

Genetic aspects linked to production and use of forest reproductive material (FRM)

Collecting scientific evidence for developing guidelines and decision support tools for effective FRM management



Dušan Gömöry, Katri Himanen, Mari Mette Tollefsrud, Claes Ugglå, Hojka Kraigher, Sándor Bordács, Paraskevi Alizoti, Stuart A'Hara, Aline Frank, Gunnar Friis Proschowsky, Josef Frýdl, Thomas Geburek, Monique Guibert, Mladen Ivanković, Ana Jurše, Stuart Kennedy, Jan Kowalczyk, Heike Liesebach, Tiit Maaten, Andrej Pilipović, Roberta Proietti, Volker Schneck, Alain Servais, Brynjar Skúlason, Christoph Sperisen, Frank Wolter, Tefide Yüksel and Michele Bozzano.

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PREFACE

The genetic makeup of Forest Reproductive Material (FRM) is directly affected by the decisions made by the various actors involved in its production chain. These decisions, which often ignore the genetic perspective, have a major impact on the survival of future forests.

There are still gaps and uncertainties in the current body of knowledge on the matter, such as which FRM should be recommended for a given site and what the adaptive potential of forest tree populations will be. This makes it difficult to prepare detailed guidelines for the production and use of FRM. These gaps are being partly addressed by ongoing research, which is generating scientific evidence to reinforce the development of decision support tools for assisting in the production and use of FGR.

In November 2015, the EUFORGEN Steering Committee established a working group to unpack these research efforts and identify the genetic aspects in each link of the chain for the production and use of Forest Genetic Resources (FGR).

The working group reviewed relevant literature and capitalised on the results of a previous EUFORGEN Network, known as the *Forest Management network*, which was active from 2005 to 2009¹. The group also built upon the publication, *Use and transfer of forest reproductive material in Europe in the context of climate change*². Furthermore, the working group integrated the discussion points derived from the GenTree³ stakeholders' consultation which took place in Madrid (Spain) in October 2016. The working group met three times: in November 2016 (Madrid, Spain), June 2017 (Warsaw, Poland) and November 2017 (Rome, Italy). During the 13th Steering Committee meeting (June 2018), the draft report was presented along with recommendations stemming from it for specific target groups. An extended circle of experts from EUFORGEN community then received the document for peer

¹ <http://www.euforgen.org/about-us/history/phase-iii-2005-2009>

² The report, *Use and transfer of forest reproductive material (FRM) in Europe in the context of climate change* is available at the EUFORGEN website: <http://www.euforgen.org/publications/publication/use-and-transfer-of-forest-reproductive-material-in-europe-in-the-context-of-climate-change/>

³ <http://www.gentree-h2020.eu/>

review. Finally, a Task Force (Katri Himanen (Finland), Paraskevi Alizoti (Greece) Sándor Bordács (Hungary), Mari Mette Tollefsrud (Norway), Dušan Gömöry (Slovakia), Hojka Kraigher (Slovenia) and Claes Uggla (Sweden)) reorganised the report in November 2018 (Oslo, Norway) and developed its first draft. In 2019 Silvio Oggioni, as a member of the EUFORGEN Secretariat, contributed to the coordination and the finalisation of this report. Several rounds of peer reviewing within the EUFORGEN community followed until June 2020.

This work is the result of an international collaboration rooted in EUFORGEN for more than two decades. It is built on the firm belief that the genetic element is decisive for the creation of a resilient forest capable of surviving threats and adapting to changes, thus enabling the evolution of ecosystems and the conservation of the productive landscape.

Michele Bozzano,
EUFORGEN Coordinator,
December 2020

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ACRONYMS

AGF	Assisted geneflow
AM	Assisted migration
BWB	Breeding without breeding
CBD	Convention on Biological Diversity
cpDNA	Chloroplast DNA
CREA	Research Centre for Forestry and Wood, Italy
DCU	Dynamic Conservation Units
DMSO	Dimethyl sulfoxide
DUS	Distinctness, Uniformity and Stability
EAFRD	European Agricultural Fund for Rural Development
EFI	European Forest Institute
EUFORGEN	European Forest Genetic Resources Programme
FAO	Food and Agriculture Organisation
FOREMATIS	Forest Reproductive Material Information System
FSC	Forest Stewardship Council
FSGS	Fine-scale spatial genetic structure
FVM	Marketing of Forest Reproductive Material
GPA-FGR	Global Plan of Action for the Conservation, Sustainable Use and Development of Forest Genetic Resources
GTTN	Global Timber Tracking Network
IBL	Polish Forest Research Institute
IDS	Invigoration, drying, and separation
IUFRO	International Union of Forest Research Organizations

MPBS	Multiple Population Breeding System
NDA	National designated authority
NIR	Near infrared spectroscopy
OECD	Organisation for Economic Cooperation and Development
ONP	National Poplar Observatory, Italy
PEFC	Programme for the Endorsement of Forest Certification
RNBM	National Register of Basic Materials, Italy
RPBC	Radiata Pine Breeding Cooperative
SE	Somatic embryogenesis
SFI	Slovenian Forestry Institute
Skogforsk	Forestry Research Institute of Sweden
SMP	Supplemental mass pollination
SNPFs	Semi-natural planted forests
SSR	Single sequence repeat
UPOV	International union for the protection of new varieties of plants
VCU	Value of Cultivation and Use tests

EXECUTIVE SUMMARY

The increasing human population will continue to generate a corresponding increase in demand for forest goods and services, requiring even more intensive and sustainable management of forests in Europe and elsewhere. The production, trade and use of appropriate Forest Reproductive Material (FRM) is therefore of utmost importance for the future of forests, where the genetic characteristics of FRM are essential to ensure the long-term adaptive potential and increased productivity of forests, especially in the context of climate change. FRM genetic makeup is directly affected by the decisions made by the various production chain actors. These decisions, which often ignore the genetic perspective, have a major impact on the survival of future forests.

Currently, for the eight main forest tree species alone, about 30 million plants and 400 metric tonnes of seeds are traded annually in Europe. This trade occurs within an EU framework of regulations related to FRM: the OECD Scheme for the Certification of the Forest Reproductive Material Moving in International Trade controls the international trade of FRM, while EU Council Directive (1999/105/EC) on the marketing of FRM sets the minimum requirements for the approval of different types of basic material intended for production and use at the European level.

There are still gaps and uncertainties in the current body of knowledge on FRM-related matters, such as which FRM should be recommended for a given site and what the adaptive potential of forest tree populations will be. These gaps are being partly addressed by ongoing research, which is generating scientific evidence to reinforce the development of decision support tools for assisting in the production and use of FGR.

Against the background of the EU framework of regulations, a working group established by the European Forest Genetic Resources Network (EUFORGEN) assessed the research on the chain of production and use of FRM in order to better understand the potential genetic influences inherent in each link.

In bridging forestry and science, this report synthesises EUFORGEN's research review and its decades-long international collaboration, built on the firm belief that genetics is critical for the creation of a resilient forest capable of surviving threats and adapting to changes,

thus enabling the evolution of ecosystems and the conservation of productive landscapes. It advocates a chain of custody from the origin of a seed used at a certain planting site to all goods produced over the rotation time (accumulated value) such that forest management would benefit, where FRM could significantly improve forest stability and productivity.

The report summarises the current state of knowledge in key areas and in the final chapter offers 38 recommendations arising from the review. Its two overarching recommendations are to:

- ➔ Create an online information system to contain geo-referenced records of the origin, movement and use of FRM.
- ➔ Balance the goals of production and of genetic diversity conservation in all FRM management.

Report Contents

The first chapter describes the different categories of Forest Reproductive Material (FRM), considers the strategic choice between artificial and natural regeneration in the face of climate change, and articulates the need for and purposes of FRM. Chapter 2 outlines the FRM production chain, in terms of: i) FRM approval, management, collection and certification (seed sources, stands, orchards, plus trees and clones others); ii) Testing standards and examples; iii) Effect of seed and seedling material not intended for use as FRM on forest genetic resources; iv) Breeding effects on basic material including conservation strategy; v) Harvesting, processing and storage of seeds; vi) Nursery practices; vii) Certification and Traceability; and viii) FRM Trading and Transport. Chapter 3 examines FRM with regard to: i) Forest regeneration strategies – especially considering climate change, and ii) Assisted migration and available decision support tools. Chapter 4 provides a detailed overview of forest establishment methods, and chapter 5 looks at keeping records of seed origin to improve forest management. The chapters can be summarised as follows:

Comprehensive overview of FRM categories

The six types of basic FRM (Seed source, Stand, Seed Orchard, Parents of Family/-lies, Clone, Clonal Mixture) intended mainly for forestry and agroforestry can be certified by a Designated Authority (an official national body) under one of the following broad categories: a) Source-Identified, b) Selected c) Qualified, and d) Tested. (OECD, 2018). All FRM will fall into one of the 24 cells in the matrix of six types by four categories (OECD, 2018).

Strategic choices between artificial and natural regeneration in coping with climate change

Foresters can both rely on the natural capacity of tree species to cope with environmental changes and actively help forests to survive the various stresses associated with climate change. Active assistance takes the form of directly influencing the genetic composition of forest stands by applying silvicultural measures selectively and by choosing specific FRM for reforestation. Each carries risks and the choice of either, or of a combination of both, depends largely on the adaptive genetic variation in the populations and the time frame under consideration.

The need for and purposes of FRM production

The report looks at six categories of forestry that make use of FRM: multifunctional forestry, quality and energy wood production (plantation forestry), climate change adaptation and mitigation, and land and forest restoration. The decision to use FRM produced in nurseries will depend on the kind of project, the main socio-economic and ecological objectives of the future stand, legal and voluntary certification obligations, and risk management with respect to climate change adaptation and mitigation.

Production of basic material

The working group examined all steps in the production of the six different types of basic material in the four categories of FRM, bearing in mind that foresters may not be fully aware of the differences between FRM and the basic material they purchase. Basic material must be approved by testing before FRM can be collected. Sources and stands must be mentioned in forest management plans and any management measures assessed for the possible effects on the FRM produced by the stand. Certification of FRM must accommodate nationally-specified procedures to ensure the rights of the basic material owner and to allow the FRM to be traced from production to market.

Effect of material not intended as FRM on forest genetic resources

Seeds not collected from approved sources, as well as trees used for decorative purposes, short rotation biomass production or erosion prevention and other uses, may contribute unintentionally to forest genetic resources as a result of the movement of pollen or seed. Foresters and management need to be aware of this possibility.

Decision tools to support choice of FRM for climate change

Natural regeneration and assisted migration, or a combination of the two are available as regeneration strategies in the context of climate change. The choice will depend on the available FRM and existing knowledge about the adaptive potential and plasticity of the material. Each country has its own decision support tools to help choose FRM, based on different parameters, predictions and other factors.

Record keeping for improving forest management

Forest managers face a variety of issues regarding the establishment and management of forests, which will have an impact on the differential survival and juvenile growth of individual trees, thus potentially influencing the genetic structure of the new stand. These effects make it essential to maintain records on seed sources. Good records benefit several levels, from policy to practical management. Ideally, records should track the complete chain from the origin of seed used at a site to all goods produced over the entire rotation cycle.

Recommendations

The review content helps better inform conserving and managing the diverse FRM resources and to more effectively use these to help meet increased demands for forest products and services and mitigate any negative impacts of climate change and other stressors. As well as the overarching recommendations cited above, the authors have included detailed recommendations relating to policy, research and FRM management as articulated in chapter 6 and summarised as follows:

→ Policy

- i) Increase knowledge on potential benefits from not exclusively using natural regeneration in response to climate change;
- ii) Communicate the importance of choosing appropriate FRM;
- iii) Promote planting with genetically variable material;
- iv) Comply with obligations to share information about FRM movement between EU Member States;
- v) Highlight the importance of phytosanitary regulations and raise awareness on pests and diseases;
- vi) Keep national and international registers updated;
- vii) Keep records of the origin of forest stands;
- viii) Develop decision support tools for transfer of FRM in response to climate change, and
- ix) Improve FRM production, use and conservation by sharing good practices

→ Research

i) Disseminate information about field trials online and in English; ii) Create genetic models to identify the most suitable FRM; iii) Emphasise adaptive traits in future studies; iv) Develop tools to rapidly identify pathogenic species and share information; v) Study and elucidate the role of epigenetic phenomena in adaptation and diversity; vi) Study symbiotic interactions; vii) Improve marker-based certification; viii) More provenance trials for rare species, and ix) Study the effects of environment and management on genetic diversity of FRM

→ Management: General

i) Record the origin and movement of FRM for traceability; ii) Disseminate knowledge about the best use of FRM; iii) Increase the variety of approaches for testing FRM; v) Increase knowledge of phytosanitary issues in FRM; vi) Seed companies are encouraged to keep reference samples of FRM for traceability, and vii) Maintain genetic diversity of FRM in artificial forest regeneration and forest management.

→ Management: Basic Materials

i) Plan management activities in seed stands to assess and conserve genetic diversity; ii) Favour collections during mast years and improve FRM harvesting techniques; iii) Make available as much information as possible on Source-Identified and Selected Stand FRM; iv) Manage seed orchards to promote genetic diversity and genetic mixing; v) Produce high-value FRM from plus trees and trees included in breeding populations to widen the range of regeneration options; vi) In clonal stands, encourage clonal mixtures and controls on clone status, and vii) Stricter controls on collection of FRM for own use and for purposes other than forestry.

→ Management: Seed Production and Nursery

i) Avoid seedling production steps that decrease genetic diversity; ii) Promote genetic diversity by controlled mixing of seed lots of different years; iii) Discourage seed fractioning to maintain genetic diversity; iv) Consider carryover effects of nursery activities, and v) Consider inoculating seedlings with beneficial mycorrhizae.

1. INTRODUCTION

Alizoti, P.

Forest ecosystems and their contribution towards climate-change mitigation are becoming increasingly important, as they represent critical components of the global carbon cycle. Forests remove vast quantities of anthropogenic carbon per year via their net growth, and store large reservoirs of carbon, holding more than double the amount of carbon in the atmosphere (Canadell and Raupach, 2008). However, forests also have to cope globally with intensified human-related stressors; direct (logging, deforestation, change of use, fragmentation, increased demand for forest goods and services) or indirect novel ones (climate change, air pollution, invasive biotic factors) (Trumbore *et al.*, 2015; FAO, 2016; Adams and Pfautsch, 2018). Forests remain resilient in the face of disturbances. However, the unprecedented speed and amplitude of novel stressors like climate change may seriously threaten forest health and existence (Gauthier *et al.*, 2015), therefore appropriate management options to mitigate those threats need to be adopted. Multipurpose forestry is expected to be the main forest management approach to mitigating these threats, as it offers an extensively broad array of ecosystem services compared to other wood production options (i.e., forest plantations).

Given the above-mentioned challenges, the production, trade and use of genetically and adaptively appropriate Forest Reproductive Material (FRM) is thus critical for the future of forests and the goods and services that they produce.

Given the significance of production, use and trade of FRM, the EU Council Directive (1999/105/EC) (hereafter only “Council Directive”) on marketing of FRM has been established in Europe since 1999 (based on modifications from the two previous directives from 1966 and 1971). The Council Directive has thereafter been implemented in all Member States’ national legislations, to ensure that FRM supplied for any site within the EU is suitable for the geographic location of that site. For the same important reasons, the international trade of FRM has been facilitated on a global scale since 1967, when the Organisation for Economic Cooperation and Development (OECD) established its first Scheme, that was fully revised in 1974. The second full revision was completed in 2007 and became the ‘OECD Scheme for the Certification of the Forest Reproductive Material Moving in International Trade’ released in its amended form in 2018.

According to the Council Directive, FRM is ‘the reproductive material of tree species and artificial hybrids which are important for forestry purposes in all or part of the Community’, and which can include seed units, parts of plants, planting stock. Meanwhile, OECD (2018) gives an equivalent definition for FRM, defined as ‘the reproductive material of genera and species of forest trees and shrubs’, which can include seeds, parts of plants and plants.

Within the above-mentioned framework, there are six types of **Basic Material** (trees or vegetative material from which reproductive material is obtained): **seed source, stand, seed orchard, parents of family, clone, and clonal mixture**. The FRM intended mainly for forestry and agroforestry is divided into four broad categories: a) **Source-Identified**, b) **Selected** c) **Qualified**, and d) **Tested**.

In accordance with the Council Directive, each EU Member State holds a national register of different types of approved basic material, and all the FRM marketed within the EU must come from an officially registered basic material. Once collected, the FRM is issued a Master Certificate by the Designated Authority certifying that it is derived from approved basic material. The certificate provides additional relevant information, such as the type of basic material, its origin and its phenotypic and genetic quality. All approved basic material appears in national registers, while information regarding all existing FRM categories and types per EU country, as well as any tree species of interest, is held in the Forest Reproductive Material Information System (FOREMATIS) database⁴. A Master Certificate issued in one EU Member State is valid throughout the whole of the EU. When FRM is transferred between Member States, the authority of the providing Member State must inform the authority of the recipient Member State of the identity and amount of traded FRM. Therefore, the company or individual trading FRM across Member States shall notify the authority of the providing country about the trade. The import of “source-identified”, “selected” and “qualified” FRM into the EU from third countries is regulated by the EU Council Decision 2008/971/EC and Decision 1104/2012/EU, according to which the imported FRM should possess the same assurances as the material produced within the EU. The EU Council Decision states that any FRM certified in Canada, Norway, Serbia, Switzerland, Turkey and the United States under the OECD certification rules fulfils the equivalent EU requirements. Furthermore, Commission Decision 2008/989/EC and Commission Implementing Decision (EU) 2015/321 additionally regulate imports (for the “tested” category and for some other third countries), while additional imports are allowed based on the decisions of the Working group on FRM at DG SANTE (G1 plant health).

⁴ <https://ec.europa.eu/forematis>

Within this EU framework, about 30 million plants and 400,000 kg of seeds from eight main forest tree species alone [Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), maritime pine (*Pinus pinaster*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), sessile oak (*Quercus petraea*), common oak (*Quercus robur*) and northern red oak (*Quercus rubra*)] are traded annually in Europe, with coniferous FRM trade being dominated mainly by Northern European countries, and broadleaved species being traded to a larger extent in Central Europe (Jansen *et al.*, 2019).

The scope of the present report is to provide a detailed review of the genetic aspects of **collection and storage, production, approval, management, certification, traceability, trading, transport and use** of the different types of basic material and the four Categories of FRM, as well as recommendations for various steps of the procedures that need to be followed in all the above phases.

1.1 Categories of Forest Reproductive Material

Alizoti, P.G., Kraigher, H.

The EU Council Directive (1999/105/EC)⁵ follows an equivalent scheme (in effect since 1999, provisioning and defining all the categories and types of basic material) to that of the OECD. It applies to EU Member States and sets the **Minimum Requirements** for the approval of different types of basic material intended for production and use at the European level. More stringent requirements, however, can be set-up by the individual EU Member States⁶. The amended ‘OECD Forest Seed and Plant Scheme’ was launched in 2018 and includes equivalent types and categories of basic material to the ones provisioned by the Council Directive.

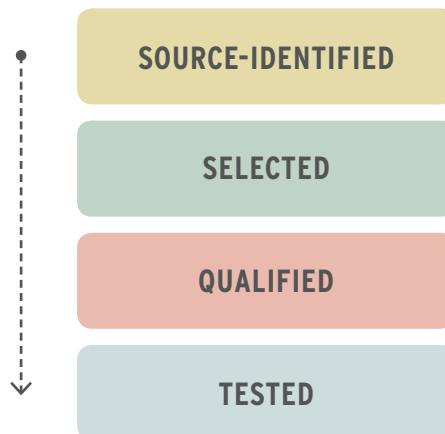
According to the Council Directive, the four categories of FRM (Figure 1) used by the Official Bodies to characterize the FRM are as follows:

⁵ <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A31999L0105>

⁶ <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31999L0105&from=EN>

- **Source-identified:** the minimum standard permitted. Basic material which may be either a seed source or stand-located within a single region of provenance. The location and altitude of the place(s) from which FRM has been collected must be recorded; little or no phenotypic selection has taken place.
- **Selected:** basic material consisting of a stand located within a single region of provenance, which has been phenotypically selected at the population level.
- **Qualified:** basic material which shall be seed orchards, parents of families, clones or clonal mixtures, the components of which have been phenotypically selected at the individual level. Undertaking or completing testing is not required.
- **Tested:** basic material consisting of stands, seed orchards, parents of families, clones or clonal mixtures. The superiority of the reproductive material must have been demonstrated by comparative testing, or an estimate of the superiority of the reproductive material calculated from the genetic evaluation of the components of the basic material.

FIGURE 1. Categories of Forest Reproductive Material according to the Council Directive (1999) and the 'OECD Forest Seed and Plant Scheme' (OECD, 2018). The direction of the arrow indicates the selection level of the FRM (also see Appendix 1).



The definitions of terms related to Forest Reproductive Material are provided in Box 1.

BOX 1. Definitions of terms related to Forest Reproductive material (FRM) (Council Directive, 1999).

TERM	DEFINITION
a. Forest reproductive material (FRM)	Reproductive material of those tree species and artificial hybrids thereof which are important for forestry purposes
Seeds	Cones, infructescences, fruits and seeds intended for the production of planting stock
Parts of plants	Stem cuttings, leaf cuttings and root cuttings, explants or embryos for micropropagation, buds, layers, roots, scions, sets and any parts of a plant intended for the production of planting stock
Plants	Plants raised from seed units, from parts of plants, or from plants from natural regeneration
b. Basic Material	Trees from which reproductive material is obtained
Seed Source	Trees within an area from which seed is collected
Stand	A delineated population of trees possessing sufficient uniformity in composition
Autochthonous stand	A stand or seed source which has normally been continuously regenerated by natural regeneration. It may have been artificially regenerated from reproductive material collected from the same stand or seed source, or from autochthonous stands or seed sources within close proximity
Indigenous stand	An autochthonous stand or seed source or a stand or seed source artificially raised from seed, the origin of which is situated in the same region of provenance
Seed Orchard	A plantation of selected clones or families which is isolated or managed, so as to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant and easily harvested crops of seed
Parents of Family/Families	Trees used to obtain progeny by controlled or open pollination of one identified parent used as a female, with the pollen of one parent (full-sibling) or a number of identified or unidentified parents (half sibling)
Clone	Group of individuals (ramets) originally derived from a single individual (ortet) via vegetative propagation, for example, via cuttings, micropropagation, grafts, layers or divisions
Clonal mixture	A mixture of identified clones in defined proportions
c. Origin	For an autochthonous stand or seed source, the place in which the trees are growing. For a non-autochthonous stand or seed source, the origin is the place from which the seed or plants were originally introduced. The origin of a stand or seed source may be unknown

BOX 1. continued

TERM	DEFINITION
d. Provenance	The place in which any stand of trees is growing
e. Region of provenance	For a species or sub-species, the area or group of areas subject to sufficiently uniform ecological conditions in which stands or seed sources showing similar phenotypic or genetic characters are found, taking into account altitudinal boundaries where appropriate
f. Official body	An authority established or designated by the EU Member State under the supervision of the national government. It is responsible for questions concerning the control of marketing and/or the quality of forest reproductive material; the authority designated by, and responsible to, the Government of a country participating in the OECD Scheme; or an EU Member State for the purpose of implementing the OECD rules on its behalf

Table 1 summarises the permitted combinations of types of basic material and FRM categories (Council Directive, 1999; OECD, 2018).

TABLE 1. Categories in which the various types of basic material can be certified when fulfilling the respective requirements (Council Directive, 1999 & OECD, 2018).

TYPES OF BASIC MATERIAL	CATEGORIES OF FRM			
	Source Identified	Selected	Qualified	Tested
Seed source	√	-	-	-
Seed Stand	√	√	-	√
Seed Orchard	-	-	√	√
Parents of Family/Families	-	-	√	√
Clone	-	-	√	√
Clonal Mixture	-	-	√	√

1.2 Strategic choice between artificial and natural regeneration under climate change

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In a frequently cited review, Aitken *et al.*, (2008) presented three options for tree populations under climate change: *migration* into more suitable habitats, *adaptation* allowing local persistence, and *extinction*. The future fate of tree populations is not only relevant for trees as determining components of forest ecosystems, but also for a broad spectrum of other organisms, co-defining forest ecosystem functioning. Forest owners and forestry professionals can therefore choose one of two approaches: they can either rely on the natural capacities of tree species to cope with environmental changes, or actively help them to survive various types of stresses associated with climate change. The latter can be achieved by influencing the genetic composition of forest stands by applying silvicultural measures or choosing certain reproductive materials for reforestation (Konnert *et al.*, 2015). Each of these alternatives is associated with risks of its own, and the choice of either, or a combination of both, depends largely on the adaptive genetic variation in the populations and the time frame under consideration.

In addition to the survival of tree populations to maintain ecosystem functioning and the delivery of its services, foresters must also ensure optimal wood production for timber and pulp, and for carbon sink purposes. These objectives will all greatly influence the choice of strategy.

In general, high fecundity, large population sizes, broad geographic distributions, spatially extensive dispersal, phenotypic plasticity and rapid generation turnover (or short generation duration), are factors supporting population persistence in changing environments (Aitken *et al.*, 2008; Anderson, 2016). For the majority of forest trees, all these characteristics apply, except for generation turnover, which is typically very long.

Persistence of tree populations in their existing habitats relies on a trade-off between two more or less complementary strategies: local adaptation through natural selection, and flexible response through phenotypic plasticity (Anderson, 2016; Schlichting & Smith, 2002). The crucial issues are the limits of these strategies, the time frame within which they operate, and the extent of loss in production and other functions of forests with which they are associated.

The strategy of local adaptation via natural selection requires long time frames, due to the longevity of forest trees, their late start to effective fecundity and the resulting long

generation turnover time. Population genetics models predict that the allele frequency change under natural selection will be quite slow, unless selection is strong (Wright, 1931). The speed of adaptive response of forest populations, however, depends on population size, heritability of traits related to fitness⁷, interconnection with other populations and the intensity, direction and duration of the selection pressures (Alfaro *et al.*, 2014). However, depending on the extent of the within-population adaptive genetic variation, severe environmental stress causing high selection pressure may provoke high mortality and/or reduced biomass production, which is associated with a loss of forest ecosystem services. Therefore, relying on local adaptation may be a high-risk approach if the pace of climate change is too fast and the frequency of extreme climatic events too high for natural selection to cope with, also depending on the species. From an evolutionary point of view, however, mortality can promote the evolutionary adaptation of forest trees to climate change, if the population is large enough and of high evolutionary potential (Kuparinen *et al.*, 2010). Phenotypic plasticity is a typical feature of forest trees, as it is favoured in heterogeneous environments or under temporally unstable climate. Both spatial and temporal heterogeneity are typical for forest tree populations. In stand-forming species, their large distribution ranges always cover a variety of microsites, frequently contrasted in terms of climate, soil and biotic communities, etc. On the other hand, in rare species, the ranges are typically disrupted and differentiated small fragments are formed.

Temporal heterogeneity is common in trees, because they are frequently exposed to environmental fluctuations during their long lifespans. There is a consensus that plasticity is heritable and may thus be subject to selection (Nicotra *et al.*, 2010), but the molecular basis of plasticity is largely unknown; the background may be both genetic and epigenetic⁸ (Schlichting & Smith, 2002). Vegetative phenology is a good example of an important adaptive trait which may be at least partly under epigenetic control (Vanden Broeck *et al.*, 2018). In European beech (*Fagus sylvatica*), budburst date associations with climate seem to result from local adaptation; however, they also show significant genotype-by-environment interactions, which indicates phenotypic plasticity (Kramer *et al.*, 2017). In conifers, spring flushing as well as growth cessation, and consequently frost hardiness, are determined by photoperiod and temperature during embryogenesis (Johnsen *et al.*, 2005; Skrøppa 1994). Even

⁷ *Fitness* - the ability of a genotype to contribute to the gene pool of the offspring generation- in nature, fitness depends on the viability and fertility of a genotype.

⁸ *Epigenetic change* – hereditary change which is not caused by an alteration of the sequence of bases in the DNA, but rather by chemical modification of the DNA (e.g., cytosine methylation) or DNA-associated histone proteins, or interactions with small RNA molecules.

changes of phenology from the parental generation to the offspring were observed in Norway spruce provenances transferred over several degrees of latitude (Skrøppa *et al.*, 2010). This newly acquired expression pattern caused by epigenetic change may be transferred across several generations (Pigliucci *et al.*, 2006). Climate at the site of early growth may induce similar shifts of budburst (Gömöry *et al.*, 2015). All these examples illustrate that generation turnover makes the population accommodated to the changed climate, as phenological adaptation does not necessarily require genetic change. However, empirical evidence for epigenetic carryover effects in forest trees is only available in association with phenology and frost resistance; whether they also apply to other climate-relevant traits, such as drought tolerance, has been less well studied (Brodrribb *et al.*, 2020).

Provenance experiments, especially those containing large numbers of provenances repeatedly planted across large geographic and climatic gradients, have documented that while tree populations can support a much broader range of abiotic environments than their current distribution ranges, they are limited by biotic interactions (symbiosis, competition, and pests and diseases). In terms of the best performance (growth, survival), tree populations - except those on rear edges of distribution ranges - tend to inhabit climates colder than their climatic optima (Rehfeldt *et al.*, 2002; Wang *et al.*, 2006; Gömöry *et al.*, 2012). Practical experience in forestry shows the same tendency: trees have been successfully grown far beyond their natural environments, more frequently in warmer than in colder climates (Norway spruce being an illustrative example, see Spiecker *et al.*, 2004). This indicates that, except for trees on southern limits, climatic constraints play a minor role in determining the distribution of tree species compared to biotic interactions, such as competition, pests or pathogens, which all can (to different extents) be affected by silvicultural practices. The gap between inhabited and optimal climates thus means that silviculture has a relatively broad playground in which to mitigate the effects of climate change.

Potential and limits of silvicultural measures and enrichment planting to enhance adaptation of tree populations to climate change

Silvicultural measures have the potential to influence evolutionary processes such as gene flow and selection both in positive and negative ways by acting simultaneously on species demography and local environmental conditions (Finkeldey & Ziehe 2004; Lefèvre *et al.*, 2014). Therefore, silvicultural practices may alter the properties of adaptation and adaptability in a forest stand. Given the fast pace of climate change and the large uncertainties in climate projections, forest practices should be oriented concomitantly towards accelerating genetic adaptation and maintaining or increasing

genetic diversity; the former would help trees cope with expected future environmental conditions, while the latter would serve as a basis for tree populations to respond to unexpected changes.

As mentioned above, genetic adaptation occurs when new advantageous genotypes emerge - either through recombination of alleles present in a local population or through the introduction of alleles by gene flow – and are able to spread before the population reaches a critical limit of maladaptation and goes extinct (Aitken *et al.*, 2008).

Within the framework of evolution-oriented adaptive forestry, three main objectives have been formulated: i) to increase the chance of emergence of ‘innovative’ genetic combinations, ii) to facilitate the spread of the best-adapted genotypes, and iii) to preserve genetic diversity for long-term response to selection (Lefèvre *et al.*, 2014). Accordingly, current forest management is faced with the challenge of not only considering multiple genetic processes, but also different time frames.

Silvicultural practices can influence demographic parameters of tree populations in multiple ways, depending on the species and silvicultural systems used. Whether applied systematically or selectively, thinning reduces population sizes and increases the intensity of genetic drift in small populations, which may reduce the number of rare alleles. Thinning also affects the mating system by shaping the spatial structure of genetically-related reproducing trees and their contribution to reproduction (Finkeldey & Ziehe 2004; Lefèvre *et al.*, 2014). The removal of neighbouring and related individuals through thinning may reduce inbreeding through the reduction of spatial genetic structure (Dounavi *et al.*, 2002). Furthermore, thinning allows managers to specifically enhance pollen flow within a stand or among single trees of rare species (Lefèvre *et al.*, 2014). The spread of potentially advantageous alleles is thus facilitated (Kremer *et al.*, 2012).

At the stage of regeneration felling, diverse cutting regimes can influence genetic variation by providing different ecological niches and, as a consequence, variable selective pressures on trees (Brang *et al.*, 2014). The effects that naturally regenerating silvicultural systems have on genetic variation in the offspring generation are mostly quite small: in general, no negative impact of various silvicultural practices on gene diversity or inbreeding, and a small loss of alleles at the stand level compared to old-growths have been reported for temperate and boreal trees (for review, see Ratnam *et al.*, 2014). Ideally, diverse silvicultural treatments should be implemented at the landscape level to create areas of both old-growth forest in which trees contribute to regeneration for a long time, and areas of juvenile and young forest in which short rotation periods

favour regeneration, lower disturbance risks, and allow for faster species transitions (Brang *et al.*, 2014; Westergren *et al.*, 2015; Schelhaas *et al.*, 2015; Fady *et al.*, 2016). In practice, areas for wood production and areas for evolution could be separated, given the sufficient gene flow occurring between them (Lefèvre *et al.*, 2014).

Silvicultural practices also alter the local environmental conditions in forest stands; silvicultural measures usually dampen the natural selective pressure of environmental stress. Thinning, for example, reduces the number of trees requiring water in a specific area and enhances the amount of precipitation reaching the forest floor. Consequently, such measures help trees in dry regions - at least in the short-term - to cope with limited water availability, as has been shown for Scots pine (*Pinus sylvestris*) in the Valais (Elkin *et al.*, 2015). As a result of reduced selection intensity, the genetic improvement from one generation to the next is slowed down (Lefèvre *et al.*, 2014). Therefore, sufficient time should be left for natural selection to act on the juvenile trees before thinning measures are taken.

Evidence from observational, experimental, and theoretical studies indicate that many tree populations may suffer from adaptation lags⁹; particularly marginal populations at the rear edge of species ranges and at ecological boundaries within species ranges (Fady *et al.*, 2016). Hence, there is good reason for forest managers to also use silvicultural measures and enrichment planting in naturally regenerated forests for speeding up evolutionary processes, particularly in forests with high risks from climate change (e.g., drought or disturbances). Yet their success depends on the right choice of provenances and species, which is a challenging task. Making decisions regarding suitable forest genetic resources is impeded both by the uncertainty of climate change projections (and future pressure from known and unknown pests) and the (still) limited knowledge from provenance and species translocation trials. While climate-change models have significantly improved in the last decade, the number of experiments studying the translocation of FRM has not evolved to the same extent. In addition, large differences in site conditions occur over the lifespan of individual trees, which makes it even more difficult to select appropriate FRM for enrichment planting (Alfaro *et al.*, 2014).

Given the limited knowledge of the suitability of FRM for assisted gene flow and migration in many countries, these management strategies are still difficult to put into practice locally. It is therefore essential to make headway in transferring and applying existing knowledge locally, even if it is limited. Moreover, the potential risks and failures

⁹ *adaptation lag* – disparity between the performance of a population on its local site and in its climatic optimum

involved in moving FRM may deter forest managers from applying assisted gene flow and migration measures. In particular, the movement of tree species into entirely new areas is hotly debated due to potential resulting disturbances to indigenous flora and fauna and large failures arising from the use of ill-adapted genetic resources (Alfaro *et al.*, 2014). Therefore, enrichment planting measures should make better use of existing within-species diversity and rely on better adapted provenances of native species instead of non-native ones. Depending on local conditions, planting strategies may involve a mix of provenances alongside the current population, and involve not only single provenances and species, but rather multiple provenances and species to reduce risks (Konnert *et al.*, 2015). Ideally, various management and silvicultural approaches should be applied in an integrative manner (Brang *et al.*, 2014).

Legislation, voluntary forest certification schemes and public perception of forest policies all put additional constraints on the variety of practically feasible mitigation measures. Currently, certification under the Programme for the Endorsement of Forest Certification (PEFC) and the Forest Stewardship Council (FSC) is adopted for a substantial part of European forests¹⁰. In certain countries, certification schemes (especially FSC) tend to exclusively favour natural regeneration, which can impede the possibility of speeding up forest adaptation to climate change by introducing potentially better adapted FRM from other locations. The position maintained by forest certification schemes in these countries neglects a very complex reality that requires different solutions, based on the local environmental and socio-economic conditions. Policymakers need a more nuanced perception of the benefits and challenges of adding enrichment planting of transferred FRM to natural regeneration, which also need to be communicated across society. Moreover, one should assert that natural regeneration should be just one component of close-to-nature forestry, rather than its final aim. Other issues needing to be addressed include managing the structural diversity of stands and maintaining low-growing stocks and optimum species mixtures (Brang *et al.*, 2014), thus enhancing the resistance and resilience of forest ecosystems to climate change. In popular wording: natural regeneration is still *alpha*, but not *omega* in close-to-nature forestry (Božič and Kraigher 2012).

Current forest policies often restrict the transfer of FRM to provenance regions and across legal boundaries, hence limiting the application of assisted gene flow and migration in practice. Trees do not recognise borders – a fact that should be integrated into current recommendations for transferring FRM.

¹⁰ e.g. 89 % of the forests managed by the members of the European State Forest Association are certified.

Potential of breeding to cope with climate change

The choice between using natural or artificial regeneration (Figure 2) is a matter of finding a balance between ecology and economics. Artificial regeneration with genetically improved FRM allows the planted crop to be chosen, enabling prior selection for traits of biological and economic importance, such as improved growth and survival, quality, and resistance to biotic and abiotic stresses, such as disease and drought respectively. However, increasing the frequency of desirable traits through genetic selection ultimately leads to a reduction in stand diversity as a result of the promotion of the selected genes. From a commercial point of view, the less stand variability there is, the greater the potential gain will be. Nevertheless, there must be a trade-off between the increased yield from tree improvement and the loss of ecosystem services resulting from potential loss of adaptability to changes in climate. In the same way there must be a compromise between the simplification of forestry technologies, practices, stand structures, and potentially lower forest resilience as a risk to forest health, associated with outbreaks of pests and diseases.

FIGURE 2. Artificial (A) vs natural (B) regeneration. (Photo credit: Pacific Southwest Region 5/Flickr (A), Stanislav Kucbel (B)).



The concept of forest resilience in response to threats, such as climate change, has existed for some time, and it is recognised that the more diverse the forest, the more likely it will be able to withstand such threats, known or unknown (Thompson *et al.*, 2009). It is also well-recognised that forest plantations will be more susceptible to risk compared to primary forest due to their reduced biodiversity (Burdon, 2001). However, when considering this elevated risk, the loss incurred if such risks are not taken must also be evaluated. Sometimes a single species dominates commercial forestry; for example, Sitka spruce (*Picea sitchensis*) in Ireland and the United Kingdom, and radiata pine (*Pinus radiata*) in New Zealand. Site suitability has often meant that no other species comes close to matching the growth potential of the dominant commercial species. To plant other species would not only incur a loss of productivity, but could also lead to failure due to biotic and abiotic stresses, resulting in the planting of large areas of monoculture despite the risks involved (Burdon, 2001). Furthermore, potential growth gains of over 40 %, which can be made by deploying selected clones (Sutton, 2002; Sorensson, 2006), can considerably reduce risks from a changing environment by shortening the rotation length, in spite of limited genetic diversity.

Selecting for something today that may be better suited to future conditions is dependent upon the accurate prediction of changing climate and potential threats; even the best models are likely to only predict broad trends over a 40–80-year rotation period and there will still be uncertainties related to risk level. Therefore, breeding programmes must target selection for plasticity and adaptive traits (Alfaro *et al.*, 2014) to buffer them from such threats. Breeding programmes must also include measures for conserving genetic diversity in order to cope with any future uncertainties (Eriksson *et al.*, 1993). It will be important to be able to deploy new material quickly in response to events by relying more on vegetative propagation methods as a means of deployment. Rapid response to changing events will also place greater emphasis on early selection, employing DNA sequencing and genotyping to increase the accuracy and speed of selection. In addition, a broader assortment of species should be considered alongside main commercial species to replace existing planted forests in case of failures or to be deployed when models predict that future conditions will be unfavourable to the current species.

Regeneration strategy in Europe today

In Europe, large regional differences exist in forest regeneration practices. Artificial regeneration by planting genetically improved plant material dominates - and is currently proportionally increasing - in Sweden, Norway and Finland, partly because the method leads to rapid reforestation and works well on most sites. In other European

countries, the common practice for regeneration has changed a lot in the last few decades. Natural regeneration has become increasingly popular in regular forestry in central Europe, because it is cheap and accords with close-to-nature forestry. In Slovenia, for example, 95 % of regeneration is natural regeneration, and only large-scale disturbances which took place in 2014 (ice-sleet) and 2015 and 2016 (widespread bark-beetle outbreaks on Norway spruce) have re-initiated support for regeneration (enrichment planting) by planting and sowing, and an increasing number of species is being planted. In Germany, natural regeneration dominates, with a figure of 70-80 % today, whereas the proportion was inverse 30 years ago; this is connected to a change in the composition of tree species used for reforestation. Mixed stands are expected to be more resistant to various weather extremes, such as strong winds, as well as to biotic threats, such as fungal and insect attacks (Knoke *et al.*, 2008). In Germany, there is currently a preference for mixed stands with a majority of deciduous trees.

Throughout Europe, in response to a large-scale decline in Norway spruce caused by drought and bark-beetle outbreaks, Norway spruce monocultures have begun to be gradually replaced by stands which have a tree species composition appropriate for the site (Spiecker *et al.*, 2004). However, this is often only feasible with planting.

1.3 The need and purposes of forest reproductive material

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Bordács, S., Wolter, F., Servais, A., Gömöry, D.

The planted forest area, including forest plantations and semi-natural planted forests (SNPFs), in 2005 was almost 261 million hectares in 61 countries (Carle and Holmgren 2008). This area comprised about 95 % of the world's then total Planted Forests, and comprised 128.1 million hectares of Forest Plantations and 132.4 million hectares of SNPF. The total area of planted forests comprised about 7 % of global forests and 2 % of global land use which may already have contributed to around 70 % of the world's recent production of industrial roundwood. The role of planted forests in industrial wood production is likely to increase in the future, in 2020 this area was already nearly 300 million hectares (FAO 2020).

BOX 2. FRM and sustainable forest management

The production of FRM is an integral part of sustainable forest management, aiming to provide suitable reproductive material for reforestation and afforestation. While **forest regeneration** might rely on natural means, many **reforestation** activities need reproductive material specifically collected and produced for that purpose. In the case of **afforestation**, the use of FRM from external sources is typically the only option. The production of FRM is therefore of utmost importance for the forest sector and for sustainable forest management. The decision to utilise FRM depends on the different possible opportunities and options available to the forester/landowner, which include: kind of project (afforestation, reforestation, restoration of degraded sites, multipurpose plantations, agroforestry, etc); main socio-economic and ecological objectives of the future stand (quality wood production, energy production, protection forest, recreational forest, etc); legal or voluntary certification obligations; and risk management regarding climate-change adaptation and mitigation. These various purposes are usually all, to varying extents, integral parts of forestry practice. Most purposes are also particularly important in the political and social sphere of the forest sector.

Multifunctional forestry

Multifunctional forestry aims to combine the fulfilment of a maximum number of forest functions and services. In a broad sense, most European national forestry strategies are designed for multifunctional forestry. In a narrower sense, it mainly occurs in well-adapted ecosystems and appears to have become the mainstream forestry model in many highly populated European regions. While the silviculture associated with this mainstream model tends towards close-to-nature practices (including natural regeneration) in many areas of Europe, it is still important to use appropriate FRM when renewing forest stands, particularly for increasing species diversity and site adaptation, or following large-scale disturbances, or to meet other objectives, such as recreational purposes. In these areas, the use of produced FRM is mainly linked to the regeneration needs of species that cannot be regenerated naturally. In other areas of Europe, however, the use of FRM artificial regeneration is simply due to economic reasons, as it allows improved material with higher productivity to be used, and it has a shorter regeneration time and a more rapid and simple workflow. In multifunctional forestry, there are high expectations regarding the versatility of FRM and its ability to ensure long-term fitness and adaptation to local ecological conditions in combination with an expected wood production capacity.

In order to fulfil these expectations, FRM with high genetic variation must be produced. By using genetically diverse material with high adaptive potential, it is possible to increase a forest stand's suitability for undergoing close-to-nature forestry

management, or at least its ability to naturally regenerate (Jump *et al.*, 2009; Thomas *et al.*, 2014). When using autochthonous material (FRM with local or regional origin), it is recommended to opt for seed sources from the categories 'source identified', 'selected' and 'qualified'. If a non-autochthonous basic material has (already) been positively tested for any given local conditions, then the tested FRM can also be suggested for use in forestations. When choosing tested FRM, material from tested seed stands, multiclonal seed orchards or clonal mixtures can be recommended as basic materials.

Plantation forestry: wood and biomass production, multipurpose plantations, agroforestry

With an expected continuous increase in demand for wooden materials in Europe and little income for other forest goods and services, forestry with wood production as a main purpose - sometimes known as plantation forestry - is widespread and popular. (Figure 3). Due to the intensification of agricultural land use, plantations are also often used for multiple purposes, such as for fruit-tree cropping (nuts, wild fruits, etc.), mushroom production and agroforestry. Wood production forestry is often linked to high investments and the need to generate profits by producing high yields and/or high-quality products over short or medium rotation periods. Risk acceptance is usually medium to high.

FIGURE 3. Planted forest. (Photo credit: CIFOR/Flickr).



When using FRM for wood production only, it is important to have sound knowledge of its genetic and performance potential and minimum-targeted information about growth capacity, wood quality, resilience, amongst other aspects.

Generally, 'Source-identified' and 'Selected' basic material (seed sources and seed stands) are not suitable for plantations due to the high genetic variation of individuals; however, the category 'Selected', can be used if the individuals of seed stands are particularly homogenous for the purposes of the plantation (e.g., straightness, biomass growth volume and wood fibre quality). Both vegetative (clonal) and generative FRM can be used as planting material, depending on the purpose of use.

There is high ecological and economic risk associated with the use of clonal material which has originated from one or few genotypes, due to the limited genetic diversity of the plants used for plantations, such as cuttings or *in vitro* plants. Multiclonal forest plantations (Intimately Mixed Plantations) have been established in the past with good results when specific interactive clones were planted in good sites (Ahuja & Libby, 1993), however, despite the relatively low biological risk, the costs of establishment, management and harvesting were high, and the produced material (harvest of wood/end product) was not uniform. Mixtures (mosaics) of monoclonal plots can be both biologically wise and economically prudent (Ahuja & Libby, 1993). On the contrary, monoclonal plantations may be more advantageous in terms of economy, management and silvicultural and pest control techniques, but they are characterised by a major increase in the risks involved (i.e., mass failures due to biotic and/or abiotic factors).

Family forestry is another type of plantation forestry which is carried out to a large extent in the USA. In these plantations, open-pollinated, polycross or full-sib families - derived from tested seed orchards and raised separately - are planted. According to Carson (1986) and Libby (1990), family forest plantations incorporate all or some of the advantages of clonal plantations, and for this reason they are often an attractive alternative. While family forestry is not currently traditional in Europe, it is a potential future alternative.

As using clonal FRM approved in the category 'Qualified' (i.e. untested plant material) includes a high economic and ecologic risks, using it only in in the case of short rotation plantations where cultivation and harvesting methods are optimised for short-term use (1-10 years) reduces those risks. The use of planting material in the category 'Tested' can reduce the ecological risks of plantations if the clonal genotype has been tested under the local conditions at the site of the plantation. In the case of non-clonal plantations, planting materials are generally produced in traditional seed orchards with a low number of clonal components. It is advised that clonal components be tested on sites in which FRM is to be planted.

Climate change adaptation and mitigation

Most climate change adaptation and mitigation strategies refer to forestry as an important sector for action. Many measures for implementation are now related to the production and use of FRM. Previous reports have concluded that both the selection and the production of suitable FRM have gained new importance, as trees are long-living organisms which, during the course of their life, will be subject to rapid climate change which is expected to alter the environmental conditions in which they grow and reproduce (Koskela *et al.*, 2007).

Local provenances may not always be the sole best source of FRM. The transfer of species and provenances is a valuable complementary option when adapting forests to climate change. However, there may be legislative or environmental limits to transferring FRM, which can only be carried out with functional and traceable FRM production chains.

Maintenance of genetic diversity is also perceived as an effective solution for risk management in the context of climate change. While it can be sustained by appropriate silvicultural techniques within the stands, the additional enrichment of diversity with FRM from the production chain could be a valuable option as well.

The genetic requirements of FRM for climate change purposes may therefore differ greatly depending on the precise objective: if it is adaptation, then high genetic diversity, heterozygosity, and adaptive capacity are expected; if it is mitigation, then the requirements are quite similar to those in wood production forestry (see previous subchapter on climate change adaptation).

Land and forest restoration

Whether aiming towards naturally diverse ecosystems and landscapes, greening or other purposes, the restoration of land and forest remains a very busy sector in Europe. Land restoration is usually needed on former mining areas or on eroded/degraded lands, making use of tree and shrub plantations for which high genetic variation of the starting population is advised, due to strong local selection pressure and relatively low survival rate. According to this crucial requirement, the basic materials must originate from large stands, preferably growing on marginal sites that are characterised by comparable stress factors and bearing alleles for tolerance and resistance to the limiting factors (Thomas *et al.*, 2014). A mixture of reproductive materials from different but selected origins could also be used to increase the genetic

variation of the starting populations. In this case, the mixture should also include basic material from stands representing a larger area.

In the case of forest restoration, FRM requirements may differ depending on the final objective and/or the stage of the project. For heavily degraded forest, the requirements in the initial stage of restoration may be similar to those of land restoration, whereas the final stages of restoration usually require well-adapted autochthonous species.

Links with gene conservation

The production of forest reproductive material offers both risks and opportunities in terms of gene conservation. The risks are mainly associated with a loss of genetic variation and the genetic pollution of autochthonous genetic material when using or planting maladapted or inappropriate FRM. Naturally, the magnitude of such risks increases with increasing selection intensity and increasing spatial scale at which the products of selection are used for reforestation. Whenever the products of intensive breeding replace natural or non-intensively managed forest stands, there is a danger of losing rare or unique genes or genetic lineages occurring in local populations. Such a risk is obvious in clonal forestry, in which the number of genotypes used within a country typically ranges from tens to hundreds, depending on the species. In the case of seed orchards, the genetic and genotypic diversity of seed crops is often comparable to that of natural populations if the number of unrelated clones is large enough to represent the gene pools of the populations of origin. However, when seed orchard progenies predominate in reforestation, genetic richness may be in peril, at least regionally. Even in approved seed stands where selection intensity is the lowest, common seed-harvest practices may lead to a loss of genetic variation (Hussendörfer, 1996).

Dynamic *in situ* gene conservation is thus an inevitable complement to artificial forest regeneration. Only a sufficiently dense network of Dynamic Conservation Units (DCU) may secure the conservation of evolutionary processes in forest tree populations in order to maintain their adaptive potential and to face future stresses. On the positive side, basic materials represent valuable genetic resources and the selection underlying their approval and establishment generally focuses on traits of practical interest, such as growth rate, quality, wood properties and disease resistance, while taking into account overall health condition and natural regeneration success (the long-term survival of the population in its environment) as a general indicator of adaptedness. The collection of clones for both vegetative propagation and mass production of improved progenies (parents of families), seed orchards and progeny tests, etc., is not only of interest for the production of FRM, but can also be considered as a kind of *ex situ* conservation measure.

2. PRODUCTION CHAIN OF FOREST REPRODUCTIVE MATERIAL

The Council Directive compels the member states to produce a list of national basic material, including a succinct description of seed stands and seed orchards (e.g., number and origin of the genotypes). However, forest owners purchase FRM and not basic material, and most foresters are not fully aware that a given basic material can produce FRM of variable genetic quality. In fact, the genetic composition of the crops differs from year to year, because climatic conditions can impact genotype fertility, flowering overlap and extent of pollen contamination, as well as the populations of various pests affecting trees or clones differently. Thus, deviations to panmixia are likely to fluctuate from one crop year to another for a given seed source.

Extreme deviations may result in poor adaptation to afforestation sites and may affect FRM performance, especially in the case of seed orchards composed of a limited number of genotypes (Lindgren & Prescher, 2005; Reed & Frankham 2003). Therefore, the implementation of guarantee seems necessary to avoid the commercialisation of such crops or at least to restrict their use. The measures taken in certain countries to guarantee 'sufficient' FRM diversity and/or to inform forest owners of their genetic quality are explored below, with a focus on methods that allow seed orchard managers to make a diagnosis before cone collection. *A posteriori* judgements based on gene markers are not commonly applied in forestry practice; therefore, they are not considered here.

2.1 Approval, management, collection and certification

All FRM should fall within the types and categories of approved basic material. For *in situ* basic material in the category, 'Selected', ten criteria from the EC/1999/105 are to be met, and possibly further defined by country; for 'source identified' these criteria are not regulatory according to the Council Directive, but it is still possible to further define criteria by the member state.

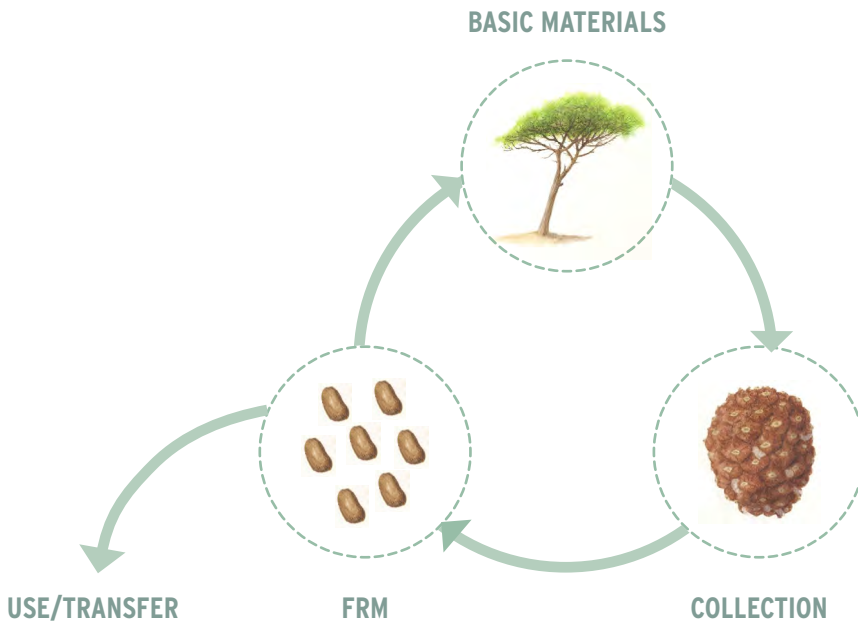
The management of approved basic material can be defined, if the sources (when in forests) and stands are mentioned or delineated in forest management plans, and any

management measures are suggested. In some countries these only apply to stands for the category 'Selected', where support for the species in question must be expressed taking into account stand stability. The removal of phenotypically inferior trees can be considered and all measures must be assessed for their possible effects on genetic diversity of FRM produced within the approved forest stand.

The collection of FRM can also be specified with a view to supporting its genetic diversity, as long as the timing of collection (in most years), methods, minimum number of trees for collection, and the spatial distance among the trees is specified.

The certification of FRM must comply with the exact procedures as stated by the country, so as to fulfil the basic material owner rights and allow the FRM to be traced throughout the production chain (Figure 4) to the market. Therefore, the Official Bodies must be nominated, the professional control and exchange of information articulated, and the information flow within and among countries defined.

FIGURE 4. Simplification of seed production chain. (Credit: C. Giordano /EUFORGEN).



2.1.1 Seed sources and stands (Source identified)

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Bordács, S., Ivanković, M., Maaten, T., Frank, A., Kraigher, H.

To produce FRM of the category ‘Source identified’, seed sources and stands are considered as basic material. The definitions of seed sources and stands (see Box 1) are such that seeds may be collected from areas other than forests and seed orchards (e.g., from urban areas, roadside plantations and parks), and seed units can be composed of seeds collected from trees in the whole region of provenance. Seed sources might be preferred in the case of rare or endangered species, especially if there are no basic materials available in ‘higher categories’. Many species (e.g., the wild fruit species from the genera *Pyrus*, *Malus* and *Sorbus*) do not occur in large stands and are often harvested in seed sources.

In addition, for some economically important tree species, such as Norway spruce (*Picea abies*), pines (*Pinus* spp.), oaks (*Quercus* spp.) and beech (*Fagus sylvatica*), seed crop collection is not regularly carried out in some regions, because it involves collecting a large amount of seeds; in such cases, the management of seed stands on a large scale, as demanded for the higher categories, is not a reasonable and pragmatic approach. Furthermore, seeds might be collected from felled trees in a big area with little or no information about the characteristics of the basic material. Nevertheless, FRM seed lots from locally-adapted stands can be more advantageous than the transfer of FRM from other regions, which would require information about tree genotype and specific stand management. In the former case only a minimum level of information is required about certain FRM seed lots that are used: tree species name and geographical location of origin. This information generally covers the minimum requirements of the category ‘Source identified’.

In general, the minimum requirements of the category ‘Source identified’ are stated in the EU Council Directive (1999/105/EC), but seed sources and stands must meet the criteria set by the Member States. The Member States are allowed to set up more stringent requirements for the production and marketing of FRM in the category ‘source identified’; e.g., the category is not accepted in some countries or only allowed for a few species, especially for those that are minor/scattered and not stand-forming. FRM production in the category ‘source identified’ is allowed in all EU countries, but there are additional legal restrictions for use in eleven EU countries (Konnert *et al.*, 2015). This category is traditionally used for afforestation of large clear-cut areas, mostly in Northern or South-eastern parts of Europe, primarily because of low associated costs (Ahtikoski *et al.*, 2013).

It is also advantageous in case of forest or landscape restoration after for example, forest fire, wind or snow damage in large areas.

When using FRM seed lots from 'source identified' stands, it is possible for the genetic variation to be higher - especially allelic richness - in comparison with FRM from clonal seed orchards with a low number of clones (Sønstebo *et al.*, 2018). In Furthermore, in scattered or rare tree species, the mixing of seeds collected from single trees or groups of trees from the whole region of provenance might be beneficial for genetic diversity. However, it is also possible for just a single or a few trees to be the source of FRM in this category. The seeds for a given seed lot may also be collected from several stands and marketed under a single master certificate. This high variation can ensure better adaptability and stability for forestation. However, the lack of information about the quality traits and genetic composition of basic material is disadvantageous in multifunctional forestry where wood quality is of importance.

Collection and harvest

The way the seeds are collected is crucial when FRM is used in the category 'Source identified'. Large stands are usually harvested in a different way to seed sources (a single tree or group of trees) or smaller stands. Large stands are preferably harvested during mast years and are also used for the economically beneficial collection of a huge amount of seed; consequently, most of the stand area is harvested, in which case, seed lots must represent the genetic variation of whole stands. In contrast, seed sources or smaller stands are composed of only a few genotypes and therefore a small amount of seeds should be used to increase the effective population size. Admixing of seed lots is allowed by certification schemes when basic materials are located in the same region of provenance and the procedure of admixing is documented; for example, register references of seed sources harvested and reference numbers of master certificates issued for composite seed lots. However, it is also advised that geographic data of original basic material (point versus polygon coordinates and altitude) be documented. Admixed seed lots can also be managed and marketed more economically. Nevertheless, seed collection procedures are similar to those for seed stands, as detailed in the following section.

2.1.2 Seed stands (Selected)

Ivanković, M., Gömöry, D., Kraigher, H.

Approval or establishment

According to the Council Directive, seed stands can be approved for production of FRM in the category 'Selected' when pre-defined criteria for approval are met. Selected FRM includes seed material, plant parts and plant material from forest trees originating from seed stands within a certain provenance region; the stands must be phenotypically different to populations from other provenance regions and superior to other forest stands within the region.

Typically, existing adult stands are approved as seed stands, and they represent the best parts of available forest stands in a given area. The criteria for their choice are formulated in Annex 3 of the Council Directive in quite a general manner, while detailed criteria are left to be determined by Member States. The general criteria include commercially important heritable properties, such as volume production, wood quality and form, and growth habit. Uniformity of these traits is desirable and should be noted at approval. It is recommended that phenotypically inferior trees be removed from the stand to avoid their genetic contribution to the seed crop. Stands must also be of an age that allows their properties to be judged in a reliable way and any proposed management measures to be implemented.

Another important aspect is adaptedness: the stands need to be adapted to the environmental conditions prevailing in the respective region of provenance. This is demonstrated by a stand's long-term persistence in the environment, which is shown by its reproduction (generative or vegetative); i.e., whether the trees of the species in question show regular flowering and fructification, whether the seeds germinate, and whether the seedlings / saplings are able to survive. Furthermore, the stands should be in good health; visible pest attacks and diseases should be absent and it should be possible to note resistance to natural stress factors as an exceptional positive trait. Finally, the last group of criteria focuses on genetics: the origin of the population, isolation from potential inappropriate pollination sources (poor stands, presence of related species able to hybridise, non-autochthonous stands surrounding an indigenous seed stand, etc.), and population size. This guarantees sufficient genetic diversity in the seed crop, thus securing the adaptability of the next generation stand (established from FRM produced within the stand in question) to future environmental fluctuations.

In some countries, the way in which seed stands are intentionally established ensures that the best approved seed stands can be reproduced (so-called '*uprawy pochodne*' in Poland, also see chapter 5). Seeds are collected from a large number of maternal trees in an approved seed stand (a minimum of 40, but 100 is recommended in Slovakia), and a plantation is established with the intention of being used as a seed source once it starts full fructification.

The ways in which the general selection criteria of the Council Directive are implemented in practice vary. Some countries (The Czech Republic and Slovakia) have formalised procedures of phenotypic classification based on forest mensuration results, performed within the framework of forest management planning: only the stands classified in the best phenotypic categories can subsequently be approved as seed sources by the forest authority. However, the process of approval is mostly a formality, without closer inspection of the proposed stands. In contrast, a commission nominated by the forest authority is responsible for the approval of seed stands in Germany, Slovenia and the UK, where the decision is more subjective, although stand data from forest management plans may be available (as in Slovenia), and is based on an immediate on-site judgement about the quality of the proposed stands (Anonymous 2005; Forestry Commission, 2007; Kraigher *et al.*, 2019).

Population size, spatial arrangement, and adaptedness to environmental conditions at a certain age and/or developmental stage, are the main factors determining genetic diversity of seed crop and future adaptability. The Council Directive requires seed stands to consist of one or more groups of trees that are well distributed, sufficiently numerous, of a sufficient density, and already flowering to ensure adequate inter-pollination and prevent inbreeding. Again, these general requirements are specified in diverse ways in national legislation. For instance, the German decree on the approval of FRM specifies minimum age (ranging from 20 to 70 years), stand size (0.25–2.5 ha) and number of individuals of the target species in the stand (20–40) for each tree species separately; whereas in Slovakia, minimum numbers are applied to all species (60 years, 0.5 ha, and 10 % of the target tree species with a minimum number of 40 trees respectively). In Slovenia (Figure 5) the minimum size of the approved seed stand (5 ha) and number of reproducing trees (70) is demanded for five stand-forming species (Norway spruce (*Picea abies*), silver fir (*Abies alba*), beech (*Fagus sylvatica*), pedunculate and sessile oaks (*Quercus robur* and *Q. petraea*)), and 10 trees is the minimum for other tree species. In other cases (UK, Belgium and Sweden), the legislation itself adopts the exact wording of the Council Directive in terms of the minimum requirements, and their interpretation is left to the judgement of the approving body. The recommended area of a seed stand (consisting of one or several compartments) is typically moderately

larger than the minimum set by legislative norms; usually a few hectares, and less frequently tens of hectares.

Even though gene conservation is not the primary aim of seed stands, they still represent valuable genetic resources, and, providing they reach internationally required minimum criteria, can often form part of genetic forest reserves/dynamic conservation units. In light of this, the preservation of the genepool of the maternal stand in the seed crop is relevant. With minimum numbers of parental trees as mentioned above, the genetic diversity loss is 1-2.5 % (Koskela *et al.*, 2013), provided census and effective population size are the same (which is not realistic). However, such small populations are rarely approved in practice, except in the case of minor species.

FIGURE 5. Forest seed stand in Dvor, Žužemberk, Slovenia. This stand was part of the LIFEGENMON workshops on the importance, development and implementation of conservation of genetic diversity in Slovenian forests. (Photo credit: LIFEGENMON project).



Management

Neither the Council Directive nor national forestry legislations give instructions concerning the management of seed stands. However, the fact that a stand is approved as basic material is usually taken into consideration within the framework of forest management planning. In some countries (Croatia and Slovenia) seed stands have a specific or more specified management plan than common commercial stands, prescribed in general within the Decree on approval of such a stand which becomes an obligatory part of the general forest management plans for the area.

There are some general practices for seed stand management, which are widely applied across Europe. As seed stands are mostly (but not necessarily) intended for producing FRM during a longer period, the rotation period is often extended - at least in the most valuable stands - if the owners agree. Superior trees are intentionally favoured in silvicultural treatments. Crown thinning is applied to release their crowns and thus promote fructification. For this, the stability of the forest stand must be ensured and, in the case of tree species in which seeds are collected from the forest floor, ground cover must not favour excess light (to reduce both natural regeneration and weed development, which would render seed production difficult or incur additional costs related to ground preparation). Thinning is also carried out to ensure the early removal of trees that exhibit heritable adverse traits, such as forking and spiral grain. Sometimes such trees are also removed from surrounding stands. Final fellings, especially in coniferous stands, are planned in a year of heavy seed crop to facilitate cone collection from the felled trees. Target diameter felling is avoided, as it may remove beneficial alleles if fast-growing trees are removed before their reproductive maturation (Finkeldey & Ziehe, 2004).

The genetic effects of such silvicultural interventions are not completely known. In general, the genetic structure of a stand has mostly been found to be affected to only a minor degree when subject to different thinning regimes, unless thinning intensities were too high (which is usually not the case with seed stands). The irregular shelterwood system, applied in an approved beech seed stand in Slovenia, was found to have negligible effects on the young regeneration centres, similar to the natural regeneration processes in a virgin forest reserve (Westergren *et al.*, 2015). The often-used future-tree oriented thinning is beneficial for the conservation of gene diversity, provided that a high number of future seed-bearing trees are selected (Finkeldey & Ziehe, 2004; Konnert & Hosius 2010; Kavaliauskas *et al.*, 2018). Thinning from below in high intensities, which may result in a loss of rare genetic types (Konnert & Hosius, 2010), is rarely applied in seed stands. Rare alleles may also be removed by

selective thinning in older stands, if the inferior phenotypes which have been removed systematically are related to particular genotypes (Hosius *et al.*, 2006; Kavaliauskas *et al.*, 2018; Ratnam *et al.*, 2014). Of course, the value of such rare alleles may be a matter of dispute, as they tend to be detrimental (Loewe and Hill, 2010). However, their adaptive value to future environmental stresses can never be excluded, which means that the conservation of as much of the genetic spectrum as possible is generally desirable.

Specific rules apply to seed stands located in gene reserves or dynamic gene-conservation units. These are frequently categorised as special-purpose forests, and hence the variety of silvicultural approaches is broader, or more specified, than in common commercial forests. To ensure the conservation of extant genetic diversity, silvicultural systems based on natural regeneration are preferred in these stands. When artificial regeneration is necessary, FRM originating from the same genetic conservation unit or from nearby autochthonous stands should be used (Koskela *et al.*, 2013).

Collection

The main concern associated with the collection of FRM is the preservation of the species-specific genepool and prevention of genetic erosion in seed lots as compared to the stand of origin (Blanc-Jolivet & Degen, 2014). The total size of the parental population which is reproduced in artificially regenerated stands depends on the number, size and structure of seed stands used for seed harvesting, as well as the number of maternal trees harvested per stand. Approved seed stands usually represent less than 10 % of mature forests stands (Hosius *et al.*, 2006). Frequently, only a small part of these stands is repeatedly harvested and the collected seed lots used for reforestation, due either to their phenotypic superiority or to their comfortable access. Harvesting will also be worthwhile when a certain part of the approved seed stands exhibits sufficient fertility and seed-bearing cones. In the eastern EU in particular, this usually concerns rare tree species, and harvesting focuses repeatedly on a few reliably fructifying stands.

Minimum population size is defined in the laws or decrees regarding FRM in many European countries: for example, 10 to 20 maternal trees per stand, depending on species, need to be harvested in Austria, Germany or Slovakia; 30 trees in the UK; 50 or at least 25 trees for stand-forming species in Slovenia. In Austria, FRM labelled as 'material with increased genetic diversity' must be collected from at least 25 to 50 trees per stand (again, depending on the species). However, parental balance is only

explicitly required in British regulation (Forestry Commission, 2007). Experimental and simulation studies suggest that the minimum numbers used may be enough to preserve genetic diversity of the source stand (Blanc-Jolivet & Degen, 2014; Heinze & Fussi, 2017). However, the proportion of crop trees changes from year to year and, consequently, the genetic composition of seed lots from the same stand differs from year to year (Konnert & Behm, 2000). Moreover, the degree to which the genepool of a maternal seed stand is reproduced in the collected seed lot depends on mating patterns, which in turn are determined by many factors, such as fine-scale spatial genetic structure (FSGS) (Hoebee *et al.*, 2006; Pandey *et al.*, 2012; Mosca *et al.*, 2018), stand density (Jolivet *et al.*, 2011), clonality (Suvanto & Latva-Karjanmaa, 2005; Blanc-Jolivet & Degen, 2011; Jankowska-Wróblewska *et al.*, 2016), pre-zygotic incompatibility (Stoeckel *et al.*, 2006), and flowering phenology (Thomasset *et al.*, 2014; Moracho *et al.*, 2018).

These aspects are generally not tackled in national legislations and may not be respected in practice. A group of phenotypically superior trees may induce the forest personnel to harvest them all, although it may represent a family or even a clone (e.g., in wild cherry (*Prunus avium*)). When collecting conifer cones from standing trees, climbers sometimes swing from the crown top and jump onto the neighbouring tree instead of climbing down and up; this practice, although strictly prohibited by labour security regulations, is not exceptional in central and eastern Europe, and again may result in an excessive proportion of half-sib relatives in the collected seed lots. Therefore, a uniform harvesting of as many trees as possible across the whole area of a seed stand and mixing of seed lots collected from one stand in different crop years are to be recommended, although the latter is not allowed under many national legislations. Official bodies and state forestry authorities also need to make use of their competence, and control the FRM collection.

In the case of tree genera represented by several mutually crossable species, the species purity and proportion of hybrids in a seed lot may be an additional relevant issue. In oaks, where the levels of interspecific gene flow are generally high (Chybicki & Burczyk, 2013; Gerber *et al.*, 2014) and seeds are difficult to distinguish, increased attention must be paid to the choice of maternal trees in mixed seed stands, and the proportion of hybrid seeds needs to be controlled. Therefore, additional insight into these cases would be beneficial.

2.1.3 Seed orchards

Gömöry, D., Kowalczyk, J., Liesebach, H., Philippe, G., Himanen, K., Uggl, C., Alizoti, P.

For several economically important tree species in certain parts of Europe, seed orchards are a major source of seeds. They thus represent 'synthetic' populations in which superior individuals (known as 'plus trees') are planted together so that mutual mating can occur. As such, they may be composed of vegetative copies of plus trees typically obtained by grafting (clonal seed orchards) or, less frequently, generative progenies of plus trees (seedling seed orchards). Seed orchards are also considered to be important tools for forest tree improvement, because a) they represent collections of valuable materials and are expected to yield improved seeds, and b) further breeding activities are frequently performed there (e.g., artificial crossing and collection of material for progeny testing). In addition, special seed orchards may help the reproduction of scattered and endangered species that do not reproduce well in forest stands. The reason for establishing seed orchards is mainly to improve a) the reproduction of phenotypically superior parents in order to yield well-shaped offspring that are expected to grow well, and b) the reproduction of scattered and endangered species which do not reproduce well in forest stands.

Seed orchard establishment

→ Choice of plus trees

The choice of plus trees for seed orchard establishment (both clonal and seedling seed orchards) depends on the objective of the seed orchard and the tree species. There are several main categories for different purposes as described below.

Effective production of a large amount of high-quality forest seed: Seed orchards with selected, or even tested, plus trees are often established for tree species with high economic importance in forestry. In Europe, these are mainly stand-forming tree species that are usually managed in long- or mid-rotation. High genetic quality of seed crop can be achieved by selecting plus trees which fulfil a number of selection criteria; for example, growth performance, vitality and disease resistance, and quality traits such as stem form. Therefore, seed orchards have the potential to produce higher quality seeds in comparison to those harvested in approved seed stands.

Information regarding suitable geographic regions of origin for materials to be combined in a seed orchard can be obtained from numerous provenance tests

and field experiments. Field tests established by forest research institutes in many European countries provide long-term observation data on growth performance, quality traits and disease resistance in different deployment regions or breeding zones. The derived knowledge of large-scale differentiation can help to define regions for plus tree selection; moreover, the selection of plus trees can be carried out directly in older field trials (Lieseback *et al.*, 2013). This practice has some advantages, as the seed sources are known, and the trees are identical in age and spacing after planting. Therefore, there is a lower chance of random effects compared to plus tree selection in unevenly aged forests. The possible collection of too many related trees (members of half sib families or other levels of co-ancestry) must be considered in both field trials and naturally regenerated forests.

The within-orchard diversity transferred to the seed crop should be adequate for the rotation period. The longer the planned rotation period, the more diversity might be necessary, taking into account adaptability to changing environments. This means that a balance should be struck between improved performance based on selection (genetic gain) and broad diversity as a prerequisite for long-term stability.

The genetic diversity of seed orchards is mainly determined by the number of clones or, considering possible relatedness, their effective number (see below for details about the aspect of effective population size). For example, Libby (1982) recommends mixtures of 7–25 clones to provide a robust and optimal structure. Based on their computer simulations and probability theory, Bishir & Roberds (1999) found that situations requiring more than 40 clones are not prevalent. In practice, clonal seed orchards in Europe often consist of about 20–30 and up to 50 clones, rarely more. Accordingly, Stoehr *et al.*, (2004) reviewed the average effective population sizes of seed orchards and found them to have between 20 and 50 (range 2.5–110). Current regulations in Germany require a minimum of 40 and 20 clones in order to establish a new seed orchard for main commercial tree species and secondary tree species respectively. In Poland, the minimum number of clones is 40 for the important coniferous forest tree species (Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), European larch (*Larix decidua*)), while it is 30 for the other species. In Slovakia, the current minimum number is 50, but the recommended census population size is 100 genotypes. Clone numbers in such magnitude are considered to be efficient for both genetic gain and genetic diversity (Ivetić *et al.*, 2016). Some studies evaluate the genetic diversity of seed orchards with those from natural populations of the respective species considered as a reference line (examples for Sitka spruce (*Picea sitchensis*) and white spruce

(*Picea glauca*): Chaisurisri & El-Kassaby 1994; Fageria & Rajora 2014, and Norway spruce: Sønstebo *et al.*, 2018). The results generally showed that genetic diversity in seed orchard crops was similar, sometimes even higher, than in natural stands, and genetic distances to source populations were small; i.e., allelic structures did not substantially differ. A comprehensive review of seed orchard genetics is given by Funda and El-Kassaby (2012). i.e., allelic structures did not substantially differ. Spatial distance between plus trees is a factor that should not be neglected, as proximately located trees tend to belong to the same half-sib family or even clone and may share recessive lethal or S-locus¹¹ alleles (de Cyuper, 2008). The excessive use of related clones in a seed orchard reduces genotypic diversity and increases the proportion of offspring suffering from inbreeding depression or empty seeds. The majority of breeding programmes is currently at the stage where most seed orchards established from plus tree selections in natural forests between the 1950s and the 1990s still exist and are still producing seeds. They are to be renewed in the near future with materials that have undergone genetic tests (Pâques 2013; Jansson *et al.*, 2017). Selection intensity and population size usually consider the breeding strategy. Currently the multiple population breeding strategy is generally preferred (Burdon & Namkoong 1983). The size of the breeding population is regarded as optimal when the annual increase in group merit is maximised, under consideration of potential budget constraints. High heritability of traits targeted by selection, efficient breeding strategy, high additive variance at the age of maturity, low annual budget, expensive testing methods, and a low value assigned to gene diversity - all these factors favour a small breeding population size (Danusevicius & Lindgren 2005).

The question can be posed as to whether seed orchard crops meet the high expectations of forestry practice. In the Nordic countries, which have a long history of conifer breeding, timber yield of offspring from 1st generation seed orchards is estimated to have increased by about 10 % compared to unimproved FRM, while this figure is estimated to increase by up to 25 % in the second round of Norway spruce seed orchards (Jansson *et al.*, 2013, 2017). For Scots pine the mean annual yields have been modelled to be 24% higher in 1,5-generation seed orchards compared to unimproved stock (Haapanen *et al.*, 2016) Previous results obtained from ongoing field experiments in Germany showed that some, but not all, offspring from 1st generation seed orchards have better growth performance compared to progenies from other seed sources. However, offspring from seed orchards mostly

¹¹ *S-locus* (*self-incompatibility locus*) – a locus containing few closely linked genes preventing self-fertilisation in plants

reveal superior quality traits (Rau and Schulzke, 2001; Grotehusmann, 2014); this might be expected as quality traits are commonly higher in terms of heritability than quantity traits.

Gene conservation seed orchard for scattered or endangered species: These seed orchards gather small fragmented populations or relics of scattered individuals to form sexually reproducing communities (example for Blackthorn (*Prunus spinosa*) in Kleinschmit *et al.*, 2007). While gene conservation is the main objective, these seed orchards also have a production purpose in order to provide the material for enrichment plantations of endangered species. Within the given gene conservation area, as many individuals as possible should be collected to avoid inbreeding depression and bottlenecks (Vander Mijnsbrugge *et al.*, 2010). Species-related information regarding the large-scale or small-scale differentiation pattern of phenotypic or genetic traits (example for hazelnut in Leinemann *et al.*, 2013) is not always available. However, climate maps and landscape classifications can be used to initially define the appropriate conservation areas (Johnson *et al.*, 2010).

Special seed orchards for seed production from certain crossing combinations: This type of seed orchard often consists of two or a few clones and may include introduced species without gene conservation aspects; for example, hybrid aspen (*P. tremula* × *P. tremuloides*) and hybrid larch (*Larix decidua* × *L. kaempferi*). Here, the high breeding level is per se associated with reduced genetic diversity. Typically, such hybrids are cultivated under short- or mid-rotation management and can be restricted to certain areas.

→ Effective population size

In contrast to seed stands, clonal seed orchards established from vegetative copies (ramets) of phenotypically-selected or -tested plus trees generally contain much fewer genotypes. Diversity in seed orchard crops depends on effective population size, which is affected by the number of clones in the seed orchard, seed orchard design, fecundity and pollination from the outside. The way in which seed orchards are established and managed needs to ensure maximum genetic and genotypic diversity in the produced crops. In addition, inbreeding (generally associated with decreased viability of offspring) and random deviations of the allelic structures of the seed crop from those of the parental population (genetic drift) need to be avoided.

To meet this objective, a seed orchard should ideally represent a randomly mating Mendelian population; i.e., the mating probability for any pair of parents

should be independent of their genotypes (equal fertility, absence of any mating preferences), and the viability of all produced offspring genotypes should be equal (Lindgren *et al.*, 1996). However, parental imbalance is a rule (Lindgren & Mullin, 1998). Some clones can even be sterile, either because the plus tree was sterile or because grafts were taken from inappropriate part of the crown. Such clones are to be identified and removed from the seed orchard. The production of male and female gametes is commonly unbalanced, as plus tree clones are frequently represented by different numbers of ramets and they differ in fecundity due to genetic, epigenetic and physiological variation, partly due to grafting. Differences in flowering phenology also result in unequal seed yield coming from crosses of particular clone pairs. The spatial arrangement of clones is another problem: if it is not truly randomised, some parental combinations may become overrepresented - and others underrepresented - in the seed crop, due to preferential mating among neighbours. Finally, the viability of embryos from particular mating pairs may differ due to genetic incompatibilities. This is a problem which mainly arises in angiosperms in which prezygotic self-incompatibility systems frequently occur (Rosaceae, Betulaceae); however, recessive lethals also induce postzygotic crossing barriers in gymnosperms. Attention is usually only paid to spatial design, clone balance in terms of the number of ramets and pollination from the outside; while the other aspects (phenology, fecundity variation, incompatibilities), along with their genetic implications, are often neglected, because they are difficult to evaluate during seed orchard establishment.

Knowledge about the genetic consequences of the processes linked to genetic variation in seed orchard crops can be acquired using two general approaches: *a posteriori* and *a priori*. The *a posteriori* approach is based on the comparison of genetic structures of a seed orchard and its crop by using gene markers. In spite of a significant drop in prices and increasing automation of laboratory work during the recent years, marker studies are still too laborious and expensive for routine screening of seed orchards to make management decisions. The technically and financially more affordable option is the *a priori* approach based on the assessment of mating patterns reflecting female and male fecundity, and eventually flowering phenology and spatial design. This approach involves the estimation of various types of effective population sizes (Gregorius 1991; Lindgren *et al.*, 1996). The effective number relates the state (i.e., inbreeding, coancestry, or genetic drift) of a real population (e.g., a seed orchard) to that of an ideal panmictic population. In other words, the effective population size in a seed orchard quantifies the number of clones which have equal reproductive success, the same inbreeding or coancestry coefficient, or variance of gene frequencies, as a real seed orchard population. This

is commonly expressed in a relative manner; i.e., as a fraction of the number of clones. An overview of the different types of effective population numbers and their specific meanings in the case of clonal seed orchard crops can be found in Kjær and Wellendorf (1997). Many studies have been carried out on various tree species with varying outcomes, in which the relative effective number ranges from 18 % to 98 % of the census population size (Machanská *et al.*, 2013; Ózel & Bilir 2015), depending on the species and especially on the sources of deviations from panmixia that were taken into account.

Generally, asynchronous flowering seems to affect the effective population size more seriously than variation in fecundity (Codesido *et al.*, 2005; Gömöry *et al.*, 2008, Alizoti *et al.*, 2010). Genetic incompatibilities, such as those caused by sharing S-alleles in self-incompatible species, may also cause deviation from panmixia (de Cyuper 2008). Damage by insects and pathogens to seeds and surrounding structures can also lower the effective clone number as their damage can be asymmetric, affecting certain genotypes (Nikkanen 2002; Glynn & Weslien 2004). Nevertheless, the assessment of factors such as phenology, genetic incompatibility and male flowering is labour-intensive and costly. The effective number of clones (Kang *et al.*, 2001a) - relying only on the numbers of ramets per clone - is an easy-to-use option for the control of the genotypic diversity in seed orchard crops, which is the basis for adaptation to environmental stresses. Moreover, pollination from the surrounding stands can affect the dimension of a seed orchard, thus increasing effective population size.

→ **Spatial configuration: random and row designs**

The spatial structure of a seed orchard affects both the ease of management practices and the productivity of the orchard, as well as the level of inbreeding. From a management point of view, the arrangement of clones in a row- or group-design is advantageous, because it is easy to apply clonal treatments (gibberellic injections, pesticide treatments, cutting, etc.) and to organise clonal collections. However, a high level of inbreeding can arise from this type of design. A random pattern, in which the clones are distributed so that ramets of the same or related clones are not placed in close proximity, favours outcrossing. This may increase the yield of full, viable seed and can decrease inbreeding. It must be noted that while inbreeding in some species does not automatically decrease the seed yield, it is still detrimental to the genetic quality of seed crops (Koski, 1980).

In western hemlock (*Tsuga heterophylla*), El-Kassaby (2003) observed higher outcrossing in a random-design seed orchard compared to a row-design, although

high outcrossing levels have also been documented in a row-design seed orchard of Norway spruce (*Picea abies*) (Pakkanen *et al.*, 2000). El-Kassaby *et al.*, (2007) found a difference in the outcrossing level of random- and row-design interior spruce (*Picea glauca/engelmannii* complex) seed orchards, but suggest that under intensive management row-design may produce sufficient genetic quality.

The importance of clonal arrangement, as well as other factors influencing the movement of pollen and level of inbreeding, such as ramet density and height, varies among species. Poska and Pidek (2010) found that the majority of European silver fir (*Abies alba*) pollen was deposited at a distance of 50 m from the tree, while for *Fagus sylvatica* and *Pinus sylvestris* the majority of pollen was found within 300 m and 1000 m respectively. Burczyk *et al.*, (2004) report that for *Norway spruce*, 83 % of successful fertilisations occurred with pollen from sources within 20 m away. For Douglas fir (*Pseudotsuga menziesii*), Erickson and Adams (1989) found that very little pollen was dispersed over 30 m in seed orchard conditions. For insect pollinated species, such as wild cherry (*Prunus avium*) and *Tilia* spp., pollen dispersal and pollination success depend on insect/tree interactions and varying environmental factors, which all need to be taken into account in seed orchard management. The problem of self-incompatibility in many woody angiosperms also needs to be considered.

→ Choice of location

There are several aspects that need to be considered in the choice of a suitable location for a seed orchard.

One aspect is purely organisational: seed orchards need to be maintained and managed, and this requires personnel and, preferably, access to machinery; in view of this, seed orchards should be concentrated in one place in the proximity of seed extraction units or nursery centres. Pollen exchange between seed orchards designed for different provenance regions or different altitudinal zones may produce maladapted offspring. The same applies to background pollination, which may eventually significantly contribute to seed orchard crops (Torimaru *et al.*, 2013): if the surrounding stands are of poor quality or do not match the origin of seed orchard clones, the average performance of the offspring may decrease.

Local environment is another factor to be taken into consideration when choosing a location for a seed orchard: the topography and aspect should produce a favourable microclimate and poor soils should be avoided (Eriksson *et al.*, 2013). To achieve proper flowering, seed orchards are sometimes transferred several degrees

of latitude to the south or to lower elevations, especially in Fennoscandia¹². Such transfers are at risk of epigenetic after-effects: for example, seeds in Norway spruce developing under warmer climates and shorter days have reduced hardiness compared to progenies of the same genotypes in the original stands (Johnsen *et al.*, 2005; Skrøppa 1994). The effect of temperature during embryogenesis has also been investigated in seed lots produced in years with contrasting temperature sums (Solvin and Steffenrem, 2019.). A shift in bud set in seedlings, as well as in the timing of flushing and cessation of leader elongation in saplings are observed depending on the accumulated temperature sum in the specific seed year. Currently, the timing of bud set in tree crops in every seed orchard is tested and compared prior to recommendation of deployment region in Norway. Seed production in various climatic regions may thus be important to enhance the adaptedness of Norway spruce.

FIGURE 6. Silver birch (*Betula pendula*) greenhouse seed orchard in southern Finland. (Photo credit: Katri Himanen/Natural Resources Institute Finland).



¹² The region including the Scandinavian Peninsula, Finland, Karelia, and the Kola Peninsula. The term usually covers the countries Finland, Norway, and Sweden in their entireties. It also includes a part of Russia.

Pollination from the near stands should also be taken into account, since it generally reduces genetic gain and is thus regarded as genetic contamination of seed orchard crops. One option for eliminating pollination from outside sources is to establish the seed orchard inside a greenhouse or cover the grafts with an isolation tent during flowering and pollination (Lepistö 1973; Torimaru *et al.*, 2013). In Finland, Silver birch (*Betula pendula*) and curly birch (*Betula pendula* var. *carelica*) seeds have been produced in plastic greenhouses on a production scale for several decades (Figure 6). In Sweden, tent isolation of Scots pine seed orchards has proven to be effective in excluding external pollen from the grafts, thus increasing self-pollination (Torimaru *et al.*, 2013), and a similar approach is currently being established for Norway spruce. Supplemental mass pollination can be used as a method to secure genetic diversity in such settings (Funda *et al.*, 2016).

Climate warming may enhance seed production at northern latitudes in some species (Caignard *et al.*, 2017). In Norway spruce for instance, seed set requires two consecutive warm summers, and mast years occur with an interval of 3 to 10 years (Børset, 1985). However, drought can be a limiting factor for reproduction success, which has been shown to decrease seed crops in white spruce (*Picea glauca*) (Roland *et al.*, 2014). Frost events due to more unstable weather may also kill flowers. There is therefore no simple correlation between climatic warming and seed production in conifers.

→ Designing new seed orchards to meet future needs

The question of how to design seed orchards to maximise genetic and economic benefits has received much attention in the literature. Many tree improvement programmes are now in advanced generations of breeding and orchard design considerations have become more complex (Hodge & White, 1993). Advanced-generation orchards may either contain offspring selected from a new base population (forward selections) or original first-generation selections (backward selections), or mixtures of both (Lstibůrek *et al.*, 2015).

The selection of plus trees and the subsequent establishment of seed orchards with selected clones is always based on phenotypes developed in the past as an interaction of genotype and environment. To meet future needs, the expected shifts of climatic parameters and added uncertainty need to be accounted for by using a broad genetic base.

Forest seed orchards can be designed in several possible ways. Already existing seed orchards can be used by extending or adapting the recommended employment

regions (Berlin *et al.*, 2016) or new ones can be established containing higher diversity with larger or more diverse regions of clone origin. In the latter approach, sexual recombination between genotypes originally adapted to different environments is possible. In principle, such offspring could express higher adaptability, but outbreeding depression¹³ is also possible. Therefore, further research on assisted migration is needed.

In addition, the effect of epigenetic changes should be taken into account. The example of the influence of local environmental conditions on the adaptive properties of Norway spruce progenies in Norway (Johnsen *et al.*, 1996, 2005) has already been mentioned (see 2.1.3. *iv Choice of location*). A second example, from the Austrian research project 'AdaptTree', describes the influence of temporal changes of environmental conditions between drought and moist years of seed ripening in the same stands, by comparing the drought stress reaction of seedling populations derived from seed lots from Norway spruce, Scots pine and European larch (*Larix decidua*) (Schueler 2014, unpublished¹⁴). Therefore, taking into account these epigenetic effects, seed orchards could be established outside of the region where the material was originally selected and the year-to-year variation of climatic conditions during seed development could be exploited to recommend seed lots for certain purposes.

In general, breeding populations and clonal seed orchards from more-or-less natural populations and silviculturally managed forests are managed separately. However, in the long term, they need the potential of genetic resources existing in the species as a whole, including permanent adaptation processes as far as is possible. In view of this, sustainable breeding programmes always include measures for preservation of genetic resources (Gregorius, 2001; Kleinschmit, 2004; Dempfle *et al.*, 2016).

Management

→ Flowering balance and treatments for flower induction

The abundance of flowering has a genetic component (e.g., in Norway spruce (*Picea abies*) and the distribution of the flowering frequency is skewed left, meaning that trees or clones with low flowering frequency are most common (Koski &

¹³ *outbreeding depression* – decrease in fitness caused by crossing between phylogenetically distant genetic lineages.

¹⁴ <https://bfw.ac.at/rz/bfwcms.web?dok=9280>

Tallqvist, 1978; Nygren *et al.*, 2017). Nikkanen & Ruotsalainen (2000) also observed that the flowering abundance of seed orchard clones in different years was usually positive and significant: the clones flowering prolifically do so in consecutive years. Differences in flowering and other aspects of reproductive phenology are also evident in many species. Differences in Scots pine reproductive phenology between clones have been closely examined by Bhumibhamon (1978). Nikkanen (2001) reported a difference in female flowers' receptivity of 2–4 days between the earliest and latest clones in a Norway spruce seed orchard, while the entire duration of receptivity varied from 2.6 to 4.0 days between years. Even larger phenological lags have been observed in French seed orchards of Douglas fir (*Pseudotsuga menziesii*) and larch (*Larix spp.*). (Philippe and Baldet 1992). Therefore, the selection of clones, as well as the management practices affecting flowering abundance and phenology, have a genetic bearing.

Management measures such as pruning, fertilisation, and sometimes irrigation are commonly used to enhance seed production in seed orchards. As they are usually applied to the whole seed orchard area in a uniform way, they are unlikely to have significantly different effects on the various clones, and thus do not contribute to parental imbalance.

Flower stimulation techniques aim to create artificially favourable conditions for reproductive bud initiation and differentiation. In the Pinaceae, which have been extensively studied, these techniques have influenced the development of young undifferentiated meristems and pushed them preferentially into the sexual path instead of the vegetative and latent paths (Allen & Owens, 1972). Though the underlying physiological mechanisms remain largely unknown, several techniques have proved their effectiveness in conifers. Properly timed cultural treatments (nitrogen fertilisation, stem girdling, root pruning) and hormonal treatments (gibberellin 4/7 application (GA4/7)) have enhanced female and/or male flowers in many coniferous species (e.g., Bonnet-Masimbert & Zaerr, 1987). The best results can generally be obtained with a cultural treatment combined with GA4/7 injection.

Flower stimulation treatments are not as widely used in seed orchard management as in tree breeding, even though their cost-effectiveness has been demonstrated in some species like Douglas fir (Ross & Bower 1989; Philippe *et al.*, 2004). This may arise from fear of injuring the trees and lack of registered gibberellins. Nevertheless, some treatments are commonly used in some countries, such as France, where Douglas fir seed orchards are systematically girdled every third year. GA4/7

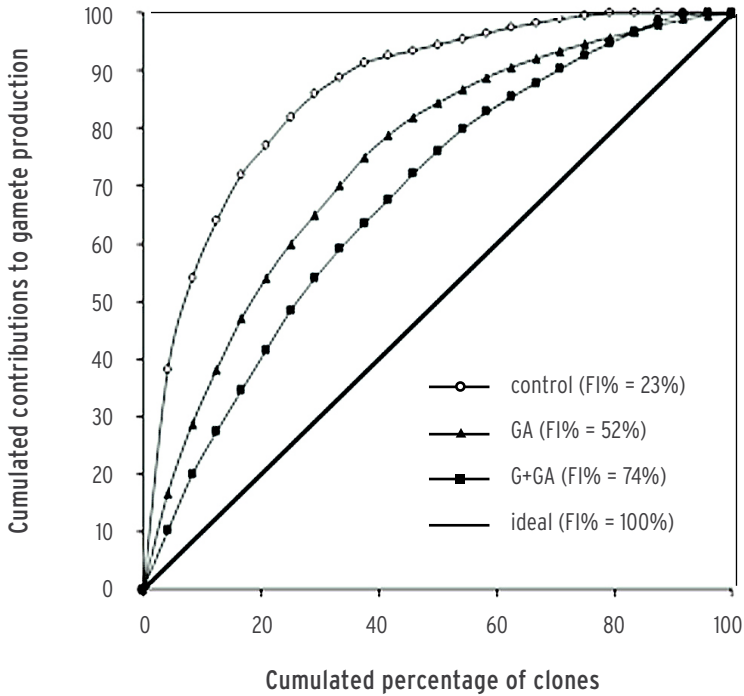
injection has started being used in Norway spruce seed orchards in Sweden and Finland in recent years.

When establishing seed orchards to provide forest owners with sufficient amounts of FRM of high genetic quality, it is important to ensure that the quantitative and qualitative objectives are not antagonistic. Divergent opinions have been expressed concerning flower stimulation. On the one hand, induction treatments can be considered to favour panmixia, because they increase the number of flowering genotypes, and thus the number of contributors to the seed lot (Wheeler *et al.*, 1985). On the other hand, it has been reported that these treatments are particularly effective for genotypes with good flowering ability; however, this comes with the risk of treatments increasing the parental imbalance of fertility and the deviation to panmixia (Sweet & Krugman, 1977).

The result of those conflicting effects has been studied in dozens of trials for three species: Douglas fir and Maritime pine (*Pinus pinaster*) (Philippe, 2005; Philippe *et al.*, 2006). Parental contributions for gamete production were assessed by visually estimating the male and female flower production of each tree. The deviation to even-fertility in treated and non-treated populations was displayed graphically (see Figure 7 below) or characterised using a fertility index (FI). This index corresponds to the status number for unrelated and non-inbred orchard parents (Lindgren & Mullin, 1998) and is often used as an indicator of orchard crop diversity.

For both Douglas fir and Norway spruce, flower stimulation consistently attenuated the differences in production among parent clones. This positive effect was particularly pronounced the years when natural flowering was scarce, but it still persisted in good flowering years. In addition, it differed in magnitude according to the treatment. The most effective treatments for flower production also resulted in the highest homogeneity of clonal contributions (see Figure 7).

FIGURE 7. Clonal contributions to gamete production in a Norway spruce seed orchard after gibberellin 4/7 injection, applied alone (GA) or in combination with stem girdling (G+GA). The diagonal represents the ideal situation where all the clones contribute evenly to gamete production (From: Philippe, 2005).



The pattern was somewhat different for maritime pine, a species that flowers abundantly and much more regularly: natural flowering was more evenly distributed among parent genotypes than in Douglas fir and Norway spruce, and the effect of flower stimulation treatments (N-fertilisation and GA4/7 injections) was limited.

Although the impact of flower induction on parental contributions depended on species, years and treatments, no antagonism was found between quantitative and genetic objectives in any orchard. On the contrary, treatment effect may even be increased by two factors: first, highly flowering trees produce smaller cones with lower seed potential, which tends to homogenise parent genotype contributions; and second, the treatments which promote female flowering effectively also favour pollen production in most coniferous species. This results in more even contributions from the parent genotypes for both female and male gamete production.

Most studies show that flower stimulation does not have any deleterious effects on seed set and seed viability (Wheeler *et al.*, 1985; Beaulieu *et al.*, 1998; Philippe *et al.*, 2006). However, this point has been disputed; for example, reduced seed weight and/or germination rate have been observed in the offspring derived from GA treated clones of Scots pine (Eriksson *et al.*, 1998) and Norway spruce (H. Liesebach, unpublished).

Ideally, panmixia should assume that: all genotypes produce the same number of gametes, the genotypes mate at random, and the orchard is not subject to pollen contamination. The first assumption is not affected by flower stimulation. Conversely, increased within-orchard pollen production will lead to reduced pollen contamination (Kang *et al.*, 2001b) and a lower selfing rate. Flower stimulation treatments are highly recommended, because of their positive impact on seed production and genetic quality, both in terms of diversity and genetic gains (better respect of the assumptions made for the calculation of genetic gains).

The choice of site where a seed orchard is established can itself be considered as an induction treatment. In indoor orchards, heat and drought have been shown to stimulate flowering for several species of Norway spruce (Ross, 1988) and European larch (*Larix decidua*) (Philipson, 1995). In addition, the fertility index described above appears to be strongly and positively correlated with the percentage of flowering grafts in a Norway spruce seed orchard (Philippe, 2005; Philippe *et al.*, 2006). Therefore, the deviation to panmixia is reduced when environmental conditions are favourable to flower initiation, pollination and seed development (Sedgley & Griffin, 1989). Such sites should be sought when establishing new seed orchards, especially if managers do not intend to use flower stimulation treatments or if the species does not respond to standard induction techniques. Nevertheless, high seed production does not necessarily mean 'good' seed production from a genetic point of view. Very warm sites should be avoided because of epigenetic after-effects (see above) being likely to produce maladapted FRM.

Choice of genotype might also be seen as a kind of flower stimulation treatment. It follows logically from the above results that panmixia, and thus genetic quality, will be enhanced when selecting phenologically compatible genotypes with a good ability to produce female flowers, pollen and sound seeds.

→ Supplemental mass pollination and controlled pollination

Supplemental mass pollination (SMP) is the wide application of pollen to strobili that are not isolated from wind-borne pollen. A potential benefit of supplemental

mass pollination in conventional intraspecific tree improvement programmes is the increase in yield of sound seeds and in realised genetic gains (Bridgewater & Trew, 1991). In specific cases, supplemental pollination is also used to overcome differences in flowering phenology; for example, in hybrid seed orchards of *Larix decidua* and *L. kaempferi* (Bonnet-Masimbert *et al.*, 1998).

Controlled pollination is another way of increasing genetic gain in seed orchards and reducing pollen contamination, mostly used to cross genotypes with a high specific combining ability or to produce hybrid seeds. Traditional crossing procedures require a skilled operator and specific equipment, as the isolation of female flowers or strobili needs to be pollen-proof. Even though this has been routinely done in some North American seed orchards, the related costs render it impractical (Bridgewater *et al.*, 1998). A variant using simple paper bags and simpler installation is referred to as controlled mass pollination. In this case, a certain level of contamination of the crops by outside pollen is accepted in return for lower overall expenses and higher labour productivity (Bramlett, 1997).

Any artificial pollination requires experienced personnel. All operations associated with pollination (pollen collection, extraction, storage, viability testing, assessment of the receptivity of female flowers/strobili and optimal time to pollinate, pollen preconditioning and finally application) influence the final outcome. This may be one of the reasons why very different success rates have been reported, mainly in the case of supplemental mass pollination, which is quite a common procedure in some parts of Europe (Eriksson *et al.*, 1994, 1995).

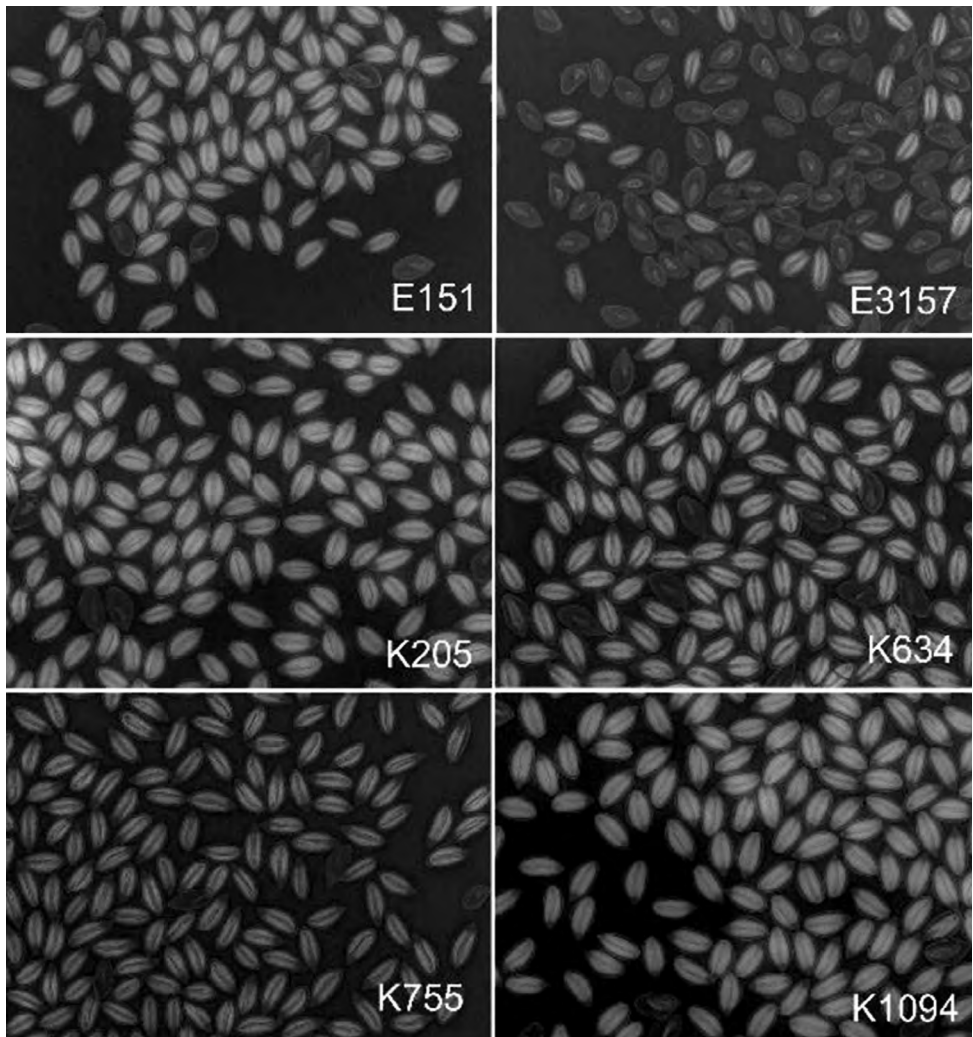
Artificial pollination can be carried out using both fresh and stored pollen. In the former case, male trees need to flower before females sufficiently early to permit pollen collection and processing, whereas males and females need not be located in the same place. A seed orchard with appropriate male clones located in a warmer climate provides the advantage of increasing the time lapse between pollen collection and female receptivity. The latter strategy requires the expertise for proper processing and storage of large quantities of pollen (Bramlett & Matthews, 1991).

→ Parental balance among clones

For many reasons (clone relatedness, improper clonal arrangement in the seed orchard resulting in selfing, flowering asynchrony, etc.) a high number of seeds may lack a viable embryo and/or surrounding internal structures. These seeds are usually called 'empty' seeds. In species such as Scots pine and lodgepole pine (*Pinus contorta*) unfertilised ovules are typically aborted and therefore empty seeds

rarely occur (Owens *et al.*, 1981); whereas in Norway spruce, unfertilised seeds develop further, resulting in high proportions of empty seeds. In some tree species, a high proportion of empty seeds is considered an adaptive trait which reduces the effects of seed predation (Perea *et al.*, 2013). Consequently, empty seeds often appear similar to full and viable seeds and thus cannot be recognised during collection.

FIGURE 8. Clones in a seed orchard may contribute differently to the pool of viable seed in a seed lot, even if they contribute evenly to the cone crop. The X-ray image of Scots pine seed collected from a seed orchard in central Finland shows that clone E3157 contains a large proportion of empty seed. (Photo credit: K. Himanen/Natural Resources Institute Finland).



The formation of empty seeds can occur for different reasons and may affect clones or individual trees in different ways; therefore, the actual contribution of different maternal genotypes to a given seed lot may differ from the apparent cone or seed crop (Figure 8). In Norway spruce, Himanen *et al.*, (2016) found that the proportion of full seed varied from 33 % to 79 % between different clones in a seed orchard. Observations of the imbalance between the size of cone crop and seed yield (the amount of full seeds obtained) and the differences between clones in the seed yield have also been made in Sitka spruce (*Picea sitchensis*), (Chaisurisri & El-Kassaby 1993).

Parental imbalance may also occur from the paternal side. The differential paternal mating success of individual clones (due to phenological asynchrony), variation in male fertility and postzygotic barriers, can reduce the effective population size (N_e) of male parents compared to the census number of the population (Alexander & Woeste, 2017; Hansen, 2008). Buiteveld *et al.*, (2001) found that in a common oak (*Quercus robur*) clonal seed orchard, where only 19 out of 56 potential pollen donors contributed to pollinations, the number of pollinations roughly corresponded to the number of ramets per clone in the orchard, and to the differences in crown level or cardinal directions of the crown. However, they detected no differential reproductive success among the contributing pollen donors. On the other hand, Alexander & Woeste (2017) found that the largest influence on the pollination success of northern red oak (*Quercus rubra*) was flowering phenology and distance.

→ Pest management

The level of pest damage to seeds and surrounding structures and the resulting significance to the genetic diversity of seed crops differs between tree species. Cone and seed pests pose a considerable problem to the seed production of some important tree species in European forestry, such as Norway spruce for which infestation rates of cone insects have been reported to be as much as 95 to 100 % (Nikula & Jalkanen, 1990; Bakke, 1955; Seifert *et al.*, 2000), although typically the level of losses is less severe. The most common pest species include spruce seed moth (*Cydia strobilella* L.), pugs (*Eupithecia* spp.) and cone gall midge (*Kaltenbachiola strobi* (Winn.)). Cherry-spruce rust (*Thekopsora areolata* (Fr.) Magnus) and inland spruce cone rust (*Chrysomyxa pirolata* Wint.) are the most significant fungi, damaging Norway spruce cones and seed during their development with varying intensity from year to year. Almqvist and Rosenberg (2016) report that 70 % of cones in studied Swedish seed orchards were damaged by cherry-spruce rust in 2006.

From a genetic point of view, the most crucial issue lays in the observations that pest damage affects some genotypes more than others. Glynn and Weslien (2004) report a significant effect of genotype on spruce seed moth infestation rates. Himanen *et al.*, (2016) noted a similar effect with both spruce seed moth and seed infesting spruce seed chalcid (*Megastigmus strobilobius* Ratz.) damage in a mature Norway spruce stand when cones of individual trees were examined. However, in the same study, the difference in infestation rate of cones by spruce seed moth in different clones in a seed orchard did not vary. These observations can be explained by the great variance of insect populations from year to year and the production environments (Annala, 1981; Kaitera, 2013), as well as by the differences in flowering phenology discussed in the previous chapter. For example, spruce seed moth lays its eggs in open, female flowers; therefore, it can be hypothesised that certain clones are more susceptible to these pests, because the peak period of ovipositing is synchronous with some genotypes' female flowering patterns and not with others.

Finding effective pest management practices could thus have a positive effect on both the productivity of seed orchards and the genetic diversity of FRM. Available management practices are currently limited. Given indications that the geographical distribution of some seed insects, along with the severity of their damage, will change due to climate change, and given the appearance of new, alien pest species, such as the western conifer seed bug (*Leptoglossus occidentalis* Heidemann) in Europe (Lis *et al.*, 2008), the impact of seed pests on FRM production needs to be studied further.

Seed collection

→ Examples of general rules for the collection of seed crop

Beyond the general wording of the Council Directive, there are no absolute legal restrictions in Europe regarding the number of clones to be harvested or seed lot size per clone. Typically, all the seed crop is expected to be harvested. However, such restrictions have been imposed in some national regulations (e.g., Austria and Slovenia). In several European countries, there are simple regulations aimed at maintaining some basic levels of gene diversity in seed orchard crops; for example, in Slovakia, cone or seed collection in seed orchards is only allowed when more than 50 % of clones flower.

Regulation in British Columbia (BC, Canada) imposes the use of FRM with high diversity and good genetic quality on forest companies which exploit and reforest public land. A seed lot rating protocol has been developed for this purpose

(Woods *et al.*, 1996; Stoehr *et al.*, 2004). Genetic diversity is assessed on the basis of clonal contributions to male and female flowering, using the concept of effective population size (N_e) or status number. It corresponds to the number of unrelated and non-inbred clones in an ideal seed orchard with a panmictic reproduction regime. All seed lots must exceed a minimal threshold set ($N_e = 10$) to be used for public land reforestation. Crossing (im)possibilities are not taken into account, because the added cost and effort required to collect phenological data are not considered justified in most of the seed orchards (Xie *et al.*, 1994).

The genetic worth of orchard seed lots is estimated by taking into account the breeding value of each clone, clonal contributions to male and female gamete production and, if necessary, supplemental mass pollination (SMP) efficacy. In some orchards at risk (risk assessment based on genetic quality of both the orchard and the surrounding stands), the calculation will also include the proportion of contaminant pollen during the period of receptivity of female flowers (Woods *et al.*, 1996; Stoehr *et al.*, 2004).

Similar protocols have been implemented in New Zealand with the aim of rating radiata pine (*Pinus radiata*) seed lots on the basis of their breeding value (GF rating followed by GF Plus rating) (Vincent 1990, Carson *et al.*, 1992; RPBC, 2003). However, they do not take genetic diversity into consideration.

The system used in French public seed orchards was inspired by the procedure used in British Columbia for genetic diversity assessment - albeit with some differences - and is implemented by an expert committee. In this system, genetic diversity is not assessed systematically, but only for crops that are likely to cause problems in terms of diversity; i.e., when the percentage of flowering grafts is low or medium. If litigious cases are identified during the systematic orchard surveys carried out at flowering time, male and female flowering are assessed for each ramet in order to estimate the effective population size. Vigilance is higher for seed orchards with fewer clones, but all the French public orchards, except the hybridisation orchards of larch, have a high genetic basis (N_e calculated with the number of ramets per clone > 50).

The threshold for effective population size is set at $N_e = 15$. This increase appears justified for the following reasons:

- **Questionable reliability of flower counting:** visual assessment from the ground tends to minimise the differences of fertility between genotypes with high and

low production, which results in overestimated N_e (Philippe *et al.*, 2006). This problem can be overcome by using a drone.

- **Many factors that contribute to a general loss of diversity are not taken into account:** a phenological lag making some crossings impossible; the development of ovules into seeds; and cone, seed and seedling processing in seed plants and nurseries, particularly that involving the sorting of seed and seedling
- **Little visibility for the future:** unlike in Canada, forest managers in Europe cannot ensure that the plantations created with the current crops will not be regenerated naturally. Therefore, the danger of further erosion of diversity in the next generation cannot be eliminated.

If the number of effective clones is less than 15, the coordination committee either prohibits the seed dealers from collecting the cones or obliges them to mix the crop with a previous seed lot. The option of setting fixed volumes of cones to be collected from each genotype has proved to be unfeasible, because cone collection control would be too time-consuming.

Intuitively, in terms of representativeness of the basic material, a given N_e (for example, 15) will not be the same, if the seed orchard is composed of 20 or 100 genotypes. It would probably be advisable to set another threshold for the ratio, ' N_e based on clonal contributions to flowering: N_e based on the number of ramets per clone'; but given the lack of scientific references on the topic, this ratio is not taken into account.

A flowering assessment may also be demanded by the coordination committee in particular circumstances; for example, when the orchard production greatly exceeds the seed dealer's needs. Collecting the cones from the most productive grafts is not desirable, because they are likely to belong to a limited number of genotypes. In such cases, the data from flowering assessments are used to delimit a restricted area in which all the cone bearing grafts will be harvested.

Hybridisation orchards are another particular case. According to EU regulation, seed lots may be marketed with any hybrid percentage, provided that this is specified. However, seed orchard managers, policymakers and foresters should question commercialising and planting so-called 'hybrid' FRMs with a low hybrid percentage. For hybrid larch (*Larix ×eurolepis* or *×marschlinsii*), a minimal threshold of at least 6070 % should be set (Philippe *et al.*, 2016). This would ensure improved

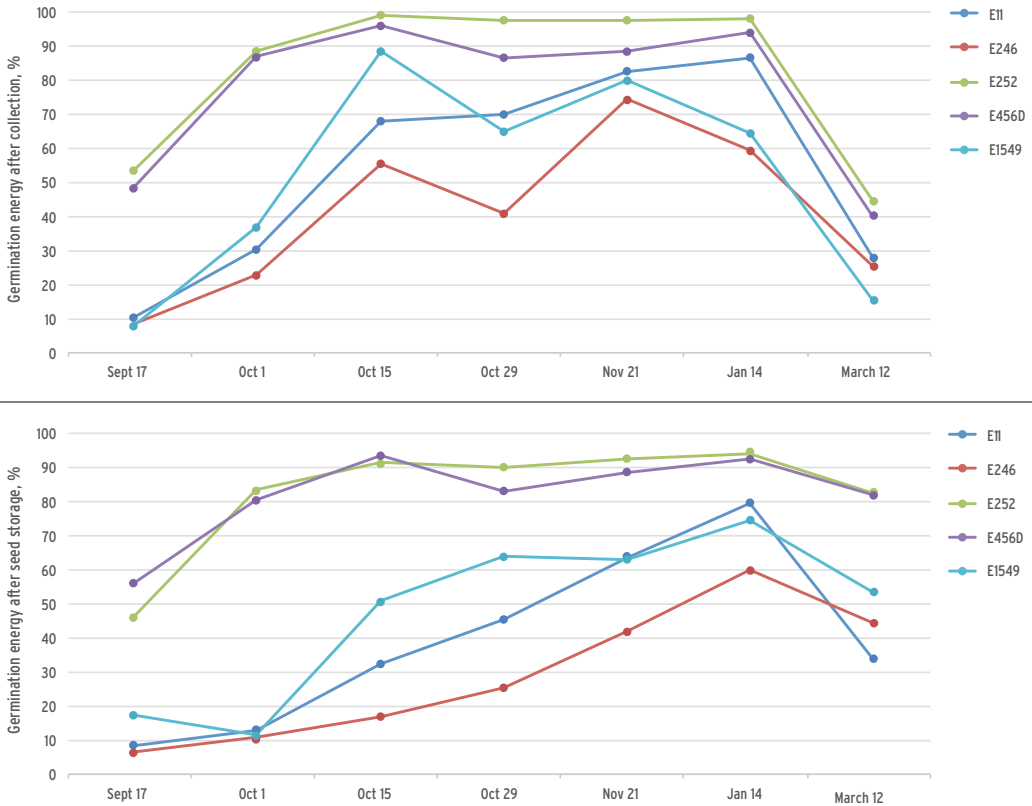
and more homogenous FRM performance and avoidance of commercial disputes and lawsuits (Myking & Skroppa, 2006). In larch open-pollinated hybridisation orchards, it is recommended to monitor flower development and to estimate flower abundance and the flowering overlap of the genotypes of the two parent species (and possible pollen contamination). It would be wise to avoid collecting the cones if there is not a good chance of obtaining a large proportion of hybrid seeds. An early verdict before cone collection time can be obtained using molecular markers (Philippe *et al.*, 2016).

→ **The effect of differences in seed maturation timing on parental balance**

The level of seed maturation affects germinability, especially germination energy, and viability of the seed in storage. Immature seeds are also more susceptible to pathogenic or saprophytic fungi (Sutherland *et al.*, 2002). The physiological maturity of seeds may also affect their germination response to different environmental conditions and change their need and response to dormancy breaking treatments. This was demonstrated in Scots pine in Nygren (1987): the later the seeds were collected in the autumn, the higher their germination percentage when seeds were germinated in the dark, while germination tests in the light showed no difference according to seed maturity.

For these reasons, high seed quality depends on the collection of seeds only upon maturity or the use of appropriate after-ripening techniques needed for some species. Different genotypes, however, vary in the timing of seed maturation (Nygren 1987, Daws & Jensen 2011). The phenomenon of finding genetic differences may be more pronounced in extreme populations of the species distribution range, where full maturation does not always occur (Harju & Ruotsalainen, 1996). In Norway spruce, clonal differences in seed maturation timing have also been found (Figure 9): the early maturing clones reached high germination energy weeks or months before the slowly maturing clones. Thus, inappropriate – or single – collection times for all clones or individual trees may result in an uncontrolled reduction in effective population size, because immature seeds lose their viability in storage and perform poorly during seedling production, compared to seeds from early-maturing genotypes.

FIGURE 9. Laboratory germination percentage on day 7 of five Norway spruce clones (E11, E246, E252, E456D and E1549) collected on seven different occasions from a first-generation seed orchard in Finland straight after collection (upper figure) and after 2.5 to 8 months storage at +4 °C (lower figure). (From: Katri Himanen, Natural Resources Institute Finland, unpublished).



2.1.4 Plus trees/Parents of families

Gömöry, D., Kowalczyk, J.

Neither the Council Directive nor the OECD Scheme uses and defines the term ‘plus tree’. Nevertheless, it is widely used by practitioners and sometimes by national legislations and in guidelines for FRM. Plus trees (Figure 10) can be defined as trees with desirable phenotypic characteristics (Eriksson *et al.*, 2013); i.e., outstanding trees selected for their phenotype and which exceed other trees on comparable sites in terms of traits of interest (typically growth rate, stem quality, health, etc.). Generally, their genetic value has not been proved via progeny tests. They can serve as parents of families during the initiation phase of a breeding programme.

FIGURE 10. “Plus tree” of Scots pine (*Pinus sylvestris*) in Dubrava virgin forest reserve, Lithuania.
(Photo credit: mapio.net)



Parents of families are a legally recognised type of basic material. They are defined as trees with known breeding value, and are used to obtain progeny via the controlled or open pollination of one identified parent (used as a female) with the pollen of one or more identified or unidentified male parents. This definition implies that in most cases, procuring FRM from parents of families is associated with additional effort and costs, and thus only makes sense when the genetic value of the maternal tree is known. Therefore, parents of families are certified solely in the category ‘Tested’ in most (but not all) countries.

Approval and management

Plus trees can be selected using several different approaches, the choice of which is influenced both by the genetic information available and the time and cost involved. Phenotypic selection is the oldest method of selection; the best individuals are selected on the basis of their appearance without knowledge about the genetic background of the traits of interest (Giertych 1995; Kowalczyk, 2005). This method is simple, fast and effective, but relies on the (not necessarily realistic) assumption that a good phenotype is associated with a good genotype.

Phenotypic selection can be performed using several methods. The simplest one is based only on the personal judgement of the breeder who compares several traits in a tree. In this case, quality traits are generally preferred. In some cases, general rules regulating phenotypic selection are defined. In the tree comparison method (Ledig, 1974), some restrictions are imposed on the number of neighbouring trees which are to be compared to the chosen ones. A similar comparative approach is used in the selection of oak plus trees in Croatia, where quality traits of candidate trees are graded, and a weighted average of grades is used as a sort of selection index (Bogdan *et al.*, 2018). In Slovakia and the Czech Republic, selection indices relying on growth and stem form functions have been developed for the main commercial species and are derived from age, height and trunk diameter, and subjectively assessed crown form is also considered. The selection thresholds are then differentiated by site quality.

A problem with the selection of plus trees in mature stands is that imperfections in the bottom part of the stem may remain hidden inside the trunk. In Sweden, this has been avoided since the 1980s by carrying out new selections in planted stands which have reached a third of their rotation age. Stem quality selection is thus based on the economically most valuable part of the stem (Eriksson *et al.*, 2013).

The selection criteria for most plus trees are general ones (e.g., growth, quality and health), but in specific cases the target traits can be modified and adapted to the objective of a particular breeding programme. The prerequisite is that the trait of interest is heritable. A good example is curly birch: this silver birch variant, with highly decorative curly-grained and brown-figured wood, occurs naturally in northern Europe and parts of eastern and central Europe. Curliness is a trait with simple Mendelian inheritance (Kärkkäinen *et al.*, 2017), thus selection for such a trait makes sense.

Growth and quality criteria are also often disregarded in species or local populations under a strong abiotic or biotic environmental pressure. In such cases, resistance to stress becomes the main (or even the sole) criterion for the choice of a plus tree; for example, the selection of conifer plus trees for polluted areas in the Ore Mts. (Braun, 1998) or Ash (*Fraxinus*) trees tolerant to the ash dieback fungus *Hymenoscyphus fraxineus* (Bogdan *et al.*, 2018).

Eventually, the quantity and quality of fruit production may become the main target of selection; for example, in the case of chestnut, walnut and fruit trees. The method for phenotypic selection of stone pine (*Pinus pinea*) plus trees is exceptional, aiming only at cone production and relying on an analytical approach to choose the best trees (Carrasquinho *et al.*, 2010).

Thousands of trees have been selected by phenotypic selection in more-or-less mature forests in Sweden, Poland, Germany, France and other countries, so this approach is frequently used. However, selection criteria were updated during the last decades of the previous century and at the beginning of the current century. Moreover, numerous progeny tests involving both individual clones and seed orchards as units have been carried out in most European countries, where selection criteria have also been subject to revision (Frýdl & Šindelář 2005; Šindelář *et al.*, 2007).

In most European countries, parents of families are only used as basic materials in the category 'Tested'. The Council Directive, as well as national legislation in many European countries, allows the use of plus trees in their original locations as parents of (open-pollinated) families. However, this option is rarely used in practice. Progenies are mostly produced by artificial pollination from plus-tree clones located in seed orchards or specific clonal collections or clonal archives. In this case, the Council Directive requires that the objective, crossing design and pollination system, components, isolation and location must be approved and registered with the official body. This formulation is implemented in most national legislations.

Mass controlled pollination is a common practice in North America or Australia (Bridgwater *et al.*, 1998; Harbard *et al.*, 1999). However, it is not widely used in Europe to produce FRM, but mostly for testing or establishing breeding populations within breeding programmes. In the latter case, the numbers of families produced is usually large (tens or hundreds), which ensures sufficient genotypic diversity of seed crops (Rosvall & Mullin, 2013).

Collection

Not many seed collection restrictions are applied to plus trees used as parents of families produced by open pollination in the original stands. One restriction is the number of plus trees that can be harvested; for example, in Slovakia a minimum of 10 plus trees must be harvested and the seed lots are subsequently mixed. This does not automatically imply a balance between families, but climbers usually choose trees with abundant crops; consequently, per-tree seed-lot sizes are mostly quite similar. If the distribution of trees is to follow the requirements of the Council Directive, they must originate from the same seed zone (region of provenance) and altitudinal zone this does not necessarily imply origin from the same population. The risks for the performance of the offspring itself are unlikely to be bigger than in the case of other basic materials. Genetic diversity in such mixed crops may even be higher than in a seed-stand crop, as the paternal parent set is much broader. Hypothetically, a risk of outbreeding depression could exist in the over-next generation, as differential

locally-adapted populations could occur even within a single seed zone. However, the collection of seeds directly from parent plus trees is so rare that there is no experimental evidence either supporting or contradicting this expectation. For example, in Slovakia a minimum of 10 plus trees must be harvested and the seed lots are subsequently mixed. This does not automatically imply a balance between families, but climbers usually choose trees with abundant crops; consequently, per-tree seed lot sizes are mostly quite similar. If the distribution of trees is to follow the requirements of the Council Directive, they must originate from the same seed zone (region of provenance) and altitudinal zone - this does not necessarily imply origin from the same population. The risks for the performance of the offspring itself are unlikely to be bigger than in the case of other basic materials. Genetic diversity in such mixed crops may even be higher than in a seed-stand crop, as the paternal parent set is much broader. Hypothetically, a risk of outbreeding depression could exist in the over-next generation, as differential locally adapted populations could occur even within a single seed zone. However, the collection of seeds directly from parent plus trees is so rare that there is no experimental evidence either supporting or contradicting this expectation.

In the case of families produced by controlled crossings (mostly certified in the category 'Tested') the Council Directive again requires that the identity, number and proportion of the parents in a mixture be approved and registered with the official body. National legislations of guidelines usually take over this wording. However, there is generally no exact quantification for minimum population size. Sometimes, this is regulated in national legislations; for example, in Austria, the minimum number of maternal trees is 20. As the crossing design and pollination system also underlie official approval, the risk of inbreeding is almost absent in this case.

2.1.5 Clones and clonal mixtures



Bordács, S., Pilipović, A., Kennedy, S., Skulason, B.

Vegetative propagation is a traditional method used for clonal reproduction of selected plant genotypes. Clonal propagation techniques, such as grafting, budding, cutting and layering have been used for thousands of years, traditionally in horticulture to reproduce grape or fruit plants with outstanding qualities. Plants for forestry purposes were traditionally produced by seed and use of clonal material was common only in the case of fast-growing trees, such as poplars or willows. Recently, forest nurseries have been adapting all the knowledge used in horticulture, including reproduction techniques, procedures for treatments and maintenance.

The advantages of clonal forestry often include increased genetic gain based on improved utilisation of additive genetic variance and more uniform products. The main drawback of clonal forestry is lack of genetic diversity. Due to low genetic diversity, the use of clonal forestry is associated with high ecological and economic risks. The risk level usually depends on how many clones are used, the genetic diversity among the clones, how the clones are mixed in the plantations, and landscape.

Due to growing demand for wood material, intensive plantation forestry has increased over the past 5080 years. Consequently, greater emphasis has been placed on the use of superior clones for forestation. Genetic variation is deliberately excluded to maximise the desirable characteristics of a particular individual or individuals. Limits on the minimum number of clones to use in plantings are set at the country level. There are many risks associated with using clonal material planted over large acreages; for example, abiotic or biotic hazards can easily destroy large monoclonal stands composed of one single genotype. Mutation of stock plants or mislabelling of plants may also often result in inappropriate use of FRM.

Clonal material from a lot of different tree species also has a relevant role in agroforestry and greening of farmland areas. *Ex situ* gene conservation used to preserve the genetic resources of many tree species is also based on clonal reproduction techniques.

Specialities in vegetative propagation

In contrast to generative reproduction, the whole clonal propagation chain needs different approaches and priorities. According to international rules (OECD, EU legislation), the clonal material must be clonal (genotype) identical, homogeneous and stable in all phases of production and use. The requirements regarding these characteristics are summarised in the DUS (Distinctness, Uniformity and Stability) criteria published by UPOV¹⁵ (1981), an international organisation which provides common standards for plant varieties, including poplars and willows. To test clonal materials there are many molecular markers available; for example, common SSR (single sequence repeat) molecular markers are adapted to and optimised for testing clonal identity or clonal purity.

Vegetative plant propagation is specified in much detail by certification systems such as the OECD Forest Plant Scheme and EU legislation, in which the technical requirements for specific issues (e.g. clonal identity, homogeneity, selection and testing for production

¹⁵ International Union for the Protection of New Varieties of Plants

categories, etc.) are summarised. For instance, clonal reproductive material shall only be marketed in the category 'Qualified' or 'Tested', which are classified by minimum requirements related to applied genetics (e.g., phenotypic selection, testing approaches, genetic evaluation, types of basic materials, etc).

The choice of clonal FRM differs depending on the purpose of the clones. In the case of plantation forestry for producing either biomass or timber, single clones are preferable for unifying silvicultural techniques and specific cultivar technologies. For restoring natural forests, such as riparian forests, the use of a clonal mixture of poplars and willows is preferable. Clonal mixtures should contain greater number of genotypes in order to secure a significant genepool for ecosystem sustainability. Furthermore, in the case of dioecious species, maintaining the appropriate sex ratio in clonal mixtures should also be considered.

Propagation methods and techniques

Clonal reproductive material can be produced by using cuttings, grafting or *in vitro* methods. The production of cuttings is the simplest and most common way of multiplying clonal plants of fast-growing broadleaved species, such as poplars and willows (Figure 11). Cuttings are traditionally produced in stool beds established and maintained in nurseries. This production method has many advantages, such as cost-effective mass-production and traditional cultivation techniques with well-defined standards and simple procedures.

Grafted material can be used for clonal multiplication of species that have no ability to reproduce auto-vegetatively. Although the production of such material is more complex and expensive, it has the advantage of increased phenotypic variability of grafted material, due to the interactions of the scions and rootstocks. In addition, dependence upon environmental conditions can be overcome by selection or suitable rootstocks. Traditional methods such as grafting, budding and layering have never been used in the mass production of FRM, due to low effectiveness and high production costs. The use of grafted plants on various forestation sites is also limited, since they are often threatened by biotic stress factors or infections. Nevertheless, grafting is often the only available method for establishing field (*in vivo*) genebanks or breeding collections of selected plus trees.

In vitro culture is used for the propagation of species (e.g. conifers) which prove difficult to multiply in other vegetative ways, or for preserving the existing genepool of endangered tree species, and increasing their variability by thus capturing any somaclonal variability. Furthermore, the *in vitro* plants can be used for establishing basic materials to produce FRM in the next phase of the production chain. Tissue culture can also be used for producing virus-free clonal material that can be used in common vegetative propagation.

FIGURE 11. Willow (*Salix* spp.) cuttings for clonal mixtures in England. (Photo credit: Rothamsted Research/Flickr).



Establishment of clonal basic materials

As previously discussed, most technical criteria of clonal materials are specified in certification schemes. Type of basic material may differ depending on various aspects, such as species, purpose of use and cost effectiveness. The most common types of basic materials used in forestry are stool beds, stock plants and *in vitro* culture-propagated plants via organogenesis or somatic embryogenesis.

The use of common clonal propagation techniques (as described above) on many tree species is often uneconomical. Clonal reproduction could be an adaptive gain for pioneer species when colonising new habitats. A combination of vegetative and generative reproduction systems can successfully be applied to some broadleaved tree genera (e.g., Poplar (*Populus* spp.) or Willow (*Salix* spp.)) so that they are optimally adapted to colonising specific riverside habitats. A plant's natural ability for clonal reproduction is traditionally exploited by humans and many tree species can be propagated economically by using cuttings. This reproduction ability is also used for establishing clonal collections. The establishment of *ex situ* genotype collections

has been advised in many EUFORGEN publications by the *Populus nigra* Network (and REFOCUS project) to preserve fragmented or endangered populations of black poplar (*Populus nigra*) (Lefèvre *et al.*, 2001, Vanden Broeck 2003). These publications also include the genetic criteria for conservation and restoration, such as selection of genotypes and minimum number of clones per population. Many studies emphasise that genetic variation of *ex situ* clonal collections is crucial for preserving the genepool of black poplar populations in a representative manner (Storme *et al.*, 2003., Smulders *et al.*, 2008b.).

A recent case study (Bach *et al.*, 2017) has reported a relevant selective reduction of genotypes in black poplar restoration programmes in Hungary. In these programmes, many old trees were selected on various sites, but their genotypes could not be multiplied for clonal collections or for planting stocks, probably due to aging symptoms linked to physiological constraints. Restoration stands in which a clonal mixture (composed of 4067 clones) of black poplar genotypes had been used for planting were also monitored. Poplar stands with an age of 1215 years included only 1217 % of clones planted in clonal mixtures. As a result, it was proposed that the number of selected trees/genotypes be as high as possible to minimise loss of genetic variation arising from local selection effects.

The purpose of clonal collections is also crucial for establishment. In general, clonal collections for breeding purposes include clones which have been selected for phenotypic traits with economic importance, such as straight stem, fast and high growth, and resistance to frost or drought. Clonal collections and stool beds for genetic conservation purposes should include genotypes which have been selected using no-breeding selection criteria. The selection of genotypes (natural ortets) should be carried out with systematic screening to cover the distributional area, without any selection criteria related to phenotypic traits. As a result, the *ex situ* collections should include a high number of clones with at least a minimum of phenotypic information.

Clonal basic materials should be established, maintained and documented by genotypes separately. Nurseries can often make a mistake and mix clones (un) intentionally in stool beds or in an early phase of production. The clonal composition of clonal mixtures is generally fixed according to legal or breeding requirements, (e.g. 'Max 1'-'Max 5' poplar clones used as a clonal mixture of 5 clones). Therefore, clonal components should be mixed during the final phase of production, whether their ratio is fixed or random.

Special requirements for basic materials and vegetative propagation of clonal reproductive materials in Hungary

Due to the production of large numbers of genetically identical individuals, plants produced by vegetative propagation are at greater risk of microbial infections (bacteria, fungi or viruses) or mutagenic effects during seedling production or in general. In Hungary, to minimise these risks, a specific propagation system for clonal FRM has been adopted and developed from horticulture (Parry 1990, Carlile & Coules 1995). The system is well adapted to poplar (*Populus* spp), willow (*Salix* spp) and black locust (*Robinia pseudoacacia*) clones and is based on three certification grades (Super elite, Elite, Commercial) for clonal propagation required by national legislation. By regenerating material from the previous grade, the original genotype (ortet) can be maintained in the long-term, and mass production from nurseries effectively provides clone-identical (non-mutated) clonal materials.

Elite and commercial plants are only produced upon commercialisation of a specific clone. The commercial grade is managed very intensively in order to produce a high number of commercial plants economically. For these purposes, fertilisers and chemical treatments are intensively used to minimise microbial infection, which can promote mutagenic effects; therefore, the maximum rotation period of plant stocks is limited to 10 years or even less. The stool beds are then renewed by sealed 'Elite' cuttings (A2 ramet plants) only, to avoid vegetative propagation of mutated (non-clone-identical) plant stocks. (Table 2).

TABLE 2. Basic elements and minimum requirements of a clonal propagation system required by FRM legislation in Hungary (Ministerial Decree 110/2003 (X.21.) FVM)

Generation	Grade of plants	Minimum number	Maximum rotation (years)	Function of material	Renewed by sealed cuttings
A0	Ortet (parent)	1	No limit	Founder plus tree	-
A1	Super elite	510	15	Preserve genotype	(Yes)
A2	Elite	1001000	15	Commercial stool bed	Yes
A3	Commercial	No limit	10	FRM for planting	Yes

The optimal balance between intensive management (mechanical cultivation, fertilising, spraying) and economically effective cutting production is generally clone (genotype) specific. The use of the propagation grading (super elite, elite and commercial) system is a practical tool for finding a balance using clones. In the case of resistant and less mutative clones the super elite and elite plant stocks can be maintained to a minimum number and the commercial stool beds can be maintained and managed more intensively (up to 1050 hectares production area, 1015 rotation years, high cutting production per stocks). In the case of more mutative and/or less resistant clones, it is advised to reduce the number of plant stocks, production area and rotation periods.

Regarding *in vitro* culture, the same propagation system should be implemented as that for cuttings. The A1 generation ramets should be preserved in a clonal genebank and A2 generation *in vitro* plants (tissue culture plants) regularly renewed with A1 generation ramets from the clonal gene bank. The maximum rotation period of A2 ramets is not limited; it basically depends on the species' sensitivity to mutation, which is species-specific. In the case of high genetic variability, the *in vitro* plants must be regularly monitored for clone identity and, if needed, the A0 and A1 generation plants must be propagated to renew ramet plants for *in vitro* culture.

Post control of clonal varieties and clones

Clonal varieties in agriculture and horticulture, such as wine grape or fruit, are usually maintained and produced worldwide. In order to satisfy the minimum requirements associated with their clonal identity, the planting materials must be regularly compared by means of post-control tests (as comparative field trials) to evaluate both clonal identity and vigour of planting materials. During a long rotation (vegetative production) period the clonal genotypes are produced under strong mutagenic pressure and their genome can be significantly modified.

The main reason for post-control tests on planting material lots is to be able to compare the homogeneity of the basic materials for clonal identity and plant vigour in the place of origin. Clonal identity is usually evaluated according to DUS criteria by comparing each item of planting material to the clone (variety) description. The minimum requirements of testing methods are usually summarised by certification schemes (e.g., OECD Seed Scheme) and might be species specific (wheat, potato or apple tree). The plant vigour can be simply tested by the survival (rooting success) rate, which is an indicator of clonal stability and indirectly of health and resistance.

Should there be any significant discrepancy in the clonal (genotype) identity of propagated plants, both plant lots and basic materials will have to be officially investigated at their place of origin by the designated FRM authority. The results of post-control tests on poplar stool beds in Hungary (*Populus × euramericana* 'Pannonia' and *P. × euramericana* 'Agathe-F') pointed to a statistical variance in plant vigour for both poplar clones, due to heterogeneous rooting and the survival rate of cuttings (Bordács 1995). The basic materials (stool beds) with insufficient plant vigour were therefore re-inspected and re-sampled. Based on the results of a repeat post-control test, the stool beds with low plant vigour were withdrawn as a basic material. Due to the strict propagation grade system in Hungary, no stool bed was withdrawn as a result of questionable clonal identity (Bordács 1995).

Production of clonal material

In general, the whole production chain of clonal reproductive materials is regulated by certification schemes, including rules of collection and production of vegetative plant parts. According to legislative rules, the vegetative propagules (the parts of plants such as cuttings and layers, etc.) must be harvested from registered basic materials (stool beds, stock plants) under the control of designated authorities. For all the legal requirements in the production chain to be fulfilled, the vegetatively propagated materials must be appropriately homogenous (clone-identic) and the plants optimally vigorous.

According to legislative rules, the basic material of clones must be maintained to guarantee the clonal identity of all propagated plants. Therefore, clones must be separated throughout the whole production chain; for example, bunches of cuttings are marked by clones to avoid undesirable mixing of genotypes. In the case of clonal mixtures, the planting material must be heterogeneous, as required by breeding and registration documents, which also require information such as number of component clones, mix ratio of clonal components and technique of mixing (systematic vs. random). From a genetic perspective, it is advised that the clonal components be kept separately as much as is possible, and that they be mixed only in the last phases of the production chain (e.g. bunching of rooted cuttings before transport).

The random mixing of component clones can result in appropriately heterogeneous planting material and plants that are optimally adapted to local site conditions. Inappropriate mixing of component clones, however, may lead to patchiness in the afforestation site, with differently sized 'monoclonal plantations', instead of a homogenous mixture of various genotypes. Inappropriate mixing of clones can be disadvantageous, especially on sites with diverse soil conditions.

Use of clonal FRM

The use of clonal material for multifunctional forestry purposes is not common in Europe; most clonal FRM is used for plantation forestry. Its use in multifunctional forestry is limited by risks originating from the discrepancy between the disadvantages and advantages of clonal materials, such as high breeding value versus low genetic variation. According to a review on the benefits and risks of using clones in forestry (Wu, 2019), theoretically, 530 clones provide as much “safety” as would be experienced in infinitely large populations, and the optimum level of diversity might be around 18 clones, with a minimum of around 6. However, genotypic, as well as allelic diversity in such stands is drastically reduced, producing several purely practical problems; for example, the deployment of clones and clonal mixtures is much more complicated in genotype-by-environment interactions than in sexually propagated materials, and a pathogen infestation could have more serious consequences than in a genetically diverse stand. On the other hand, in terms of loss of adaptability of a clonal stand as a whole, reduction in diversity is not necessarily a problem, provided enough natural or close-to-natural stands are preserved. In plantation forestry, the risks associated with the disadvantages (for example, limited genetic variation, high ecological or environmental risks by use) are mitigated by appropriate management. To mitigate the effects of climate change, the use of clonal FRM should be carefully considered, and remains an option for the diversity of actions in regeneration.

Clonal FRM is common in Europe especially for poplars, willows and eucalyptus (introduced), followed on a minor scale by other tree species, such as Norway spruce, wild cherry, etc. In general, the objectives of the management tools and measures applied in clonal forestry are as follows:

- To maintain an assortment of tested clones, ensuring the optimal application of appropriate clones for certain sites. In many European countries (e.g., Austria, France, Germany and Hungary) the forestry sector is encouraged to enlarge the list of registered/approved clones. (Figure 12).
- To restrict the use of clones which have not been tested locally or regionally. To limit use of ‘non-tested’ clones (e.g. maximum plantation size and use for testing purposes exclusively).
- To use a varied set of regional and local clones. It is not advised to mix clones within a stand, but in a larger area the use of a varied set of clones can minimise ecological risks and increase landscape diversity.

- To regulate plantation forestry with national legislation and common rules. In many countries national regulation regulates plantation forestry, including production and use of reproductive material; for example, in Hungary, wood plantations can only be established using certified FRM and clones must be listed in the National Clone Register.

FIGURE 12. Test plot established by the national authority in Hungary to test applicant clones for approval. (Photo credit: S. Bordács).



A recent study (Lelu Walter *et al.*, 2013) surveyed EU member states to evaluate legislative rules and restrictions on the use of clones in forestry. In Germany and France, only tested material can be vegetatively propagated and it must be planted in clonal mixtures. In Finland, not more than one million copies (two million for birch) copies of forest reproductive material in the category ‘Qualified’ can be vegetatively propagated. For the ‘Qualified’ material, more than 11 clones must be used to plant clonal mixtures, and with the ‘Tested’ material a minimum of four clones is needed in mixtures. There are no restrictions on minimum number of plants propagated. In Sweden, only up to 5 % of the estate (20 ha always allowed) can be planted with one or more clones. In Denmark only the use of *Populus* clones is regulated.

2.2 Testing standards and examples

Schneck, V., Kowalczyk, J., Bordács, S., Pilipović, A., Uggla, C.

According to the Council Directive, seed stands, seed orchards, parents of families, clones and clonal mixtures can be approved as basic material for producing FRM in the category 'Tested' after a testing procedure. The Directive applies general wording when describing testing procedures and Member States implement the rules slightly differently according to their national legislation and, in practice, to their specific needs.

One common rule is that the whole procedure of evaluation and genetic calculation must be approved by an official national body.

The amount of basic material approved as "Tested" varies considerably between Member States. According to the FOREMATIS database¹⁶ tested material is approved in seven EU countries with 383 entries. Most of the material comprises clones and seed orchards.

In this chapter, examples are given for the procedures for testing and approval of the material in several countries.

Poland

Until now, most FRM in Poland comes from phenotypic selection. Historically, the selection of seed stands and plus trees, alongside other silvicultural activities, has considerably increased the quality of forest stands. Of course, plus tree and seed stand selection was only phenotypically based, and thus their high quality may have been the consequence of environment and special treatments, but not genotype. It was therefore necessary to test the phenotypically selected trees to know their breeding value. This was done by provenance and progeny testing and improving the seed orchards by removing the worst performing clones based on the test results (Kowalczyk, 2007). Clone breeding value was recognised by progeny testing plus trees and by taking measurements of the seed orchard seedlings. Some results from the oldest provenance tests are commonly known and have been published (Giertych, 1984, 1991).

From a genetic perspective, the way in which tested material represents the provenance region is important: it depends on forest stand diversity and the method

¹⁶ <https://ec.europa.eu/forematis>

of seed sampling during the tests. In the older provenance tests, seeds were generally sampled from several trees after harvesting and logging. Such test series for Scots pine and Norway spruce have proved efficient and it still has operational value for tree breeding. The results have been used for delineations of the provenance region and for establishing seed transfer rules. Testing of broadleaved tree species' provenances started in the 1980s.

Old provenance experiments were designed for the genetic evaluation of FRM components, thus helping to recognise patterns of variability and to identify standards. It is important for the old test plots to be maintained in a functional state, because they give reliable results from mature trees. It is not certain which purposes will emerge in the future, but the cost maintaining old experiments does not differ much from the costs of cultivating normal forest stands. Documentation should be kept and relevant marking in the forest maintained.

An important FRM testing programme, managed by the Forest Research Institute (IBL) was officially launched in 2004 by the Polish State Forest department. (Chałupka *et al.*, 2011). Seed stands have undergone comparative testing and the genetic evaluation of components of basic material have been used for progeny testing. The idea is to establish a second generation of seed orchards in the next stage, based on better material which has already been genetically tested. The genetic aspects of these activities are taken into consideration in the regulation of testing procedures. In seed stands, the trees for seed collections are selected randomly along a transect with a minimum distance of 30m and permanently marked. The same quantity of seeds is sampled from each tree and is mixed in order to be representative of the tested seed stands. The seed collections are performed in mast years or in the year of a good crop. The whole process is supervised and monitored, and the seeds are stored in Kostrzyca Forest Gene Bank. Molecular marker techniques are used to validate seed samples and origins. The tests must be large enough for observations to be made for at least 20 years. They are usually established in single tree plot designs (plus tree testing) or randomised blocks (seed stands testing). In Poland, the county standards which have been set up on the basis of provenance research are performing very well, and after 10 years hardly any FRM shows superiority for a character or set of characters.

Until now, practical application of the results from this programme has been limited to promoting the harvest of seed from the best in-stand trials. Furthermore, the results from the testing of the mother trees have not yet been compiled in an appropriate way for recording the best trees. Considering the effort involved in setting up and maintaining the test area, the proportions between provenance (stands) and family

tests should be considered when revising the programme. Once the results have been obtained, it will be possible to set up the next generation of seed orchards and move on to the next selection stages. The current programme should be monitored and adjusted to changing needs (Kowalczyk, 2016).

Germany

In Germany, the parliamentary act on FRM is based on the Council Directive, covering all regulations in connection with the approval, production and marketing of FRM. The minimum requirements for all categories of basic material are set down in the ordinance upon approval of the FRM. Details regarding the procedure of approval of tested FRM are given in the guidelines of the joint expert group for legislation regarding FRM (Federal Office of Agriculture and Food 2012).

For the category 'Tested' the two test methods, comparative testing and genetic evaluation of components, are allowed. The general approaches and steps are regulated and described for the testing in this ordinance and for the guidelines. Furthermore, minimum requirements are delineated for the different kinds of basic material like seed stands, seed orchards, parents of families, clones, and clonal mixtures. Until now, all tested material has been approved after comparative testing, with standards or trial means being significantly outperformed. No examples exist for the genetic evaluation of components. According to the joint expert group guidelines, material can only be tested if the seed sample is representative; therefore, the seed should be harvested in a year with very good flowering and seed set. Furthermore, seeds must be collected from a sufficient number of trees; for stands, the guidelines recommend between 10 and 20 trees. A test must be established at two or more test sites. The climatic and edaphic conditions at the test sites must be similar to the conditions at the future deployment site of the tested FRM. The trials must be designed to allow effective analyses with common statistical tools.

A national list of standards for testing has been developed by an advisory board for tested reproductive material. This list is published online and updated periodically. The advisory board is responsible for all topics in connection with tested FRM and examines all applications for its approval. A prerequisite for approval is that the tested material outperforms either the standard, the trial mean or any other comparable material. The significant statistical superiority must be verified. The duration of the tests is between 10 and 20 years depending on the species, during which at least two assessments must be carried out.

Special requirements are set down for parents of families, clones, and clonal mixtures in the ordinance on approval of FRM. Clones should be described with distinguishable characters easy to detect. The original trees (ortets) of clones must be selected according to their outstanding phenotypes. A minimum age depending on tree species is required for selection and propagation. The suitability of a clone must be proven. Clones are approved at most for ten years, with possible renewal for another ten years. For the renewal no new tests are essential. It is also possible to define a maximum number of ramets per clone. This avoids one or a few clones being produced and planted over a longer time span for large-scale production. Furthermore, efforts should be promoted to breed new clones. For clonal mixtures the clones must to be propagated separately and the percentage of single clones should be nearly equal. An approved clonal mixture should include at least 15 clones; a clonal mixture is marketable if it includes more than three-quarter of the original number of clones

According to the EU directive, for parents of families, the purpose, crossing and mating design, isolation and place of production must be approved by a designated authority. For artificial hybrids, the percentage of hybrids within a seed lot must be detected before marketing.

Despite most FRM used in German forestry coming from selected seed stands and qualified seed orchards, the amount of basic material in the category 'Tested' has increased in the past few decades. Mostly seed stands and seed orchards have been approved in the category 'Tested' for Scots pine, Norway spruce, larch, poplars, beech and oak. Parents of families are approved for hybrid larch and aspen. Clones and clonal mixtures are listed for poplars, aspen, wild cherry, birch, Norway spruce and Douglas-fir (Federal Office of Agriculture and Food).

Serbia

The use of tested material in Serbia was implemented after the law on FRM management was updated in 2004, in accordance with Council Directive. In common practice, tested material mainly refers to clonal material obtained from poplars and willows both before and since the enforcement of the law. The use of tested FRM from seed stands and seed orchards is not yet common in forestry practice, since corresponding tests have not been carried out. Seed orchards, provenance and progeny trials have only been established for Serbian spruce, common oak, Scots pine and Austrian pine, and mostly for scientific purposes.

The testing of clonal material of new poplar and willow cultivars was initiated in Yugoslavia and was conducted by the Poplar Research Institute (now the Institute of Lowland Forestry and Environment) from Novi Sad. This resulted in 14 and 6 registered poplar and willow cultivars respectively. Three more poplar cultivars have been tested and registered since the implementation of the new legislative in 2004.

The testing should be conducted on at least three established trials with different site conditions, designed as a randomised block design with a minimum of three replications. The number of plants in each replication should ensure correct assessment of the properties for which the testing is conducted. The testing period should last at least 10 years, although testing duration is decided by the testing commission named by the Ministry. In the tests, the same standards must be used for comparison, which are either commonly-used cultivars with well-known properties that are object of the testing, or in the case of hybrids, parent trees. During the test, diameter and height are measured annually, while volume increment and total volume are analysed at the end of the test. Finally, the results are processed and analysed by the commission. In the case of early indication of the superiority of the tested material, it can be registered on the condition that its superiority is confirmed within 510 years.

Hungary

In Hungary, the whole agricultural seed and propagating material sector is regulated by Act of Parliament No. LII./2003. The Act covers all activities regarding gene conservation, registration of plant varieties, and multiplication and marketing of seed and propagating material, including FRM. In total, eight ministerial decrees are enacted to regulate seed, fruit, grapevine, vegetable plants, and even forest reproductive material sectors. The minimum requirements regarding materials and methods to test forestry plants are summarised in Decree No. 110/2003. (X.21.) FVM (Marketing of Forest Reproductive Material) and Decree No. 40/2004. (IV.7.) FVM (State Registration of Plant Varieties). Both decrees are enacted on the basis of EU legislation concerning testing methods.

Genetic evaluation is a relatively new element in legislative requirements for testing reproductive materials. Comparative trials are traditionally used to test genotypes (clones and clonal plant varieties or clonal components of seed orchards) or basic materials (seed stands, seed orchards, parents of families). Clonal forestry is common in the Hungarian lowlands, and is generally based on poplar, willow and black locust clones and registered in the category 'Tested'.

In accordance with the approved methodology required by EU legislation, the national designated authority (NDA) prescribes the establishment of a minimum of two trials (the number of trials differs from country to country). The testing process comprises a combination of DUS (Distinctness, Uniformity and Stability) and VCU (Value of Cultivation and Use) tests (Figure 13). The VCU trial plots must be established in at least three geographical/ecologically distinct regions where the applicant clone is to be used and must include a standard (reference) clone for common evaluation of test results. The trials must be designed in random blocks (repeated at least three times), and the minimum size of a single plot must be 500 m². The evaluation time period depends on the tree species being tested; for example, for poplars it is 815 years. However, in practice, the time period is not longer than the mean rotation period.

Based on the test results, the NDA summarises the significant differences between the reference clone and the applicant clone. In the case of significantly positive VCU results, such as higher growth capacity and better wood quality, a proposal must be made by the NDA. The proposal is evaluated by an advisory board (National Council of Variety Registration), if the applicant clone was registered in the category 'Tested' on the National List of Clones (clonal plant varieties). Only clones listed in this category can be used for forestry purposes in Hungary. Clones in the category 'Qualified' can only be used for non-forestry purposes, such as short rotation plantations, re-cultivations, and gene conservation forestry.

FIGURE 14. VCU-test plantation of poplars in Hungary for testing clonal characters, (Photo credit: S. Bordács).



Basic materials (seed stands, seed orchards, parents of families) can also be tested for character selection (mostly phenotypic). In order to do this, offspring plants must be tested for quantitative and qualitative characters in comparative trials as required by FRM legislation. The minimum requirements for tests and methods are similar to those for clonal testing; for example, random block design with at least three repetitions and use of comparative standards. A comparative standard from the same type of basic material which is being tested shall be selected, i.e. when testing a stand or a seed orchard it shall be a stand or seed orchard. Basically, the offspring plants within a test block must be measured individually, but it is advisable to calculate and statistically evaluate the mean values of each block in order to compare the results of trials established at different sites.

Sweden

Testing procedures for approval in tested category shall be in accordance with the regulation on production and marketing of FRM (SKSFS 2002:2). At present, there are more than 40 seed orchards approved in this category, corresponding to approximately 20% of the total number of seed orchards in Sweden. Most of the seed orchards in the category 'Tested' comprise Scots pine, but there are also a number of tested seed orchards for the production of Norway spruce and silver birch (*Betula pendula*) seed.

The testing of clones for seed orchard deployment is integrated into the breeding programmes, which means that an analysis is made on both selection for deployment and selection for further breeding. Skogforsk (the Forestry Research Institute of Sweden) is responsible for the Swedish tree breeding programmes, including testing for the approval of basic material. Skogsstyrelsen (the Swedish Forest Agency) is responsible for the procedure for approval of basic material.

One such breeding programme involves Scots pine in 24 separately managed breeding populations and with specified profiles related to temperature (degree-days) and photoperiod (latitude) regimes. Each breeding population comprises approximately 5060 clones, indicating that there are more than 1000 founder Scots pine trees in Sweden. In addition, breeding programmes in Finland and Sweden exchange Scots pine breeding material, such as seeds or clones, and have developed a mutual web-based tool, 'Planter's Guide', for deployment guidance. In every separate breeding population, each trait (height, diameter, seedling survival, various quality traits, damage, etc.) is measured in 45 field trials (i.e., more than 100 tests in total per trait, species and generation). Trials are established for either progeny or clonal testing, and sometimes a mix of the two strategies is applied for optimal outcome. Field trials are measured at the

ages of 1020 years and results extrapolated to full rotation time. In addition, artificial freeze tests are conducted to test seedling survival. An important tool in the management and analysis of the data from all the trials is the genetic evaluation system TREEPLAN®. This tool makes breeding and selection more effective and allows more parameters and longer data series to be included in the analysis.

The breeding programmes for Scots pine and Norway spruce are currently in the first or second cycles of selection, depending on the breeding population. The third round of seed orchards established entirely on tested material for producing tested seed is now in the procedure of approval. As these new seed orchards of tested clones are moving into production phase, they will replace older seed orchards of clones in the improved category, and the proportion of tested seed produced in Sweden will increase in the coming decades.

Italy

The commercialisation of FRM is regulated by ‘Legislative Decree n. 386/2003’. Established by the Ministry of Agriculture and Forestry (Decree n. 17132/2015), the National Poplar Observatory (ONP), is the representative body for Government, regions, producers, industry, and universities responsible for approval of basic material in the category ‘Tested’ (poplar clones in particular), which is reported in the Decree n. 74738/2015¹⁷. Public or private parties wishing to register clones or poplar clone mixtures in the National Register of Basic Materials (RNBM), must submit an application to “Direzione Generale per la promozione della qualità agroalimentare e dell’ippica del Ministero delle politiche agricole, alimentari e forestali (Directorate General) no later than September 30 of each year. –With the support of the Research Centre for Forestry and Wood (CREA,) the ONP is required to evaluate the characteristics of clones. In order to be registered as “Qualified” materials, the clones, or mixtures of clones, must have distinctive characters and their value must be demonstrated by experience or sufficiently prolonged experimentation. Minimum requirements for the approval of basic material and data on growth performance and resistance/tolerance to major biotic stressors must be fulfilled. In compliance with the conditions set out in Legislative Decree 386/2003, the ONP notifies the Directorate General that “Qualified” material can be registered in a “definitive way” as RNBM Clones. Poplar clone mixtures may be registered in a “provisional way” as “Tested” materials for a maximum of 10 years, and these materials can also be marketed if the documentation meets the minimum requirements for admission. For the ONP Technical Expert Group to monitor compliance with the requirements for “Tested” materials, the selector or applicant must

¹⁷ <https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/9406>

provide a list of at least two comparative plantations; these must mature and suitable for evaluating the genotype-environment interaction. The Technical Expert Group approves the clone in the category “Tested” when both of the following conditions are fulfilled: a) significant superiority for one or more characters listed in Article 4 of the Decree 386/2003; and b) absence or significantly inferior value for one or more characters listed in Article 4 of the Decree 386/2003, which may cause harm to crops and/or species to which the clone belongs. For each “Tested” clone the conservation of its purity is noted; the prototypes of all the clones registered in the RNBM must also be maintained in at least two arboreturns managed by the Research Centre for Forestry and Wood (CREA). The clones listed in the National Register are available at: <http://www.fao.org/forestry/ipc/69637@204274/en/>

2.3 Effect of seed and seedling material not intended for use as FRM on forest genetic resources

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Seeds that are not accepted in any of the basic material groups are sometimes used for forestry purposes. These include, for example, seeds that have not been collected from approved seed orchards, seed stands or seed sources. In addition, trees used for decorative purposes, short rotation biomass production or for erosion prevention, or similar, may contribute unintentionally to the forest genetic resources of natural or cultivated forests and wooded areas. This will happen when, for example, their pollen or seed spreads to the latter areas resulting in trees reaching reproductive maturity. This chapter describes the risks involved in the use of this material.

Collection for own use

In several countries, it is legal for forest owners to collect cones and seeds for use in their forest for forest regeneration. The Council Directive applies to the marketing of FRM only, and therefore does not cover this use. In most countries, no clear statistics are available regarding the volume of this type of collection or use of seed, but the amount is thought to be rather small. However, in some countries, such collection is also carried out in state forests, and since collection and use of seeds (e.g., oak acorns) from and in neighbouring stands can reach tens of tons, state foresters need to be educated about the importance of genetic quality. Evidencing this use in each country would be necessary.

If seed material is collected from a nearby site of the regeneration area, or even from the same site, provenance problems are not likely, as the forest owner will have had

a chance to follow the health and growth of the stand prior to collection, and hence to assess the quality of phenotypic properties of the mother trees. However, if these collections are done without guidance and mostly by laypersons, the seed material may be collected from a limited number of trees or from a stand in which self-pollination is common. The guidelines described in chapter 2.1.2 on stand seed collection with regards to choosing trees for collection and ensuring sufficient genetic diversity may also be applied to the collection of seed for own use.

Unregulated seed and seedling trade

In addition to forestry, tree seeds are collected, and seedlings produced for several other purposes: Christmas tree or bough production, energy wood plantations, and decorative tree cultivation, all signify in the economics of some European areas. This type of plant material may be used as FRM deliberately or by accident. For example, the information about the origin and handling history of a seed or seedling lot may be lost or misinterpreted when crossing borders, especially if it is written in a national language and not translated. Cases of forgery have also been revealed on rare occasions when the FRM lot does not correspond to the information on the official documents, such as plant passports or master certificates.

There are several risks associated with the marketing and use of unregulated plant material. The seeds or seedlings may be poorly adapted to the climate of the site, and their genetic diversity and quality may be questionable. In decorative plants, traits that are undesirable in forestry may be favoured and these traits often only show in mature trees. In the case of poor out-planting results, or disease problems arising from the use of seedlings not intended as FRM, it is not possible for forest owners to be compensated for losses and any public benefits of future forests are lost.

It is the duty of official bodies in each country to prevent the trade of material of unknown origin. Illegal trade could also be prevented by raising public awareness about the legislation and the importance of the right provenance, and by making sure the legal market of FRM is healthy and functional.

The effect of trees used for landscaping and biomass plantations on forest genetic resources

Forests in many parts of Europe are fragmented and are surrounded by buildings, roads and other infrastructure, especially in densely populated areas. In urban and semi-urban areas, decorative plantations are in close contact with natural environments and

forests. Thus, trees planted in parks, gardens and energy wood plantations, for example, with often unknown and uncontrolled provenances (Sjöman & Watkins 2020), may contribute pollen and seeds to forests. If these trees are poorly adapted to local climate, or have undesirable properties from a forestry perspective, the effects on forests can be detrimental. Seed orchards and seed stands should not be approved and established in these surroundings.

Woody species are also widely used for erosion prevention and as sound barriers on roadsides and road banks. In many countries, the provenance and genetic diversity of these plantations are not covered by legislation, and they could therefore contribute unfavourably to forest genetic resources. Extra care should also be taken when choosing planting material for roadsides in the proximity of conservation areas and populations of particular genetic value. In some countries, such as Slovenia, the Act on FRM also covers the FRM intended for use in all kinds of plantations outside of forests, including roadside or short-term plantations.

FIGURE 14. Trees whose origin is not governed by FRM legislation are often used in roadside plantations or for decorative purposes in urban areas close to forest stands. Over time they may contribute unintentionally to forest genetic resources. (Photo: Katri Himanen).



2.4 Breeding effects on basic material including conservation strategy

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Forestry has become increasingly important for mitigating climate change through carbon sequestration, and for meeting society's need for increased biomass production as a renewable replacement for oil-based products. In conjunction with forest management, forest tree breeding is looked upon as an efficient way to improve tree species to meet society's ever-increasing demand for timber and other forest products, as well as to develop forest reproductive material for current and future environment (Pâques, 2013).

Over the last 70 years, forest tree breeding (Figure 15) in Europe has successfully generated genetically improved FRM, mainly focusing on major economically important forest tree species and on growth potential.

FIGURE 15. One-year-old seedlings of Scots pine in the forest tree nursery of Smorgonsky experimental forestry enterprise, Belarus. (Photo credit: Nosnikov V.V./Researchgate).



The intensity of breeding activities dramatically differs among the regions of Europe, as does the perception of tree breeding by the general public. A comprehensive review of the state of the art of breeding for the main forest tree species in Europe is published in the book, 'Forest tree breeding in Europe' (Pâques, 2013), and 'Best practice for tree breeding in Europe' has been summarised as a part of the EU funded NovelTree project in Mullin and Lee (2013). In this chapter, we look at the potential for breeding for key adaptive traits relevant to climate change, how breeding may affect genetic variation in basic material, and the different strategies for maintaining genetic diversity in breeding schemes.

Forest tree breeding a strategy to address climate change challenges

Given the speed at which climate change is occurring and the huge uncertainty of climatic scenarios, the choice of a proper strategy will be a fundamental issue which breeding will inevitably face.

One option is to identify potential stress drivers and predict their future level and breed for specific traits essential for the survival of the trees in their future environment to obtain improved material that is well-adapted to future conditions. The most frequently mentioned effects of climate change on forest ecosystems include i) drought stress resulting from the increasing occurrence of heat waves and lack of precipitation during the growing season; and ii) shifts in timing of vegetative processes resulting in damage caused by early and late frosts. Temperature increase may also facilitate a spread of non-native pests and pathogens, which will be enhanced by the weakening of trees and disturbances in forest ecosystems, caused by various effects of changing climate. Thus, tree breeding programmes could focus on key adaptive traits for drought tolerance, cold hardiness, vegetative bud phenology, and resistance to pests and pathogens.

Developing drought-tolerant tree varieties is considered a viable option for climate-change mitigation. Drought tolerance can be defined as the ability to survive, and sometimes grow, during periods of water shortage. Drought tolerance is a complex trait comprising many underlying physiological mechanisms, including avoidance of hydraulic failure and decreased cellular water potential, prevention of cellular damage in the case of low water content, management of carbon reserves, and changes to anatomical structures (Moran *et al.*, 2017). These mechanisms have a strong genetic background, making drought tolerance a heritable trait (Newton *et al.*, 1991), and hence not only a potential target for natural selection, but also for breeding, as reflected in several current breeding programmes (even though mostly outside Europe, Harfouche

et al., 2014). The genetics of drought tolerance in conifers and the potential to breed for drought tolerance is reviewed in Moran *et al.*, 2017.

Vegetative phenology is regarded as a highly heritable trait whose genetic background is intensively studied in both quantitative and molecular genetics (e.g., Gömöry *et al.*, 2015; Lalagüe *et al.*, 2014; Müller *et al.*, 2015). At northern latitudes, the selection of traits related to phenology is central to both climatic adaptation and growth potential. Among Norway spruce provenances for instance, there are large differences between the timing of bud flush in the spring and that of bud set in late summer. The effects of selection on these traits can be accelerated by latitudinal transfer of the selected clones, which is common practice in breeding programmes in Nordic countries. However, artificial selection for desirable timing of bud burst or frost hardening may be complicated by the inheritance of phenology having a strong epigenetic component (Skrøppa *et al.*, 2010).

Utilising naturally occurring genetic resistance towards pests and pathogens may provide a solution that will foster continuous coevolution of the affected tree species and the damaging pathogens. Traditional breeding programmes have focused on pathogens that appeared a long time ago, such as Dutch elm disease (Yanchuk, 2012), for which the breeding programme has been successful. Since 1928, breeding efforts in Europe and North America have provided more than 20 clones with good to very good tolerance to Dutch elm disease. These are available on the market and new promising unreleased varieties are awaiting adaptation trials. Mostly obtained by crossing and backcrossing native and Asian elm (*Ulmus* spp.) species, these clones are primarily intended for use in urban forestry (Martín *et al.*, 2018). A forestry-related example is Scots pine, which has been shown to have a genetically inherited resistance to blister rust (*Cronartium flaccidum* and *Peridermium pini*) which causes serious damage to forests in Northern Sweden (Samils *et al.*, 2011). Seed orchard genetic thinning has thus been performed to reduce the infection of the offspring. The development of applied programmes and use of resistant FRM is still in a relatively early phase for most species (see review by Sniezko & Koch 2017).

The long generation times, late flowering, long breeding cycles and variable juvenile-mature correlations of trees all pose considerable challenges to forest tree breeding. Genetic dissection approaches such as quantitative trait mapping and association genetics¹⁸ have been the focus of genomics research in forest tree breeding for a long

¹⁸ The identification of gene components and variants in candidate genes associated with adaptive traits

time (e.g., Neale & Kremer 2011). The implementation of marker-assisted selection in forest trees has so far been very limited, however, largely because of the complex multifactorial inheritance of most traits of interest. The convergence of high-throughput genomics and quantitative genetics has nevertheless enabled the development of new approaches. A very promising approach is genomic selection, which could accelerate breeding cycles, increasing selection intensity and improving the accuracy of breeding values (Grattapaglia *et al.*, 2018). Genomic selection relies on phenotyping and high density genotyping of such a large sample of the breeding population (i.e., the training populations that have undergone at least some breeding and that have been carefully phenotyped for the desired trait), that the majority of loci that contribute to a quantitative trait are closely linked to one or more markers. The effects of all markers are then estimated simultaneously and used to predict genomic breeding values in a test population without the need for phenotypic data (Resende *et al.*, 2012). Genomic selection is currently being developed and tested in several forest tree species (e.g. Norway spruce, Lenz *et al.*, 2019; Ash (*Fraxinus excelsior*), Stocks *et al.*, 2019).

Another promising approach is 'breeding without breeding' (BWB) which comprises the use of i) genotypic or phenotypic pre-selection of superior individuals, ii) informative DNA markers for fingerprinting and pedigree reconstruction of offspring to assemble naturally created full- and half-sib families resulting from mating among selected parents, and iii) quantitative genetics analyses to identify elite genotypes for further genetic improvement or the establishment of production populations (El-Kassaby & Lstibůrek 2009). By avoiding the costliest step in breeding programmes, namely the controlled crossing, BWB can substantially speed up progeny testing and allow tests to be performed on larger scales.

An alternative to focusing on specific adaptive traits is to focus on increasing the resilience of future forests to environmental fluctuations by maintaining as much genetic variability as possible. The target of selection in this case will not be particular traits, but rather adaptability, often expressed as phenotypic plasticity and high genetic diversity in the improved material, at the expense of genetic gain. Widespread and economically important species may benefit from intensive breeding programmes which develop specific FRM for specific uses; whereas rare species, species with limited genetic variation or phenotypic plasticity, species at the trailing edge of climate change, and fragmented populations, may all benefit from breeding and conservation programmes focusing on enhancing genetic diversity and resilience. The choice of the strategy will probably depend on the species, local traditions and preferences.

Breeding effects on genetic variation in basic material

High selection intensity may produce a large genetic gain, but it may also increase inbreeding and reduce genetic variation, especially if selection is based on traits with high heritability within only a few families. It is possible for genetic variation to get lost with any kind of phenotypic selection; not only in genes underlying the target trait, but also in closely linked genomic regions (associated selection) and, when breeding populations are small, across the whole genome (genetic drift) (Godt *et al.*, 2001). Growth and wood quality traits are often negatively correlated (Gräns *et al.*, 2009; Jansson *et al.*, 2016) and selection intensity for growth traits will always have to be balanced with quality traits. Another example is the relationship between the timing of shoot elongation and the frequency of lammas shoots in Norway spruce. Materials with an early growth start and early growth cessation are more prone to developing lammas shoots than those that have a later development. Lammas shoots increase the probability of forking, which is a negative factor in the production of high-quality timber (Skrøppa & Steffenrem 2016). In some cases, selection may produce unexpected effects, for example in lodgepole pine, selection for growth has been associated with the delay of growth cessation by 1–10 days (MacLachlan *et al.*, 2017).

The amount of genetic gain obtained in the seed orchard crops depends on the number of parents and their breeding value. Reducing the number of well-performing clones will increase genetic gain; however, this will also reduce the genetic diversity, and, in some cases, the vitality of the seed orchard crops. Typically, production populations of long-term cyclic tree breeding programmes are represented by seed orchards containing phenotypically or genetically selected trees that will produce seed and seedlings to meet specific needs. The breeding populations, however, are broader populations, from which genotypes included in the production populations (seed orchards) are selected. For several tree species, seed orchard seeds are used as predominant, sometimes even exclusive, materials for reforestation, at least in some European countries (Lindgren *et al.*, 2007). Therefore, in these specific cases, attention must be paid to diversity within seed orchard crops, which can be increased in several ways. Clone collections used to establish first-generation, and especially advanced-generation, seed orchards need to be systematically enriched by newly selected plus-trees; supplemental mass pollination may be used to introduce new genes; and crops from different years, and even different orchards, may be mixed (Kolström *et al.*, 2011). However, national legislations in several countries prohibit such measures.

The biggest loss in genetic diversity occurs in the use of clones in forestry. Clonal propagation is common in Europe in poplars, willows and introduced eucalyptus, but

is also applied to other tree species, such as Norway spruce, wild cherry, black locust, etc. Some clones, especially poplar clones, are frequently planted in monoclonal cultures covering large areas (Vanden Broeck 2003), and the risk is higher when new bred clones come from the same genepool (having the same resistance gene). In other species, multiclonal mixtures are used; however, they also rarely consist of more than a few tens of genotypes (Muhs 1993). The use of clonally propagated FRM in Europe is not generally positively perceived by the public (Häggman *et al.*, 2013).

A specific problem associated with cloning in breeding programs (especially when *in vitro* cultures are used) is somaclonal variation. High doses of phytohormones and additives used in cultivation media may cause mutations in cell lines, which subsequently spread across the growing plant. For example, dimethyl sulfoxide, used as a solvent and a protective substance in cryo-conservation of *in vitro* propagated material or somatic embryos, is mutagenic (Aronen *et al.*, 1999). However, in woody plants, somaclonal variation has mostly been addressed by molecular studies; the relevance of this problem in terms of phenotypic and fitness effects is largely unknown.

Tree improvement, also desirable from the point of view of commercial forestry, may lead to problems in the conservation of genetic resources. Black poplar (*Populus nigra*) in Europe is a good example, for which mass propagation of clonal cultivars with desirable properties started as early as in the mid-18th century (White, 1993). Moreover, hybrids of the native black poplar and the North America eastern cottonwood, *P. deltoides*, exceed the domestic species in terms of growth and wood production, and monoclonal plantations have replaced most natural stands, especially on highly productive sites. The variety of commercially used cultivars is limited; for example, only three cultivars (*I-214*, *Robusta*, *Pannonia*) are currently used in Slovakia. In addition to risks associated with limited genotypic diversity, this type of strategy leads to introgression, causing contamination of native genepools by *P. deltoides* genes, and thus the disappearance of native poplar populations, not only due to planting, but also to the fitness advantage of interspecific hybrids (Aas 2006, Vanden Broeck 2003, Smulders *et al.*, 2008a).

Breeding schemes and conservation of forest genetic resources

Forest tree species are at a very early stage of domestication: so far, little is known about all the potential variation that exists. It is thus a general desire to conserve the genetic diversity represented in the existing populations to ensure sufficient genetic variability for future progress. Maintaining genetic diversity is therefore a stated objective of most breeding programmes. This may involve the maintenance of natural

levels of diversity to the identification and preservation of rare alleles in *ex-situ* collections (Mullin and Lee, 2013).

In Nordic countries, breeding programmes aim to breed for climate change while increasing growth and conserving genetic diversity. This is clearly expressed in the objectives of the Swedish breeding programme, for example: “The objective of the Swedish long-term breeding programme is to improve commercially important tree characteristics like adaptation, productivity and wood quality, to adapt to climate change and to maintain genetic diversity without compromising future selection response” (Mullin and Lee 2013).

An effective forest tree breeding scheme aiming both at the genetic improvement of forest trees and the conservation of their genetic resources in the face of climate change is the Multiple Population Breeding System (MPBS), developed by G. Namkoong (Namkoong 1984; Eriksson *et al.*, 1993). In this system, the breeding population of a breeding programme is subdivided into smaller subpopulations that are established over a spectrum of different environments and conditions. The trait of interest for improvement may be identical across subpopulations or emphasis for specific traits of interest can be put into some of the subpopulations. The MPBS capitalises on the disruptive selection which occurs among subpopulations, thus keeping most of the additive genetic variance within all the subpopulations, while the among subpopulations additive genetic variance increases. Splitting the breeding population into many subpopulations allows each subpopulation to be included in the next breeding cycle earlier or later than the rest of the subpopulations, depending on the site conditions or the breeding goals of the individual subpopulations. The MPBS may simultaneously capture the total genetic variation (by establishing genetically variable subpopulations, and thus conserving the forest genetic resources of interest), meet breeding goals, and secure faster evolution within each subpopulation for given environments. According to Eriksson *et al.*, (2013), the MBPS has been adopted in Sweden for silver birch, lodgepole pine, Norway spruce and Scots pine breeding programmes, and has also been adopted globally in several breeding programmes on forest tree species.

Another system for long-term breeding is nucleus breeding, in which the breeding population is split into a smaller nucleus containing a few tens of trees and a larger subpopulation of a few hundred trees. Gene conservation and long-term gain are expected to be guaranteed in the larger subpopulation, while the most intensive breeding occurs in the nucleus. The difference between the two subpopulations increases over the generations. Sometimes material is transferred from the larger

subpopulation to the nucleus, mainly in order to avoid inbreeding (Eriksson *et al.*, 2013; Wu *et al.*, 2016).

Many European countries have conservation strategies for forest genetic resources, to which EUFORGEN has contributed considerably by establishing dynamic conservation units of forest trees. Dynamic conservation of genetic diversity means that not only are specific forest genetic resources conserved, but that the evolutionary processes within the conservation units are safeguarded to maintain their potential for continuous adaptation. In addition to the gene conservation units, different countries have other strategies for *in situ* conservation of forest trees, such as protected areas and national parks. *Ex situ* conservation includes seed collections, clone archives or other living archives, seed orchards and progeny tests. In Norway, the Genetic Conservation Centre, a forest owner organisation, and some forest owners are currently collaborating to dynamically conserve FRM from different seed orchards of Norway spruce. In this programme, the forest owners manage the selected forest stands as usual and most importantly, they carry out seed collection before harvesting.

2.5 Harvesting, processing and storage of seeds

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Introduction

It is important that genetic diversity does not unintentionally decrease during the processing steps from the original collected seed lot to obtaining pure commercial seed. However, in practice, seed processing may lead to a decrease in genetic diversity when the presence of some families in the seed lot is reduced. Seeds of different families may differ in morphological and physiological characteristics and some families may be subject to selective pressures, which implies the possibility of them being discarded from the final seed lot. (Ivetic *et al.*, 2016). Seed handling does not inevitably lead to loss of genetic diversity, but it is important for the seed practitioners to cater for the necessity of using methods for reducing these losses.

However, genetic diversity can be intentionally decreased or directed by harvesting a subset of clones in a seed orchard or a small number of trees in a stand. Procedural steps of refining seed lots, such as grading and fractioning, may also lead to a reduction in or the separation of genetic variation. Such intentional influence on genetic diversity should be in line with the regulations; the Council Directive requires that seed orchards

be managed, and seed be harvested in such a way that the objectives of the orchards are attained. This should also be carefully documented, and the information be kept along with the FRM for the end user.

During seed processing, as well as during seedling production as described in the following chapter - genetic diversity can only be lost, not gained.

Harvesting

In the FRM production chain, seed collection is of utmost importance to keep the entire genetic diversity of the basic material within the commercial seed lot. Cones, fruits or seeds are commonly harvested in various ways, depending on factors such as species, maternal stand, weather course during the growing season, and even local habits (Gordon, 1992). In conifer seed stands, cones are usually collected from standing trees or from fallen trees by climbing, while in seed orchards, cones are mostly hand-picked from the ground, ladders or hydraulic platforms. It is rare to climb standing broadleaves: seeds or fruits are commonly hand-picked from fallen trees or from pruned fruit-bearing branches, stripped, pulled down by rakes or hooks, or shaken (there are also mechanised tree shakers, but they are not commonly employed). For beech or oaks, seeds are collected from the ground after they have fallen, typically using close mesh nets or sheets laid out on the ground. Beech seeds can also be collected using a vacuum cleaner or common yard tools. In some regions in eastern Europe, beechnut or oak acorn supplies made by rodents are dug out.

In principle, whatever technique is used, it is unlikely to have any detrimental effects on the genetic variation of the progeny. Nevertheless, labour-intensive and costly collection practices are always associated with the risk of collection rules defined by legislation or guidelines not being obeyed, in particular when the monitoring of collection by official bodies or competent authorities is not stringent enough. In some countries, the attention of official bodies focuses on main commercial species, while rare tree species are monitored less scrupulously. Consequently, less-than-prescribed heavily fruiting trees (only one in extreme cases) are felled and harvested, if the crop covers the local needs. Only total obedience of the rules specified in chapter 2.1 can ensure sufficient genetic diversity and adaptability of the produced forest reproductive materials.

The choice of appropriate timing for harvesting is another relevant factor when considering genetic diversity. For example, for oaks, the collection date may affect the dryness of the seeds, whether the black rot fungus *Ciboria batchiana* has already infested

them, and whether any germination has started, all influencing the susceptibility for thermotherapy as well as the possibility to store these seeds over more than one winter. Moreover, harvesting seed in a narrow time frame may result in unintentional selection and may reduce genetic variation. Harvesting seed towards the beginning or end of seed maturity may also favour a certain subset of genotypes and result in genetic shifts in the collected crop (Thomas *et al.*, 2014).

Extraction, cleaning and sorting

Extraction, sorting and cleaning are usually part of an integrated process that aims to prepare the seed for marketing. International standards, and OECD and EU regulations, give standards for ensuring traceability and for the physical and biological attributes in marketable seed lots. Seeds need to be cleaned of debris and inert material, and the number of dead or damaged seeds needs to be kept to a minimum.

In addition, the smallest or largest viable seeds are sometimes removed from seed lots and may be discarded or used separately, because seeds that are homogeneous in size are often preferred in seedling production to ensure the effective functioning of sowing machines. As seed size may affect germination rate and the size of the seedlings (Dunlap & Barnett 1983; Sorensen & Campbell 1993), small and large seed are sometimes considered troublesome in nursery production.

The necessity and technique for extraction and cleaning of seeds depends on the form and type of fruiting bodies of the tree species (especially for hardwoods). Most of the fruits of broadleaved species require dry extraction of seeds with removal of unwanted parts, such as bracts and cupules, while in species with juicy fruits the removal of pulp requires soaking, maceration and flushing.

Most conifer species require exposure of cones to heat for seed extraction, wing removal and sorting, with exceptions like larches, cedars and junipers, which require additional operations, such as mechanical opening of cones, water flotation or maceration. After extraction of seeds and removal of the wings and other unnecessary parts, remaining debris can be removed by sieving larger parts or by winnowing smaller particles, either by hand or in special machines with airflow or vibration tables. Special machines for cleaning seeds (e.g., Vincent-type grain cleaner) can remove empty and badly developed seeds, as well as debris. One common method is the IDS treatment that was developed by Simak (1983), which involves invigoration, drying, and separation of dead seed from vigorous seed.

As with the case of seed collection, it is necessary for the seed producer to have a good understanding of the biology and characteristics of the seeds/species to be treated. The technical process must be appropriate for the character of the seed, so as not to reduce the viability of the seeds. For taxa with recalcitrant seeds, which do not withstand drying during seed storage, the procurement and handling must be organised differently to species with orthodox seeds tolerant to drying. For example, attention must be paid to using the appropriate temperature when heating and drying FRM, and to avoiding cleaning techniques that could damage the seed coats. Fleshy fruits are also very susceptible to fermentation. Appropriate techniques should be used, not only to maximise the yield, but also to avoid (or at least to minimise) systematic losses of genes, genotypes or families during the process.

Seed size and seed weight are subject to strong maternal effects. For example, in conifer seeds, the megagametophyte storage tissue that comprises the main part of seed weight is haploid; there is maternal tissue, and therefore a genetic relationship between the mother tree and seed weight, exists (Reich *et al.*, 1994; Roach & Wulff 1987). Removal of some seed size categories from a seed orchard harvest the heaviest or lightest seeds can thus reduce the genetic diversity of the seed lot by sorting out certain mother clones and may cause unintentional selection for other traits.

Besides genetic factors, other factors affecting the variability of seed dimensions and phenotypic characteristics include (Regent, 1980): (i) position of fruit in the crown of trees and position of seeds in the fruit; (ii) factors representing the development stage or age of the parental tree, and (iii) factors related to habitat conditions affecting shape and size of fruits and seeds.

Himanen *et al.*, (2016) examined the source of seed weight variation in Norway spruce in material collected from both a forest stand and a seed orchard. In both cases, over 80% of seed weight variation was explained by within cone variation, indicating that the mother tree effect on seed weight was less than is commonly perceived.

Mixing or keeping separate different seed lot fractions

To ensure that a seed lot is well-mixed, it is often necessary to sort and to separate seeds using different grids of varying sizes. To preserve genetic diversity, the different parts obtained at the end of the process need to be mixed. However, from a commercial point of view, it might be attractive for seed companies not only to remove the smallest and biggest seeds as described above, but also to sort seed from a single seed lot into weight or size fractions. This may optimise economic gain when marketing the seed

by offering very homogenous sub-lots of seed based on size or weight. The resulting seed lots would have differing properties and vigour, and potentially reduced genetic diversity or a different genetic profile, but they would still be identified with the same Master Certificate Number. It is questionable if such seed fractioning would be in line with the Council Directive (Annex IV, 1 (e)) and article 12.1.

In some cases, seeds are graded/calibrated for standardisation or containerised seeding, at the request of nursery staff. This could also have a genetic impact with a reduction in genetic diversity.

It is important to highlight the difficulty in mixing seeds of some species due to their big size, such as those from oaks, beech and chestnut (Figure 16). Due to the lack of homogenisation of the lot, the marketed seed lot may include a smaller number of genotypes compared to the collected number. Moreover, other nurseries activities, such as plant grading or culling, could further increase this reduction.

The very long lifespan of these species could be detrimental to the adaptation of the future stand and increase the risk of genetic drift in the next generation when using natural regeneration.

FIGURE 16. Example of difficult mixing, due to size, of acorns for a large seed lot of sessile oak (*Quercus petraea*) before commercialisation in Belgium (Forest tree seed centre of Wallonia).



In accordance with the EU Council Directive (1999/105/EC), all the seed lots must be kept separate during processing. However, some definite mixings are permitted, such as the mixing of seeds coming from two or more seed stands within a region of provenance, or those collected from the same basic material in different years.

In making use of this legal opportunity, the genetic diversity of these marketed seed lots could be easily increased, which could be of a certain value in the case of seed lots from a single basic material that varies in genetic composition or viability from year to year. Some countries, such as Belgium (Wallonia) encourage this procedure.

Chemical treatments

The chemical treatment of seeds prior to storage can be performed either to prevent the development of pests and diseases during storage, or to influence their dormancy. Seed dormancy is defined as a state in which the seed does not germinate, despite favourable environmental conditions. The association of plant disease organisms with seeds was reported over two hundred years ago and was doubtlessly recognised by farmers and other growers long before that (Crocker & Barton, 1957). Disease prevention or control treatments with chemicals vary depending on whether the seeds belong to an orthodox or recalcitrant group of seeds. Since the orthodox seeds can be stored in hermetically sealed containers with low moisture content, dry or wet application of different pesticides is possible. The application of pesticides on recalcitrant seeds should be performed with higher caution due to their higher moisture level. In most cases, recalcitrant seeds are treated with powder formulated pesticides. In addition to chemical treatments, or as a substitution, thermotherapy is used to prevent the development of diseases, or the further spread of existing ones, during storage of recalcitrant seeds.

The application of chemicals in order to affect seed dormancy can prevent germination during storage, or neutralise seed dormancy and stimulate germination. Some chemicals used for the improvement of germination (e.g., inorganic salts, fertilisers, growth stimulators and herbicides) can act dually depending on the dosage: low concentrations can stimulate, and high dosages can inhibit germination.

Storage

The genetic structure of seeds (genetic factors influencing seed quality and characteristics) is one of the most important components affecting the seed storage process (Justice & Bass, 1978). This is also valid for seed maturity; in fact, it is well known that mature seeds fare better in storage than immature ones (Bish & Ahlawat,

1999). Preserving seed germination capacity in the storage process is important for ensuring the persistence of genetic diversity. A variation in viability may eliminate seeds of low relative viability (Schmidt, 2000). This is important in the case of long-term storage, or even short-term storage for recalcitrant seeds. Treatments for the breaking of dormancy may also induce such irregular seed survival.

A loss of viability in storage will decrease the seedling number and could therefore narrow genetic diversity of the seed lot (Roberts 1972, Barner 1975).

Storage procedures differ among tree species depending on whether they are orthodox or recalcitrant. When dealing with recalcitrant species, such as oaks, chestnuts, hazels and sycamore, special attention should be paid to maintaining their moisture level above the threshold for their vitality. According to Suszka, (1996), moisture levels are 40-48 % for oaks and 24 % for sycamore. Such high-water content favours development of seed diseases and enhances respiration of seeds which can affect their vitality. Therefore, the storage of recalcitrant seeds requires the fulfilment of two constraining conditions: (i) a relatively high level of humidity must be maintained in seeds, and (ii) metabolic processes must be minimised. These opposing conditions can be fulfilled by storing the seeds at low temperatures (around 0 °C) together with sufficient ventilation. On the other hand, most of the orthodox seeds can be stored at low temperatures, and hermetically sealed at a low percentage of humidity for many years. This makes sense in the context of the present report in that conservation of seed vitality in storage differs largely among families (Schmidt, 2000). All storage factors (moisture, temperature, duration and seed maturity) affect genetic diversity of a seed lot by maintaining or reducing seed viability. In Kazdaği fir (*Abies equi-trojani*), different moisture contents (8 %, 6 %, 4 %) and temperatures (+4 °C, -6°C, -20 °C) over 2 years, resulted in very different germination percentages between families for the same seed lot after storage (Yüksel, 2017). The same result was also found for oriental beech (*Fagus orientalis*) (Yılmaz & Dirik, 2007).

Stratification

Stratification is one of the most effective tree seed dormancy breaking methods for many temperate species, which allows seeds to germinate more rapidly and in higher ratios (Schmidt, 2000). It is known that response to stratification is under strong genetic control (Ivetić, 2016). Although germination capacity may not change, germination speed clearly increases after stratification. Damaged or weak seeds could easily deteriorate during stratification (Leadem, 1986).

Stratification removes the variation in germination capacity between Turkish Pine (*Pinus brutia*) provenances and families. Işık (1980) sowed seeds of 60 Turkish Pine families from six different provenances in seedbeds without stratification, and he also germinated samples in parallel in the laboratory after 30 days of cold stratification. In the nursery, he found statistically significant provenance and family effects on germination. On the other hand, in laboratory conditions, no significant effect was found at both provenance and family levels. One interpretation of this is that stratification may help to ensure that the genetic diversity of the original seed lot is not reduced in the germination phase.

In the Kazdağı fir (*Abies equi-trojani*), an endemic species of Turkey, different reactions to pre-chilling at the provenance level are very clear. An ideal pre-chilling duration for both optimal and extreme populations was found to be 30 days, and for intermediate populations it was 15 or 45 days. This point clearly highlights the inefficiency of using standard pre-treatment duration (Yüksel 2017). Significant differences were found at tree level in an optimum Kazdağı fir population in terms of germination capacity after 30 days pre-chilling duration. Pre-chilling enhanced the germination capacity in all families, but it was not able to eliminate the family level variation (Yüksel, 2017).

Many broadleaved tree species in the temperate zone exhibit dormancy phenomena which are often greater than those encountered in conifers (Suszka *et al.*, 1996). Although seed of different forest tree species are generally divided into dormant and non-dormant seeds, some species can be placed in both or intermediate categories. For instance, birch and alder seeds remain dormant until exposed to light, or hazel seeds can develop secondary dormancy in the case of drying. Dormant seed can be stratified and/or scarified differently depending on the type of dormancy (undeveloped embryo, impermeable shell, presence of inhibiting compounds in seeds). The stratification procedure should mimic natural conditions of the seed biology which varies between the species. For species with fruits which ripen and fall early in the summer (e.g. wild cherry), or for species that require warm periods to end embryo development (e.g. lime, hornbeam and ash), the stratification process should include both warm and cold stratification. For species that require only cold stratification (e.g. maple and beech) stratification is performed in a moist medium, or with a controlled moisture content, at temperatures of between 0 and 6 °C for a period of 2 weeks until they begin to germinate, depending on the species and the seed lot.

Seed testing

In germination tests, it is important to obtain homogenous and reliable results (Aldhous 1972), which reflect the genetic diversity of the seed lot in question

Germination tests should be conducted using pure samples obtained by carrying out purity tests. Samples should be prepared with randomly selected seeds from completely mixed test pieces, otherwise the germination percentage and energy of the seed lots may be wrongly estimated due to variation in germination capacity between tree families and individuals (Funda *et al.*, 2012).

Seed lot selection based on germination test results may only narrow the genetic diversity of FRM. On the other hand, seed vigour tests offer new opportunities. The variation in germination value according to heat or water stress continuum reveals the germination performance stability of seed lots in the bare root production of black pine (*Pinus nigra* subsp. *pallasiana*) (Çalikoğlu & Tilki, 2002). The seedbeds used for the production of this species in Turkey have very heterogeneous moisture levels: a strong correlation was found between the germinability of a seed lot in a 6-bar water stress test and the performance of a black pine seedling in bare root production (Çalikoğlu, 2002). Thus, the seedling production of drought-tolerant black pine for afforestation in semi-arid regions could benefit from seed lot stress tests and the seed lots for seedling production can be chosen accordingly.

2.6 Nursery practices

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The production chain of seedlings, originating either from seeds or vegetatively, may have an impact on the genetic diversity of the FRM finally reaching regeneration or afforestation sites. These effects are easily overlooked and uncontrolled. Nursery practices are diverse and seedling production often takes place on a scale and in conditions that make controlling difficult. The effects of nursery practices on seedling survival and genetic diversity of cultivated forests may be noticed after out-planting, as production practices may, for example, affect the growth rhythm of the seedlings. In addition, the desire for both uniform germination and seedling morphology in practical seedling mass production encourages limited genetic diversity. In the following chapters, we describe some steps that may need to be addressed to make sure genetic diversity is not narrowed during the production process of seedlings.

Selection of seed lots based on their properties

Seed lot price and germinability are the key attributes affecting choice of seed lots for seedling production irrespective of the production methodology (bare root versus containerised seedling production) and tree species. In bare root production, for which seedlings are produced in outdoor fields, lower germinability and purity of seed lots can be accepted compared to container seedling production, for which seedlings are grown in greenhouses. Germination capacity the final germination percentage affects sowing density and seed consumption, and germination energy and rate – indicating germination speed affect the evenness of seedling crops, and thus seedling quality and cull percentage (Boyer *et al.*, 1987; Himanen & Nygren 2015). In addition, legislation and regulations on provenance selection in forest regeneration drive the choices of seed lots in nurseries: seedlings with suitable origins for a given area must be produced.

Germinability variation is caused by genetic factors, as well as by the production chain of a seed lot. Germination capacity and energy vary both between and within tree populations (Thompson & El-Kassaby 1993; Baskin & Baskin 2014). Therefore, the choice of seed lot for nursery production has genetic implications. High germination capacity originating from genetic factors will boost the chance of superior families participating more effectively in seedling crop, while problems in germinability originating from the processing conditions could lead to elimination of those superior families (Ivetic *et al.*, 2016).

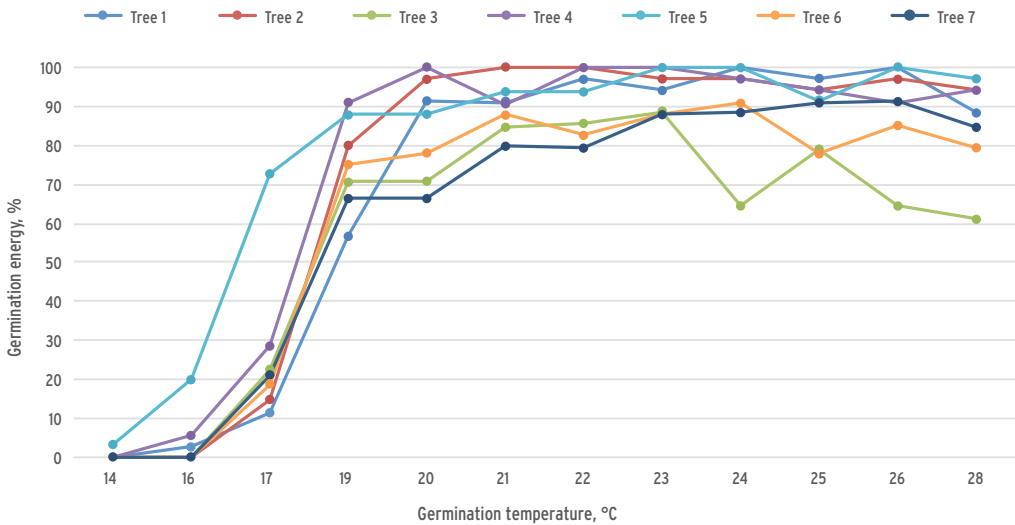
Edwards and El-Kassaby (1996) point out that using multiple seed sowing and subsequent thinning – a normal practice with low-germinability seed lots – may work as unintentional directional selection in containerised seedling lots. When multiple seeds germinate in a single container cell, the smallest are thinned and seed from parents producing, for example, fast germinating less-dormant seeds are left to grow. The quick early development does not, however, directly indicate genetic superiority of the remaining individuals and in any case, genetic diversity is reduced compared to the original seed lot.

Germination phase

In nursery conditions - especially in greenhouse production - germination temperature and growth media attributes (e.g., pH-value and moisture content) can be controlled to ensure an equal starting point for the growth of all seeds and cuttings. In practice, this regulation of conditions is often difficult, resulting in patchiness in the seedling crop. From a genetic point of view, the differences in response between provenances, families and clones to the same growth conditions are of more interest. For example,

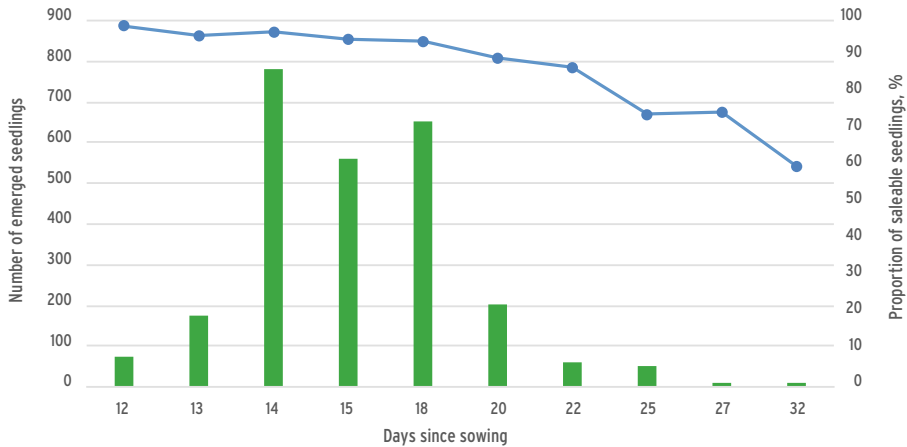
while a fixed temperature can be optimal for the germination of seed from certain families, it can result in slow germination in others, or even cause secondary dormancy which will prohibit germination until the dormancy is broken, despite the favourable conditions. As can be seen in Figure 17, the temperature response of germination differs in seeds collected from different individual Scots pine trees: seeds of some individuals germinate readily at 17 °C, while others need higher temperatures to germinate. These genotypic differences in germination response can also result in differences in seedling emergence and development in a seedling crop.

FIGURE 17. Germination percentage (day 7) in different temperatures of Scots pine (*Pinus sylvestris*) seeds collected from 7 individual trees in Central Finland. (From: Markku Nygren, Natural Resources Institute Finland).



In a one-year-old seedling crop of Norway spruce it has been observed that slowly emerging seedlings are more likely to die during seedling production or become cull seedlings than to become rapidly emerging ones (Figure 18) (Himanen & Nygren, 2014). Slowly-emerging individuals suffer from competition from the surrounding seedlings, or growth measures in the nursery can be ill-suited to their stage of development, causing stunting a greater likelihood of succumbing to pests. Therefore, the differences in germination response to different environmental cues of seeds from certain families, or differences in their dormancy, may cause unintentional under-representation of some families in seedling crops. However, slow germination may also be an indication of a genetic defect, in which case the high probability of being culled is linked to these issues rather than to growth measures.

FIGURE 18. Seedling quality in relation to emergence time in containerized seedling production. The proportion of 1-year-old Norway spruce seedlings qualified for sale (blue line) in relation to the day of emergence (green bars). (From: Himanen and Nygren, 2014).



Pest management and fertilisation practices

Different clones and provenances may differ in their susceptibility to plant diseases and other pests during seedling production (Mutikainen *et al.*, 2000). Poteri and Rousi (1996) found that silver birch (*Betula pendula*) clones varied in their resistance to birch rust (*Melampsoridium betulinum*) in nursery conditions. This indicates that diseases may change the composition of seedling crops as some families and clones, etc. are more likely to be culled due to poor quality. In the same study, a clone × fertilisation interaction in the susceptibility was also observed. This indicates that certain management practices may either enhance or dampen the clonal effects in susceptibility to pests.

Seed lots with different breeding values may also respond differently to the same management practices. In a study on slash pine (*Pinus elliottii*), seedlings of fast- and slow-growing families were grown in a greenhouse for one growing season under 10 nitrogen regimes (Dewald *et al.*, 1992). In optimal and high nitrogen concentrations the fast-growing families had higher needle nitrogen concentrations and higher shoot/root ratios compared to the slow-growing families, while no differences were

observed in low nitrogen regimes. This indicates a fertilisation level x breeding value interaction. Li *et al.*, (1991) also report that nitrogen use efficiency is under genetic control in loblolly pine (*Pinus taeda*) seedlings, with family differences in nitrogen use.

In northern Europe, growth cessation is a key seedling attribute, as frost damage is possible both in the nursery and in autumn plantings. The timing of the growth cessation is controlled through fertilisation among other management practices in the nursery. Ill-suited fertilisation regimes as regards the level of genetic gain in the FRM can therefore increase poor out-planting results, as well as economical losses for nurseries and forest owners. Similarly, the different responses of families to fertilisation may alter the genetic composition of seedling lots, as these differences may change seedling size and thus their chances of becoming cull seedlings.

Transplantation and undercutting

Transplantation and undercutting are common nursery techniques, especially in Central and Eastern Europe, which are used to optimise shoot/root ratio of seedlings by stressing and mechanically modifying the root system. Due to the treatments, the seedlings develop a shorter taproot and more intensively developed lateral and hair root system, and the seedlings are expected to have higher survival rates after out-planting.

Transplantation is a traditional nursery technique to grow optimally developed 2-4 years old bare root or container seedlings. Undercutting is a cost-effective technique used to intensify root development via mechanical trimming in bare root production. Consequently, 2-year-old seedlings are similar in conditions and outlook to transplanted seedlings at the age of 2-4 years and have an optimal shoot/root ratio (Rook 1971). Undercutting has become a more popular nursery technique in recent years. Both techniques are used for broadleaves, mainly species of oaks (*Quercus* sp.), beech (*Fagus sylvatica*), elm (*Ulmus* sp.), ash (*Fraxinus* sp.), maple (*Acer* sp.) and lime (*Tilia* sp.), as well as for pine species (*Pinus* sp.), spruce (*Picea abies*) and other marginal species, such as *Malus*, *Pyrus*, *Sorbus*, and *Prunus*.

The success and effects for the cull percentage of both techniques depend on many factors (e.g. soil conditions, watering, fertilisation, air temperature and humidity) and may vary depending on the species. Under optimal conditions a 5-20 % loss of seedlings is typical. The selection on the seedlings' genepool can be asymmetric, especially if the conditions during the treatments are not optimal.

FIGURE 19. 2-year-old undercut seedling. Taproot is drastically cut and lateral roots are intensively developed to take over functions of tap-root. (Photo Credit: Beáta Pintér).



Both nursery techniques are often optimised for profitability which might also have genetically negative selection effects on the seedling lots. Many nurseries try to sell 1-year-old seedlings, thereby selecting out the best growing seedlings and leaving the ‘waste products’ to grow one to two seasons more in the nursery. At the end of the growing season the best dimensional seedlings are lifted out from the seedbed, and the substandard seedlings are left in the seedbed or transplanted to grow further and be marketed later.

The local conditions of forestation sites might be a strong selection factor in the seedlings’ genepool; therefore, survival rate is a crucial factor in artificial regenerations. Schultz and Thompson (1997) reported better survival and growth when red oak and black walnut undercut seedlings were planted. Seedlings with more optimal shoot/root ratio, represented by a smaller shoot size and intensified root system, had a significantly better survival rate. For both species they found a significant correlation between survival and number of secondary side roots.

These nursery methods can also be disadvantageous. In the case of many tree species, such as oaks, walnuts and lime, which are adapted to growing on deep soils, the long and intensively growing taproot is beneficial in the juvenile (seedling) phase. In general, seedlings are adapted to finding groundwater resources deep in the soil as fast as possible. However, while the modified root system of undercut seedlings can positively influence the survival rate in the first years (Figure 19), there is no information regarding how the root system grows and uses the groundwater resources in later phases.

In suboptimal conditions, such as inappropriate timing or depth adjustment, undercutting can negatively influence seedling adaptability. In their recent study on chestnut-leaved oak (*Quercus castaneifolia*) seedlings, Mostafaloo & Aliarab, (2017)

showed that late root undercutting can negatively affect seedling survival and performance on planting sites.

The possible genetic drift or uncontrolled selection caused by transplantation or undercutting techniques, both in the nursery and after out-planting, needs to be studied further.

Mycorrhization practices

Mycorrhiza (see Smith and Read 2008) comprises the symbiotic organ formed by the plant root and a mycorrhizal fungus. Each plant species forms the symbiosis with specific fungal species and the host trees may alter the symbiotic partners as they age. Mycorrhizal mycelia enable uptake and re-translocation of water and nutrients to and among plants, and receive and re-translocate plant photo-assimilates within their common mycelial networks (Kraigher *et al.*, 2013). In comparison to non-inoculated plants, it has been shown that beech (*Fagus sylvatica*) seedlings inoculated by *Paxillus involutus* in the nursery are more resistant to water stress, frost and damage by deer, and are colonised more quickly by native fungi and thus adapt more quickly to the planting area (Kottke & Hoenig *et al.*, 1998; Herrmann *et al.*, 1992). In a study on Norway spruce, seedlings with well-established mycorrhizae were less susceptible to *Heterobasidion* root rot, but more susceptible to *Gremmeniella abietina* causing needle damage (Velmala *et al.*, 2018). It is known that excess fertilisation and use of pesticides in nursery practices greatly reduces mycorrhization potential (Molina & Trappe, 1984).

While mycorrhizal fungi often spontaneously spread to seedling beds during seedling production from the surrounding environment, symbiosis does not always develop, and the species may not be optimal for the seedlings and for supporting out-planting success (Figure 20).

Mycorrhization – the introduction of desired fungal species to seedlings to instigate symbiosis – has been widely applied in nursery practices in the last three decades (Molina & Trappe 1984; Kottke & Hoenig, 1992; Aldhous & Mason, 1994), and is still largely applied, especially in arid climates (Ndoye *et al.*, 2012; Oliveira *et al.*, 2010) or after large-scale clearcutting (Pickles *et al.*, 2015).

FIGURE 20. *Laccaria* sp. mycorrhizal fungi in a Norway spruce seedling. The fungus commonly appears in containerised seedlings in Finland but is not considered an optimal mycorrhizal species. (Photo: Erkki Oksanen, Natural Resources Institute Finland).



To produce mycorrhizal seedlings, it is possible to apply soil inoculum, “nurse” seedlings, spores and sporocarps, and pure fungal cultures (Molina & Trappe, 1984; Lazarević *et al.*, 2012). In addition, seeds can be coated in mycorrhizal spores. Bare root and containerised seedlings differ in mycorrhization best practices, but regardless of the practice, functional compatibility among the symbionts is a prerequisite (Gianinazzi-Pearson 1984; Hazard *et al.*, 2017). Different species and strains of fungi have been shown to be useful in different ways when in symbiosis with different species, populations or clones of forest trees, thus influencing the performance in growth, size and phenology of seedlings in the nursery. Velmala *et al.*, (2013) found that Norway spruce seedlings showed clonal differences in the formation of mycorrhizas and in the mycorrhizal community, the tree genotype explaining a quarter of the variation

in the mycorrhizal species composition. Thus, the level of mycorrhizal colonisation in the seedlings - affected by the host genotype composition - may alter the morphology and other attributes of seedling lots, and subsequently the grading of the seedlings in culling, as well as establishment success after out-planting (Klaviða *et al.*, 2017).

Culling

Morphological features, such as root collar or stem diameter, stem length and root mass, are related to the survival and growth rate of the seedling after planting (Puttonen, 1996; Ward *et al.*, 2000; Aphalo & Rikala, 2003). Weak seedlings are thus culled from marketable seedling lots and these characteristics are often used as criteria for grading. The Council Directive and the related national legislation also require that only healthy seedlings of an appropriate size are marketed. In addition to ensuring good out-planting results, seedlings may be culled for practical reasons. Small seedlings are difficult to plant, especially mechanically, and tall seedlings pose a problem in packaging and in transportation.

The genetic component in the variation of the characteristics used for culling varies. Some biotic and (especially) abiotic damage (such as mechanical damage to seedlings), has little or no genetic background. Seedling morphology, however, has a clear genetic component, which is demonstrated in many tree species and studies (e.g., Kormanik *et al.*, 1998, Lamhamedi *et al.*, 2000, Himanen & Nygren 2014). The effect may be more pronounced in the initial years of the tree's development (Ununger *et al.*, 1988). Therefore, grading or culling practices based on size (seedling height, stem diameter, etc.) are likely to influence the genetic composition of the seedling lot: culling small - but otherwise healthy - seedlings can cause unintentional reduction in genetic diversity. However, compared to sturdier plants, seedlings with small stem diameters for example, are more likely to suffer damage from, pine weevil (*Hylobius abietis*) attack for example, after out-planting (Thorsén *et al.*, 2001). As a result, they could be excluded from the final forest stand, despite being part of the planting stock.

Seedling storage

When storing seedlings, it is imperative to have knowledge of and to provide optimal conditions for different tree species, in order to ensure minimum mortality and to prevent uncontrolled losses of genetic diversity. In Turkey, Çevik (1996) found that the seedling survival ratio of Austrian pine (*Pinus nigra* subs. *pallasiana*) - a species widely used in the highlands of Turkey's semi-arid regions - was 98 % after 4 months of cold storage at +3 °C.

Taking certain measures prior to storage – in addition to the actual storage conditions – may also ensure viability during storage and increase out-planting success. Boydak and Dirik (1990) found that drought stressing Lebanon cedar (*Cedrus libani*) seedlings before lifting and storage resulted in the development of significantly more and longer roots once planted in semi-arid regions. Uğurlu (1989) found that dipping the roots of Lebanon cedar in the commercial hydrophilic gel Agricol® before planting increased survival by about 30 % after one growing season.

The timing of seedling storage is also essential to storage success and more importantly it may alter the genetic composition of a seedling lot. Growth cessation and frost hardiness development is under genetic control (Savolainen *et al.*, 2004; MacLachlan 2017), and thus ill-suited timing in starting storage, especially freezer storage, may affect certain genotypes negatively compared to others. In bare root nurseries, poorly timed lifting, which precedes storage, may also affect seedling survival and impact genetic structure in seedling lots (Campbell & Sorensen, 1984).

The role of production environment

Bare root production and containerised seedling production share many production methods but differ in the growing techniques applied. Typically, requirements regarding seed quality and germinability are higher in containerised production than in bare root production. Bare root production may offer a growing environment which, in terms of soil quality and microbial interactions, mimics forest conditions more closely; thus, possibly contributing to good out-planting success. On the other hand, greenhouse production offers better possibilities for monitoring and altering growing conditions. In more centralised nursery production this can have the advantage of being able to manage the growth and characteristics of seedlings originating from propagation material from wide- ranging geographies. Irrespective of production method – bare root or containerised – genetic diversity is maintained or lost in the various production phases and affected by the level of skill with which the growth measures are applied.

In many European countries, small acclimatisation nurseries are being abandoned and nursery production is gradually becoming concentrated within large nurseries, in which plants are grown under controlled and optimised conditions. Although this makes sense in terms of cost minimisation, concentration of labour and other aspects, such practice is risky from the point of view of potential epigenetic effects. The vegetative phenology of conifers has been shown to be substantially affected by their germination and early-growth environment (Gömöry *et al.*, 2015). There is increasing

scientific evidence of epigenetic effects occurring in plants (e.g., Nicotra *et al.*, 2015), which may also occur in trees and in adaptively relevant traits.

The production of vegetatively propagated plants

The mass propagation of selected material by vegetative propagation is used by nurseries to bulk up material of genetic value through cuttings, somatic embryogenesis (SE), organogenesis and micropropagation. Whilst these practices deliberately reduce genetic variation by propagating a specific clone or family, genetic factors in production can further influence the composition of subsequent planting lots or the viability of propagating a promising clone at all. Furthermore, carry-over effects of the hormonal composition of the culturing media may influence the adult plant development (Jones *et al.*, 1995).

SE provides a means of propagating recalcitrant material from mature trees by establishing embryonic cultures from seed, thus enabling infinite copies to be produced. The seedlings produced from such embryonic cultures are known as emblings. The process involves multiple stages in which substantial losses within cell lines (clones) can occur at each stage (Table 3). Typically, 75 % to 90 % of cell lines can be lost before acclimatisation in soil media (Hogberg *et al.*, 1998, 2015; Fenning 2017). Entire families can fail to produce successful plant material. Fenning (2017) reports failure in a third of families, while Hogberg *et al.*, (1998) managed to propagate cell lines for most families tested, but the number of clones per family and plants per clone varied considerably. During acclimatisation from laboratory to nursery conditions, further losses of cell lines can be expected (Figure 21). At the genetic level, this can be due to disease or quality of initial planting stock; for example, insufficient root formation of the seedlings.

Embryogenic calluses -tissue masses which are maintained for a long time - can be prone to genetic instability. In maritime pine (*Pinus pinaster*), phenotypic abnormalities have been detected in 33 out of 52 emblings from four cell lines, and phenotypic abnormalities did not appear to be linked to the 5 emblings identified as having genetic mutations (Marum *et al.*, 2009). However, only a small part of the genome was investigated, so a link to mutation cannot be discounted. It is also possible that epigenetic factors are brought about by stress during the SE process. Whatever the cause of phenotypic abnormalities - such as loss of apical dominance and plagiotropism - the affected trees are culled out during subsequent grading.

TABLE 3. Approximate number of viable cell lines remaining, starting with 1000, after each step of the SE process in Norway and Sitka spruce (From: Hogberg, 1998 and Fenning, 2017 respectively).

Step in SE process	No. of cell lines Norway spruce		No. of cell lines Sitka spruce	
Initiation	1000	(100 %)	1000	(100 %)
Proliferation	500	(50 %)	630	(63 %)
Germination	250	(25 %)	360	(36 %)
Cryopreservation	250	(25 %)	100	(10 %)

FIGURE 21. Three different clones produced using SE displaying clonal variation after transplanting. (Photo: Stuart Kennedy, Coillte, Ireland).



Factors affecting clone viability, alongside potential genetic advantage, can help determine if a particular cell line will become commercially viable. The lower the production efficiency, the more expensive the plant. Particularly productive cell lines can end up accounting for over 50 % of the individuals making up a particular cross, due to differences in clonal receptiveness to SE. One of the biggest challenges of clonal propagation is getting all the required elements to stack up in your favour. Using emblings as stock plants from which to harvest cuttings is one way to reduce plant costs and increase the volume of available material. Cuttings from juvenile material provide a high success rate: >90 % in spruce species (*Picea sp.*) (Bentzer, 1981; Mason *et al.*, 2002). Rooting success does not appear to be greatly influenced by genotype. Differences have been found in rooting percentage and rooting score between eight clones of *Sitka spruce* (*Picea sitchensis*), but they were not evident in all years. Where differences did occur, root score was a greater factor, suggesting that the amount of root development may be genetically controlled (Mason *et al.*, 2002). Overall rooting success in the loblolly pine (*Pinus taeda*) is much lower: 44 %. Clonal differences in *P. taeda* were found to account for 10 % to 17 % of the total variation in rooting success (Baltunis *et al.*, 2005). Egertsdotter (2018) recommends that in SE production of conifers, the cell line variability in the steps of the production process must be adjusted to ensure an appropriate genetic representation.

2.7 Certification and Traceability

—

Lieseback, H., A'Hara, S., Bordács, S., Kraigher, H.

Legal regulations: the formal certification system

According to the Council Directive, FRM must be identified throughout the whole production chain (from seed collection, processing, storage, nursery production and transportation to the end user) to be able to verify that the reproductive material is the same as the declared provenance. This is because FRM must be adapted to its planting site, particularly in terms of its stability, resistance to disease, adaptedness and adaptability, as well as the productivity and diversity of the future forest.

The national regulations further define the minimum requirements for the approval of basic material, the creation of national lists of tree species and basic material, and a national register of suppliers to be held. The EC Regulation 1598/2002 defines the exchange of information between Member States, and the Commission decision 2005/871/EC defines the requirements for the National lists to be submitted to FOREMATIS- the EC Forest Reproductive Material Information System (see Box 3).

BOX 3.**The EU list of basic material per country - FOREMATIS**

In 2016, the Commission Working group on legislation on plant reproductive material - Forest Reproductive Material section - set up a common database of approved basic material for the production of FRM (to improve the standardisation of the national list submissions), with some further updates in the past few years. The national list can only be submitted and validated by a specified person per country, but it can be viewed by several users.

FRM can only be marketed if it belongs to one of the four categories the Council Directive specifies, and if the basic material is approved. Basic material is officially approved and inspected regularly. The quality criteria vary depending on the category. There are also criteria relating to species purity in fruit and seed lots. After collection, a master certificate (Figure 22) is issued with a unique register reference for information and traceability.

Based on national registers, the Commission has drawn up an EU list to ensure that the scheme operates smoothly throughout the EU. The Commission's Forest Reproductive Material Information System (FOREMATIS) provides a search tool for forest breeders, forest nurseries, experts and the general public, functioning as a repository linked with Member State data of planted forest tree species. FOREMATIS provides access to the data of national registers, containing details of approved basic material, including area and geographic location, which are essential for determining if a particular material is suitable for a particular site.

According to Regulation 1597/2002/EC the database contains the following fields of information: **Member country, Tree name, Tree category, Identity code, Location name, Latitude, Longitude, Altitude, Type of basic material, Area (ha), Origin, Origin for non-autochthonous/non-indigenous basic material, Purpose code** (any text in **Remarks**), **Remarks** (if tree species is *Populus nigra*, or if purpose is Other), Comments (the last column - any other text).

In the last few years, the following fields have been added: Primary key, which identifies each record based on completed mandatory fields (Year, MS, UPOV Code and Region category; the template generates the PK automatically after clicking on 'Adopt'), and UPOV code, which identifies tree species and plant variety provided by the international Union for the Protection of New Varieties of Plants (5 first digits of GENUS name and 3 first digits of SPECIES name: GENUS_SPE)

Currently, FRM traceability involves generating an audit trail from collection to user in the trade and is based on several documents that must be completed for each commercial harvest of FRM. This starts with an intention to collect and leads to the drawing-up of a master certificate (Figure 22). Each country has a national Official Body tasked with ensuring compliance with the regulations. These documents

(Master certificate, Supplier's document, Information document) accompany the FRM material through the trade chain to the end-user. No corrections can be made to the Master certificate after it has been issued, but if FRM has been collected as cones or fruits intended for trading immediately after collection, the quantity of cones/fruits may be stated, while the quantity of seeds acquired after the extraction process can be added based on the records on seed processing procedures. Information about FRM lots which move between EU member states must be accompanied by these documents and be exchanged by national authorities.

In general, national authorities use an electronic database to store all data about FRM suppliers (nurseries, traders, forest owners), basic materials, issued master certificates, field control protocols made by the authority and supplier documents. Documents containing data and information from this database are then issued, but usually with a certain time gap which can often be a few months, when FRM lots have already been planted in the forests by the end-users. To minimise this time gap between EU internal trade and end-use of the FRM lots, national authorities often communicate and exchange the information directly, thus minimising the use of undocumented FRM lots.

The general principles of seed certification within the chain of forest seed production were summarised by Matthew in 1964, when genetic identity could not yet be verified by marker-based techniques. He emphasised, "The object of seed certification procedures is to make available to the forester seed and plants that are true-to-name and satisfy certain minimum requirements of quality. The object of the certification of tree seed and plants is to maintain and make available to the practising forester sources of seeds, plants and other propagating materials of superior provenances and cultivars so grown and distributed as to insure the genetic identity and high quality of the seed and plants".

FIGURE 22. Model Master Certificate of identity for FRM derived from seed sources and stands.
(From Annex VIII of the Council Directive).

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ANNEX VIII

PART A

MODEL MASTER CERTIFICATE OF IDENTITY FOR REPRODUCTIVE MATERIAL DERIVED FROM SEED SOURCES AND STANDS

(Certificate must contain all the information outlined below, and in the exact format)

ISSUED IN ACCORDANCE WITH DIRECTIVE 1999/105/EC

MEMBER STATE:	CERTIFICATE No EC/(MEMBER STATE CODE)/(No)
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It is certified that the forest reproductive material described below has been produced:

- in accordance with the EC Directive
- reproductive material under transitional arrangements

1. **Botanical name:**

2. Nature of reproductive material:	
Seed unit	<input type="checkbox"/>
Part of plants	<input type="checkbox"/>
Planting stock	<input type="checkbox"/>

4. Type of basic material:	
Seed source	<input type="checkbox"/>
Stand	<input type="checkbox"/>

3. Category of reproductive material	
Source-identified	<input type="checkbox"/>
Selected	<input type="checkbox"/>
Tested	<input type="checkbox"/>

5. **Purpose:**

6. **Country register reference or identity of basic material in National register:**
..... / Mixture:

7. Autochthonous Non-autochthonous Unknown
 Indigenous Non-indigenous

8. **Origin of basic material (for non-autochthonous/non-indigenous material, if known):**

9. **Country and Region of provenance of basic material:**

Provenance (Short title, if appropriate):

10. **Altitude or altitudinal range of site of basic material:**

11. **Year in which seeds ripened:**

12. **Quantity of reproductive material:**

13. Is the material covered by this certificate the result of a subdivision of a larger lot covered by a previous EC Certificate?		Yes <input type="checkbox"/>	No <input type="checkbox"/>
Previous certificate number	Quantity in initial lot		

14. Length of time in nursery:

15. **Has there been subsequent vegetative propagation of material derived from seed?** Yes No

Method of propagation Number of cycles of propagation

16. **Other relevant information:**

17. Name and address of supplier

Name and Address of Official Body:

Stamp of Official Body:
Date:

Name of Responsible Officer:
Signature:

Misgivings about the validity of FRM documents repeatedly arise, and there have been some past cases of FRM mislabelling which have become public. Usually, they are not disclosed in scientific journals, but a few detailed descriptions for detected mislabelling of FRM have been given by Konnert (2006) for Sycamore (*Acer pseudoplatanus*) and wild cherry (*Prunus avium*), by Finkeldey *et al.*, (2010) for acorns, and by Westergren *et al.*, (2017) for beech nuts. In these cases, the origin of seeds or seedlings could be excluded by observed mismatches to the progenitor seed orchard clones, the mother trees of the seed stand or to reference samples. These situations highlight the need for additional means of control, over and above the current international and national regulations. In addition to a better enforcement of existing regulations, representatives from forest owners, private enterprises and inter-trade organisations from many European countries, together with members of research institutes and universities, have clearly ascertained the necessity for further development in the field of marker based verifications of FRM origins at several stakeholder meetings in the past few years.

Tracking using marker-supported systems

→ Potential of genetic markers

The Council Directive requires the national register to include information about seed stands, seed orchards and clone mixtures. A unique reference is provided on the master certificate after each harvest; this is a key element for tracing FRM. The legal control regulations imply the monitoring of the delivery papers (e. g. the master certificate), but in order to be effective, this must be conducted very frequently, which is highly staff- and cost-intensive. Such plausibility controls based on the monitoring of delivery papers are therefore not 'safe' enough. The development of genetic markers (biochemical and molecular) offers possibilities for more precise and efficient control on the basis of comparison of the genetic composition of seed stands, seed and seedlings within a traceability system. In other words, seedlings and seed material can be assigned to a provenance and/or to stored reference material related to a master certificate. Reference material can comprise seed lots or plant tissue from each tree sampled during harvest in seed stands, or a sample of each clone in the case of seed orchards.

A molecular traceability system in forestry can, for example, identify species-level FRM, trace back the origin of FRM to the respective seed stand/provenance or seed source/region of provenance, improve the quality of planted material by clone identification, protect wild species from introgression by cultivated relatives, and prevent illegal logging via identification of wood samples. Additionally, the same system can be applied to check and safeguard genetic diversity of the produced

FRM. Several techniques are now commonly used in fields outside forestry to track the movement of goods, such as in food quality control. One such technique is the analysis of stable isotopes to identify the region of origin of agricultural products. This method is based on the premise that the isotope signatures of plants and animals can, to a degree, be influenced by the regional growth conditions, including moisture and nutrient availability. Similarly, genetic markers are now routinely used to identify and check many products; for example, the species composition in meat products and the purity of honey.

Regarding the traceability of FRM, stable isotopes could be used to identify the region of seed origin or the region of the nursery in the developmental stage of young plants at a relatively low spatial resolution. In the past, attempts have been made in a research project to test the suitability of isotope analyses for checking the origin of seed lots of beech (*Fagus sylvatica*), Alder (*Alnus glutinosa*), sycamore (*Acer pseudoplatanus*), Norway spruce (*Picea abies*) and Silver fir (*Abies alba*) (Gebhardt, 2008; Gebhardt *et al.*, 2008). Despite initial promising results, it proved impossible to establish the method in practice, due to the considerable technical input required and the necessity of having a library of comprehensive reference samples. Another physical technique, NIR (near infrared) spectroscopy, was applied to assign Norway spruce seeds to different European regions (Farhadi *et al.*, 2017). Currently, the Global Timber Tracking Network (GTTN) initiative is creating a database combining stable isotope and DNA marker information to allow high value and rare timber products to be tracked (Dormontt *et al.*, 2015). A reference database is always necessary to assign unknown samples to a certain region, but creating a comprehensive reference is a major hurdle when assigning the origin of material. In the case of nursery-grown plants or propagated clones, the origin of seeds, or the identity of the respective clones, cannot be checked due because they are being grown outside of their area of natural origin.

In contrast, genetic markers are intrinsic to an individual and can be used to verify the origin of FRM independently of their growing region. Noncoding (neutral) regions of DNA are frequently used for identification purposes since the functional importance of the variation is of minor or no concern in this context. Many small case studies have demonstrated the potential of genetic markers. Large-scale studies have been carried out using plastid DNA to help study historic re-colonisation routes of different species since the last ice age. While genetic variation is better conserved in chloroplast DNA in comparison with nuclear DNA, differentiation among geographically distant populations is often much higher for maternally inherited markers, since the dispersal of genetic information via seed trees is far

less efficient than dispersal via pollen and seeds. The detailed knowledge about phylogeographic variation patterns of chloroplast DNA (cpDNA) haplotypes in European oaks (*Quercus* spp.; Petit *et al.*, 2002) makes cpDNA markers excellent tools for inferring the origin of oaks (Gailing *et al.*, 2003; Gailing *et al.*, 2007) and for identifying misclassified oak seedlings at a geographic/regional scale. Studies over the last decades have used DNA markers on both a fine scale (to examine the current genetic diversity of many trees' species and populations) and a much broader scale. Reviews covering the current state of knowledge have been given by Gaudal *et al.*, (2014), Ivetić *et al.*, (2016) and the EU project Trees4future¹⁹.

Neutral marker variation is useful for assessing levels of diversity (particularly important in approved seed stands, as reported by Westergren *et al.*, (2012), Eusemann *et al.*, (2019) and Wojacki *et al.*, (2019)), analysing parent-offspring relationships, and determining the numbers of harvested parents. There are clear uses for neutral genetic markers when checking output from seed orchards, in terms of the degree of individual component contribution and the assessment of potential external pollen contamination (e.g., Scots pine, Funda *et al.*, 2015). Furthermore, genetic markers are very informative for species and hybrid classification, as well as for clone identification and evidence of parentage, as has been demonstrated for the genus *Populus* by Liesebach *et al.*, (2010, 2015), for *Quercus* by Guichoux *et al.*, (2011), for beech trees by Lefèvre *et al.*, (2012), for black locust by Liesebach and Ewald (2012), and for ash by Westergren *et al.*, (2013). Suitable markers as nuclear microsatellites are available, but there is a need for more standardisation within different laboratories. Substantial effort in species-specific marker development need to be made in order to benefit from the application of genetic markers. The application of DNA fingerprinting methods requires well-equipped labs, relatively expensive consumables, and well-trained staff. The development of cost-effective markers and genotyping methods based on high-throughput sequencing may result in the rapid expansion of knowledge of the geographic partitioning of genetic variation in tree species.

→ Examples of the application of genetic markers in tracking FRM

In Germany, two FRM certification systems have been set-up by private seed collecting enterprises and nurseries, which have established a control procedure based on genetic markers. This additional control procedure is not required by national legislation. Methods and markers with the potential to be used in tracking

¹⁹ http://www.trees4future.eu/uploads/t4fdeliverables/284181_T4F_D7.1_Methods_identification_web.pdf

procedures have been developed by research institutes and tested for their practical suitability in close cooperation with the associations and certification bodies. The existing certification systems work with very similar principles. Detailed information (in German only) is available on the websites for “Zertifizierungsring für überprüfbare forstliche Herkunft Süddeutschland e.V. (ZüF)”²⁰ and “Forum forstliches Vermehrungsgut e.V.”²¹.

The first step involves the documentation of a respective seed harvest in a comprehensive database with a record of the amount of seed collected. At the same time, the first seed sample for storage must be taken directly from the stand (reference sample 1), as well as a sample of plant tissue from every tree used for seed collection (reference samples 2). Further information, (e.g. amount of seeds after different processing steps or results of germination testing) is added to the database as soon as it is available. Simultaneously, representative samples of seeds at every step of processing (reference samples 4) are collected and stored as references for possible future genetic analysis. When the seed lots or young plants with a certificate come on the market, an initial plausibility check is possible by comparing the estimated maximum number of plants - as extrapolated from the database - and the actual number offered in the market. A randomly selected number of samples (reference samples 3) will then be analysed using genetic markers and checked for their accuracy by comparing them to the retained reference samples. In addition, in the case of any doubt, samples can be analysed to check that labelling is correct.

All members of a certification system pay a membership fee and must submit their data to the database and their reference samples to storage, according to a detailed procedure. They are aware that their samples could be among the randomly selected samples for the genetic check. This kind of certification system reassures the customers and end-users that they will receive the correct FRM product. Some incidents involving detected mislabelling, the mixing up of samples, and other mistakes, have been published anonymously without dates, names and places. This acts as a reminder to the members of the certification system to be diligent in all steps of their work in order to sustain customer trust. Recent evaluations indicate that the application of FRM certification within these two systems in Germany is well-established and standardised, and implemented as a routine operation. Its presence on the market shows an increasing tendency towards such systems.

²⁰ <https://zuef-forstpflanzen.de>

²¹ <https://ffv-zertifikat.com/en>

Similar, but compulsory systems exist in Slovenia and Austria as part of their national regulations: a sample from every tree that seeds have been collected from must be submitted to the respective national forestry research institutes by the on-the field control person. The material can be a certain number of seeds/cones per tree, or dormant buds per tree, and is immediately freeze-dried, or DNA is extracted, for storage in a DNA library. This makes it possible to check not only the number of harvested trees and the correct provenance identification, but also the descendants of FRM from the sampled mothers.

In Slovenia, the Slovenian Forestry Institute (SFI) is the designated authority that checks the origin of FRM according to national legislation requirements and the forms provided by on-site control persons, and/or (in the case of doubt) the molecular marker comparisons, before issuing the master certificate for any FRM intended for forestry use. In a recent study (Westergren *et al.*, 2017), the origin (provenance) and genetic diversity of FRM collected from four beech (*Fagus sylvatica* L.) seed stands in 2016 were analysed and compared to samples stored in the Slovenian Forest Gene Bank, resulting in the suspicion of (intentional) mislabelling.

From a technical point of view, the selected 16 markers proved to be appropriate for individual tree identification, evaluation of genetic diversity, and identification of the alleged origin. The results also emphasised that FRM should be collected from the number of trees required by the decree on approval of the seed object, issued by SFI, in order to safeguard the genetic diversity of the FRM. The genetic diversity of smaller samples (seed collected from less seed trees) was significantly lower. Bayesian clustering methods and phylogenetic trees correctly determined the origin of reference samples, while the resolution of the principle component analysis was lower. The results from analyses using all methods for the identification of the alleged origin of seed samples unambiguously proved that one of the samples submitted for certification was not collected in the stated seed stand, and it also differed from the rest of the analysed beech genepool in Slovenia. In this case study, the applied methods for the molecular identification of FRM origin proved to be necessary to prevent an introduction of inappropriate FRM into Slovenian forests.

In Hungary, a specific system has been used for inspection of Forest Genetic Resources and FRM of black poplar (*Populus nigra* L.). The system is based on a combination of inspection phases using morphological, phenological and molecular markers (Bordács & Bach, 2014). As a result of the molecular marker supported inspection system a complex restoration programme was carried out in Hungary, including *ex situ* gene conservation and FRM production of *P. nigra*.

Prospects for further developments

Further research is needed to improve the existing marker-based certification methods:

- The list of tree species with an available set of genetic markers should be increased by developing new species-specific markers.
- More and better markers to obtain more reliable results are required, including technical developments to obtain faster results. In the future, next generation sequencing methods for marker development and genotyping should make this far less onerous. First examples have been given by Plomion *et al.*, (2016) for *Pinus pinaster* and by Godbout *et al.*, (2017) for *Picea glauca*.
- Further development in statistics and data evaluation methods for assigning test samples to reference data is needed, including the assignment of test samples to certain seed stands or seed orchards without retained samples. An effort should be made to standardise the approaches and outputs of the results of the next wave of tree population analyses based on many thousands of markers, both neutral and adaptive. An open access common/central database of results would also be of great benefit. However, the handling and storage of these huge datasets would require bioinformaticians and suitable infrastructure for computers.

The basic principles of the existing certification schemes could be used elsewhere. They are open to further development in terms of certification scheme and DNA library organisation, as well as laboratory techniques and scientific background. The biggest hurdle is the cost: schemes such as these could only be mandatory if backed with sufficient resources from the official control body in each country. Therefore, a cost-benefit analysis per species and region should be conducted, considering forest growth with all its functions, or its deterioration, and comparing it to the costs of the analysis used to check for any mislabelling.

It is necessary to educate end users about the dangers of using inappropriate FRM and the possibilities for its certification and tracking. Case studies need to be compiled on the negative consequences or loss of forest functions - including wood production - due to misuse of FRM and/or its mislabelling during the production chain.

2.8 Trading and Transport

Bordács, S., Uggla, C., Tollefsrud, M.M.

FRM can be critically affected during trade and transport, which may have a negative genetic impact on the FRM lots planted. These impacts may not be easy to detect during trade and transport, but can arise at a later stage, for example, sometime after planting.

The EU Commission Regulation EC 1598/2002 implies that the record keeping of FRM transfer is needed on a national basis, alongside collaboration between national authorities. However, harmonised EU documentation has still not been established (Jansen *et al.*, 2019). Due to such lack of documentation and central recording of the FRM trade by the EU, only national statistics are available in certain countries, which vary greatly in content (Jansen *et al.*, 2019).

Local transportation

In the past, forest nurseries were located within or next to areas of forest where the FRM was used; therefore, the transport of FRM was short, both in distance and duration. Nowadays, nursery production is usually separated from both silvicultural management and forestation sites; for example, in Central-European countries the usual transport distance from the local nursery to the sites of planting is 10-150 km. In the case of short distance and duration of transport, seedling lots do not need to be specifically handled, packed or stored. However, some deficiencies and imperfections can negatively affect the plants' viability, physiological conditions or even traceability.

One of the riskiest phases for seedlings is the on-site storage interval before planting. This type of storage could be crucial for bare root seedlings, if it is longer than 1-2 days. According to Luoranen *et al.*, (2019) Norway spruce and Scots pine container seedlings should not be stored in the field in cardboard boxes for more than a week. There might be selection pressure on sensitive genotypes, basically affected on radix by frost, drought, solar radiation. All these abiotic factors can reduce the genetic variation of seedlings planted in a one-dimensional, tendentious way.

Insufficient labelling or separation of FRM lots could cause undesirable mixing, leading to potentially invalid genetic information on the documents for the FRM lots. In the case of local transportation there is a low risk of intentional deficiencies, falsification

or illegal trade. Partners within the marketing chain are interested in direct contacts and partnerships, often in personal contacts as well, thus limiting the intentional deficiencies of FRM lots. Due to these direct partnerships and short transport distances, unintentional deficiencies, such as mislabelling and mixing of FRM lots, can be simply detected and clarified.

Regional and continental transport

Recently, the international movement of FRM lots has been rapidly growing across Europe. Due to general EU regulations on the European market, international trade barriers are simplified or eliminated between EU member states. There are no legal barriers for the transportation of FRM lots and in many cases forest nurseries and suppliers sell their products on the European market. The absence of barriers also allows nursery companies to produce seedlings by subcontracting growers in other member states, which means that seed and seedlings are transported across the EU during both the marketing and production phases.

Both European and OECD legislation on FRM include minimum requirements for documentation and labelling to ensure traceability of FRM lots traded. Suppliers and trading companies using modern techniques (e.g., insulated trucks and cold storage) have easy access to the continental FRM market. In general, seed and bare root seedling lots are preferred for minimising the costs of international trading. As a result of international trade, there is a more diverse choice of marketed FRM lots available to the end-users. On one hand, the diversification of the FRM market has many positive impacts on afforestation and silviculture, but on the other hand, foresters and silviculturists have an increasing responsibility when they choose FRM and its genetic value (e.g., provenance, origin and category).

In contrast to local market situations, FRM lots in international trade are transported hundreds or even thousands of kilometres, and the journey usually lasts 2 to 3 days or longer. Therefore, attention must be paid to specific requirements during the handling, packaging and storage of the seedlings, such as cooling, watering or isolating. Container and bare root seedlings often have differing needs. Any deficiencies or imperfections caused during transport could significantly reduce the seedlings' viability.

Seed

The importance of storage conditions for seeds is dealt with previously in this report. The same environmental conditions suitable for seed storage generally apply during

transport. It is often impossible to avoid suboptimal storage conditions at all stages of transport, but they should be avoided for extended periods of time. Transport should therefore be planned carefully. Seeds of recalcitrant species such as oaks are most sensitive during transport.

With regard to the genetic identity of seed, the most important issue during transport and trade is to ensure sealed packaging that has a consistent identification label in line with the applicable legislation.

Seedlings

Seedlings are vulnerable to stressful environmental conditions during transport and care should therefore be taken in planning the transport. Seedlings transported in the dormant state are generally less sensitive. Seedling storage prior to planting is often a challenge, because of lack of cold storage facilities and exposure to sunlight, wind and temperature fluctuations. The planting season and metabolic state of seedlings also have a general influence on environmental requirements.

In Nordic countries, seedlings are often stored frozen, then transported and intermediately stored in refrigerator conditions. In this system, seedlings will thaw slowly during transport and intermediate storage, and they should not be planted until completely thawed. This means that a very short time between freezer storage and planting is not always preferred. In southern Europe, seedlings are planted in late autumn. Containerised seedlings are preferable for the most xerothermic regions as the planting period can be prolonged and, despite being more costly, the survival rate is high. Bare-root seedlings are used in favourable sites or higher altitudes. In order to transfer and preserve the bare root seedlings, the root system is protected by either covering it with soil or wet fabric or using hydrogels, which are more effective. Guidelines for the treatment of seedlings from the nursery to the planting site need to be developed and followed taking into account national practices and regional environmental conditions.

Containerised seedlings are less sensitive than bare root seedlings to environmental stress during transport, because the most sensitive part – the root system – is protected, and the medium surrounding the roots (peat) acts as a water reservoir, as long as it is not frozen. Containerised seedlings should therefore be carefully protected from sun and light exposure when the roots are frozen.

When seedlings are subject to transport or intermediate storage under suboptimal conditions, the variability in their response to any given environmental factor or stress

is likely to result in genetic selection pressure on the genotypic range of seedlings; this will penalise genotypes (e.g., offspring from certain mother clones) that are more sensitive to that stress factor.

Certification, labelling and phytosanitary issues

The verification of origin and control systems of FRM according to Council Directive, and the OECD Scheme for the Certification of Forest Reproductive Material, is covered elsewhere in this report.

In the EU, there are also requirements for phytosanitary certification. On the one hand, phytosanitary requirements and restrictions may impact the genetic aspects of FRM, because they often prohibit the import of plants, parts of plants, and sometimes seed from certain regions outside or within Europe, thus reducing the possibility of moving genetic material in those directions. This can have a substantial impact on species with recalcitrant seed²². On the other hand, phytosanitary restrictions aim to reduce the risk of introducing diseases that will potentially have strong genetically selective pressure on FRM. One example of such a disease is ash dieback, with the ash tree species (*Fraxinus excelsior*) showing strong genetic variability in resistance, resulting in high mortality in many sensitive genotypes.

Importing seedlings for silviculture from non-EU countries is subject to phytosanitary restrictions. In the case of conifers, there is a requirement for a health certificate (Phytosanitary Certificate) for plants originating in Europe outside the EU, whereas the import of conifer plants from outside of Europe is prohibited. The phytosanitary certificate certifies that the plants comply with EU phytosanitary import rules on the absence of regulated pests, in accordance with the EU Council Directive (2000/29). A new EU regulation (2016/2031) on protective measures against plant pests has been adopted and has been applicable to all EU Member States since 14th December 2019. We do not explicitly deal with the new regulations in the present report, but in brief, the new EU regulation aims to focus more on preventing the spread of certain pests and broaden the requirements for importing plants into the EU and their movement therein. Furthermore, it introduces new obligations and responsibilities for professional operators. A phytosanitary certificate will be required for most plants entering the EU and more plants and plant products will require a plant passport. Plants listed as “high risk” will be prohibited from entering EU.

²² Seeds that do not survive drying or freezing and are only viable for a short time; these are typically tropical species.

Invasive forest pathogens have exponentially increased in the past four decades, with new pathogens introduced from North America and recently from Asia (Santini *et al.*, 2013). According to an analysis of a large database of invasive forest pathogens developed to investigate the patterns and determinants of invasion in Europe, the countries which hosted the most invasive forest pathogen were those that had a wider range of environments, higher human impacts or international trade (Santini *et al.*, 2013).

Importing live plants has historically been the most common pathway for the introduction of non-native forest insect pests and pathogens (Liebhold *et al.*, 2012). For example, nearly 70% of damaging forest pathogens established in the United States between 1860 and 2006 most likely entered on important live plants (Liebhold *et al.*, 2012). Today we have examples of alien fungal and fungal-like pathogens that are killing millions of trees and almost eradicating tree species: for instance, sudden oak death *Phytophthora ramorum* (Rizzo *et al.*, 2005, Rizzo & Garbelotto, 2003) and chestnut blight *Cryphonectria parasitica* (Anagnostakis, 1987) in North America, Dutch elm disease *Ophiostoma ulmi* and *O. novo-ulmi* and hybrids, in both North America and Europe (Dunn, 2000), and ash dieback *Hymenoscyphus fraxineus* (McKinney *et al.*, 2014) in Europe. In Europe, ash dieback is currently the most acute forest pathogen problem (e.g., Semizer-Cuming *et al.*, 2018). In North America, ash forests are rapidly being lost to another introduced species from Asia, the wood boring beetle known as the emerald ash borer. Currently it is in Russia, spreading 12 km each year to the west and south of the Moscow area. Observations in Europe have shown that a low percentage of ash trees can withstand the infections of *H. fraxineus*, but far greater losses are expected when the emerald ash borer meets ash dieback (e.g., Semizer-Cuming *et al.*, 2018).

Although regulations have been in place to prevent the introduction and spread of forest pests via transport and trade with the EU Council Directive (2000/29), they have not been sufficiently effective to prevent the spread of pathogens. Hopefully, the new EU regulations will help prevent the entry and spread of plant pests, at least within the EU. Regulations should also be continuously updated with science-based knowledge, awareness raising and proactive actions against invasive alien species as recommended by Semizer-Cuming *et al.*, 2018.

Intentional falsification, illegal trade

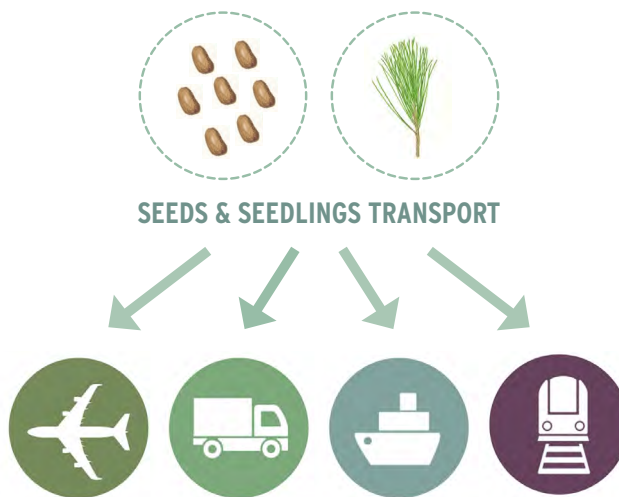
As previously mentioned, insufficient or unintentional labelling, or separation of, FRM lots could cause undesirable mixing during transport and interval storage. As a result, it will not be possible to identify the mixed FRM lots for their genetic origin. Due to the missing or misunderstood genetic information, the FRM lots might not correspond to the environment of the planting site.

There are significant differences in costs and price between local and regional FRM markets in Europe. FRM suppliers are therefore interested in trading from lower-cost regions to higher-premium regions. In specific cases, suppliers or end-users may wish to intensify their profits and are therefore interested in the falsification of documents or labels, by modifying information on the suppliers' documents. Genetic information of FRM lots, such as origin, provenance region, seed source and category, can be intentionally falsified at any stage of transportation.

There are available tools and techniques for detecting falsifications. Many molecular markers make it possible to identify the basic materials, individuals, populations or even provenances, as source of FRM lots. The use of molecular markers can help to control the whole production and marketing chain of FRM. As a result of the development of molecular markers, specific certification systems (e.g., ZÜF in Germany) have been developed for which markers are used as a complementary control tool to check control deposits of certified FRM lots (see Chapter 2.7 Certification and Traceability).

In conclusion, the trade and transport phase are crucial parts of the FRM production and marketing chain. Due to intensified trading activities across Europe, the traceability of FRM lots should be improved, involving tighter control and better management of accompanying documents and use of control tools by national authorities, such as molecular markers and efficient information exchange.

FIGURE 23. Transport of Forest Reproductive Material. (Credit: Seeds/Seedlings: C.Giordano/EUFORGEN; Transports: all-free-download.com).



3. USE OF FOREST REPRODUCTIVE MATERIAL

The EUFORGEN report by Konnert *et al.*, (2015) put forward possible approaches, identified critical factors and made recommendations for the use and transfer of FRM in Europe in the context of climate change. It recommended that decisions regarding the selection of suitable material for forest regeneration should now be made taking into account climate projections for the next 30-200 years. It also recommended utilising within-species adaptive genetic diversity, and it identified FRM transfer as a valuable option for adapting forests to climate change, especially in those areas that are most severely threatened by it. The report highlighted that local material does not always demonstrate optimum fitness and is not always best for regeneration purposes, depending on various biotic and abiotic factors. Moreover, it emphasised the importance of the conservation of local genetic resources when deploying FRM.

In this chapter, different management strategies for choosing FRM in the context of climate change are outlined, and the potential of using assisted migration in adaptive forestry is explored. Decision support tools currently available for choosing suitable FRM in the face of climate change are described, and several examples on the legal basis for the use of FRM are given, including examples of recommendations regarding national provenance.

3.1 Regeneration strategies - choosing forest reproductive material in the context of climate change

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Regeneration strategies relevant in the context of climate change can include both a natural regeneration strategy and an assisted migration strategy using single provenance or a composition of provenances. Among other elements, strategy choice will depend on the type of reproductive material that is available, and existing knowledge about the adaptability and plasticity of the material.

Natural regeneration strategy

Under a natural regeneration strategy, forest management is used to improve conditions for adaptive processes. Natural regeneration arguably constitutes a much larger base population than is typical for plantations (Fady *et al.*, 2016). Natural regeneration may also lead to a much stronger selection process than that of regeneration based on cultivation, especially planting; this is because natural regeneration will ensure a much higher seedling density than can be obtained by planting, and the strong selection acting on natural regeneration will ensure adaptation (Fady *et al.*, 2016). However, genetic diversity and adaptive potential may vary along the species range and will depend on local ecological conditions and demographic constraints, which could severely limit the capacity of populations to evolve under the most severe climate changes. Management scenarios based solely on locally existing forest material and genetic resources may thus prove risky and ineffective, particularly at range and ecological edges (Fady *et al.*, 2016).

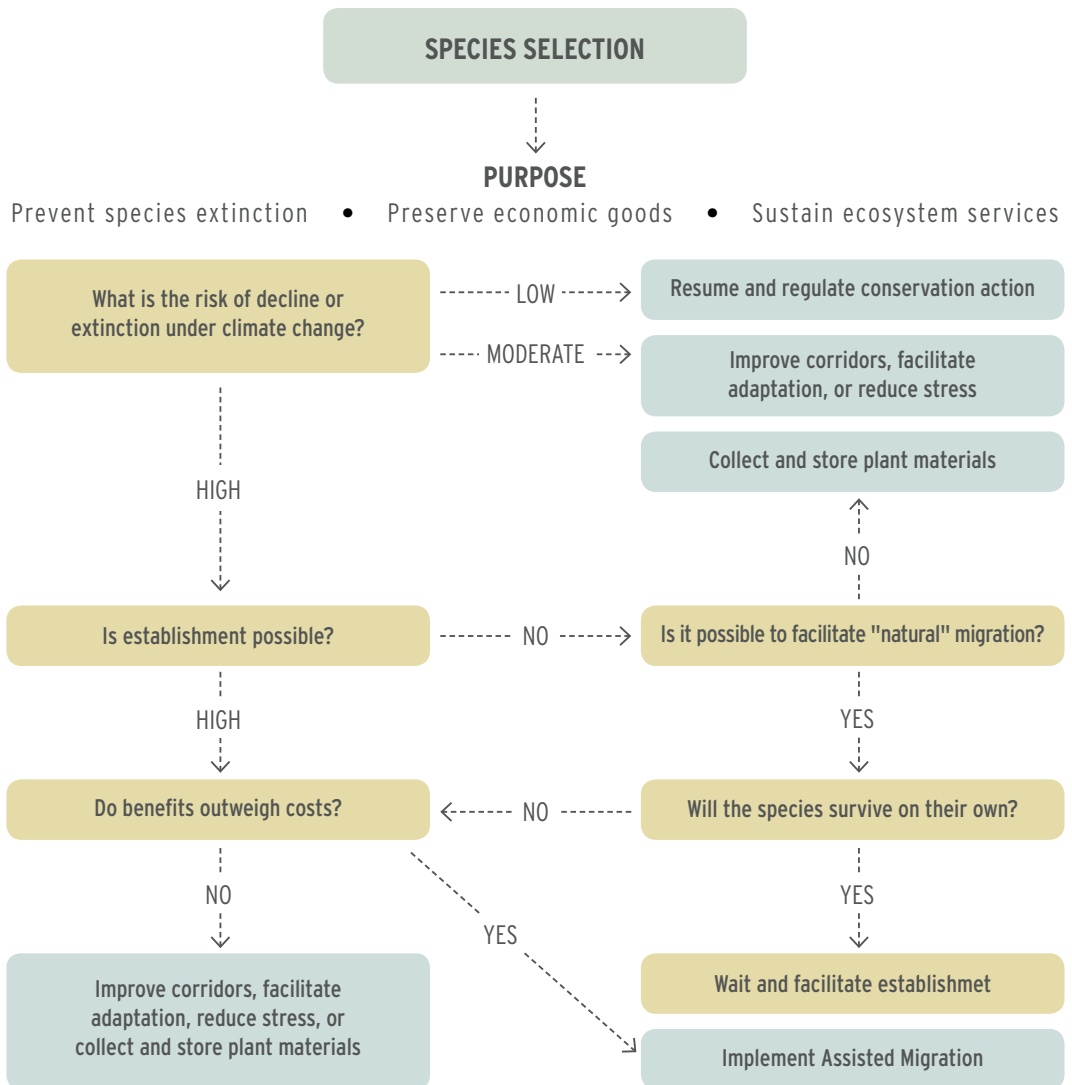
Assisted migration strategy

With climate changing more rapidly than populations can adapt or move, one potential tool for aiding adaptation to a new climate is assisted migration (AM), or assisted gene flow (AGF). Whereas assisted migration is the intentional translocation of individuals within or outside the natural range of a species, assisted gene flow has a narrower definition and refers to the intentional movement of individuals within the range of a species (Aitken & Whitlock 2013). In this report, we do not differentiate between the two terms and use assisted migration for both. When a population is facing challenges due to a new climate (e.g., drought or warmer and longer growth periods), the genotypes best adapted to this new climate may occur at higher frequencies elsewhere in the species range. The single provenance strategy is based on choosing a provenance which is expected to be suitable for the predicted future climate. Aitken & Bemmels (2015) recommend the adjustment of seed zones and seed transfer rules, in order to use genotypes that are pre-adapted to near-future conditions for reforestation and restoration.

Shared adaptive clines in height growth potential and fall events show that the patterns of adaptation along climatic gradients are often very similar among species (Aitken & Bemmels 2015), thus justifying an assisted migration strategy. However, any attempt to transfer material already adapted to a projected future climate will be accompanied by a risk of “prognostic error” (Kolström *et al.*, 2011), and success will depend on the right choice of provenances and species. The latter is a challenging

task and is impeded both by the uncertainty of climate change projections and by the (still) limited knowledge acquired from provenance and species translocation trials. Therefore, assisting gene flow must rely on considerations about how to mitigate risks and capture benefits, and it is not recommended to transfer FRM more than a few degrees of latitude (Aitken & Bemmels 2015).

FIGURE 24. A decision framework from Hoegh-Guldberg *et al.*, (2008) to determine adaptation strategies of a plant species or population that has conservation, economic or social value. Adapted from Williams and Dumroese (2013).



A decision framework like the one presented by Hoegh-Guldberg *et al.*, (2008) (Figure 24) could serve as a guide for identifying the species or populations at risk, and for assessing the feasibility of assisted migration under climate change. According to this tool's guidelines, assisted migration can be implemented if the following three requirements co-occur for the species/populations of interest: i) they are at high risk of decline or extinction under climate change, ii) they can be successfully translocated to and established in the new environments, and iii) the benefits of their translocation outweigh the biological and socioeconomic costs and constraints (Williams & Dumroese, 2013). In the remaining cases, a range of actions can be considered and implemented - depending on the level of risk of decline or extinction involved, and after taking into account the possible restrictions on each particular population or species. These actions include, for example, *in situ* conservation, facilitation of adaptation, reduction of stress factors, opening of corridors to enhance gene flow and natural migration, collection and storage of plant genetic material (seeds and plant parts, etc). To effectively navigate through the decision tool, all available genetic information, bioclimatic models, historical records and results from current assisted migration experiments need to be considered (Lawler & Olden 2013, Williams & Dumroese, 2013).

Provenance and species choice for assisted migration strategies should ideally be based on the results from range-wide and long-term reciprocal transplant studies. In the United States, what was probably the most comprehensive provenance trial was performed for lodgepole pine (*Pinus contorta*) in British Columbia testing 140 populations at 60 test sites (Illingworth 1978). In Europe, the International Union of Forest Research Organizations (IUFRO) 1964/68 inventory provenance trial for Norway spruce studied 1100 populations from Europe and Siberia at 20 test sites in 13 countries (for results from the Hungarian test site, see Ujvári-Jármay *et al.*, 2016). Other extensive provenance tests have been made on: i) 110 Scots pine populations from the species' northern range studied at 47 test sites in North America and Eurasia (Rehfeldt *et al.*, 2002); ii) 116 sessile oak (*Quercus petraea*) populations from across Europe studied at 23 test sites in six European countries (Sáenz-Romero *et al.*, 2017); iii) 279 Norway spruce populations from several European countries studied at 29 test sites in Austria (Kapeller *et al.*, 2012), and iv) 60 beech (*Fagus sylvatica*) field trials located in 19 European countries as part of the International Beech Provenance Trial, with a total of 200 provenances (Frýdl *et al.*, 2010). Such datasets allow us to develop functions representing climate-related provenance responses; i.e., population response functions and population transfer functions (Aitken *et al.*, 2008). These data are very useful for predicting the performance of natural tree populations or plantations under

climate change, and for selecting the most suitable populations for plantations under particular climate-change scenarios.

Population response functions depict the phenotypic responses of a population to the climatic conditions at various test sites (e.g. Rehfeldt *et al.*, 2001, Wang *et al.*, 2006), and are also valuable for estimating the phenotypic plasticity of a population (Aitken *et al.*, 2008). Meanwhile, population transfer functions relate population performance of planted trees to geographic or climatic distances between population and common garden locations (Rehfeldt *et al.*, 1999). In both cases, the large provenance tests needed to develop precise response and transfer functions are rare, because they are time- and cost-intensive and often require scientific collaborations at the international level to cover species ranges. Currently, transfer models are typically based on fitness proxies such as survival or height growth. Although such traits are of primary interest for practical forestry, they are genetically complex and their relationship with the environment is multifactorial. To obtain a reliable picture of adaptive responses of populations and their geographical patterns, more emphasis needs to be given to physiological traits in the future.

In the past, provenance trials were mostly established for stand-forming conifers and broadleaved tree species. In the case of noble hardwoods and rare stand-forming tree species, not many provenance trials have been established and evaluated. The implementation of assisted gene flow or assisted migration is thus still difficult to put into practice for many species at a local level. It is therefore essential to ensure the transfer of existing knowledge to local and applied levels. Today, both the genetic aspect and the ecological balance associated with potential risks and failures when moving FRM often deter forest managers from applying assisted gene flow and migration measures. Additionally, the movement of new tree species into entirely new areas is intensively debated. This is due to factors such as potential unknown disturbances to or interactions with indigenous flora and fauna, use of ill-adapted genetic resources potentially leading to large failures (e.g. possible maladaptation of southern populations to extreme cold events in the north), or, more commonly, the introduction of pests and diseases (Alfaro *et al.*, 2014). Weaknesses also include insufficient scientific justification for the translocations, and difficulties associated with implementing and migrating enough genetic diversity, which can cause significant economic losses, as has happened in the past (Hewitt *et al.*, 2011). Caution has been advised when using extensive assisted gene flow, as the different responses will not only affect the trees, but also the whole forest community in which they are planted (Fady *et al.*, 2016).

Composite provenancing

In their review and synthesis paper, Aitken & Bemmels (2015) argue for a portfolio strategy or composite provenancing, using multiple seed sources in one generation to increase genetic diversity and buffer against future climate uncertainty (e.g., Kremer 2010, Aitken & Bemmels 2015). Composite provenancing could comprise a proportion of FRM adapted to current climate; for example, using more local seed sources and in mixtures with seed sources which are adapted to future climate. However, for temperate/boreal forest tree species which have a rotation length of 40-100 years, the challenge lies in determining which life stage to focus on: how to ensure both survival during the first years under the present climate and good growth during mid-rotation? FRM that may be well-adapted to future climate may suffer heavily from selection under the current climate (e.g. spring frost damage). Phenotypic plasticity can, however, buffer selection or help a population overcome stressful conditions via plastic changes during, for example, its early life stages. Aitken & Bemmels (2015) showed that most populations from warmer provenances had a consistently greater growth potential over a longer growing season, set bud and developed cold hardiness later, and underwent bud burst earlier or later in comparison to populations from cooler provenances. Seedlings which are moved from milder to cooler locations via assisted gene flow should then match the new temperature and moisture regimes better than local populations.

A molecular study in Germany (Gailing *et al.*, 2007) reported the positive effects of assisted migration that was carried out in the past. Pedunculate oak (*Quercus robur*) stands of Slavonian origins were introduced into Germany in the 19th century. The Slavonian oak has not only been acclimatised to local conditions in Germany, but an admixture with local oak genepools has been registered (Gailing *et al.*, 2007). This kind of genetic combination could be beneficial, especially in Central Europe where Slavonian oak stands have been established and where intensive climate warming is predicted in the next 50 years. Introduced “southern” genes for traits such as drought and heat tolerance may help local oak populations adapt to the changing climate.

3.2 Assisted migration and available decision support tools

Kowalczyk, J., Guibert, M., Friis Proschowsky, G., Uggla, C., Kraigher, H., Alizoti, P., Gömöry, D.

Adaptive forestry and assisted migration

The human-facilitated adaptation of forests to new climate conditions could be a tool for maintaining functional systems, minimising any economic climate-related risks to production forests and protecting genetic biodiversity (Konnert *et al.*, 2015; Schueler *et al.*, 2014). Forest ecosystems naturally adapt to environmental changes, but their natural adaptive ability may not be fast enough for the speed at which climate is expected to change in this century. In particular, natural adaptation does not secure wood production of the commercially important tree species, but rather survival of the fittest, regardless of species.

If forestry systems are to adapt to climate change, a framework for designing adaptive strategies to sustain or enhance forest growth, health, and ecosystem services in new climates is needed (Chmura *et al.*, 2010). Within such a framework, two approaches can be considered: the adaptation of decision-making processes in forest management and the adaptation of forest ecosystems. To meet future challenges, forest managers will need to apply multiple spatial and temporal measures and adopt more adaptive and flexible management approaches (e.g. shorter rotations and species diversification).

When considering measures to be applied in the adaptation of forest ecosystems, one of the options much deliberated by scientists and stakeholders is the intentional translocation of species, populations or genotypes to compensate for observed or future climate changes (Benito-Garzón and Fernández-Manjarrés 2015). AM, 'managed relocation', 'translocation', 'population or genotypes reinforcement' and 'assisted colonisation' are all terms used in the literature for these management activities. In forestry, AM is mainly carried out to maintain forest productivity and health and ecosystem functionality under climate change, as well as to potentially protect species and prevent extinctions. Current scenarios of climate change are based on models that are continuously revised and knowledge about climate change is lacking at every location (Lindner *et al.*, 2014). Uncertainty in predicting future climate, particularly with regards to precipitation, water balance and aridity, can be a major factor in determining future tree growth (Dobrowski *et al.*, 2013). AM can be an effective tool in maintaining productive forests, but genotypic diversity aspects, unknown resistance

patterns to pests and calamities, and uncertainty about the future climate need to be considered. Adopting the conservative approach of not applying the same AM regime on all forest land is one way to handle these uncertainties. For example, in order to spread out the risks and obtain more regional variability, it is possible to use alternative AM models side by side and a wider range of native and exotic tree species, as well as apply various kinds of natural regeneration and enrichment planting.

Of course, all risks associated with the introduction of exotics need to be taken into account. In addition to enhancing the spread of pests and diseases (Santini *et al.*, 2013), the invasive potential of the introduced species needs to be considered. Several introduced tree species have recently been recognised as invasive only after being commercially utilised for a long time for horticulture, forestry or agroforestry (Koskela *et al.*, 2014). To avoid important negative impacts on native forest ecosystems, such as in the cases of black locust (Vítková *et al.*, 2017) or the Australian Acacia species (Gaertner *et al.*, 2009), the possible invasiveness of exotics needs to be considered in plans for assisted migration.

In conclusion, human-facilitated adaptation of forests (e.g., by assisted migration) can be split into two main goals: first, if deemed necessary, to mitigate the effects of rapid climate change on the ecosystem for biodiversity reasons, and second, to adapt species composition and the genetic properties of trees to maintain or increase wood or biomass production, thus securing the economic value of forests and forestry. These main goals often overlap, and one adaptation activity may contribute to both goals; however, any human-facilitated adaptation strategy must be evaluated against both in order to avoid backlash in either biodiversity or economic productivity.

Potential to use assisted migration

Around the globe, the area of planted forest now accounts for 7 % of total forest area, with the highest proportion in Asia and Europe (almost 20 % and 8 % respectively; FAO 2014). AM could offer larger prospects and economic benefits for countries in Europe, where the large amount of new forests planted each year suggests a high potential for this practice. Results of simulation studies show that the European forest system is very inert and that it takes a long time to influence the populations and species distribution by replacing them after final felling (Schelhaas *et al.*, 2015). It is expected that under business-as-usual management, on average only about 36 % of the area expected to have decreased forest suitability will have changed in population or species structure by 2070 (Schelhaas *et al.*, 2015). This underlines the urgency for acting and the need for alternative management. However, shorter rotations and species changes based on

expected climate trends is estimated to only slightly increase the area with changed population and species structure (Schelhaas *et al.*, 2015). In addition, close-to-nature silviculture and the active promotion of natural regeneration of selected populations has been advocated as being the possible approach to managing forests to cope with future climate change (Brang *et al.*, 2014).

Large-scale transfer of reproductive material is common practice in forestry and is not only limited to recent history (Pâques 2013). Large imports of non-native FRM has occurred from the 19th century onwards, partly due to prior deforestations connected with charcoal production, extraction of timber, production of tar and pitch, and activities of big seed trading companies (Myking *et al.*, 2016; Jansen *et al.*, 2017). Cases of extensive failure of introduced non-native FRM, awareness of adaptive traits which were developed increasingly in the 19th century, and knowledge about provenance variation in the 20th century, have led to more targeted imports of FRM, aiming specifically to improve climatic adaptation, trait qualities and growth. This prompted the development and update of national regulations and guidelines to control the transfer and use of FRM. Due to the geographical scales involved, transfers of FRM have undoubtedly affected local genepools, especially for species such as Norway spruce, Scots pine, common beech, and sessile and pedunculate oak, although relatively few examples of adaptive failures due to transfer have been reported (Myking *et al.*, 2016).

Available decision support tools for FRM transfer

The transfer analysis of forest reproductive material (i.e., modelling and forecasting responses based on provenance trials, studies on former transfers and field trials) is a useful tool for investigating the effects of environmental changes on adaptive responses, growth and health. As part of this analysis, the phenotypic response of a provenance across test sites is interpreted as a norm of response to changing climatic conditions or to a transfer function, and a combination of both as a universal response function (Koralewski *et al.*, 2015; Chakraborty *et al.*, 2016, Rosvall 2011). Unfortunately, on a practical scale only a few systems utilise genetic information and developed functions. The Swedish-Finnish collaboration on transfer effect models for Scots pine takes climate change predictions into account in the models, including growth and survival predictions depending on geographical coordinates and altitude. The models use high-resolution gridded climate data and can predict performance in future climatic conditions. These are now implemented in the deployment recommendations. Other tools are based on general information and rely on genetic tests developed for the present climate conditions. Some of the recommendations have been published as classical documents for particular species and provenances

(e.g., in France and in Poland) and some have been developed as web applications. In France, it is planned to continually update these recommendations, adapting them to the developing knowledge on the adaptive capacities of different FRM and integrating any developments in climate scenarios.

Advanced decision-support tools can be very specific in their recommendations and rankings of various basic materials on both a species and genetic level (e.g. ranking of different basic materials suitable for a specific site). One risk associated with a very strict compliance with such recommendations is that the most highly-ranked basic material will dominate in reforestation activities in a certain deployment area. This could reduce diversity and increase the risks connected with increased homogeneity.

At present the counselling systems are only accessible in national languages. For example:

- In Denmark (plantevalg.dk) is a web-based user oriented advisory system. Recommendations are linked to location and specified site conditions. Information is available on all certified Danish seed sources and commonly used imported seed sources. The information and recommendations are based on trials and experience, and cover the present climate. They are made by the University of Copenhagen. No transfer functions or schemes for assisted migration have been developed or implemented in the system.
- The *Planter's Guide*²³ is a tool developed for Sweden that helps the user to choose seed orchard basic material of Scots pine, Norway spruce, lodgepole pine and silver birch based on the latitude, longitude and altitude of the intended site. Recently it was extended to include Scots pine recommendations for Finland and Sweden together. The tool is an application of the breeding and transfer programmes for the species concerned. There are several sources of detailed information on the structure of breeding programmes and how geographical transfer is used in them in order to optimise the genetic gain; i.e., plant survival and stem volume production.
- TREES4FUTURE climate matching tool²⁴ uses a method modified from the approach published by Broadmeadow *et al.*, (2005). It is based on the assumption that, by knowing where a species occurs, it is possible to infer suitable climate space and

²³ <http://www.skogforsk.se/produkter-och-evenemang/verktyg/plantval>

²⁴ <http://193.185.149.20/t4f/cmt>

thus estimate the potential geographical distribution using seasonal phenology and relative abundance. The tool identifies locations around the world where the current climate is most similar to the future climate of a selected European site for a range of possible climates.

- In Slovenia, a set of EUFORGEN Technical guidelines for conservation of forest genetic resources has been translated, and for each item a Slovenian addition has been prepared introducing recommendations for their production and use in Slovenia²⁵.
- On the regional scale of Central Europe, a complex decision-support system for the transfer of FRM under climate change has been developed within the project SUSTREE²⁶. The project aims to integrate into a single system detailed high-resolution climatic data, climate-change scenarios, national registers of Basic Material and transfer models, relying on the results of the provenance experiments. In addition to transnational seed zones, a smartphone or web-based tool (SUSselect) has been developed allowing the forest manager to choose the most suitable FRM for any particular reforestation site across the whole region under the consideration of future climatic conditions. Currently the tool focuses on seven ecologically and commercially most-important tree species. Pilot implementation is planned in Austrian Federal Forest Enterprise, but after the testing phase the system should be applicable in the whole region covered by the project.

While following EU, national and regional laws, European countries apply their own methodological approaches for the delineation of provenance regions, and consequently seed transfer, to ensure the use of the most appropriate FRM in terms of productivity, quality and adaptability. The countries are divided up into provenance deployment zones or regions, within which recommendations for the use of FRM have been established. National differences can seriously interfere with the development of strategies for tree species-wide conservation and for transnational adaptation of forest ecosystems under climate change. Future European/Regional/National regulations therefore need to take climate change into account, and should make use of widely-available genetic and phenotypic data for more science-based recommendations.

²⁵ <http://www.euforgen.org/member-countries/slovenia>

²⁶ <https://www.interreg-central.eu/Content.Node/SUSTREE.html>

Some examples of national provenance recommendations

→ Germany

In Germany, the forest administration of each federal state is responsible for the recommendations concerning FRM use. These recommendations are based on the comparison of the site conditions where the basic material originates from and where the FRM will be used. The results of provenance trials and progeny tests for only a few species have been used to develop more sophisticated recommendations, in which both geographic transfer and use of improved material are covered (Liesebach, 2020).

→ Norway

In Norway, the Forest Act defines the framework for the use of FRM, while the National Designated Authorities specify its use. Almost 90 % of FRM comes from seed orchards, and its transfer and the choice of deployment regions are based on field trials and progeny tests. In addition, the growth rhythm for new seed harvests is tested for epigenetic effects when determining the deployment regions for the material. A web-based tool for selecting FRM is also available on the Norwegian Seed Centre website (http://www.skogfroverket.no/side.cfm?Id_kanal=7).²⁷

→ Slovenia

In Slovenia, the Forest Act (1992 with subsequent modifications) defines the use of FRM, while the subordinate regulations recommend provenance regions and elevation zones in which the (less appropriate, appropriate, most appropriate, or exceptionally appropriate) FRM can be used for planting and sowing. The recommendations for the use of FRM are regularly adapted to new data (first based on Kutnar *et al.*, 2002, modified based on Westergren & Kraigher, 2007, and further modified based on expertise information from Westergren & Kraigher, 2017, pers. comm. on FRM from neighbouring countries).

→ Italy

Because of its genetic hotspot status, Italy is one of the richest countries in terms of species and intraspecific diversity of forest tree species. Several items in the national law recommend that genetic aspects be accommodated during FRM selection and production. This is an important tool for properly managing genetic resources, especially those from marginal populations in view of adaptation needs.

²⁷ <http://www.skogfroverket.no>

The national Legislative Decree 386/2003 delegates Regions for managing FRMs. Genetic aspects are also considered in the definition of provenance regions. In order to highlight possible within-species differentiation patterns, the delimitation of provenance regions therefore ensures both ecological homogeneity and collection of genetic information for the following main species: *Abies alba*, *Prunus avium*, *Quercus* spp., *Pinus* spp., *Fraxinus excelsior* (Ducci *et al.*, 2005). Special attention is now paid to the effect of climate change on the isotherm shift and on provenance region modifications (Marchi *et al.*, 2013, 2016). Particular attention is thus paid to the FRM production of endemic and/or endangered species and populations, such as *Pinus heldreichii* H. Christ. (1863), *Betula pendula* Roth, *Abies nebrodensis* (Lojac.) Mattei or/and *Zelkova crenata* (Pall.) C. Koch and many others (Ducci *et al.*, 1999; 2004).

→ Greece

In Greece, FRM production is regulated by the Presidential Decree 17/2003, in compliance with the Council Directive. Furthermore, Law 1564/1985 regulates the organisation of the production and trading of plant reproductive material, while Ministerial Decision 303206/1997 defines the requirements for granting the within-country trading of plant reproductive material. The country is a biodiversity hotspot with around 5.800 plant species; 400 of these are forest tree species, many of which are endemic (Alizoti and Aravanopoulos, 2019). Rear edge peripheral and ecologically marginal populations can be recognised for all the species growing naturally in the country. Several species meet their natural distribution limits in Greece, such as Aleppo pine (*Pinus halepensis*), Turkish pine (*Pinus brutia*), Scots pine, common beech, silver fir (*Abies alba*), Norway spruce, chestnut (*Castanea sativa*) and wild cherry (*Prunus avium*) (Curtu *et al.*, 2017). For all the above reasons, one third of the land mass of the country is covered by various types of protection status. The autochthonous forests of the country are sustainably managed and naturally regenerated. When artificial regeneration is necessary, FRM of the same origin is used. In the unfortunate case of mass destruction of populations due to stochastic events (e.g., mega-fires), the closest provenance will be used. In any other case, the most appropriate provenance will be planted, based on the results of existing provenance trials (if the variation among species populations has already been tested) and genetic data (Alizoti *et al.*, 2019).

More examples of national provenance recommendations can be found in Konnert *et al.*, (2015).

4. FOREST ESTABLISHMENT METHODS

Proietti, R., Schneck, V., Pilipović, A., Ugglá, C., Himanen, K., Maaten, T., Bordács, S., Kraigher, H., Tollefsrud, M.M., Friis Proschowsky, G., Yüksel T., Sperisen, C.

Forest establishment and management methods may strongly influence the genetic structure of forest tree populations. The first choice of method is between natural or artificial (active sowing or planting) regeneration, an aspect that has been discussed in the introduction of the report. The many choices for producing and selecting FRM or artificial regeneration are described throughout this report. This chapter on establishment methods discusses the different issues that forest managers face regarding site preparation, number and density of seedlings planted, plant protection against pests, insects and herbivores, tending the young stands, and enrichment planting. These are all factors that may have a strong impact on tree survival and juvenile growth, thus potentially influencing the genetic structure of the new stand. For the survival of the seedlings and for the economical outcome of the operations, it is always important to choose the regeneration method (natural, direct seeding, planting) and the FRM in accordance with the site at hand. Site fertility, topographical and climatic conditions need to be considered in silvicultural decision making, in the choice of tree species regenerated, the choice of genetic characteristics of FRM and in the size of planted seedlings.

Site preparation

Removing remnants of wood harvest and tillage from the site promotes both artificial and natural regeneration. Site preparation using various forms of soil scarification and mounding will contribute to planting success, direct seeding and active natural regeneration. However, removals could also have a negative effect on the establishment of natural symbioses on seedling roots. The principle effect of soil scarification is the exposure of mineral soil by removing the humus layer or by creating a mineral soil layer on top of the humus layer (Sutton, 1993). There are several factors which contribute to the generally positive effect of soil scarification on plant survival and growth:

- A decrease in pine weevil (*Hylobius abietis*) damage (Örlander & Nilsson 1999; Hannertz *et al.*, 2002)
- An increase in root-zone temperature (positive in northern countries, but negative in southern regions)
- An increase in soil root-zone porosity (depending on soil type)

- An increase in nutrient decomposition and availability (but also the leaching of nutrients)
- A decrease in competition from other plants (but also the facilitation of plant growth promoting microbiome and mycorrhizae)
- A reduction in night frost caused by outward longwave radiation
- The optimisation of water supply (but it can also induce faster drainage and therefore possible drought conditions)
- The simplification of plantation work

In Mediterranean regions water availability is often a limiting factor, and climate change could reduce and change the seasonal quantity and distribution of rainfall. In these regions soil preparation is crucial to optimising the infiltration and storage of precipitation in subsoil, and to increasing water availability for young plants (Garfi *et al.*, 2002). In mountainous areas, tillage can also prevent surface water runoff, protect from erosion and channel rainfall (water harvesting techniques). Since the effects of soil scarification can have negative consequences - such as higher mineralisation and transpiration, and elution of nitrogen - it is not common practice in all regions. Deep soil tillage could reduce the biological activity present on surface layers and upset nutrient cycles (reduction of organic matter or its transfer to deeper layers). In Germany, the forest certification systems do not allow the exposure of mineral soil during regeneration operations, due to the aforementioned negative effects. In addition, conservation tillage (techniques that reduce the overturning of soil horizons) can reduce erosion caused by water and wind (Tan *et al.*, 2015).

In an Aleppo pine plantation, in a semi-arid environment, adding organic matter along with scarification was found to improve the nutritional status and development and post-planting survival rate (Querejeta *et al.*, 2008). Superficial tillage (maximum depth of 30 cm) could be conducted after ploughing or scarification, to facilitate contact between root and soil, or to remove any weeds.

On superficial and poor organic soils, tillage can only be applied in areas where young plants are planted (Baldini, 1992), in order to open planting holes (with dimensions of at least 40×40×40 cm, to allow the young roots to rapidly reach deep soil moisture). In this way, the loss of mineral nutrients is reduced, especially in hot arid environments, and there is better soil protection from erosion (Garfi *et al.*, 2002). In mountainous areas with soil that is not excessively clayey, and slope not more than 30-40 %, tillage usually follows the land contours, and superficial work is carried out along the slopes to avoid landslides. On slopes with a gradient higher than 40 %, terraces (width of 1.5 to 3 m) often follow the land contours. This type of tillage allows a better retention of meteoric waters, avoiding rushing and erosion down the slopes. Soil conditions are

often altered during the preparation of sites for planting, resulting in increased seedling survival and thus minimal loss of the genetic variation that was initially stored in the seedling genetic material. However, in some cases, depending on the site (e.g., with steep slopes and shallow organic soil depth), site preparation may have negative results, such as oxidation of organic soil and loss of minerals, nitrogen and nutrients. In such cases, the survival of those seedlings having the genetic potential to adapt to the above conditions will be favoured, influencing the genetic variation of the remaining seedling population. For this reason, as well as to avoid soil erosion and depletion, intensive site preparation is not allowed on steep slopes in several Mediterranean countries, nor in the Alps (Puhe & Ulrich, 2001), and planting in planting holes is applied. Intensive site preparation methods can minimise the local population's genetic effects due to the low survival rate of the individuals (seeds, saplings, vegetative parts, etc.) which remain from previous stands. On the other hand, the complementary natural regeneration of various species is often promoted as a result of site preparation, and may thus increase diversity on the genetic and species levels. Consequently, depending on the effects of site preparation on already existing individuals and on complementary natural regeneration, the resulting genetic variation on the inter- and intraspecies level may be altered in either way.

Plant protection

A range of herbivores pose a well-recognised threat that may lead to severe damage after out-planting or other regeneration measures. A genetic variation in the resistance of seedlings to pine weevil (*Hylobius abietis* L.) damage has been observed. In Norway spruce, an insect-damage study of 52 open-pollinated families in two progeny trials revealed a consistent variation between the families across the trials and under different feeding pressures (Zas *et al.*, 2017). A strong genetic variation in pine weevil damage has also been found in radiata pine (*Pinus radiata*) seedlings (Zas *et al.*, 2008). Thus, the genetic diversity and quality of FRM plays a role in ensuring out-planting success and in protection from pests.

A particular concern is wildlife browsing, which may prevent both natural regeneration and planting success in areas in which ungulates live in high densities. Protection from such browsing can take the form of individual protection (Figure 25), usually by using rolls or spraying repellent, and area protection by installing fencing.

The use of appropriate progenies for forest regeneration is also important in preventing browsing damage. In a silver birch trial in southern Finland (latitude 60°), different progenies ranging from latitudes 53°–67° were compared: it was observed that the level of moose damage was more severe in trees with origins the furthest from the

site (Viherä-Aarnio & Heikkilä, 2006). The average proportion of trees browsed by moose varied between 6 and 86% among the seed origins, with the lowest levels of browsing in local and slightly more northern progenies, possibly related to changes in digestibility and palatability.

Direct seeding

Direct seeding is an effective reforestation method used to establish forest stands with a high adaptive potential. It generally causes less reduction in genetic variability compared to using seedlings, whose genetic diversity may decrease along the nursery chain as a result of nursery conditions and production methods (Ivetić *et al.*, 2016). Due to 'on-site' seed germination, the genetic diversity of the starting population is only affected by the selection pressure of local site conditions. The success of direct seeding depends on the species and the site conditions. Difficult site conditions, drought periods during germination, and competition by weeds can all exert strong selective pressure on the population. Furthermore, wild boars, rodents, birds and even insects cause major losses in all the tree species used in direct seeding (e.g., Nystrand, 1998; Madsen & Löf, 2005). Therefore, only a small proportion of the seeds in direct seeding are likely to successfully develop into seedlings.

Direct seeding is widely applied in the (sub-) Mediterranean regions of Europe after forest fires, and in some countries (such as Slovenia) it can involve seeds of more than 30 forest tree species per site. The direct seeding of beech and oak is an important regeneration method in forestry in central Europe. In Germany, it is used to establish deciduous species in older pine and spruce stands as a method for forest conversion. The advantages of direct seeding include the undisturbed development of the root system of the young plants and the fact that the adaptive potential of the offspring is not bottlenecked by improper handling or storage of seedlings.

In northern Fennoscandia, direct seeding is a common practice for Scots pine, although its popularity has decreased in Sweden, where it now represents only three percent of the reforested area. This method is mainly applied in the northern-most part of the country, where it represents about 10 % of the reforested area. In Finland, direct seeding on pine is done annually on 20,000 ha - one fifth of the annual regeneration area - and consumes two thirds of the annual tree seed use in the country. Systems currently under development include so-called seed pads and seed pucks: a conifer seed is baked into a pad or a puck which will be put in the uppermost layers of exposed mineral soil. These techniques reduce the amount of seed used for forest regeneration compared to classical direct seeding, thus allowing the use of improved seed in larger areas.

FIGURE 25. Individual plant protection of one of the last 30 natural Sicilian fir trees (*Abies nebrodensis*) in the Madonie regional park, Palermo, Sicily. The fence is made to protect the renovation from pasture and accidental human trampling. (Photo credit: Francesco/Wikimedia)



While this system may have a negative effect on genetic variability compared to classical direct seeding, the selected quality of the material can promote adaptation to specific local conditions or to climate change effects.

Plant density and minimum number of plants

The number of plants in a stand will affect their genetic structure and diversity. As forest tree species are characterised by long biological cycles, high genetic diversity of used FRM is also recommended in productive plantations to improve their resilience over time, even if this could lead to a production decline. A high level of genetic diversity is necessary in material used for stands with non-productive functions (Thomas *et al.*, 2014). Furthermore, growth performance and wood quality are influenced by spacing (planting density). The initial number of plants used for establishing new forest stands differs significantly between regions in Europe and depending on the tree species.

Many European countries regulate the minimum number of plants used for artificial regeneration or afforestation. For example, in Hungary, the minimum number of plants is stated in forest legislation. The numbers differ depending on the species and regeneration methods used. With a higher number of plants, adaptive potential is expected to be better for offspring populations.

In Sweden, around 90 % of plantations meet the requirements for minimum number of plants according to the forest act, often thanks to supplements of naturally regenerated plants. In accordance with the law, a minimum number of acceptable plants is set based on site index. Naturally regenerated plants of certain species may be accepted if they perform well. The minimum number varies between 900 (poorest sites) and 2300 plants (richest sites) per hectare. In general practice, between 2000 and 2500 plants are planted per hectare.

In Germany no regulations exist for the number of plants for reforestation. Recommendations are given for the minimum number of plants used in state forests and afforestation measures. Depending on the species these numbers differ significantly (e.g. 10-12 for Scots pine to 2000 for Douglas fir).

Enrichment planting

Enrichment planting is a valuable approach for maintaining or increasing the biodiversity of tree species or within-species genetic variation in forest stands (Brang *et al.*, 2014). With respect to establishment, the term enrichment planting is used when

referring to the planting of seed or seedlings in gaps in an already growing forest stand where natural regeneration has failed (Wickneswari *et al.*, 2014).

In Turkey, for instance, enrichment planting is principally used in gaps and patches where the natural regeneration success is lower than 80%. Site conditions permitting, other species are planted to create mixed stands, such as enriching natural regeneration of Austrian pine (*Pinus nigra*) with oriental beech (*Fagus orientalis*) (Odabaşı, *et al.*, 2004). In Central Europe, the underplanting of mature stands with local, site-adapted species, such as common beech and silver fir, is frequently carried out to convert adult monocultures of Norway spruce into mixed stands (Spiecker *et al.*, 2004), or to transform even-aged stands into multi-layered forests with a richer vertical structure (Puettmann *et al.*, 2012).

Enrichment planting can also be seen as an option to increase within species genetic diversity on which natural selection can work (Kolström *et al.*, 2011). In a framework of evolution-oriented adaptive forestry, enrichment planting aims to facilitate the emergence of new genetic combinations and to facilitate the spread of the best-adapted genotypes, as well as to secure the conservation of genetic diversity to enable long-term selection (Lefèvre *et al.*, 2014).

Tending of young stands

The number of individuals during the establishment phase of a forest stand is much higher than at the end of a rotation. This is true for planted stands and even more so for natural regeneration or direct seeding. In the case of natural regeneration, several tens of thousands of seedlings per ha are possible, and in that of planted stands the initial number of plants can be between 1000 and 10,000 seedlings per ha. In order to maintain sustainable growth performance and to ensure stability, regular tending and thinning are necessary during the lifespan of a stand to give the trees enough space to grow. Such silvicultural activities not only improve the seed productivity of trees, but can also increase their genetic variability. However, an excessive reduction in parental tree number will decrease the amount of genetic diversity within a new stand (Lyngdoh *et al.*, 2013), which could lead to a decrease in the ability of the future forest to adapt, resist biotic and abiotic stress and survive in the long-term (Ivetić *et al.*, 2016).

Janßen and Nowack (2001) investigated the influence of different thinning regimes on the genetic structure of beech stands. In their study, the genetic diversity of thinned stands (Figure 26) was higher than in unmanaged control plots. In a study about the genetic effects of harvesting on old stands of eastern white pine (*Pinus strobus*), genetic diversity was reduced significantly after removal of 75 % of the trees (Rajora

et al., 2000). The number of alleles had decreased by 26% with a significant loss of rare alleles. On the other hand, in a study on the effects of pre-commercial tending and commercial thinning of younger Scots pine stands, Danusevicius *et al.*, (2016) found that both methods had a minor influence on the heterozygosity and allelic diversity, but caused a significant loss of rare alleles. They assumed that it could be possible to identify the margins for a slow rate of rare allele loss.

In the case of monoclonal plantations, tending is not a crucial factor in terms of genetic considerations. Tending could be important if a stand has been established by use of clonal mixture. However, the actual clonal composition within the stand may have resulted from processes related to local adaptation to site conditions; the selection of individual trees (clones) should focus on maintaining as high as possible clone numbers. A survey made on black poplar forest restoration stands showed that a clonal mixture composed of 40 individual clones was reduced to 4-6 clones after 10-15 years of planting (Bordács & Bach, 2014). This reduction in clone numbers basically depends on the adaptive potential (plasticity) of clonal components and the selection pressure on local sites. Initial tending operations had been carried out within the restoration stands at the age of 10-15 years, and silviculture management had focused on maintaining clonal diversity.

FIGURE 26. Thinned loblolly pine (*Pinus taeda*) stand, 15-year old and unpruned. 220 trees per ha remaining.
(Photo credit: Scott Roberts, Mississippi State University/Bugwood.org).



5. KEEPING RECORDS OF SEED ORIGIN IN ORDER TO IMPROVE FOREST MANAGEMENT - BRIDGING FORESTRY AND SCIENCE

Geburek T., Kraigher H., Gömöry, D., Wolter, F.

In view of increasing demand for forest goods and services, a substantial part of European forests will need to undergo more intensive management, which will involve the wise and sustainable management of forest genetic resources. As the production time (rotation) from planting to harvesting of forest trees is longer than in agriculture, and as many historic failures related to the use of inappropriate FRM have been demonstrated in Europe since the late 19th century (Nanson, 2004; Jansen & Geburek, 2016; Jansen *et al.*, 2017), an adequate site-adapted choice and use of FRM is crucial for forest growth.

In order to evaluate the performance of FRM in the long term, it is necessary to keep information about its origin. At a policy level, this information is beneficial for the assessment of forest biodiversity, distribution and allocating financial (public) incentives, as well as for regulating or even restricting the use of FRM and for keeping track of an international FRM transfer. If a chain of custody from the origin of a seed used at a certain planting site to all goods produced over the rotation time (accumulated value) were to exist, forest management would also benefit, as the use of FRM could significantly improve forest stability and productivity.

In the first Ministerial Conference on the Protection of Forest in Europe, held in 1990, the Signatory States committed to implementing a policy for the conservation of forest genetic resources in their respective countries that would cover, for example, “the keeping - at least for public forests - of records covering the exact identity of the reproduction materials used for planting and regeneration” (see FOREST EUROPE, Resolution S2). However, the practical implementation of this resolution has not always been followed through. For example, in Slovakia, both state and private forest owners must keep copies of all used FRM master certificates, and they can be controlled by state forest authorities, as well as by the official body. However, this obligation only lasts for as long as the validity of the forest management plan. The records are destroyed after a maximum of ten years and therefore the local knowledge of appropriate use of FRM cannot be easily be sustained. In Slovenia, records are kept in the forestry district archives, which are not easily accessible, and the precision is not unified. On the other hand, in Poland, a concept of registered progeny plantations

(‘*uprawy pochodne*’) was introduced in 1988: large plantation blocks (up to 30 ha) are established from seeds collected in the best approved seed stands and seed orchards, and their performance is monitored. In this case, not only is the information about the origin of FRM ensured, but the seeds are also collected in such a way as to be representative of the genepool of the respective maternal stand or seed orchard. If the politically binding resolution had been correctly implemented by the Signatory States, and the performance of FRM had been correspondingly recorded, a wealth of important data would have been available. Such FRM could then have been used to address the negative aftermath of climate change

The meaningfulness of any record-keeping for FRM strictly depends on the trustworthiness of the data. Such records range from information about the basic material and the respective seed harvest to the performance of the plant material in the field. Unfortunately, the identity of seed sources in many Member States cannot be guaranteed by official documents. Although seed harvest and trade are regulated in the Council Directive, even proper documentation cannot impede fraud. It is difficult and mostly impossible for the end-user to track down the identity of FRM. Consequently, in some countries, such as Germany, organisations under private law have been established²⁸ or particular additional governmental regulations have been established. In Slovenia, for example, plant samples from each seed tree must be collected and sent by the authorised field officer to the Slovenian Forestry Institute for potential control (e.g. Westergren *et al.*, 2017). Both private and governmental systems aim to guarantee that the end-users receive the FRM which they paid for (e.g. Konnert *et al.*, 2015). Thus, an important prerequisite for improving the use of FRM is that the end-user can trust its identity.

Adaptive Management

Existing provenance recommendations are mainly based on climatic or biogeographic zonation in Europe and on practical experience; only in relatively rare cases are they based on the outcome of provenance trials. While a main objective in provenance research is still to identify vigorous and sufficiently-adapted provenances to serve as seed sources for reforestation (König, 2005), it is becoming increasingly difficult to maintain long-term field trials. This is because institutions tend to cut the budget needed to maintain the trials, and funding agencies rarely support the establishment of new experiments.

²⁸ e.g., <https://ffv-zertifikat.com>

As strong genotype \times environment interactions are typical for forest trees, provenance performance is seldom universal under different environmental conditions. This makes provenance recommendations difficult, even when based on results from provenance trials, as the number of provenances used and of the different test sites is very limited, even in international trials. As a result, even though available for the main forest tree species in most European countries, provenance recommendations are still imprecise, and effective ways to fill that gap need to be developed and deployed.

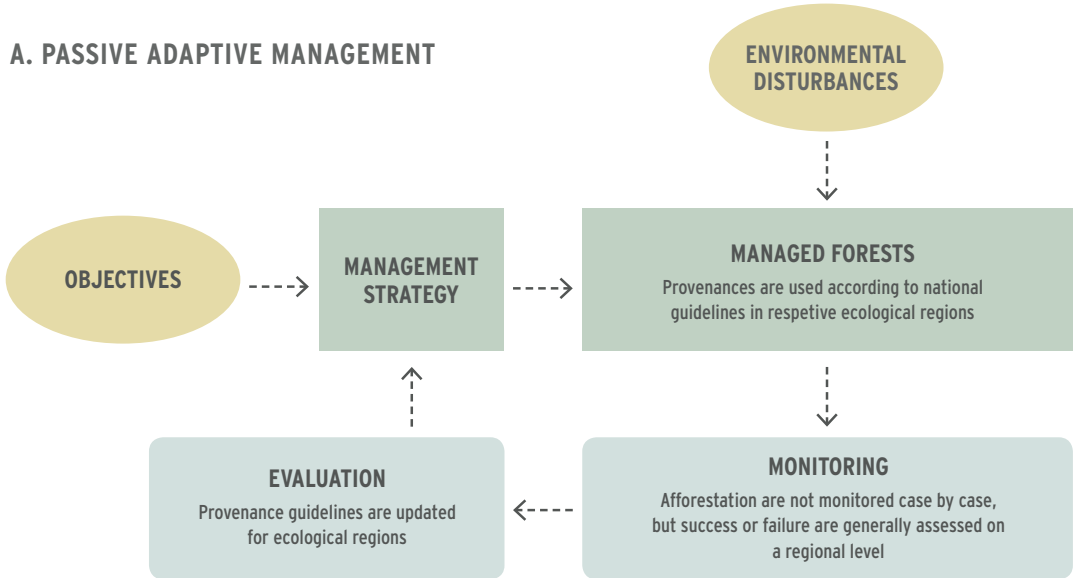
When natural resources are managed in uncertain conditions, as is the case for FRM, the concept of Adaptive Management comes into play. Originally developed by Holling (1978), the concept has often been misapplied and misunderstood. Many managers believe that management is adaptive by definition and refer to the willingness to change, while others equate simply flexible action to Adaptive Management (Halbert, 1993). The easiest way to describe the concept of Adaptive Management is to first refer to two other common approaches: trial and error ('learning by doing') and deferred action.

Trial and error is still one of the most applied approaches, which normally leads to small changes over time and is relatively inexpensive in the long run. However, it undervalues the importance of reliable information so that data are often poorly collected, if at all. Deferred action is a cautious approach in which measures are only taken after their effects are fully understood. As far as FRM management is concerned, this approach is unattractive to forest managers as it has high economic costs, because future revenues can hardly be considered. Adaptive Management is fundamentally different to these two management approaches because monitoring is a very important and indispensable element in it.

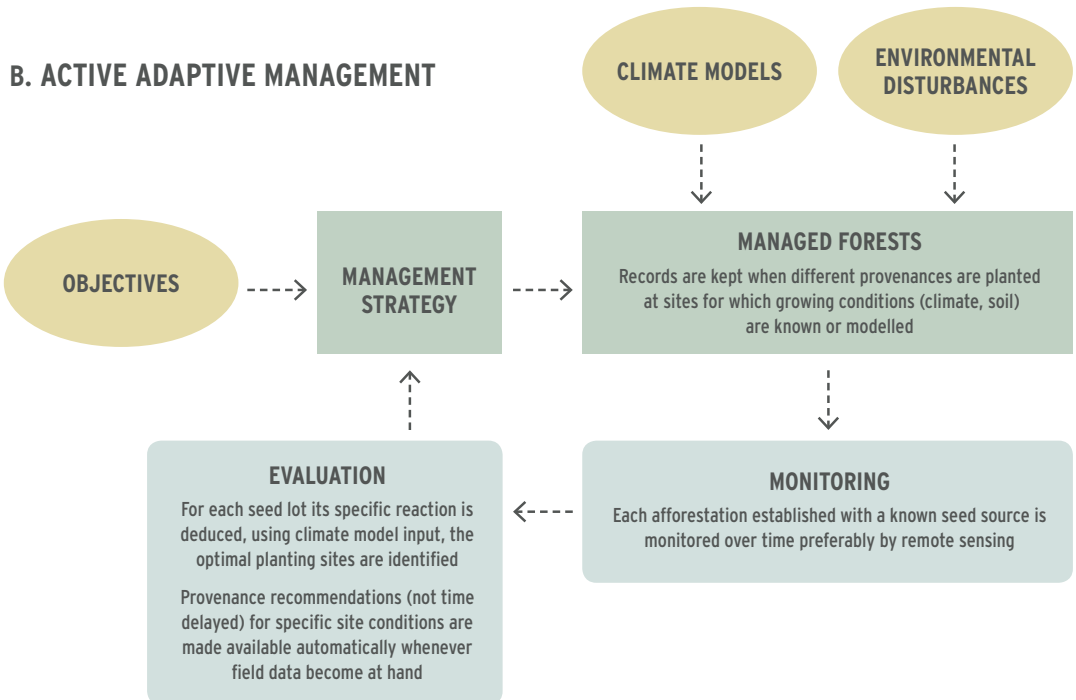
There are two different forms of Adaptive Management; passive and active. In the **passive** form, monitoring is conducted without replication and randomisation; it therefore has the flaw of not being able to identify cause-effect relationships. The **active** form is conducted in a similar way to a well-designed experiment and the results are essential for management decisions (Figure 27).

FIGURE 27. Scheme of passive (a) and active (b) adaptive management. Credit: T. Geburek.

A. PASSIVE ADAPTIVE MANAGEMENT



B. ACTIVE ADAPTIVE MANAGEMENT



Assuming that the identity of the provenance used for reforestation at many different sites is correct, and that it is possible to monitor its performance, the resulting information can be fed into a genetic model. Ideally, a transfer function for each provenance can be developed by relating performance to the transfer distance between the provenance and a given planting site; i.e., the difference between the environmental conditions at the planting site and those at the seed origin. As planting sites differ in their environmental conditions (soil properties, temperature, precipitation, orientation, and inclination, etc.) the more reforestations carried out with the same provenance, the better the precision of the transfer function. Ordinary kriging (Hamann *et al.*, 2000), multivariate regression trees (Hamann *et al.*, 2011), or canonical analysis (St. Clair *et al.*, 2005) may be used to predict zones in which the specific plant material should be planted using GIS methods. In typical provenance trials, information about whether the seed sample used for the trial is fully representative is often unavailable. Even if the number of seed trees is known, and the seeds were collected in a full mast, genetic differences between ripening years cannot be excluded (Müller-Starck and Ziehe, 1984). The proposal described here could circumvent this hindrance, as the performance of a provenance is ideally based on several planting sites with seed lots from different ripening years. The quality of the provenance assessment could increase (i) when the species is not planted in a mixture, (ii) with a higher number of planting sites and seed lots from different ripening years, and (iii) with increasing age at planting. Ideally, the genetic model should measure performance according to age, site amount, ripening year, etc., and should regularly and automatically update the modelled provenance recommendations over a certain time period (3-5 years) depending on the availability of respective performance data. Thus, provenance recommendations would probably change continuously depending on the amount of monitored data at an early stage and would later approximate the best provenance choice. The rating of provenances would be constantly re-adjusted over time relying on the availability of new performance data.

This approach has additional advantages to provenance trials, the most important of which is probably related to costs. Juvenile mortality due to competition in provenance trials is avoided, because the experimental spacing is often not identical to the spacing used in regular reforestation and competing vegetation is often rogued. Later, at an advanced age (>15-20 years), mortality cannot be assessed as experimental lots have to be thinned out, and the number of the remaining trees from each provenance may be too small to draw meaningful conclusions. The same holds true for the growth assessment in older age classes; generally, provenance data are valid for a juvenile age and provenance recommendations are based on juvenile performance assuming that the juvenile-age correlation is high. However, the reasoning given here should not be

misinterpreted: provenance trials are scientifically important but have certain limits when their results are used for FRM recommendations.

Data originating from regular reforestation are affected by differing site conditions, spacing, and mixing with other tree species. In addition, different thinning intensities may complicate the analysis; however, if recorded properly over time the total growth can still be estimated. These non-standardised growing conditions can only be compensated for by a high number of reforestation sites using the same FRM.

To date, data from regular reforestation has played a minor role when it comes to choosing provenances. Forest management often bases its choice on local experience rather than on scientific data. This is because field data collection is excessively laborious and hence resource demanding. However, more advanced technologies have drastically changed this situation: a reforestation area can now be easily digitised and documented, and its future development can be simulated - even by using smartphone applications. The most important development is in remote sensing, but only rough growth characteristics can be assessed, while information regarding adaptation to ecological conditions (which are changing rapidly due to climate change), such as flowering, fructification and survival under natural regeneration, would need thorough calibration in the field.

By far, the biggest obstacle to date has been the efficient collection of growth data at regular planting sites. Neither forest enterprises nor scientific institutions have the human or financial resources to collect these data. However, advances are being made in remote sensing (e.g. White *et al.*, 2013) in several European countries, and tree characteristics, such as height and volume, can be assessed properly and regularly with sufficient precision (Parker and Evans, 2009) and constant improvement.

In Austria, for example, regular image flights make it possible to easily estimate both volume and height throughout the whole country. Growth data for an area of up to 10 km² can be made available to forest enterprises or other end-users and costing €400 (costs in 2018). When planting sites are digitised reforestations can be easily assessed for growth (personal communication, K. Schadauer, Austrian Forest Research Centre, 2018). The Austrian image flight cycle lasts three years; therefore, new growth data will become available within a three-year cycle. Switzerland, Germany and other countries may have similar accurate data from image flights. However, weather conditions at the time of the flight (every three years for different areas in Slovenia) can hinder reliability (D. Hladnik & M. Skudnik, Slovenian Forestry Institute, personal communication.).

In addition, laser scanning data can be used for Adaptive Management. In Sweden, for instance, forest owners can easily estimate growth by using data from digitised forest areas provided by the Swedish Forest Agency (Skogsstyrelsen²⁹). Within Member States, reforestation is partially financially supported (e.g. within the framework of European Agricultural Fund for Rural Development (EAFRD; established by the EU Regulation 1305/2013), or domestic programmes) and is often based on digitised forest areas. These data can be linked to growth data provided by remote sensing, thus contributing to bridging forestry and science.

Data collection at the international level

As well as record keeping for Adaptive Management, access to international FRM data could also be useful. As a recent EU-wide analysis of the transnational transfer of FRM has shown, sound basic data are often missing when FRM is moved within the EU (Jansen *et al.*, 2019). Not all official bodies in EU Member States responsible for FRM data storage can provide the respective data on request. Nevertheless, it has been possible to show that FRM from some forest species is moved within the EU in significant amounts. As the FRM trade is likely to constantly increase due to a growing internationalised market (Koskela *et al.*, 2014), appropriate record-keeping and easy data exchange is also needed in this sector. Moreover, the use of non-local FRM (assisted migration) could enhance future socio-economic and industrial needs and is also a promising means of increasing forest stability and wood production efficiency in the light of climate change (e.g., Dumroese *et al.*, 2015).

Recent efforts of the Commission following up EC 1597/2002 to create a Forest Reproductive Material Information System (FOREMATIS³⁰) are highly appreciated.

²⁹ <https://www.skogsstyrelsen.se>

³⁰ <https://ec.europa.eu/forematis>

RECOMMENDATIONS

FIGURE 28. Forest recommendations. (Photo credit: C.Giordano/EUFORGEN).



Introduction

The previous chapters comprehensively highlight the importance of FRM, covering the genetic aspects linked to their production and use. This coverage also provides the background for collecting scientific evidence for developing guidelines and decision support tools for effective FRM management. It also offers an in-depth review of the wide-ranging literature (474 references). All of this helps better inform conserving the diverse FRM resources and to more effectively use these to help meet increased demands and mitigate any negative impacts of climate change and other stressors. The authors have thus articulated the following 38 recommendations divided into those which are overarching, and others specifically relating to policy, research and FRM management.

Overarching recommendations³¹

1. Balance the goals of production and of genetic diversity conservation in all FRM management

Climate change poses several mitigation and adaptation challenges. FRM can be used effectively to ensure the production of biomass as a replacement for fossil fuels and as a carbon sink. At the same time, however, we must conserve the ability of forests to adapt to currently unknown and predicted threats and to maintain a high-level provision of ecosystem services. This requires striking a balance between using relatively small numbers of outperforming genotypes and using broader populations which help secure genetic diversity and thus adaptability.

2. Create an online information system for geo-referenced records of the origin, movement and use of forest reproductive material (FRM)

This information system would record the entire production and marketing chain of the FRM, as well as performance data when available. It would thus be possible to assess provenance performance under different environmental and management conditions, which in turn would allow transfer guidelines to be developed indicating the most appropriate material for each site and use.

Policy

3. Increase the knowledge about the potential benefits of not exclusively using natural regeneration in response to climate change [Ch. 1.2 & 3.1]

Many European forests are certified under two certification schemes: the Programme for the Endorsement of Forest Certification (PEFC) and the Forest Stewardship Council (FSC). In many countries, both schemes, as well as others related to the forest sector, have a strong preference for natural regeneration without considering additional regeneration methods. However, this may be insufficient to effectively adapt forest to climate change. An exclusive focus on natural regeneration constrains the possibility of speeding up forest adaptation to climate change, which can be promoted by introducing better-adapted FRM from other locations. The scientific community needs to communicate more effectively so that all stakeholders learn about the potential benefits of adding non-local genetic material to the regeneration, taking into consideration the rapid rate of climate change and urgent need for mitigation.

³¹ Not prioritized list

4. Communicate the importance of choosing appropriate FRM [Ch. 1.2 - 3.1]

We encourage relevant authorities to communicate with end users in order to improve their ability to choose appropriate FRM based on its origin, provenance recommendations and genetic diversity. Furthermore, knowledge about the suitability of FRM for 'assisted gene flow' and 'assisted migration' must be improved in order to implement these management strategies.

5. Promote planting with genetically variable material [Ch. 2.4 - 4]

Large-scale reforestation and forest restoration activities are being undertaken worldwide for biomass and timber production, to store carbon for climate mitigation and to restore ecological functions in degraded landscapes. When material is planted as part of these activities, we recommend that FRM (species, provenances) be selected for its production ability, as well as for its ability to maintain or enhance the forest's genetic variability and capacity to adapt to future climate conditions.

6. Comply with the obligation of sharing information about FRM movement between EU Member State countries [Ch. 2.7 - 2.8]

Official bodies of EU Member States are responsible for sharing records about FRM that is traded between countries. The information should be compiled in a standardised Information Document. We encourage countries to ensure that the transfer of Information Documents between Official Bodies is carried out effectively, thus complying with the Council Directive (1999/105/EC). Moreover, when possible, FRM for non-forest purposes should be handled similarly, ensuring it is labelled correctly and accompanied with all the related documentation, thus avoiding any risk of introducing them into the market of FRM for forestry purposes.

7. Highlight the importance of phytosanitary regulations and raise awareness on pests and diseases [Ch. 2.8]

Phytosanitary regulations need to be effective to prevent the introduction and spread of forest pests and pathogens via transport and trade. International phytosanitary regulations for transport, travel and trade should be in accordance in order to reduce or eliminate the risk of spread of severe pests and diseases. Moreover, awareness should be raised among professionals, forest owners and policy leaders in different countries. Existing phytosanitary regulations should be continuously updated with science-based knowledge.

8. Keep national and international registers updated [Ch. 2.7]

FRM placed on the market must come from approved basic material, which must

appear in a national register. National registers are an important source of genetic information. For EU member countries, the FOREMATIS database³² holds information from national registers about all existing FRM categories and types for all listed species. We remind all EU member country national authorities, OECD members and relevant stakeholders to comply with the obligation of keeping the registers on basic material updated and to share them. In addition, EU countries should regularly report any updates to FOREMATIS, ensuring that data can be trusted and used as a basis for making decisions about the use of FRM. All countries (EU, OECD members and others) are able to read data from FOREMATIS and all national authorities with access are encouraged to upload their data in order to simplify information sharing.

9. Keep records of the origin of forest stands [Ch. 2.4 - 3 - 5]

Countries are encouraged to keep long-term records of stand-level FRM origin and performance in order to make this information available for adaptive management. Monitoring the performance of FRM of known origin in forest stands can partly replace high-cost and labour-intensive provenance trials in providing large-scale recommendations for future use of FRM and management of future forests. Such recommendations could thus be based on genetic models for adaptive management and updated regularly (every 3 to 5 years) depending on the availability of the data (see Overarching recommendation number 2).

10. Develop decision support tools for transfer of FRM in response to climate change [Ch. 3.2]

The current speed of climate change outstrips the ability of tree populations to respond solely by evolutionary processes such as adaptation, gene flow and migration. As a result, the current Regions of Provenance, further restricted by national boundaries, may become an unsuitable framework for FRM transfer. Science-based decision support tools for the transfer of FRM are urgently needed. Such tools should use the best available scientific evidence and should accommodate regional and pan-European predicted future climate scenarios in order to provide a sound basis for the use of non-local FRM.

11. Improve FRM production, use and conservation by sharing good practices [Ch. 2.7]

All national actors involved in the management of FRM and other genetic resources have developed their own procedures and processes, and each has aspects of its

³² <http://ec.europa.eu/forematis>

operation that are good and others that are less so. It would improve the overall sustainable use of genetic resources if different organisations were to share examples of good practices. We encourage the development of pan-European guidelines for a better organisation of national actors, noting too that such guidelines would benefit any country outside of Europe wishing to ensure the sustainable use of FRM under climate change.

Research

12. Disseminate information about field trials online and in English [Ch. 2.2 - 3.2 - 5]

Informed decision making on the sustainable use of FRM relies on having access to field trial information about how material is tested, phenotypic and genotypic data, production data, the interpretation of results, and other relevant knowledge. Such information, accumulated by academic researchers and forest research institutes, may already be available in national sources and in various languages. We recommend that all this information be made more easily and freely available online and in English.

13. Create genetic models to identify the most suitable FRM [Ch. 3 - 5]

The analysis of FRM performance offers a useful toolset for understanding the effects of environmental conditions and change on forest species growth, health and adaptive responses. Such an analysis is based on approaches that include modelling and the results of provenance trials, previous transfers and field trials. We recommend that additional effort be concentrated on creating genetic models as elements of decision support tools for the identification of FRM suitable for transfer in response to climate change. These models should include a wider range of species and could also include epigenetic effects. New provenance trials in extreme environments would provide valuable additional information. Models could also take advantage of geographical information on existing instances of transferred FRM. The models would have to be updated regularly.

14. Emphasise adaptive traits in future studies [Ch. 3.1]

Current models dealing with FRM transfer are typically based on fitness proxies such as survival or growth. Although such traits are of primary interest for practical forestry, the resulting models are genetically complex and the relationship of the proxy traits to the environment is multifactorial. We recommend that future studies place greater emphasis on phenology and physiological traits, to obtain a more reliable picture of adaptive responses and phenotypic plasticity of populations, and their geographical patterns.

15. Develop tools to rapidly identify pathogenic species and share information [Ch. 2.8]

Rapid diagnostic kits should be developed or strengthened to more effectively identify pathogenicity of FRM, and related guidelines published so that phytosanitary inspectors and nursery managers understand the issues and are able to make use of the kits. In addition to improving the health of FRM, this recommendation also addresses the fact that existing phytosanitary regulations are not sufficiently effective. We recommend that phytosanitary regulations make more use of scientific knowledge and be continuously updated.

16. Study and elucidate the role of epigenetic phenomena in adaptation and diversity [Ch. 2.1.3 - 2.4 - 2.6]

Epigenetic phenomena associated with adaptive variation are currently ignored. The persistent effects on phenology of photoperiod and temperatures during embryogenesis have been well documented. In addition, carryover effects caused by early-growth environment have been demonstrated in juvenile stages. The production of seedlings in nurseries, where plants are raised under optimised microclimates, may affect the later performance of the planted seedlings. Research on epigenetics in trees has to a lesser extent addressed other adaptive traits such as responses to stresses (e.g., drought or heat resistance), topics that would be of interest to practical forestry and the sustainable use of FRM.

17. Study symbiotic interactions [Ch. 2.6]

Symbiotic interactions, particularly fungal mycorrhizae, can influence the performance and phenology of seedlings after planting. Because functional compatibility depends on the species and strain of microorganism, and the species and population (or clone) of forest tree, these interactions should be considered in the production and use of forest tree seedlings. In addition, research is needed on the role of associated organisms in the adaptability of forest trees to climate change.

18. Improve marker-based certification methods [Ch. 2.7]

New species-specific genetic markers for a wider list of species are needed. These should include more and better markers for more reliable results and technical developments to achieve faster results. Next generation sequencing methods for marker development and genotyping should make certification far more reliable in the future. To complement improved markers, we also recommend the further development of statistics and data evaluation methods for assigning test samples to reference data, for example standardised protocols and formatting of the results. A central database of results would also be of great benefit, potentially leading to common certification schemes and the organisation of DNA libraries.

19. More provenance trials for rare species [Ch. 3.1]

Provenance experiments should embrace noble hardwoods and rare stand-forming tree species in addition to commercially important tree species. In the past, provenance trials were established mostly for stand-forming conifers and broadleaves. To date very few provenance trials have been established and evaluated for noble hardwoods and rare stand-forming tree species, and as a result, the use of non-local material is still difficult to introduce at the local level for many species. We recommend that additional provenance experiments be established for these species.

20. Study the effects of environment and management on genetic diversity of FRM [Ch. 2.1.3 - 2.4 - 2.6]

The genetic diversity of seeds and seedlings may be reduced both by management practices and by chance factors during many phases of production. For example, cone pests may affect certain clones in a seed orchard more heavily than others, reducing their proportion in the marketed seed lots. The level of this kind of pest damage varies annually and geographically, making these effects hard to prevent and to recognize. Management practices may decrease or increase these effects. More research is needed on determining the actual levels of genetic diversity of FRM and the effects of the different production phases on genetic diversity.

Management: General**21. Record the origin and movement of FRM for traceability [Ch. 2.7 - 3 - 5]**

We remind FRM producers and dealers of the obligation to provide sound and clear documentation to customers, which correspond at a minimum to legal requirements (master certificate 1999/105/EC). Additional documentation, such as the size of the harvested population and selection criteria, should be provided with the FRM whenever possible. We also encourage forest owners to safely keep any documentation supplied with the FRM they receive for traceability and to facilitate adaptive forestry management. National authorities are also required to keep track of the documentation/certificate number and where it is used. They should set up an EU harmonised and open web-based recording system for FRM origin and movement.

22. Disseminate knowledge about the best use of FRM [Ch. 2.4 - 2.7 - 5]

Producers, dealers and authorities should communicate with end users in order to improve their knowledge and thus their ability to choose the most suitable FRM for their needs, based on origin, provenance recommendations and genetic

diversity. We also encourage forest owners to ensure that they or their staff know enough about the characteristics and origin of FRM to make wise decisions about reforestation. The meaning of the various categories of FRM should be described in clear terms, and for 'Tested' FRM the end user should have clear information about how and by whom the tests were performed, and what the results mean for the suitability of the material at a given site.

23. Increase the variety of approaches for testing FRM [Ch. 2.2]

Progeny-testing procedures need to be adapted to rapidly changing environments, both from the point of view of which traits are tested and the testing methods. Test environments should include climatically extreme sites to assess the potential suitability of the material for future climates. Whenever possible, assessment should be based on physiological traits, which typically exhibit close age-age correlations, and thus can substantially shorten the test duration. To speed up the tests and increase their potential scale, non-conventional approaches must be considered. These could include indoor and nursery tests performed under controlled conditions, and 'breeding without breeding,' which replaces laborious and costly controlled crosses by reconstructing the pedigree by making use of informative DNA markers.

24. Increase knowledge of phytosanitary issues in FRM [Ch. 2.8]

Current phytosanitary regulations are not sufficiently effective to prevent the spread of pathogens. Official controls and inspections (under phytosanitary regulations, the EU marketing directive and the OECD certification scheme) should be intensified and better coordinated to catch up with changing trade patterns and pathways for existing and new pests and diseases. This should include increased use of rapid diagnostic kits and risk assessments for new pathogens and pests should accompany the use of non-local FRM. We advise the use of seed, rather than plants or plant parts, to minimise the risk of uncontrolled movement of pests, especially for transfer over long distances. We further advise avoiding non-local FRM for non-forestry purposes, because it may be harder to trace back to its source. Planting materials should be vigorous along the whole marketing chain. Awareness of pathogens should be raised among professionals.

25. Seed companies are encouraged to keep reference samples of FRM for traceability [Ch. 2.7]

Some national regulations include the possibility of checking the identity of traded FRM with genetic markers. Customers along the chain may also request such checks. We encourage suppliers, traders and other actors to keep well-catalogued reference samples of FRM to facilitate traceability. Specific markers have been developed for

many common species; for these to be used for tracking, the sample of traded FRM must be compared to reference samples. Where possible, we recommend the use of leaves, buds, needles or cambium of individual seed parents; failing that, samples of each seed lot should be retained as reference samples. It is becoming more efficient to store samples as dried material or extracted DNA, rather than as frozen fresh material.

26. Maintain genetic diversity of FRM in artificial forest regeneration and forest management [Ch. 4]

To ensure that the genetic diversity of FRM is maintained, we recommend that forest owners and practitioners apply the silvicultural practices most appropriate for the site. Direct seeding can increase adaptive potential, compared to the use of seedlings, even if only a small proportion of the seeds develop into seedlings and adult trees. We also encourage activities such as enrichment planting, used in gaps and patches where natural regeneration is insufficient.

Management: Basic Materials

27. Plan management activities in seed stands to assess and conserve genetic diversity [Ch. 2.1.1 - 2.1.2]

In general, seed stand management should aim to conserve as much genetic diversity as possible. All silvicultural activities (such as plant protection, thinning and tending) should therefore be carried out with the intention of conserving genetic diversity, even if it may be necessary to cull negative phenotypes. The type of thinning and its intensity should accommodate the need to ensure sufficient pollen flow and thus cross fertilisation, especially in wind-pollinated species. Avoid or minimise the use of fertilisers and fungicides in seed stands in order to preserve below-ground microbial biodiversity. Seed stands may be approved for FRM of multiple species; however, the number of target species should still allow appropriate silvicultural practices. We further recommend regular monitoring of the status of seed stands and the outcomes of silvicultural activities, both to ensure the genetic diversity of FRM produced and to keep traceable records of the silvicultural practices carried out.

28. Favour collections during mast years and improve FRM harvesting techniques [Ch. 2.1.1 - 2.1.2]

Mast years (when trees produce an abundance of seeds) should be monitored and recorded and used for the collection of large quantities of seed. The seed should be gathered from many trees and groups of trees, preferably unrelated individuals,

across the entire stand, or at least document the number of mother trees harvested. Ensure professional and controlled mixing of FRM. Finally, the quality of the seeds (including germination of the stored seeds and their genetic profile) should be checked over time, and where relevant, the presence of hybrids should be noted.

29. Make available as much information as possible on Source-Identified and Selected Stand FRM [Ch. 2.1.1 - 2.1.2]

In addition to the legislative requirements, specific, relevant information about the genetic background and the origin of basic material should be made available whenever possible. This particularly includes the effective size of the population from which it was sourced. Data about the performance of the material from the selected stand, specifically related to its provenance (i.e., ecological adaptation and health condition), are very important for further reference. General information about number of stands per provenance region (or climate zone) and elevation zone in each country is also needed when relevant.

30. Manage seed orchards to promote genetic diversity and genetic mixing [Ch. 2.1.3]

Seed orchards need to be established with a enough clones or families to guarantee a high level of genetic diversity of seed orchard crops. This recommendation also requires the actual minimum numbers as defined in legislation or commonly accepted practice to be reconsidered. Whenever possible, high-performing ('plus') trees should be used to establish them. Selecting plus trees and genotypes with similar environmental triggers to flower would avoid precocious or late flowering and thus might improve genetic mixing among clones in a seed orchard. Flowering induction management methods and pest control may prohibit the loss of genetic diversity. Collection of seed should be avoided in poor crop years. To even-out seed lots, we recommend that seeds from different years be carefully mixed. The share of seed-orchard FRM may need to be carefully monitored during reforestation and afforestation to ensure that the material does not come only from a restricted pool of genetic diversity.

31. Produce high-value FRM from plus trees and trees included in breeding populations to widen the range of regeneration options [Ch. 2.1.4]

The identification and selection of plus trees generally aims to form a breeding population and to use the plus trees as parents in a seed orchard. The production potential of plus trees can be realised with greater speed and focus by controlled or open pollination to produce half-sibling and, even better, full-sibling families. In this way, rapid production of well-defined and tested FRM can be produced, albeit in limited quantities. We also highlight here the production option of high-performance and high-value FRM in the Parent of Families category of basic

material, supported and regulated by the Council Directive (1999/105/EC) and the OECD certification scheme,

32. In clonal stands, encourage clonal mixtures and controls on clone status [Ch. 2.1.5]

Unintentional mixing of clonal genotypes in a single lot of FRM should be avoided. This makes it essential to keep meticulous records and documentation along the entire production chain. Generally, clones of the ‘Tested’ category should be preferred, although to minimise ecological risks and increase landscape diversity, we recommend using mosaics of a variety of different monoclonal stands. The vigour of vegetatively-propagated plants should be maintained at all phases of production, and we advise regular control at each stage to test clonal identity, clonal purity, and plant vigour. We also suggest that national and regional legislation, along with common standards on clonal plantation forestry, be reconsidered in line with these recommendations.

33. Stricter controls on collection of FRM for own use and for purposes other than forestry [Ch. 2.3]

The EU directive on FRM covers only the material that is marketed for forestry purposes. Countries that allow the collection of FRM for own use need to gather and keep data on collection for which they should create guidelines. In areas close to conservation areas and populations of particular genetic value, extra care should be taken when choosing planting material for roadsides, ornamental purposes, and so on, to avoid genetic contamination of valuable populations.

Management: Seed Production and Nursery Practices

34. Avoid seedling production steps that decrease genetic diversity [Ch. 2.1.4 - 2.4 - 2.6]

The production chain of seedlings will impact the genetic diversity of the FRM that finally reaches regeneration or afforestation sites. For example, size-based culling may result in the loss of a significant proportion of seedlings; if seedling size is strongly linked to genotype, the effects on genetic diversity on the final lot of FRM may be considerable. We recommend that the impact of individual nursery practices on the population structure and genetic diversity be assessed as part of optimal FRM production.

35. Promote genetic diversity by controlled mixing of seed lots of different years [Ch. 2.5]

Genetic diversity may differ considerably between seed lots harvested from the same population in different years, for example as a result of poor flowering, heavy seed damage or inter-annual environmental fluctuations. We recommend that

when a seed lot is suspected of hosting a major genetic deviation from balanced harvesting, producers use the legal opportunity provided by 1999/105/EC to mix seed lots from the same basic material of different years. In this way, the genetic diversity could be increased and equalised from year to year. (Nevertheless, possible epigenetic effects, suggest that seed lots from years of extreme weather should be tested for unfavourable phenological traits or omitted from mixing.) Buyers and users of FRM should be aware that the genetic composition of a specific FRM seed lot may deviate considerably from that of the basic material. We therefore encourage them to request information about any such major deviation.

36. Discourage seed fractioning to maintain genetic diversity [Ch. 2.5]

Sorting seed into different fractions, for example by size, weight or other properties, is common practice in the cleaning process. Such fractioning potentially reduces the genetic diversity of a seed lot or gives it a different genetic profile, but the sorted seed lots are still identified as having the same properties as given on the Master Certificate. We encourage seed producers to be transparent with customers and authorities on the possible effects of sorting seed, and at the same time encourage users to mix the different parts obtained after sorting in order to increase genetic diversity. It is also questionable whether such fractioning of seed is in line with the EU Council Directive (1999/105/EC); responsible parties should investigate this area.

37. Consider carryover effects of nursery activities [Ch. 1.2 - 2.1.3 - 2.6]

Producers need to understand and consider the potential risks associated with raising FRM under climates substantially different from those of plantation sites. Recent research has demonstrated that the adaptive traits of forest trees are affected by conditions during germination and early growth. Considering this, the production of seedlings in nurseries that make use of optimised microclimates may affect further performance of the planted seedlings. We recommend monitoring the outcomes in order to evaluate the epigenetic effects of nursery activities and to understand how genetic diversity affects them. The research community should also inform producers and others of any such carryover effects.

38. Consider inoculating seedlings with beneficial mycorrhizae [Ch. 2.6]

Many tree species form beneficial symbiosis with mycorrhizae. We encourage producers to consider the natural distribution of mycorrhizae species and strains that can influence their production of forest tree seedlings. We further recommend considering deliberate inoculation, to improve the adaptive ability and success of outplanting the seedlings.

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APPENDIX

Annex 1

Categories of Forest Reproductive Material as defined in the EU Council Directive (1999/105/EC)

- **Category 'Source-Identified':** 1. The basic material shall be as seed source or stand located within a single Region of Provenance. It shall be at the discretion of the Member State in each individual case as to whether a formal inspection is required except that, a formal inspection must be made where the material is destined for a specific forestry purpose. 2. The seed source or stand shall meet criteria set by the Member States. 3. The Region of Provenance and the location and the altitude or altitudinal range of the place(s) where the reproductive material is collected must be stated. It must be stated whether the basic material is: (a) autochthonous or non-autochthonous or the origin is unknown or (b) indigenous or non-indigenous or the origin is unknown. In the case of non-autochthonous or non-indigenous basic material the origin must be stated if known.

- **Category 'Selected':** The stand will be judged with respect to the specific stated purpose for which the reproductive material will be intended and due weight shall be given to requirements 1-10, depending on the specific purpose. The criteria for selection shall be determined by the Member State and the purpose shall be indicated in the National Register. Criteria: 1. **Origin:** It must be determined either by historical evidence or other appropriate means whether the stand is autochthonous/indigenous, non-autochthonous/non-indigenous or the origin is unknown and for non-autochthonous/non-indigenous basic material the origin must be stated if known. 2. **Isolation:** Stands must be situated at a sufficient distance from poor stands of the same species or from stands of a related species or variety which can form hybrids with the species in question. Particular attention shall be paid to this requirement when the stands surrounding autochthonous/indigenous stands are non-autochthonous/nonindigenous or of unknown origin. 3. **Effective Size of the Population:** Stands must consist of one or more groups of trees well distributed and sufficiently numerous to ensure adequate inter-pollination. To avoid the unfavourable effects of inbreeding, selected stands shall consist of a sufficient number and density of individuals on a given area. 4. **Age and Development:** Stands must consist of trees

of such an age or stage of development that the criteria given for the selection can be clearly judged. 5. **Uniformity:** Stands must show a normal degree of individual variation in morphological characters. When necessary, inferior trees should be removed. 6. **Adaptedness:** Adaptation to the ecological conditions prevailing in the Region of Provenance must be evident. 7. **Health and Resistance:** Trees in stands must in general be free from attacks by damaging organisms and show resistance to the adverse climatic and site conditions, except for damage by pollution, in the place where they are growing. 8. **Volume production:** For the approval of selected stands volume production of wood must normally be superior to the accepted mean under similar ecological and management conditions. 9. **Wood Quality:** The quality of the wood shall be taken into account and, in some cases, it may be an essential criterion. 10. **Form or Growth Habit:** Trees in stands must show particularly good morphological features, especially straightness and circularity of stem, favourable branching habit, small size of branches and good natural pruning. In addition, the proportion of forked trees and those showing spiral grain should be low.

- **Category 'Qualified':**
- A. Seed Orchards:** (a) The type, objective, crossing design and field layout, components, isolation and location and any changes of these must be approved and registered with the official body, (b) The component clones or families shall be selected for their outstanding characters and special consideration shall be given to the requirements 4, 6, 7, 8, 9 and 10 mentioned in the Category 'Selected' above, (c) The component clones or families shall be planted or shall have been planted according to a plan which has been approved by the official body and established in such a way that each component can be identified, (d) Thinning carried out in seed orchards shall be described together with the selection criteria used for such thinnings and registered with the official body, (e) The seed orchards shall be managed and seed harvested in such a way that the objectives of the orchards are attained. In the case of a seed orchard intended for the production of an artificial hybrid, the percentage of hybrids in the reproductive material must be determined by a verification test;
- B. Parents of Family(ies):** (a) The parents shall be selected for their outstanding characters and special consideration will be given to the requirements 4, 6, 7, 8, 9 and 10 mentioned in the Category 'Selected' above, (b) The objective, crossing design and pollination system, components, isolation and location and any significant changes of these must be approved and registered with the official body, (c) The identity, number and proportion of the parents in a mixture must be approved and registered with the official body, (d) In the case of parents intended for the production of an artificial hybrid, the percentage of hybrids in the reproductive material must be determined by a verification test;
- C. Clones:** (a) Clones shall be identifiable by distinctive characters which have been approved and registered with the official body, (b) The value of individual clones shall be established by experience or

have been demonstrated by sufficiently prolonged experimentation, (c) Ortets used for the production of clones shall be selected for their outstanding characters and special consideration should be given to the requirements 4, 6, 7, 8, 9 and 10 mentioned in the Category 'Selected' above, (d) Approval shall be restricted by the Member State to a maximum number of years or a maximum number of ramets produced; **D. Clonal Mixtures:** (a) Clonal mixture shall meet the requirements in points C(a),C(b) and C(c) above, (b) the identity, number and proportion of the component clones of a mixture, and the selection method and foundation stock must be approved and registered with the official body. Each mixture must contain sufficient genetic diversity, (c) Approval shall be restricted by the Member State to a maximum number of years or a maximum number of ramets produced.

- **Category 'Tested': Requirements for all tests:** (a) General - The basic material must satisfy the appropriate requirements of Categories 'Selected' and 'Qualified'. Tests set up for the approval of basic material are to be prepared, laid out, conducted and their results interpreted in accordance with internationally recognised procedures. For comparative tests, the reproductive material under test must be compared with one or preferably several approved or pre-chosen standards, (b) Characters to be examined - (i) Tests must be designed to assess specified characters and these must be indicated for each test, (ii) Weight shall be given to adaptation, growth, biotic and abiotic factors of importance. In addition, other characters, considered important in view of the intended specific purpose, shall be evaluated in relation to the ecological conditions of the region in which the test is carried out, (c) Documentation - Records must describe the test sites, including location, climate, soil, past use, establishment, management and any damage due to abiotic/biotic factors, and be available to the official body. Age of the material and results at the time of the evaluation must be recorded with the official body, (d) Setting up the tests - (i) Each sample of reproductive material shall be raised, planted and managed in an identical way as far as the types of plant material permit, (ii) Each experiment must be established in a valid statistical design with a sufficient number of trees in order that the individual characteristics of each component under examination can be evaluated, (e) Analysis and validity of results - (i) The data from experiments must be analysed using internationally recognised statistical methods and the results presented for each character examined, (ii) The methodology used for the test and the detailed results obtained shall be made freely available, (iii) A statement of the suggested region of probable adaptation within the country in which the test was carried out and characteristics which might limit its usefulness must also be given, (iv) If during tests it is proved that the reproductive material does not possess at least the characteristics: of the basic material or of similar resistance of the basic material to harmful organisms of economic importance, then

such reproductive material shall be eliminated. **Requirements for Genetic Evaluation of Components of Basic Material:** (a) The components of the following basic material may be genetically evaluated: seed orchards, parents of family(ies), clones and clonal mixtures, (b) Documentation-The following additional documentation is required for approval of the basic material: (i) The identity, origin and pedigree of the evaluated components, (ii) The crossing design used to produce the reproductive material used in the evaluation tests, (c) Test procedures - The following requirements must be met: (i) The genetic value of each component must be estimated in two or more evaluation test-sites, at least one of which must be in an environment relevant to the suggested use of the reproductive material, (ii) The estimated superiority of the reproductive material to be marketed shall be calculated on the basis of these genetic values and the specific crossing design, (iii) Evaluation tests and genetic calculations must be approved by the official body, (d) Interpretation - (i) The estimated superiority of the reproductive material shall be calculated against a reference population for a character or set of characters, (ii) It shall be stated whether the estimated genetic value of the reproductive material is inferior to the reference population for any important character. **Requirements for Comparative Testing of Reproductive Material:** (a) Sampling of the reproductive material: (i) The sample of the reproductive material for comparative testing must be truly representative of the reproductive material derived from the basic material to be approved, (ii) Sexually produced reproductive material for comparative testing shall be: harvested in years of good flowering and good fruit/seed production; artificial pollination may be utilised, harvested by methods that ensure that the samples obtained are representative, (b) Standards: (i) The performance of standards used for comparative purposes in the tests should if possible have been known over a sufficiently long period in the region in which the test is to be carried out. The standards represent, in principle, material that has been shown useful for forestry at the time that the test starts, and in ecological conditions for which it is proposed to certify the material. They should come as far as possible from stands selected according to the criteria mentioned for the Category 'Qualified' or from basic material officially approved for production of tested material, (ii) For comparative testing of artificial hybrids, both parent species must, if possible, be included among the standards, (iii) Whenever possible several standards are to be used. When necessary and justified, standards may be replaced by the most suitable of the material under test or the mean of the components of the test, (iv) The same standards will be used in all tests over as wide a range of site conditions as possible, (c) Interpretation: (i) A statistically significant superiority as compared with the standards must be demonstrated for at least one important character, (ii) It will be clearly reported if there are any characters of economic or environmental importance which show significantly inferior results to the standards and their effects must be compensated for by favourable characters.

Annex 2

List of tree species cited (in scientific name alphabetical order)

COMMON NAME	SCIENTIFIC NAME
Silver fir	<i>Abies alba</i>
Trojan fir	<i>Abies equi-trojani</i>
Sicilian fir	<i>Abies nebrodensis (Lojac.)</i>
Sycamore	<i>Acer pseudoplanatus</i>
Maple	<i>Acer spp.</i>
Black alder	<i>Alnus glutinosa</i>
Silver birch	<i>Betula pendula</i>
Curly birch	<i>Betula pendula var. carelica</i>
Hornbeam	<i>Carpinus spp.</i>
Chestnut	<i>Castanea sativa</i>
Lebanon cedar	<i>Cedrus libani</i>
Common hazel	<i>Corylus spp.</i>
Eucalyptus	<i>Eucalyptus spp.</i>
Oriental beech	<i>Fagus orientalis</i>
European beech	<i>Fagus sylvatica</i>
Common ash	<i>Fraxinus excelsior</i>
Black walnut	<i>Juglans nigra</i>
Common walnut	<i>Juglans regia</i>
European larch	<i>Larix decidua</i>
Hybrid larch	<i>Larix eurolepis</i>
Japanese larch	<i>Larix kaempferi</i>
Dunkeld larch	<i>Larix marschlinii</i>
Apple	<i>Malus spp.</i>
Norway spruce	<i>Picea abies</i>
Engelmann spruce	<i>Picea engelmannii</i>
White spruce	<i>Picea glauca</i>
Serbian spruce	<i>Picea omorika</i>
Sitka spruce	<i>Picea sitchensis</i>
Brutia pine	<i>Pinus brutia</i>
Lodgepole pine	<i>Pinus contorta</i>

COMMON NAME	SCIENTIFIC NAME
Slash pine	<i>Pinus elliottii</i>
Aleppo pine	<i>Pinus halepensis</i>
Bosnian pine	<i>Pinus heldreichii</i>
European black pine	<i>Pinus nigra</i>
Maritime pine	<i>Pinus pinaster</i>
Stone pine	<i>Pinus pinea</i>
Radiata pine	<i>Pinus radiata</i>
White pine	<i>Pinus strobus</i>
Scots pine	<i>Pinus sylvestris</i>
Loblolly pine	<i>Pinus taeda</i>
Eastern cottonwood	<i>Populus deltoides</i>
Pannonia poplar	<i>Populus euramericana 'Pannonia'</i>
Black poplar	<i>Populus nigra</i>
European aspen	<i>Populus tremula</i>
American/Quaking aspen	<i>Populus tremuloides</i>
Wild/Sweet cherry	<i>Prunus avium</i>
Blackthorn/sloe	<i>Prunus spinosa</i>
Douglas fir	<i>Pseudotsuga menziesii</i>
Pear	<i>Pyrus spp.</i>
Chestnut-leaved oak	<i>Quercus castaneifolia</i>
Sessile oak	<i>Quercus petraea</i>
Pubescent oak	<i>Quercus robur</i>
Northern red oak	<i>Quercus rubra</i>
Black locust	<i>Robinia pseudoacacia</i>
Willow	<i>Salix spp.</i>
Rowan	<i>Sorbus spp.</i>
Lime	<i>Tilia spp.</i>
Elm	<i>Ulmus spp.</i>
Caucasian/Siberian elm	<i>Zelkova crenata</i>

