sample numbers.

The phenotypic performance of the free-pollinated seedlots harvested from hybrid parents is n argument in favor of the shift of LC orchard management from control to free pollination, providing that parents are well selected (checked) F1 hybrids. This shift is under way by the FMPcoop while designing the 3rd generation LC seed orchards (LC3 varieties).

This would fit with a more realistic management of seed orchard enlightened by the knowledge of real (often very high) rather than expected low external pollen flow, leading to a gap between promised and achieved genetic gain at the level of commercial plantation. This enlightened consideration of pollen flow would at the same time lead to a reduction of the request fora very high diversity of parent trees while keeping a same expected seed genetic diversity. This would in turn improve the delivered genetic gain. At the level of the global (planted) forest area, the effective genetic diversity would be kept constant since the easier establishment of seed orchard would ease the faster renewal of varieties which is in turn enabled by new innovative breeding strategies (e.g. Bartholome, et al., 2016, Vidal, et al., 2017).

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Keywords: Interprovenance cross, growth form, simple diagnostic, DNA

ARCHIVES OF LARCH BREEDING POPULATIONS DESIGNED AS SEED ORCHARDS

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The breeding of a marginal species like larch has not been on the forest tree breeder radar for decades in Sweden. However, with a growing demand from forest owners in recent years a potential breeding of different Larch species in Sweden has received more attention. As a consequence, Skogforsk has evaluated several young field trials to support selection of individual larch trees. Selection of new candidates for further breeding activities has been done for European larch (L. decidua) in southern Sweden (Ekebo) and for Siberian larch (L. sibirica var. sukaczewii) in northern Sweden (Sävar). The selected genotypes have been grafted and have, or will be planted in archives located at Skogforsk research stations at Ekebo or at Sävar. The archives have been planned using the OPSEL tool for optimizing the number of selected genotypes per family (Mullin 2014). The spatial distribution of the genotypes in the archives has been optimized using SEEDPLAN® (Kerr, Dutkowski et al. 2007).

The proactive design of the archive as a seed orchard will enable us to use Breeding without breeding (BwB). BwB was first suggested by El-Kassaby and Lstiburek (2009), and has been exemplified by (Funda, Chen et al. 2008) for western larch as an effective breeding method. BwB could be suitable for larch in Sweden given the limited resources available for breeding. The method can be applied as soon as we have enough flowers and pollen production in the archive, which is likely to be the case within five years.

We will present the design of the seed orchards and present breeding strategies for larch in Sweden at the poster session, with hope of fruitful discussions and suggestions for improvement.

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Keywords: Larch, archive, breeding without breeding

GENETIC DIVERSITY OF NORWAY SPRUCE SEED ORCHARD PROGENIES: CASE STUDY IN LATVIA

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Planting is used as prime regeneration method for Norway spruce in Scandinavian and Baltic countries, where this tree species is of high economic importance. Notable effort had been dedicated to tree breeding and currently significant portion of seeds (>33%) is obtained from seed orchards. Active seed orchard establishment programs in most of the countries in the region suggests that this proportion will increase in future. This may rise concerns about the potential impact on genetic diversity. Therefore, aim of the study was to assess the possible influence from the use of seed orchard progenies on the first level of biodiversity – genetic diversity – of Norway spruce stands.

Since genetic diversity may vary between years (asynchronous flowering, different levels of background pollination) and orchards (number and set of clones), material for the study was collected from two seed years from progenies of Tadaine and Valgums seed orchards (51 and 20 clones, resp.) and single seed year from Remte and Katvari seed orchard (50 and 20 clones, resp.). Seed orchards are located in different regions of the country with minimal overlap between the clones used for their establishment. For comparison randomly selected trees (with distance of 100 m) or seedlings from average seed sample of the stands (including from Moricsala nature reserve) were used. Stands were randomly chosen to represent western (3 stands, 139 trees in total), central (3 stands, 144 trees) and eastern (2 stands, 96 trees) provenance regions. DNA, extracted from the needles, was analysed with 5-6 nuclear SSR markers.

Results reveal that mean number of alleles per locus ranges from 10 (UAPgAG150) to 35 (EAC7H07) and no differences in this parameter between forest stands and seed orchard progenies were observed. Total number of alleles found is slightly larger, but number of effective alleles slightly smaller for seed orchard progenies in comparison to forest stands, however observed heterozygosity (Ho) did not differ between the analysed groups (Ho=0.61 and Ho=0.59, respectively). Significant differences were not detected also in values of diversity index (DI) and Shannon's Information Index (I): for the progenies of seed orchards DI=0.92 and I=2.0, for the forest stands DI=0.91 and I=1.9. Number of alleles with high frequency (≥5%), that are important for genetic stability of stand, is slightly higher for the progenies of seed orchards than for the forest stands. Differences are more pronounced in comparison with stands from the central and eastern provenance region. The only genetic diversity parameter that was significantly different between the Moricsala population and the progeny trials was the mean number of private alleles, which was higher in the Moricsala population.

It can be concluded, that the number of clones in seed orchards (20 to 51) is sufficient to avoid possible losses of genetic diversity for seed orchard progenies. Therefore, use of seed orchard seeds for seedling production does not decrease the genetic diversity of young Norway spruce stands.

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Keywords: Number of alleles; observed heterozygosity; Shannon's Information Index

THE COMPARISON OF PROGENY FROM SEED ORCHARDS AND SEED STANDS OF BLACK ALDER – PRELIMINARY RESULTS

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In Poland various seed sources are used for seed collection. Until 2010 most seeds, about 90%, were collected in commercial seed stands, about 7% in selected seed stands, and about 3% in seed orchards. However, the contribution of particular species may differ. In seed orchards, the most seeds, about 50%, were collected for European larch, whereas only about 2.5% were collected for black alder. Currently the contribution of seeds collected from seed orchards is increasing, up to 10% annually.

In 2014 Scientific Consortium DendroGen, commissioned by the General Directorate of State Forests, started researching the optimization of using seed orchards in Polish forestry. In one study, we compare the performance of progeny from the seeds collected in the clonal seed orchards and in the commercial seed stands. In 2014 the seed sources of black alder were chosen: 8 seed orchards (1 seedling and 7 clonal), 11 commercial seed stands and 1 selected seed stand, which was one of the most valuable in Poland. Seeds were collected from all clones in the seed orchards and from 30 trees in the stands. The analysis of seed size was performed by WinSeedle program (Regent Inc.), taking at random at least 400 seeds. It was established that the seeds which originated from the seed orchards were bigger than seeds collected in the stands. However, big differences were also noted within these two groups. The mass of a single seed, the mass of an embryo and the average time of germination were defined for 3 seed orchards and 3 stands. The seeds collected in seed orchards had a higher average mass of a single seed and a higher embryo mass, however, they started germination a little later.

In spring of 2015 seeds were sowed in a nursery, where annual seedlings were grown in controlled conditions (a foiled tent). The seedlings grown from the seeds which were obtained from seed orchards where larger, but the statistically important difference was found out for the height of the seedlings and for the size of the assimilation apparatus.

The comparative studies will be continued on the experimental plots, which were established in the spring of 2016 in 4 locations in Poland. If the differences discovered at the nursery stage will maintain at the more advanced age, they will be beneficial for the planning of breeding. This will lead to the increase in using seeds originating from the seed orchards of black alder.

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Keywords: *Alnus glutinosa*, seeds, seedlings, clones, DendroGen

MATING SYSTEM ANALYSIS IN DOUGLAS FIR SEED ORCHARDS AND SEED STANDS IN GERMANY

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The objective of the joint project "FitForClim" is the production and sustainable supply of high-quality forest seeds of six economically important tree species in Germany (see also presentation by Liesebach M, Liepe KJ and Schneck V). In the frame of this project, plus trees from numerous older field trials were selected and new seed orchards for several breeding zones will be established to supplement seed production from approved stands (Liepe, at al. 2015).

In case of Douglas fir, approved seed stands need 40 adult individuals as a minimum, whereby 20 trees have to be harvested according to current regulations for forest reproductive material in Germany. Some of the already existing Douglas fir seed orchards and approved seed stands of different size should be evaluated for their genetic structure and genetic diversity. Therefore, the parent generation and the respective offspring (seed samples) were characterised by 9 highly variable nuclear microsatellite markers (Slavov, et al. 2004) to assess their mating system parameters.

Our previous results are not yet representative and will be completed in the near future. Firstly, they show that pollen distribution in closed canopy stands might be very restricted (about 80% of pollinations occur in distances below 50 m). This finding indicates that seed stands as a harvesting entity should not be fragmented into separa-ted subunits. Secondly, the number of adult genotypes in seed orchards and small approved seed stands might be similar. The repetition of genotypes in designed seed orchards, however, resulted in a better transfer of genetic diversity and more mixing of genotypes in the offspring generation in contrast to small seed stands.

Conclusions for practice in forest seed production will be prepared.

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Keywords: *Pseudotsuga menziesii*, seed orchard, genetic diversity, mating system, nuclear microsatellite markers

GENETIC VARIATION OF BUD FLUSHING IN A NORWAY SPRUCE SEED ORCHARD IN ROMANIA

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Norway spruce is one of the most important forest species in Romania, both for economic and ecological purposes. It covers about 19% of the forest area and is the main coniferous species. In last years, the surface area artificially regenerated is about 5000 ha per year, of which 41% is planted with Norway spruce (about 17 million seedlings). Seed orchards registered in the National Catalog of Basic Material are an important source of seed.

A clonal seed orchard of Norway spruce established between 1979 and 1981 at Fantanele Forest District (Eastern Carpathians) on an initial area of 15 ha was considered for this study. The seed orchard comprises the vegetative copies of 197 plus trees originating from 7 seed stands from Eastern Carpathians and is located far away from other stands of the same species.

The phenology of bud flushing was studied in Fantanele seed orchard in the spring of 2017 to invest-igate the genetic variation of bud flushing among and within Norway spruce populations. Genetic parameters, relationships among variation of bud flushing and geographical coordinates of the place of parent origin were also assessed. The potential for future selection and implications for seed production were evaluated.

There was a high genetic variation for this trait both among populations and clones of Norway spruce in the seed orchard. Differences among plus trees within the same population were statistically significant in the case of 3 populations. A high genetic control for bud flushing of Norway spruce was observed. Thus, narrowsense individual heritability estimates for three evaluations ranged from 0.37 to 0.48 while the family heritability ranged from 0.61 to 0.84. The difference in the time of bud flushing between the earliest clone and the most tardive one was 20 days. The correlations with geographic coordinates of the parent origin were also significant and indicated a clinal variation.

Keywords: Norway spruce, bud flushing, genetic variation, heritability

THE CURRENT STATE OF BREEDING FOR JAPANESE PINE RESISTANCE AGAINST PINE WILT DISEASE AND PLANNED GAIN REALIZATION BY SEED ORCHARD ESTABLISHMENT

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Pine wilt disease is caused by the pinewood nematode, *Bursaphelenchus xylophilus* and has been devastating pine forests in Japan. To cope with the pine wilt disease, tolerant clones of *Pinus densiflora* and *P. thunbergii* have been selected in western Japan since 1982. In the program, surviving pine trees within heavily infested stands were selected as candidates. The candidates were tested by artificial nematode inoculation tests, and 92 *P. densiflora* and 16 *P. thunbergii* clones were selected as tolerant clones by 1985. Tolerant seed orchards constituted by grafts of the tolerant clones have produced open-pollinated seedlings for the operational plantation. In 2016, more than 400,000 seedlings of *P. densiflora* and 700,000 seedlings of *P. thunbergii* derived from the seed orchards were planted. The realized genetic gain in the tolerance (survival rate) was estimated as to be 18% and 35% increase for *P. densiflora* and *P. thunbergii* respectively, based on the artificial inoculation test (Toda & Kurinobu 2002). Here, we show the subsequent progress of the resistance breeding in Japan. To evaluate the field performance of tolerant pine trees, we investigated the pine wilt epidemics in a mixed plantation of tolerant and susceptible families of *P. densiflora* and *P. thunbergii* for seven years. The cumulative mortality was higher for susceptible families and lower for resistant families. The results suggested that the tolerance level of the first generation tolerant pine clones may not be enough to control the disease if no other protection measures were used. A simple simulation suggested that the current tolerance level of tolerant pine families increased the probability of the disease control when some of other control methods were applied together. Because only 16 tolerant *P. thunbergii* clones was selected in the first resistance project, supplemental selection were conducted to select additional tolerant clones for the seed orchard improvement and for the advanced generation breeding. In the Kyushu region, southwestern part of Japan, seeds were collected from 437 trees in 19 disease infested sites as a candidate population. The survival rate after the nematode inoculation varied among seed trees with in a site and especially among sites. Finally, 37 additional *P. thunbergii* clones were identified as tolerant. To improve the quality and quantity of seedlings from tolerance seed orchards, we evaluated seed productivity and progeny tolerance of additionally selected clones and started to study the optimum design of a seed orchard.

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Keywords: *Pinus thunbergii, Pinus densiflora, Bursaphelenchus xylophilus*, pine wood nematode

VARIATION AMONG CLONES IN THE INTER-ANNUAL REPRODUCTIVE FLUCTUATION OF JAPANESE LARCH *(Larix kaempferi)* IN RELATION TO CLIMATIC FACTORS

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Larix kaempferi (Japanese larch) is one of the most important tree species for subalpine and subboreal forestry in Japan, and therefore its stable seed and seedling supply is an important issue. However, because *Larix* often shows a large year-to-year fluctuation in sexual reproduction (i.e. masting), it has still been difficult to control perfectly its reproduction in orchards. Moreover, because there is a possibility that genotypes originated from different climatic conditions may show different tendency in their reproductive intensity and frequency, it is important to obtain information about the degree of variation among the genotypes in the tendency of sexual reproduction.

To increase our understanding of the sexual reproduction of *L. kaempferi*, we first assessed the importance of climatic factors (weather variables) on masting behaviors. We monitored annual seed productions of 234 *L. kaempferi* clones over twenty four years. Those clones were mainly selected from the mountainous regions of central Japan (i.e. near the natural distribution ranges of *L. kaempferi*). In the analysis, the annual seed production was used as dependent variable, while monthly precipitation, monthly mean temperatures and monthly sunshine hours in the previous spring-summer as explanatory variables.

Second, we estimated the sensitivity of each clone in terms of their female and male cone productions, based on the four-years record for reproductive status of girdling and intact ramets of each clone. We analyzed the data by using a generalized linear mixed effect model with a logistic regression function, in which girdling treatment was set as fixed factor. We then portioned the variance components among clone (nested within provenance), provenance and environments.

According to the first analysis, the annual seed production fluctuated greatly from year to year, and we found that the positive effect of higher mean temperature and negative effect of precipitation of the previous summer on the seed production. Based on the second analysis for the reproductive status of producing female cones, variance components among provenances (22%) and among clones within provenance (29%) were relatively large. On the other hand, for the reproductive status of producing male cones, the variance component estimated for provenance was much smaller (0.4%). We obtained the posterior estimates for the reproductive intensity and frequency for more than two hundred *L. kaempferi* clones. This suggested that some clones/provenances had tendency to reproduce more frequently. There was also a positive correlation between the female and male functions in the sexual reproduction of *L. kaempferi*.

The findings from our long-term monitoring study will increase our understanding of evolutionary aspects of reproduction of *Larix* in relation to environmental conditions. The information about the tendency of cone production for each clone will also be useful to enhance efficient breeding program.

Keywords: Genotype-by-environment interaction, local adaptation, masting, provenance, variance component

GENETIC VARIATION AND GENETIC GAIN IN A SEED ORCHARD OF NORWAY SPRUCE IN ROMANIA

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The breeding program of Norway spruce in Romania started in the 1970s at the Forest Research and Management Institute. Selection of the seed stands, plus trees, and establishing the seed orchards were the first stages of the breeding program 1500 plus trees were selected from all forest areas in Romania and eight clonal seed orchards and one seedling seed orchard were established.

The seedling seed orchard of Norway spruce was established in 1976 at Sighisoara Forest District on a surface of 10 ha. The seed orchard is situated at sessile oak belt and is considered to be in complete isolation of other spruce stands. Plus trees were selected in nine autochthonous populations from Eastern Carpathians, being both spruce trees with resonance wood and common spruce trees. The plus trees with resonance wood were selected by phenotypic characters according with the literature in the field. In total, 117 open pollinated families were created from which 94 families were of common spruce and 23 was assumed to be spruce with resonance wood.

In this study we analyzed 43 families at an age of 34 years, 35 families are common spruce and 8 of assumed resonance spruce. The aim of the study was to determine if there is a genetic determinism for the resonance spruce characters: growth traits, stem straightness, branch and bark characters. The scientific objectives were to:

1) evaluate the genetic variation within and among the Norway spruce populations,
2) estimate heritability and
3) determine genetic gain for growth, stem straightness, branches characters and bark both for common spruce and resonance spruce open-pollinated families.

At 34-year old there was a high genetic variation for growth and quality traits among the Norway spruce families. The variance analyze highlights statistically significant differences between the two spruce groups for the following characters: diameter at 1.30m, stem straightness, bark form. The common spruce families had a greater genetic variability compared with resonance spruce families. The heritability was higher for all traits of resonance spruce progenies compared with common spruce progenies. For resonance spruce progenies, the heritability of growth traits are smaller (h2fam= 0,36 - 0,47) than the heritability of branches characters (h2fam= 0,38 - 0,67) and bark form (h2fam= 0,49).

The results have revealed a strong genetic control for branch and bark characters of resonance spruce progenies. These are the diagnosis and differentiation characters of resonance spruce and maintaining them at the progenies in other sites condition completely different from the environment of origin highlights the genetic determinism of this ecotype. Genetic gain estimated by applying both family selection and individual selection were greater for resonance spruce families than for common spruce families. Genetic gain by selection the best 10 % individuals of over all families was between 2% to 3% for growth and between 2% to 10% for branches characters.

The results are important for forest practice and in the species breeding program suggesting that selection of founders for next breeding generation could be possible.

Keywords: Norway spruce with resonance wood, progeny trial, genetic parameters, genetic variability, genetic gain

ESTIMATION OF GENETIC GAIN BY GENETIC THINNING IN BREEDING SEED ORCHARDS OF *Quercus acutissima* AND *Quercus variabilis*

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Genetic gain and gene diversity were estimated by genetic thinning methods under various thinning intensities in breeding seed orchards of *Q. acutissima* and *Q. variabilis*. The three scenarios of genetic thinning (intensities; ranged from 10% to 90%, interval of 10%) were theoretically simulated as 1) Individual thinning (superior individuals were selected for stem volume), 2) Family thinning (superior families were selected, regardless of individual performance), and 3) Family+within family thinning (family thinning first and then individual thinning from the selected family; the number of seedlings selected from each family was linearly proportional to the breeding value of the family). The gene diversity was estimated by the concept of effective population size, which considered the genetic relatedness (coancestry) among individuals. As the results, individual thinning showed higher genetic gain compared to the other methods. The family thinning gave the lowest genetic gain, so it could not be applied in these seed orchards. On the other hand, the family thinning was more effective to maintain gene diversity than the other methods. Individual thinning and family+within family thinning showed consistent gene diversity under week thinning intensities, but decreased under moderate intensities. In conclusion, individual thinning method could be effective under 50% thinning intensities and family+ within family thinning method would be appliable above 50% intensities for maximizing genetic gain and maintaining gene diversity in the breeding seed orchards of *Q. acutissima* and *Q. variabilis*.

Keywords: *Quercus acutissima, Quercus variabilis*, genetic thinning, genetic gain, effective population size

SELECTION OF PLUS TREES AND ESTABLISHMENT OF SEED ORCHARDS FROM THREE TROPICAL TIMBER SPECIES IN CAMBODIA

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The forest of Cambodia continues to play an important role in supporting economic growth and in eradicating poverty. Unfortunately, the country is facing a great challenge in dealing with the rapid decline of the forest and in restoring its condition. A production of superior seed and seedling is an essential prerequisite to restore the degraded forests of Cambodia. In the context, a landmark project from AFoCO (ASEAN-ROK Forest Cooperation) was launched to select plus trees and to establish progeny test plantations and seed orchards. This project was implemented as a part of collaboration among AFoCO, NIFOS (National Institute of Forest Science, Korea) and IRD (Institute of Forest and Wildlife Research and Development, Cambodia).

We have selected a total of 108, 105 and 100 plus trees of three economic timber species including *Dalbergia cochinchinensis, Pterocarpus macrocapus* and *Dipterocarpus intricatus* from across all ecological zones in Cambodia. The 308 plus trees were identified as mother trees so that the seeds from the 300 plus trees were collected and germinated for the establishment of half-sib progeny test planation. The progeny test plantation covers 10.8 hectares for the three species and the randomized complete block design with four replications was applied with a block size of 180 m x 150 m. One thousand and two hundred plots with a size of 6 m x 15 m were assigned to plant ten trees in a double-row plot and the seedlings were planted at a spacing of 3 x 3 m.

There was a large variation of height and diameter at root collar (DRC) among plus tress and species. The average ages of selected plus trees were 22.3, 19.4 and 17.9 years old for *Pterocarpus macrocapus, Dalbergia cochinchinensis* and *Dipterocarpus intricatus*. The averages of tree height were 16.1 (range 7.4 to 25.0), 14.8 (7.0-25.0) and 13.5 (7.0-18.0), and those of DBH were 39.9 (range 19.2 to 67.0), 25.5 (14.5-52.0) and 45.0 (20.0-135.0), respectively. In the poster presentation, we will include the genetic analysis of progeny tests from three tropical timber species in Cambodia.

The scions of selected plus trees were collected and grafted for establishing two hectares clonal seed orchards (one ha per species) of *D. cochinchinensis* and *P. macrocarpus* in 2016. The seed orchards were established with a spacing of 5 x 5 m. These seed production populations would be a source of genetically improved seed production for the reclamation of Cambodian forests in the near future.

Keywords: Selective breeding, plus-tree selection, clonal seed orchard, progeny test

HERITABLE VARIATION IN CHLOROPHYLL FLUORESCENCE, NEEDLE SPECTRAL REFLECTANCE AND PHOTOSYNTHETIC PIGMENTS IN SCOTS PINE *(Pinus sylvestris L.)*

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The existence and extent of heritable variation in chlorophyll fluorescence, photosynthetic pigments and needle spectral reflectance in Scots pine were investigated in a structured population under no apparent stress. Preselected phenotypes within half-sib progenies of seed orchards were subjected to the paternal assignment using SSRs markers.

The study was conducted on Western Bohemian pine population within two half-sib test plantings designed in incomplete blocks across two sites. On both sites, partial pedigree reconstruction was carried out providing the identification of paternal trees.

Sample collection (523 in total) and subsequent measurements of fluorescence, spectral reflectance, photosynthetic pigments and water content took place in the summer (July and August) of 2014. Spectral reflectance was measured between 350 and 2500 nm using an ASD FieldSpec 4 Wide-Res portable ASD spectrometer (ASD Inc., USA). Vegetation indices were calculated on the basis of fluorescence/reflectance data. Photosynthetic pigments (chlorophylls a and b, total carotenoid content) were extracted with N, N – dimethylformamide, their content determined spectrophotometrically and expressed as the amount of pigment per dry matter (g/kg).

The statistical analysis was based on the so-called animal model (Lynch & Walsh, 2007) under mixed linear model platform. We used a reconstructed pedigree based on SSR marker analysis to build a relationship matrix that is an integral part of this analysis.

In most studied traits, significant narrow sense heritability was found, ranging from 0.14 to 0.33. For the reflectance in single wavelengths, heritability estimates were not significant within visible region, whereas all heritability estimates for longer wavelengths were significant.

Heritability detected along the reflectance curve suggests the existence of selection pressure on reflectance properties in the visible region (arguably due to crucial importance of photosynthesis) and relatively weaker selection on spectral properties at longer wavelengths. Better knowledge of pigment- or spectral reflectance-based stress markers may help recognizing abiotic stress resistant genotypes. The spectral signals such as single wavelength reflectance or spectral indices may serve as indirect markers correlated to production or stress adaptive traits in breeding.

Keywords: Chlorophyll, foliar spectral reflectance, pedigree reconstruction, heritability, *Pinus sylvestris*

ESTIMATION OF EXTERNAL FACTORS PROMOTING CONE BUDS INDUCTION IN *Larix kaempferi, L.gmelinii var japonica* AND *Picea glehnii*

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Larix kaempferi, Hybrid larch *(L.gmelinii var japonica×L. Kaempferi)* and *Picea glehnii* are important forestry species in Hokkaido, northern Japan, and those timbers are often used as construction materials.

To enhance the wood quality genetically, we are currently implementing plus-tree breeding program for those species. However, the masting behavior in cone production and unavailability of efficient treatment for promoting cone buds have delayed the program. For the better understanding to the reproduction biology of the species, we estimated external factors that are related to the promotion of cone buds induction.

We investigated the cone production in seed orchards for seven consecutive years between 2010 and 2016 using a five levels of index in the three species. We used the Random Forest (Breiman, Leo 2001) for model construction, where the cone production were used as dependent variable and monthly precipitations, monthly mean temperatures and monthly sunshine hours from March to July in the previous year as explanatory variables.

As a result of the analysis, there was a tendency that the lower the temperature, the shorter the monthly sunshine hours in early spring, the longer the monthly sunshine hours and less the precipitation in summer of the previous year would enhance cone production in *L.kaempferi*. Low temperature in spring, high temperature in summer, shorter sunshine hours from early spring to summer have a positive effect on seed cone production in *L.gmelinii* var japonica. Since seed cone production is increased by low temperature and short sunshine hours in early spring before bud flush, vernalization may be related to the induction of cone-buds in *L kaempferi* and *L.gmelinii var japonica*.

The seed cone production tended to be increased by low monthly mean temperature in spring, high monthly mean temperature and short monthly sunshine hours in summer of the previous year in *Picea glehnii* (Fig. 1).It is likely that seed cone production is susceptible to low temperature before bud flush and high temperature after bud flush in *P. glehnii*. Vernalization may be also related to the induction of cone-buds in *P. glehnii*.

A further direction of this study will be to elucidate suitable sites for establishing new seed orchards and to develop a new cone producing system using indoor seed orchard.

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Keywords: Seed cone production, vernalization, *Larix kaempferi, Larix gmelinii var japonica, Picea glehnii*

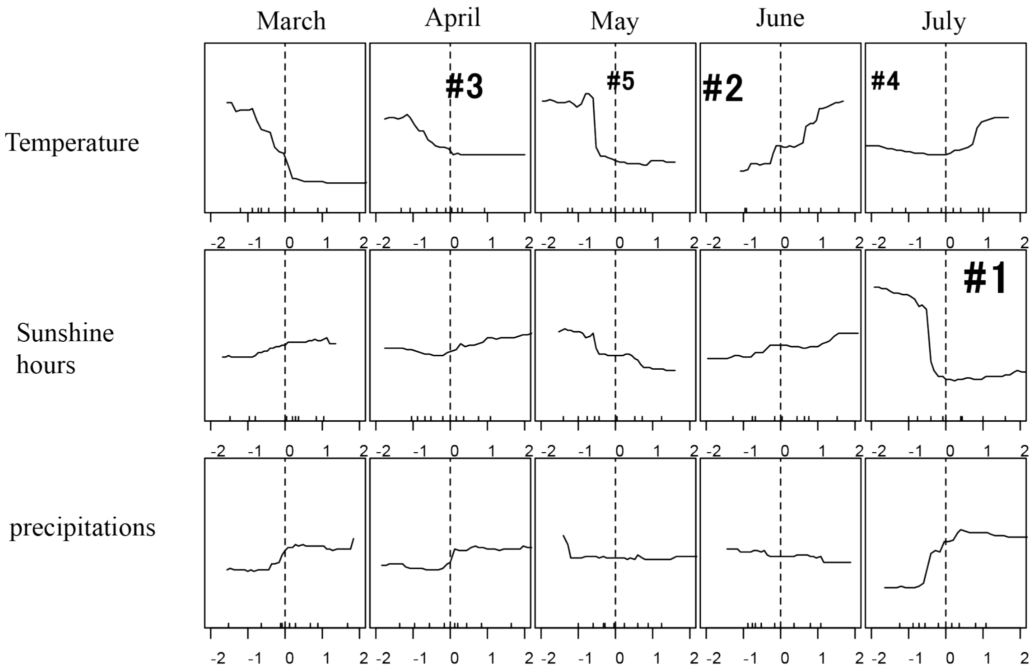

Figure 1. Effects of each climate factor in the previous year to the seed-cone production in *Picea glehnii*.

X-axis shows deviations from annual average, y-axis shows effects for the seed-cone production index and high values in y-axis indicates high cone production.

shows rank of climate factors influencing cone production.

ROOTED SHOOT CUTTINGS FROM SE DONOR PLANTS IN FINLAND – POTENTIAL MATERIAL FOR BREEDING AND PROPAGATION

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Norway spruce *(Picea abies)* is the most cultivated species of coniferous trees in Finland. Currently the testing of Norway spruce breeding materials is carried out by clone testing (Haapanen, 2009). Clonal material is propagated from seedlings by rooting shoot cuttings. Single testing cycle is approximately 25-30 years long, half of which consists of seed production and test plant production (Haapanen, 2009). Developing the production of clonal material could save time from the beginning of the testing cycle. One way to shorten the plant production time is to produce several donor plants from the same clone with somatic embryogenesis (SE) to shorten the production time of test material.

Producing large numbers of SE plants (emblings) of several genotypes from different crossings with unknown embryo production capacity is challenging, and would require large laboratory capacity (Tikkinen, et al. 2017). The number of clones needed for field testing in tree breeding is high, which makes it difficult to use emblings straight as test material (Högberg, et al. 1998). The test plant production for breeding is possible with lighter laboratory capacity, by combining SE with the production of rooted shoot cuttings (Tikkinen, et al. 2017). Key requirement for this integration is that shoot cuttings from emblings root properly.

Cryopreservation of embryogenic masses possibly enables the cessation of the ageing of the clones during the testing cycle and production (Varis, et al. 2014). Cryopreserved cell lines can also be later propagated for breeding purposes or forest regeneration material (Varis, et al. 2017). Introducing rooted shoot cuttings from embling donors requires a large scale comparison between clonal materials produced with conventional methods and modern tissue culture technologies.

Results from rooting tests carried out with cuttings originating from embling donors will be presented. Two rooting tests were carried in 2015 and 2016, and the test material consisted of 34 clones from 11 full-sib families from progeny tested plus trees from southern Finland (Tikkinen, et al. 2017). The best combination of treatments resulted in 91% rooting, on an average (Tikkinen, et al. 2017). Also estimates of demand for number of emblings to enable considerably faster test plant production compared to rooted shoot cuttings originating from seedling donors will be presented.

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Keywords: *Picea abies*, somatic embryogenesis, clone testing

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