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Paris, 9-10 december 1993

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of the European Communities

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Quality of forest reproductive material in the field of the application of European Community rules

Qualité du matériel forestier de reproduction et application des directives communautaires

Edited by : Daniel Terrasson

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Preface

The proceedings of E.C. Scientific Workshop "Quality of forest reproductive material in the field of the application of E.C. rules" are reported in this publication. The workshop was held under the auspicies of the Commission of the European Community, Directorate General for Agriculture, in Paris on December 9-10, 1993.

The participants in the workshop came from Forest Research Institutes, Universities, Forest Agencies and the Commission of the European Communities. In total, 47 forest researchers and managers from 15 countries (European Union, Norway) attended the meeting.

Objective and context

Until a recent past foresters used to think that selection realised during the life of a stand exempted themselves from paying attention to the genetic quality of forest material. But the increament of afforestation practise, and some ill-considered genetic transfers led to occasional obvious failures. These situations have stressed the necessity to take into account the origin of forest reproductive material, and to have a minimum selection for seed-stands. Therefore European Community decided to rule the commerce of forest reproductive material, which was completed through directives 66/404 and following texts.

On the other hand, because of genetic improvement achievements, foresters are now offered a choice between a "wild" material croped on natural stands, and an "obtained" material produced by more and more complex techniques.

At a scientific level, the application of the European rules raise three main issues :

- how to **identify** forest material ?
- This identification is defined in legal term by the trilogy : Distinction, Homogeneity, Stability, in which the two last words are related to the conformity with a standard ;
- how to **evaluate** the material ?

- This covers scientific legal and operational aspects with a different view for "wild" or "obtained" materials ;

- how to **use** the material ?

This issue is raised, not only by tree-planters, but also by the social community as a whole, since everybody feels strongly concerned about the stability of forest stands in the long term (conservation of biodiversity, managment of genetic resources).

Publication

This publication contains the papers presented at the Scientific Workshop. The presentations where organized into four sessions :

1. Identification of forest reproductive material
2. Evaluation of forest reproductive material
3. How to use forest reproductive matérial ?
4. Conservation of genetic resources

At the end of the seminar the participants identified gaps in knowledge and European directives, and encircled promising topics for forest research and international actions. This is reported in the synthesis at the end of the proceedings.

The commission of the European Communities thanks Mr Daniel TERRASSON, Cemagref, for his help in organizing the seminar, and the participants for their interesting contributions to the lectures and the discussions.

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Identification of forest reproductive material

Chairman : Svern M.G. DE VRIES

Use of biochemical and molecular markers for identification of forest reproductive material

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Abstract

This contribution addresses the applicability of biochemical and molecular markers for identification of forest reproductive material, originating from actual forest stands. Material originating from seed orchards is not considered. Genetic prerequisites of markers for identification purposes are first discussed. The different markers are compared for the level of polymorphism and differentiation that they reveal. Current available results are reviewed for identification of (i) single trees, (ii) populations and (iii) groups of populations. Finally, a general methodology is proposed combining genetic information of nuclear and cytoplasmic markers. It consists in a two step procedure : delineation of regional units based on multilocus analysis of nuclear markers and stand identification based on maternally inherited cytoplasmic markers.

Key-words : *Terpenes, isozymes, DNA, seed certification, forest trees, polymorphism, differentiation.*

Introduction

In the past thirty years, various biochemical and molecular markers have been developed in forest trees. These have been widely used for investigating levels and distribution of polymorphism in natural populations. For example, 935 citations were reported in a bibliographic review on isozymes in forest trees (Paule, 1990). Despite their success in genetic inventories, their application towards identification purposes has only been scarcely addressed. Among the 935 citations, only 15 concerned seed certification. It is the objective of this contribution to examine and compare critically the applicability of different markers for identification purposes : (1)

prerequisites of markers will be outlined, (2) genetic properties will be reviewed, (3) current potential applications will be stressed and finally (4) details of a proposed methodology with current available techniques will be given.

There are three different kinds of markers routinely used for genetic inventories in forest trees : secondary metabolites (terpenes), isozymes and DNA fragments. Separation techniques will not be detailed in this contribution ; they are given elsewhere (Baradat and Marpeau ; 1991, for terpenes ; Bergmann, 1991, for isozymes ; Szmidt and Wang, for DNA fragments, 1991). Only their application for identification of single trees and natural populations (or groups of population) will be addressed. Genetic units of artificial origin (seed lots from various seed orchards) will not be considered.

1. Prerequisites of genetic markers to be used for identification purposes (Table 1)

1.1 Phenotypic expression

Phenotypic expression of markers should be free of environmental influence, e.g. heritability of theses traits should be 1. In a similar way, their expression should be independent from the developmental stage of the tree. Whereas tissue specific, or development specific terpenes or isozymes have been reported, DNA markers have the great advantage to be present in any organ or tissue.

Marker	Separation technique	Inheritance	Gene action	Locus specificity	Reproducibility
Secondary metabolites	Gaz chromatography	Mendelian	Dominance	?	Fair
Isozymes	SGE or PAGE	Mendelian	Codominance	Yes	Fair
DNA Fragments					
* Cytoplasmic	RSBA or Uniparental PCR		Yes	Good	
* Nuclear	RSBA	Mendelian	Codominance	Yes	Excellent
	Targeted PCR	Mendelian	Dominance	Yes	Excellent
	Random PCR	Mendelian	Dominance	?	Poor

SGE : Starch gel electrophoresis PAGE : Polyacrymalide gel electrophoresis
RSBA : Restriction Southern Blot Analysis (leading to RFLP : restriction fragment length polymorphism)
PCR : Polymerase chain reaction

Table 1 - **Prerequisites of biochemical and molecular markers**

1.2 Inheritance

Inheritance of the markers should be well established beforehand in segregation studies based on controlled crosses. Two modes of inheritance may be considered :

- biparental, for nuclear markers. In this case, mendelian segregations must be checked.
- uniparental, for cytoplasmic DNA markers. Complete uniparental inheritance for organelle DNA markers may not be always the case ; preferential but not exclusive uniparental transmission has been reported in some cases (Wagner *et al.*,1991)

1.3 Gene action

Codominant markers permit the identification of all classes of genotypes and should always be preferred to dominant markers. However if numerous dominant markers are available, their resolution for identification purposes is as powerful as a few codominant markers. In this respect the RAPD technique (Random Amplified Polymorphic DNA), which allows to assess numerous fragments should not be discarded, even if most of them are dominant.

1.4 Locus specificity

Markers with identical phenotypic expression (for example, same length for DNA fragments) should correspond to the same chromosome location. Locus specificity arises as a problem particularly for PCR (Polymerase Chain Reaction) derived markers. When genomic amplification is used to obtain markers in two genotypes A and B, electrophoretic profiles may exhibit fragments of similar sizes in both genotypes. Similar size does not necessarily refer to the same locus. The same difficulty may occur when electrophoretic profiles comprise numerous fragments and fragments of similar size can be confounded. In either case, locus specificity can be checked using molecular hybridization with radiolabelled probes.

1.5 Repeatability of separation techniques

Identification of reproductive material necessarily implies the application of separation techniques in different laboratories. Therefore these techniques should be standardized beforehand.

2. Desired properties of genetic markers to be used for identification purposes

Optimal properties of markers to be used for identification purposes are (1) that they are polymorphic, e.g. that there are present with different variants in a species and (2) that these variants are unevenly distributed between the genetic units that have to be identified, e.g. that these units can be differentiated. Experimental results and theoretical predictions show that the current markers differ largely in the level of polymorphism and differentiation that they reveal. These two properties are independent: highly polymorphic markers are not necessarily those that differentiate genetic units and *vice versa.*

*2.1 **Polymorphism** (Table 2)*

The level of polymorphism that a genetic marker can reveal can be expressed at different levels (alleles, genotype, gamete, zygote). The corresponding parameters used to estimate polymorphism all depend on the number of the existing variants at these levels and on their frequency distribution. It is out of the scope of this contribution to review the different parameters, however the different markers currently available differ largely in their ability to reveal polymorphism.

Terpenes are limited in providing polymorphism. Quantitative assessments of these molecules only permit to identify two variants for a given locus (richness or poorness of the concentration). The separation of these variants becomes even more difficult when dominance relationships exist. Furthermore only a small number of terpenes have been so far genetically characterized : 3 monoterpenes and 2 sesquiterpenes in the case of Maritime pine (Baradat, 1988).

Whereas numerous variants can be electrophoretically separated for isozymes, their frequency distributions show that there is usually one highly frequent allele and several alleles with low frequency. As a result the effective number of alleles, for polymorphic isozymes, varies between two and three. The number of isozymes that can be assayed is also limited. In most studies, the number of loci varies between 10 and 20.

Provided that resources are available, the number of loci that can be investigated with nuclear DNA markers is unlimited, whatever current technique is used (restriction-hybridization or DNA amplification). Chloroplast DNA molecules should be considered as one single locus, if one assumes that no recombination occurs. The nuclear genome is extremely heterogeneous as regards the polymorphism. Mutation rates vary by a factor 100 from coding regions to hypervariable minisatellites and microsatellites.

These latter genomic zones are constituted by short DNA sequences that are repeated in tandem throughout the genome, or located in specific zones. Satellites vary in the number of repeats, and the polymorphism is so high that they can be used to fingerprint various genotypes.

Marker	**Mean Number of loci**	**Number of variants per locus**		**Total number of variants**		
		Alleles	Genotypes	Alleles	Genotypes	
Secondary metabolites	5	2	3	32	243	a
Isozymes	15	2	3	32768	$14*10^6$	b
DNA Fragments						
Cytoplasmic	1	10	10	10	10	c
Nuclear	?	?	?	?	?	

a) - Baradat and Marpeau, 1991
b) - Hamrick et al., 1992
c) - Demesure and Petit, personal communication

Table 2 - **Comparative levels of polymorphism revealed by each marker**

2.2 Differentiation among populations (Table 3)

Differentiation among populations is of main concern here for identification purposes of populations or groups of populations. Actual levels of differentiation among forest tree populations result from a balance between selection and genetic drift on one hand and migration among populations on the other hand. Various parameters are used to assess levels of differentiation (coefficient of gene differentiation of Nei (1973), Gst ; average differentiation of Gregorius (1987)). Levels of differentiation may vary markedly between neutral and non neutral markers.

Neutral markers

For neutral markers, levels of differentiation can be predicted from theoretical developments taking into account population sizes, and gene flow (Petit *et al.* 1993b). Forest trees are mostly allogamous and have large populations sizes. As a result, among population variation of variant frequencies of the nuclear genome is therefore expected to be low, as confirmed by experimental results reviewed by Hamrick and coworkers (1992). As shown by their review, Gst values obtained from isozyme studies in most forest trees are less than 10%. To illustrate this result, differentiation between two hypothetic populations, in the case of a diallelic locus, amounts to 9% when one allele exists with frequency 0.3 in one population and 0.5 in the other population. There have been extensive results obtained with isozymes on more than 160 species from the temperate to the boreal zones. Exceptions to the low differentiation are found for species with scattered geographic distribution, where populations may have been isolated. These expectations need however to be confirmed by experimental data. Results obtained from population surveys with neutral nuclear DNA markers are rare, however the expectations are that the level of differentiation should not be higher than with isozymes, even if the markers used exhibit higher levels of polymorphism. The reasons are that their actual levels of differentiation depend on the same evolutionary factors and that at equilibrium between these factors, an identical level of differentiation is expected, regardless of the level of polymorphism.

Marker	Pattern of population differantiation	Reference
Secondary Metabolites	Monolocus level : fixation or loss of a given allele in particular populations. Multilocus level : regional differentiation	a, b, c
Isozymes	Monolocus level : extremely low differantiation (Gst<5%) Multilocus level : regional differantiation	d
DNA fragments		
Cytoplasmic	Extremely high differantiation for maternally inherited organelles (Chloroplasts and mitochondria in broadleaves) Low differantiation for paternally inherited organelles (chloroplasts in conifers)	e, f
Nuclear	No data available on range wide studies. Expected levels of differantiation for neutral markers are similar to those obtained with isozymes. Higher levels are expected for markers linked to adaptive traits.	

a) Baradat and Marpeau, 1991 - b) Müller-Starck et al., 1992 - c) Hanover, 1992
d) Hamrick et al., 1992 - e) Petit et al., 1993 - f) Petit et al., 1994

Table 3 - **Comparative levels of differanciation revealed by each marker**

A clear difference should be made here between uniparental and biparental inherited markers. Since cytoplasmic markers are only transmitted by one parent, populations sizes corresponding to their genes, and migration rates should be lower than for nuclear genes. As a result, the former should be more affected by genetic drift than the latter and higher levels of differentiation should expected for cytoplasmic than for nuclear genes (Petit *et al.*, 1993b). The discrepancy of the level of differentiation between cytoplasmic and nuclear genes should be proportional to the difference in migration between uniparentally and biparentally inherited genes. Differentiation should be much higher for markers with maternal inheritance than paternal inheritance, since seed migrate generally at lower distances than pollen. These theoretical predictions were confirmed by experimental results obtained in oaks and pines. In oaks, chloroplasts are maternally inherited : migration of organelle genes are therefore limited by seed dispersal. On a range wide scale in *Q. petraea*, the coefficient of differentiation was 2 % for isozymes (nuclear markers) and 86% for chloroplast DNA (Kremer and Petit, 1993). In pines, chloroplasts are paternally and mitochondria predominantly maternally inherited. Levels of differentiation were much higher for mitochondrial DNA than for chloroplast DNA in Lodgepole and Jack pine (Dong and Wagner, 1994).

Chloroplast DNA does not undergo recombination; it is clonally inherited. As a result , chloroplast haplotypes can be phylogenetically ordered permitting to improve the information necessary to differentiate populations.

Non neutral markers

The magnitude of intraspecific variation, as evidenced by data of provenances tests, demonstrates the important population differentiation existing for adaptive traits in forest trees. However the molecular basis of differentiation is unknown, since most of the DNA markers currently used concern noncoding regions.

The challenge is to screen nuclear DNA markers for their ability to discriminate populations. Without any knowledge of genes involved in the control of adaptive traits, the challenge leads to a « fishing « procedure. New molecular techniques, like random amplification, permit to screen rapidly DNA markers and should ease the identification of fragments linked to genes involved in adaptation. It should be pointed out here, that for nuclear markers, much higher levels of differentiation can be obtained only if non neutral markers are investigated.

3. What genetic units can be distinguished with current available markers ?

3.1 Individual trees, clones

Isozymes can be used to identify single trees, since they show high levels of within population polymorphism. As shown on table 1, if 15 loci are routinely scored and 2 alleles exist at each locus, a total number of multilocus genotypes can be identified. This number is higher than the total number of clones being used as reproductive material for a vegetatively propagated species. Routine clonal identification, based on isozymes, is done in the case of aspen (Bergmann, 1987) and poplar (Rajora, 1989a and b).

High levels of polymorphisms were also found in the ribosomal DNA for poplars and used for identification of the different clones (Faivre-Rampant *et al.*, 1992). Future use of hypervariable DNA markers (microsatellites) is not necessary as long as the number of clones commercially used remains low (< 100) and that polymorphic isozymes exist.

3.2 Populations

It has been shown theoretically and experimentally that maternally inherited DNA shows high population differentiation. In the case of broadleaves, chloroplast DNA should therefore be a potential marker for population identification. However the polymorphism within the chloroplast DNA molecule is low. In the case of oaks, the nucleotide diversity has been estimated to 0.06% (Petit *et al.*, 1993a), which leads to a total number of existing different variants (cytotypes) of 90 for the chloroplast genome, given that its size is 150 kb. This number could be increased if polymorphism is searched in the mitochondrial genome.

It has been shown, in the case of oaks, that the majority of trees within a forest share the same chloroplast haplotype (Petit *et al.*, 1993a). But the number of existing cytotypes may be limited to allow to fingerprint each single stand. Identification may therefore be limited to a regional scale.

3.3 Group of populations (regions)

Isozymes show low population differentiation at the single locus level. If populations had to be identified with single locus information, extremely large sample sizes would be required to discriminate populations. However multilocus analysis, based on descriptive multivariate statistical methods, show geographic patterns of variation (El Kassaby, 1991, for a review ; Yang and Yeh, 1993) in forest trees. When range wide isozymic surveys were conducted (Lagercrantz and Ryman, 1990, for *Picea Abies* ; Li and Adams, 1989, for *Pseudotsuga Menziesii* ; Zanetto and Kremer (unplushihed data) for *Quercus petraea*), clear geographic groups of populations were distinguished with multivariate analysis. Range wide multilocus analysis of terpenic data distinguished also geographic regions (Baradat, 1988, for *Pinus pinaster* Ait.). The geographic grouping overlapped the pattern of variation obtained with morphological traits in provenance tests. This concordance was interpreted as the result of an « evolutionary footprint » (glacial refugia) rather than the consequence of recent convergent selective pressures (Strauss *et al.*, 1992). Similar results were obtained with several Californian conifers with multilocus analysis : these revealed statistically significant geographic patterns of variation that paralleled those of metric traits (Westfall *et al.*, 1992).

4. Perspectives

From the above survey of results obtained with different markers, various propositions can be made for developing a methodology to be used for identification purposes. I shall describe a procedure that could be implemented for most tree species in Europe.

4.1 Construction of a geographic map of genetic types

It has been shown that multilocus analysis based on actual markers (isozymes) permits to discriminate regional groups of populations. From this standpoint, contour plots of multivariate scores (principal components, canonical variables) delineate regions of similarity in multilocus frequencies over the natural range of the species. The regions of similarity will be called in the text regional units. Confidence intervals of the contour scores could be calculated with resampling techniques. This method has been used for the subdivision of breeding zones of californian conifers (Westfall and Conkle, 1992). It was earlier applied for human populations (Piazza et *al.*, 1981a, 1981b).

The building of the map requires the sampling of populations of natural origin distributed over the entire range of the species. The selected populations will be called reference populations. International provenance tests that were established, would be a recommended source of material. Additional populations may be needed for full coverage of the distribution. Optimal population sampling strategies have still to be established taking into account the actual distribution of provenance regions.

It must be emphasized here that a given map, once it is constructed, can always be improved if new markers become available. If more loci are added in the multilocus analysis, delineation of regional units will be more precise, and additional groups may be distinguished. The improvement originates from the multivariate analysis, as shown by the better discrimination obtained with multilocus analysis when compared to monolocus analysis. Therefore the reference populations should be clearly identified, and all subsequent analysis with new markers limited to this well defined sample. The regional delineation obtained with multilocus analysis of nuclear markers could be further subdivided according to the geographical distribution of maternally inherited cytotypes.With the actual technology available, the construction of a geographic map of genetic types could therefore be planned as a two-step procedure :

- delineation of regional clusters based on nuclear markers. The precision of the delineation can largely be improved with the number of markers available.

- identification of populations within regional groups with maternally inherited markers. Due to the low polymorphism in cytoplasmic genomes, the number of cytotypes may often be lower than the number of populations over the whole range. As a result, cytoplasmic markers may be more suitable for identification within regional clusters.

4.2 Identification of seed lots of unknown origin

The identification of unknown seed lots follows also a two step procedure :

- assignment to regional units

  The multivariate scores of the unknown seedlot assessed with the same markers than those used for the construction of the map, will be compared to those delineating the different regional units. Since scores are calculated with their corresponding confidence intervals, alternative hypothesis can be tested. The testing procedure may end in various conclusions .

  -Exclusion of particular regional units
  -Inference about the most likely regional unit

  Complete identification of the regional unit of origin may not always be the conclusion ; but at least, some regional units can be excluded.

- assignment to stands

  Further assignment to stands may be possible by comparison of the maternally inherited cytotype(s) of the unknown seed lot with the cytotype of the reference stands present in the regional unit.

4.3 Limits of the method

The construction of a geographic map of genetic types based on multilocus information may end up in regional groups only if systematic patterns of variation are detected. This may likely be the case if only populations of natural origin are sampled as reference populations.The inclusion of populations of artificial origin may create random « noise », and make it difficult to delineate regional groups. However, if artificial transfers were limited, the method may even permit to detect artificial origins within the reference population.

The method assumes that the seed lot of unknown origin has been collected within a given regional unit, and does not result from artificial mixtures of collections from various units. Mixtures may be misclassified with the multivariate scoring system.

Seed lots from artificial plantations will be assigned to the regional unit of the original stand, from which the plantation derived. Allochtonous origins of stands can therefore be retraced.

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The homogeneity, identification, and classification of provenances, regions of provenance, and seed orchards

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Abstract

Both international regulations on certification and marketing of forest reproductive material, the OECD-Scheme and the EEC-Directive, allow the certification of material coming from a region of provenance. The very complex matter of delimiting regions of provenance of a species is not solved properly in many cases. Thus this contribution deals with the concept of regions of provenance, with the problems of delimitation and the classification of artificial stands and seed orchards. Herein the assessment of the variation and homogeneity in space and time is important as well as the criteria for the delimitation. Genetic and phenotypic characters should be considered much more intensively and given priority over ecological conditions to the place in which the stand is growing, when delimiting regions of provenance.

Introduction

For the use of forest reproductive material, it is important to know about its suitability for afforestation of the sites given. As the suitability is not tested in most cases, for instance for reproductive material from "selected" stands, it needs to be estimated, which seems to be rather difficult or almost impossible without valid results from provenance trials. To compensate the difficulties, the concept of region of provenances has been developed and introduced in international and national regulations on forest reproductive material. The certification of reproductive material moving in trade is another reason for the adoption of the concept of regions of provenance. Since the adoption of the concept and more seriously since genetic markers have been assessed in various forest tree species, questions arose as to what extent provenances or regions of provenance should be uniform or distinctive and how to identify reproductive material derived from different sources (either provenances or regions of provenance).

While Kremer (this issue) has dealt with the distinction and more specifically with the identification tests, this contribution shall focus on some problems of the uniformity within a provenance and region of provenances. The term uniformity is more general and in the following widely replaced by homogeneity which refers to more specific characters if applicable.

1. Definitions

A provenance is "the place in which any stand of trees is growing. The stand may be indigenous or non-indigenous" (OECD), or "the area on which any indigenous or non-indigenous stand of trees is growing (EEC 66/404). Both definitions are very similar and show a pragmatic approach to make the term "provenance" applicable in regulations. Herein the provenance is a place or an area, while in forestry the term is frequently used to name a population of trees possessing outstanding characters. Both meanings are often used in literature without differentiation.

A region of provenances is defined as "the area or group of areas subject to sufficiently uniform ecological conditions on which are found stands showing similar phenotypic or genetic characters" (OECD). The definition used in the EEC-Directive is similar and must not be repeated here. The term "sufficiently uniform" is rather broad, when talking about ecological conditions, and may be defined more precisely by the designated authorities of the countries. Also the similar phenotypic and genetic characers, which shall be found within a region of provenance, have to be defined. The characters to be considered may vary by countries or even regions of provenance for certain species. While the degree of similarity within a region of provenance is not specified, the uniformity of a stand to be approved as "selected" has to fulfil the following minimum requirement: "The stands must show a normal degree of individual variation in morphological characters" (OECD, also very similar in EEC-Directive).

2. The Concept of the region of provenance

The idea of this concept is based on grouping of stands of a region showing similar characters in order to use the seed of these stands for the same forestation purposes. It has been developed in such a way that the region can be described by ecological conditions and or phenotypic and genetic characters of the stands and delimited by appropriate boundaries. The region of provenance has two major functions:

- to facilitate the trade with forest reproductive material by certification of the region where it has been collected or produced,

- to set up recommendations for the deployment of the reproductive material by regions.

The concept of the region of provenance has been introduced in both international and many, but not all, national regulations depending on the needs and special situations. It is not the place here to reflect on the advantages and alternatives of this concept, but it should be kept in mind that the countries have implemented the concept in different ways, which complicates the matter even more than can be expected from what has been said above. In this context the first function of the concept, facilitating the trade, is of interest, especially the following questions need to be answered, if reproductive material is marketed under the reference name of a region of provenance:

- Is there sufficient uniformity or homogeneity of certain traits of interest, for instance among the lots of material of a region ?

- Can the lots of material collected in one region of provenance be identified and distinguished from those of other regions ?

- How can regions of provenance be classified in general and how can artificially established stands of indigenous species or stands of non-indigenous species or seed orchards be classified ?

  It will not be possible to give exhaustive answers, but to outline the problems connected.

3. Homogeneity in space and time

Species vary in space and time. The degree of variation and its distribution depend on the mode of reproduction and the mode of adaptation, which interfere with processes like selection, gene flow and drift. Also the refuges and migration of a species, catastrophies and founder effects influence the variation of a species. Thus complete homogeneity within a species is a rarity.

On the contrary, the result of evolution will be more or less pronounced spatial patterns of variation of traits, which may be of clinal, ecotypic, mosaic or refugial types, and temporal variation, which may occur from year to year (short term fluctuation) or over a longer period or several forest tree generations (trends).

If the homogeneity of a provenance or a region of provenance is concerned, it has to be relativized in spite of the various modalities said above. As the species react differently, it may be wise not to assess all species in the same manner. That means that the variation within and between provenances of the same region as well as the variation between regions of provenance should be estimated by provenance trials and additional studies of stands in situ, before setting up criteria for measuring the degree of homogeneity and distinction. A species containing a high degree of within and even between provenance variation needs to be differently assessed than a species with low degree of variation at all levels. It seems to be possible to objectively measure the rate of heterogeneity using genetical markers, but it is generally accepted to include all characters of interest, for instance growth characters, phenological, morphological, physiological as well as biochemical and genetic traits.

Norway spruce may be an example for a complex species with a high degree of variation, showing almost heterogeneity within provenances. The most important source of variation is the refugial belonging (Dinarian-Alpine, Hercynian-Carpathian, and Nordic-Baltic), which resulted in many differences in growth and morphological characters (Schmidt-Vogt, 1977). Also isozyme studies have demonstrated highly variable provenances, in some cases significant differences between the subpopulation belonging to different refuges could be found (Bergmann, 1973, 1983).

The clinal and ecotypic variation patterns can be shown between provenances within refugial subpopulations. Adaptive traits react most sensitively and their variation has been used for distinction, for example between low and high elevation provenances by the number of buds set by August (Holzer, 1967). Also less selective traits like isozymes, show variation, especially the loci IDH-A, LAP-B, G-PGDH-B, and GDH-A are controlling isozymes (allozymes) useful for distinguishing between regions, while adjacent provenances can mostly not be discriminated (Liesebach, 1994). Flushing date is a very sensitive trait, which commonly varies with elevation, the higher the elevation of the location of the provenance the earlier is the flushing date, if the plants have grown under the same condition. Liesebach (1994) found an exception from this rule, showing a reverse trend of flushing date with elevation. The provenance from Jasina (700 m) flushed earlier than that from Dorna Cindreni (900 m) and both flushed earlier than the Brasov provenance (1050 m) of the Carpathian region. This can be explained by adaptation to local climatic conditions, which in this case may be characterized by an inverse climatic condition at lower elevations. Cold air is gathering at the bottom of the valley, while higher up the temperature is increased. So the provenances of low elevations got adapted to a very cold climate (which is equivalent to that of very high elevation above 1050 m a.s.l.) and flush earlier accordingly.

From the examples above it can be concluded that many factors are influencing the heterogeneity respectively homogeneity of provenances or regions of provenance in space. Studies of the variation with forest trees in time are not very many. They focus either on the developmental changes in composition of the population with age to find trends or on fluctuations in genotypic composition in seed collections from the same stand or seed orchard in different years. Fluctuations can be explained by different proportions of pollen and seed of a given genotype contributing to the next generation, which may vary in different years by various factors. In some cases considerable fluctuations have been found (Gregorius et al. 1986). It seems that adaptive traits vary spatially more than temporally, while marker traits like isozymes vary temporally to a higher degree. At the moment there are no sufficient data to judge about this statement. Thus it should not be generalized. But what can be found in many species and thus can be generalized is the high variation, which should be a reason to define the officially used term "sufficient uniformity" in such a way that it can be used in regulations. The distinction of lots of material derived from different regions of provenance will be difficult and independent from the definition of the term "sufficient uniformity".

4. Delimitation of region of provenance

Knowing of the huge variation within and between provenances of a species it is rather difficult to set up criteria for the delimitation of a region of provenance. This task is even more difficult, if the range of distribution of the species occupies a continuous instead of discontinnuous area. The question arises whether there are criteria at all and from what scientific basis they can be derived.

Two approaches shall be tried, a biological and a technical one. Biological approach: The species is defined, among other criteria, by having a common gene pool, which is shared by all individuals of the species. The individuals have the same ancestors in common and are capable to exchange genetic information. Unless the species has split up into subspecies or botanical varieties, the species stand out for their more or less continuous variation, which can be assessed in almost all forest tree species. In this case a boundary seperating two regions of provenance can hardly be found, which distinguishes the regions by distinct characters or by quantitative characters. Even isozymes or DNA markers fail mostly, either as distinct markers or by the differences of their frequencies. The discrimination effects of the different types of characters may vary considerably. Although it is expected that markers (DNA, isozymes) should have the highest effect, it turned out in a study using provenances from different regions of Norway spruce that traits like height growth have as good as or even better discrimating effect than isozymes (Liesebach, 1994). In conclusion, the biological approach seems to be very difficult, although it may be possible in special cases. The reasons for this are the continuous variation of most of the characters and the difficult search for characters with real discriminating function.

Technical approach: According to the OECD-Scheme the technical approach is described as follows: "Regions of provenance shall be delimited by means of administrative and geographic boundaries and, where applicable, by altitudinal and other appropriate boundaries judged to be significant in the country concerned." Using this instruction almost all regions of provenance of all species in most countries have been delimited. Although the technical approach shall consider the requirements for the definition of the region of provenance (sufficiently uniform ecological conditions, similar phenotypic or genetic characters), it seems to end up in a more artificial way of delimiting regions. The critical arguments against the technical approach are the following :

- Adjacent stands separated by a boundary and belonging to different regions of provenance often show more similar phenotypic and genetic characters and grow under more similar ecological conditions than stands located at more distant places of the same region of provenance.

- The variation of characters between stands and of their ecological conditions of the same region of provenance exceeds the variation between regions of provenance in many cases.

- There may be more arguments, but these mentioned here concerns the homogeneity mostly. It is obvious that the technical approach contains shortcomings and defects, which need to be eliminated. Those are for instance administrative and geographic boundaries do not necessarily coincide with ecological boundaries, the boundaries neglect the basic biological processes affecting variation and homogeneity (mode of reproduction, mode of adaptation a.o.), indigenous and non-indigenous species are treated the same way.

  It has been shown that the technical approach is feasible, despite of what has been said above. As long as the fundamentals outlined in the biological approach cannot be considered adequately, the technical approach is the only way to delimit the regions of provenance. At this stage the only alternative would be to omit the entire concept of regions of provenance. This would complicate the matter even more because each lot of material has to be handled separately and treated as a provenance. The numbers of lots to be kept separately would increase tremendously compared to the numbers of regions of provenance, which is far from any reasonableness for seed traders and users.

5. Identification of provenances and regions of provenance

The OECD-Scheme as well as the EEC-Directive are based on a certification system, which shall guarantee the identity of the material. For this reason each lot of material has to be accompanied by certificates. The certificates enable the designated authorities to directly control the identity. At the moment this certification system is the only way of an effective control. The assessment of a set of appropriate traits for the identification is feasible in a few cases only. But a replacement of the official certification by indirect identification methods is not possible for several reasons, which have been discussed by Kremer (this issue). Although it seems to be possible to set up tests in future, which give a good tool for checking the identity, they can only be used parallel and complete, confirm or contradict the information available from provenances.

6. Classification of artificial seed stands and seed orchards

An artificial seed stand or a seed orchard may be established using an indigenous or a non-indigenous forest tree species. Further the stand resp. seed orchard may be grown within or outside the natural range of the species. Each of these cases has its own problem of certification, if integrated in the concept of regions of provenance. The EEC-Directive has two categories (selected and tested), while the OECD-Scheme recognises four categories (source identified, selected, untested seed orchard, and tested). The region of provenance is important only for the categories EEC-selected, OECD-source identified, and OECD-selected. Seed orchard (selected but untested) are ruled differently in both regulations. While the OECD-Scheme has an own category for seed orchards, the EEC-Directive rules the seed orchard under category "selected". But both regulations have the category "tested" with identical meaning in common. If seed stands and seed orchards are tested, the concept of region of provenance is not valid any longer.

When applying the concept of region of provenance to artificial seed stands or seed orchards of an indigenous or a non-indigenous species growing within or outside its natural range, the general problem becomes obvious: all these seed stands or seed orchards do not fit into the geographic variation pattern. Thus both regulations do not allow to mix material derived from indigenous and non-indigenous stands or orchards of the same region of provenance, the lots have to be kept separately. Another problem is the meaning of the "region of provenance", for instance, of a seed stand of a non-indigenous species or of a seed stand of an indigenous species growing outside its natural range. The meaning of "region of provenance" in these cases is reduced to the information about the region, in which the trees are actually growing without the information about their origin. The latter information, therefore, is required additionally (see Certificate of Provenance), if available.

Technically the problems seem to be solved, but there are many open questions, for instance :

- What about the homogeneity of trees of artificial stands within a region of provenance, especially if they originate from various places ?
- To what extent can a change of genotype frequencies by adaptation to the new site be assumed, if compared to the original population (creation of land races) ?
- What differences can be expected between seed lots from indigenous and non-indigenous seed stands of the same region of provenance ?
- At the time information is insufficient to find an appropriate solution.

The rules for seed orchards in the EEC-Directive are somewhat curious. They define that the "region of provenance of reproductive material produced in a seed orchard is that of the basic material used for the establishment of the orchard."

This definition is also valid, if the seed orchard is growing in a region of provenances other than that of the basic material. From the point of view of the concept of the region of provenance this definition seems to be plausible, but it contradicts the definition of "provenance" in these cases (see above). Here the term "provenance" and "origin" are mixed up, where the origin is defined as follows (after OECD); "For an indigenous stand of trees the origin is the place in which the trees are growing; for a non-indigenous stand the origin is the place from which the seed or plants were originally introduced."

The rules for seed orchards in the EEC-Directive need to be re-examined, because the difinitions fit to conservation seed orchards for which they were originally set up, but do not necessarily apply for seed orchards of other types.

Conclusion

Forest tree species show a high degree of variation within and between stands. The grouping of stands consisting of trees with similar characters is based on the concept of regions of provenance, which is adopted by both international regulations (OECD-Scheme, EEC-Directive) and many national rules. Herein the term "uniformity" resp. "homogeneity" needs to be defined more precisely considering the observed variation.

The delimitation of regions of provenance are based on "artificial" boundaries, which can hardly be justified in some cases. As long as the knowledge about the biological phenomena of the spatial and temporal variation is not sufficiently understood, the technical approach delimiting the regions of provenance will dominate.

Criteria for the delimitation of regions of provenance, should be reconsidered. Ecological conditions of the site of the stand are used as main criterion so far. They are undoubtedly very important, but priority should be given to genetic and phenotypic characters of the material to be certified. Other criteria (geographic, administrative and others) should rank less. For the assessment of the variation all kinds of traits should be used like growth traits, morphological, physiological, phenological, biochemical and DNA-characters. While adaptive traits react on selection pressure, neutral traits follow different procedures (e.g. drift) more easily. Thus assessing the variation pattern of different traits does not necessarily provide the same information.

Classification of artificial stands and seed orchards are generally difficult. Information about the origin of the material in question is very useful and may be more valid than information about ecological conditions of the place in which the stand or orchard is growing.

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Synthesis of identification problems of forest reproductive material

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Abstract

This report tries to give an answer to the followig questions:

- *Should regulations on forest reproductive material continue to be based only on an administrative follow-up of the material?*
- *Problems and statutes of hybrid varieties?*
- *What about distinctness, uniformity and stability of forest reproductive material?*

With biochemical genetic methods you can answer many identification problems. The biochemical genetic methods are not yet sufficiently introduced for the control of forest reproductive material and not yet adaquately adapted to this task. There are only a few specialized laboratories which are able to do this work. The identification work is very expensive. But nevertheless these methods should be used more frequently for the control of forest reproductive material.

There are hybrid varieties with only two genera which are of some importance for marketing. Hybrids should be marketed only as tested material.

In order to protect breeder's rights to new varieties, these varieties must be new, distinct, uniform and stable. Distinctness, uniformity and stability are necessary for easy identification. In forestry it is important to have heterogenous individuals or populations in order to reduce production risks during the long production period. This is, why identification is difficult.

1. Methods of administrative and biochemical genetic identification

Identification of forest reproductive material is realized by an administrative follow-up of the material. Directives on forest reproductive material require the presentation of quite a few documents for the identification of the reproductive material. It is desirable that existing biochemical genetic methods are applied in future for identification.

1.1 Existing methods of biochemical genetic identification

There are the following three methods of biochemical genetic identification:

- Isoenzyme analysis
- Investigations of DNA
- Investigations of terpens

In principle the following questions could be answered by these methods in the field of control of forest reproductive material:

- Is the given reproductive material really the material of the species stated on the accompanying documents?

- Where is the origin of reproductive material (large provenance regions).

- Has the given reproductive material the same identity as the basic material in the case of generative or vegetative propagation?

- Was the necessary separation of different lots of forest reproductive material effected?

- Do seeds, seedlings and plants which according to the documents should be of the same origin really have the same identity?

The biochemical genetic methods can answer in principle nearly all identification problems which are not solved by morphological methods.

To state this quite clearly: These methods cannot replace the identification documents. These methods should replace the control measures to ensure that the documents and reproductive material are congruent.

1.2 Reasons for the rare use of these methods

In the Federal Republic of Germany there exists a regulation that biochemical genetic methods should be used in the form of sampling for identification. However, it must be stated that per year only one ore two samplings by biochemical genetic methods are carried out. What is the reason for this?

- All methods are still at a scientific experimental stage. They can be used to answer some special questions in the field of investigation. Researchers are able for instance to distinguish Norway spruce of eastern countries from those of western contries, but this is not sufficient for the determination of provenances.

- For biochemical genetic analysis in the field of control samples of basic material and of reproductive material are very often needed. It is very difficult to get samples of basic material. A future solution may be that laboratories gather results of basic material analysises. In that case there would be no further need to test the natural basic material again.

- Populations are difficult to identify because there is such a large number of different individuals.

- Identification work by biochemical genetic methods is very expensive.

- In Germany there exists only one research station in the field of forest reproductive material which is willing and capable to make tests by DNA methods and one station which is using terpen analysis. These stations are research stations of universities, this means they do this control work only on a voluntary basis.

- Some isoenzyme analysis laboratories have recently been established in the Federal Republic being the responsibility of the Länder. They have now started their research work. The Länder are responsible for the control of forest reproductive material and these laboratories will be involved in the task of sampling for identification of forest reproductive material.

1.3 Future actions

We should encourage researchers in the field of biochemical genetic analyses to investigate also practical problems of identification of reproductive material and to find standardized methods of identity investigation. Linkage between research and controller should be ensured.

Biochemical genetic investigation stations should collect investigation samples of different basic materials. We should accept that it will be a long way and take many years until these methods can be used to a certain extent in the form of sampling in the field of controlling the identity of forest reproductive material.

We should not exclude the idea, that in ten or twenty years' time a mobile control laboratory will control seed and plants on the premises of a seed dealer or in a plant nursery.

I guess there is no need to include these methods in the Directive. We should oblige the member states to control the identity and to use suitable methods.

2. Problems and status of hybrid varieties

2.1 Present situation

The Directives on forest reproductive material include with the exception of poplar pure species only. This is clarified by the Directive on external quality standards of forest reproductive material marketed within the Community. This directive requests a very high degree of specific purity. Poplar is included as a genus. This fact is understood in that way that pure species of poplar and hybrids between species of poplar are included.

The Directive on forest reproductive material opens the possibility for member states to cover also other species and genera by national rules. The Federal Act on Forest Seed und Planting Stock covers also the hybrids between species with all pure species covered by the law.

Presently I see only two fields where hybrids have some importance for marketing. These are:

- Populus hybrids
- Larix eurolepis

I see no chance in Germany now for growing market opportunities for these hybrids. German silviculture aims at silvicultural practices emulating nature. I guess the silvicultural situation in other member states is not completly different from that in Germany.

2.2 Status of hybrids

Hybrid forest reproductive material must be tested before it may be marketed. In some cases certain conditions must be met for reproduction. These conditions must be set by the responsible authority for approval of forest basic material.

2.3 Future actions

Regarding the marketing situation there are only two fields of hybridisation: these are Populus and Larix. The inclusion of these hybrids in the European rules is possible.

The inclusion of other genera than poplar and Larix at European level could only be regarded reasonable under the aspect of better control. I remember that several years ago hybrids of Fagus sylvatica x orientalis were imported from countries of south-eastern Europe. We mistrusted these imports because we were anxious that the material would be sold for forestry purposes. We had no possibility to prevent the seed dealer from importing this material. Now hybrids are covered by the German aformentioned Act and importation of such hybrids is controlled.

Forest hybrid reproductive material should only be marketed under the category of tested reproductive material.

National authorities should have the possibility to set special conditions for special reproductive material.

3. Role of distinctness, uniformity and stability for forest reproductive material

3.1 Present situation

The terms "distinctness, uniformity and stability" are not typical for the Directives on forest reproductive material. Annex I of Council Directive 66/404/EEC of 14 June 1966 on the marketing of forest reproductive material gives the requirements for the approval of basic material for selected reproductive material. There you can find the following special requirement for uniformity:

"Uniformity: The stands shall show a normal degree of individual variation in morphological characters."

For the terms "stability" and "distinctness" no special definition exists in the Directives on forest reproductive material, but you can find these terms in the International Convention for the Protection of New Varieties of Plants of December 2, 1961 as revised in Geneva on November 10, 1972, October 23, 1998 and March 19, 1991.

First of all I would like to clarify the aims of this Convention. The Convention is an instrument for international cooperation in the field of protection of breeders' rights. Breeders' rights will be protected by national laws following the Convention. The aims of the Convenion and the national laws are to retain the financial gains from breeding work for the breeder.

The effect of the right granted to the breeder is that prior authorisation of the breeder is required for:

- the production for purposes of commercial marketing,
- the offering for sale,
- the marketing

of the reproductive material of the variety.

If a breeder intends to market the protected variety, he can do it only in the framework of the European marketing directives, that means within the regulations of the national laws.

The breeder's right will be granted (Article 5 of the Convention) if the variety is:

- new,
- distinct,
- uniform and
- stable.

In Articles 6 to 9 of the Convention these criteria are explained in detail.

Novelty

is essential, because a breeder's right could not be granted if the variety is already marketed.

Distinctness

This means you must be able to distinguish the special variety from all other varieties. This criterion is required so that a breeder can find out that it is the protected variety by inspecting or investigating the seeds or the plants.

Uniformity

The variety is deemed to be uniform if it is sufficiently uniform in its relevant characteristics. The relevant characteristics are those which are used for distinguishing the variety. In my mind uniformity of characteristics which helps to distinguish a variety is a self-evident fact. There are millions of plants and you should be able to select for example 10 plants of the protected variety. This is only possible if the characteristics of distinction are uniform.

Stability

The variety is deemed to be stable if its relevant characteristics remain unchanged after repeated propagation. A uniform characteristic for distinctness is only useful for identification, if this uniform characteristic does not change by propagation. This is the reason for this criterion.

3.2 Assessment of these criteria for forest reproductive material

If breeders' right should be granted for forest reproductive material it is necessary to follow these regulations of the Convention. Under forest aspects some of them are not reasonable. But the need of distinctness necessarily leads to this criterion.

Our question is: Is it useful to adapt these criteria also to the identification of forest reproductive material?

Novelty is no criterion for forest reproductive materiel for marketing. Annex I of Council Directive 66/404/EEC requires for the approval of basic material of forest reproductive material that preference should be given to indigenous stands or to non-indigenous stands of proven value.

Distinctness is no criterion mentioned in our Directives. But the fact that forest reproductive material is distinct, is the basis of the regulation of the Directives. The Directives are based on the fact or on the expectation that this forest reproductive material has a genetic distinctness from other material. I would like to differentiate between two levels of distinctness: The high level of distinctness - the level of the breeders' right Convention - means, that you are able to identify a special forest reproductive material from all other materials. Also in the marketing Directive we reach this level of distinctness in many cases.

In many other cases there exists genetic distinctness which cannot be ascertained by normal methods - especially morphological methods.

- Nearly on the same level as dealed with in the Convention distinctness also exists in the case of forest reproductive material ascertained by morphological traits in the following cases: Varieties in the sense of the above mentioned Convention

- Clones

Mixtures of clones in which each clone is identified; this is only possible in :

- Species, in some genera we have nevertheless some problems for instance with Quercus and Larix

- Subspecies

- clone mixtures with a low number of clones; theoretically, it is also possible to distinguish mixtures of a high number of clones if each clone is identified. Presently it is too expensive.

The Directives demand that forest reproductive material should be separated

- if it originates from different provenance regions (selected reproductive material) or from different basic material (tested reproductive material).

- if the basic material is indigenous or non-indigenous.

In these cases you are on the other level of distinctness, where distinctness is not visible in morphological terms on the reprodutive forest material.

Starting with subspecies, clones, clonal mixtures, tested reproductive material of different basic material, selected reproductive material of different provenance regions, it becomes more and more difficult and finally impossible to recognize if the material is distinct - in a simple sense - or not. There exists also the problem that the genetic distinctness of the reproductive material is not visible at all stages of developement and that often special knowledge is required.

If you are investigating the forest reproductive material in its further development (plants, trees) or in comparative tests you can find out that there exists distinctness. Further progress in the field of distinctness by investigating only the reproductive material can only be reached by biochemical genetic methods. In applying these methods it is possible to find traits which are uniform and stable with all the individuals of the unit.

The idea in forestry is to have heterogenous individuals or populations in order to reduce the production risk during the long production period. I think it is very difficult to find some uniform characterities among different individuals of stands, seed orchards or provenance regions if in principle we intend to select, produce or propagate heterogenous individuals or populations. But on the other hand we have some research results which demonstrate that in same cases it was possible to find such uniform traits.

It is difficult to judge whether research will have in future a chance to find such characteristics. My feeling is that it is accidental if such features are found or not. In some fields there exists a possibility, in some fields there is no chance in my opinion:

- Stands as basic material of tested reproductive material, I guess it may be sometimes possible.
- Mixtures of indigenous stands as basic material for selected reproductive material. I guess it should be possible sometimes.
- Mixtures of non-indigenous stands as basic material for selected reproductive material. I guess there is no possibility.

3.3 Future action

Research by means of biochemical genetic methods should be intensified.

Summary of Session 1: Identification of Forest Reproductive Material

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1. Identification

- The requirements of identification should not influence the requirements of forestry for stands, seed orchards etc.

- Biochemical genetic methods cannot replace the documents accompanying the forest reproductive material.

- Biochemical genetic methods should ensure the congruence of documents and forest reproductive material in the control system.

- The presently existing three methods of biochemical genetic identification

  = investigation of terpens
  = isoenzyme analysis
  = investigation of DNA

have special advantages of identifying regions of provenance, stands, seed orchard clones etc., and all methods should be used and developed.

- We should encourage research in the field of biochemical genetic analysis to investigate also practical problems of identification of forest reproductive material and to find standardized methods of identity investigation

- Linkage between research and control system should be intensified in future.

- In forestry at the present stage checking purposes should have priority in the application of these methods over other purposes.

- Biochemical genetic investigation stations should collect investigation samples of different basic material. Reference populations should be determined and if possible reference maps drawn up. The problem still exists which material should be chosen as reference material: the basic material (stand, seed orchards) or forest reproductive material (seeds, plants)? Also the problem of variation by space and time should be seen.

- The same populations should be investigated by all three biochemical genetic methods. Investigations of former years should be included.

- Reasearch using biochemical genetic methods should be intensified.

2. Provenance regions

- Regions of provenance have a special meaning in regulations and in genetic research. The reason may be a lack of information about the genetic structure of our forests.

- Provenance regions are needed for the customer of forest reproductive material. In some cases it would be preferable to have information on stands or other special basic material.

- Homogenity has to be defined considering the high degree of variation observed.

- Genetic and phenological differentiations between populations and regions of provenance as a criterion for delimitation should be given priority over ecological conditions if information is available.

- For genetic differentiation traits of growth as well as morphological, physiological, phenological, biochemical and genetic characters are useful. They do not necessarily provide the same information.

3. Problems and status of hybrids

- Regarding the marketing situation there are in principle only two genera with hybridisation of some importance, these are Polpulus and Larix. There are no growing market opportunities to be seen for these hybrids.

- If there is a need for rules for such hybrids the rules must state that forest hybrid material should only be marketed under the category of tested reproductive material and that national authorities should have the possibility to set special conditions for the special reproductive material.

Stability in tissue cultures

- It is expected that usually there is a great stability in the reproduction by tissue cultures.
- On the other hand it is necessary to have control mechanisms also by biochemical genetic methods that stability is actually realised.

- Chapter 2 -

Evaluation of forest reproductive material

Chairman : R. PARDOS

National and international networks of experimentations

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1. International experimention

International standardization of experiments was discussed at an agricultural and forestry congress in Vienna as early as in 1890, two years before IUFRO was funded (Speer 1972). At the third IUFRO congress in Vienna in 1900, a committee proposed that the importance of seed orgin should be studied in international experiments for Scots pine, Norway spruce, beech and oak (Petrini 1938). The first international provenance experiment was established already in 1907-1908 with 13 Scots pine provenances at 20 localities in 7 countries (Giertych and Oleksyn 1992). Thirty years later, in 1938, new IUFRO experiments were initiated both with this species and with Norway spruce. A few years later, in 1944, the first international larch provenance trial was established with the participation of research institutes from 12 European and North-American countries (Weisgerber and Sindelar 1992). For the introduced species to Europe from North America, international trials were established with Douglas-fir in 1967 and with Pinus contorta in 1971. For the former species, seed had been distributed to 59 institutions in 36 countries by 1978 (Barner and Fletcher 1978). International experiments were also established with other important North American species.

The IUFRO networks has been the organizational framework for seed collections, planning of experiments and subsequent measurements, and in some cases also of joint evaluations and reports. For tropical species, joint provenance experiments have been established by several other international organizations besides IUFRO; e.g. Danish Forest Seed Center and Oxford Forestry Institute.

Subsets of the genetic entries included in the international trials have sometimes been used in local experiments and in more specialized studies in nurseries, phytotrons or laboratories.

In some countries, breeding and testing are done by several institutions who each are responsible for different regions. Such institutions may cooperate in joint national provenance and breeding experiments. Institutions in neighbouring countries have also established experiments with the same materials. Fewer cases are known of tests of families and clones across international borders and of joint breeding efforts involving several countries and institutions. One example of a more basic genetic study is a series of both family (9 locations) and clonal tests (9 locations) of Norway spruce established in Norway, Sweden, Finland and Denmark in 1988-90. Joint breeding populations have been proposed between Norway and Sweden for Norway spruce (Gullberg and Kang 1985).

When the early trials were established, important factors such as randomization and replications were not always considered seriously (Giertych and Oleksyn 1992). Some of the trials were also lost and forgotten. The remaining trials have, however, in spite of their shortcomings, provided important information to the participating countries. Attempts to achieve regular joint evaluations of results from the experimental series have been less sucessful (Giertych and Oleksyn 1992). At a meeting at Pont-à-Mousson in 1965 attempts were made to standardize methods for provenance research and testing (Lines 1966).

Summary results from several experimental sites have sometimes been published, e.g. for the 1938 Norway spruce experiment by Giertych (1984) and for the 1958/59 larch trial by Schober (1985). Attempts have been made to establish databanks of information from international trials, e.g. from the large IUFRO Douglas-fir series (Birot 1978; Brunet and Roman-Amat 1987). For the IUFRO 1964/68 experiment an effort is now being made to establish a database of measurement data (Skrøppa et al. 1993).

A large number of adaptive, growth and quality traits have been measured in field trials in 12 countries and serves as one example of the type of data that may be available from the international trials (Table 1). It is seeen that height and diameter growth have been measured in nearly all tests, flushing has been assessed at some locations, damage has been assessed where occurring, and several quality traits have been measured. Similar types of traits have been measured in the other international networks.

The rest of this paper will discuss international experimentation for legal regulations of reproductive material, for research and gene conservation activities.

Country	Measurements
Austria	Height, diameter
Belgium	Flushing, early height
Canada	Early height
Czech.rep.	Flushing, height
France	Flushing, height, diameter, growth cessation, wood quality
Germany	Height, diameter
Hungary	Height, diameter
Ireland	Flushing, height, diameter, frost damage
Norway	Flushing, height, diameter, quality traits, damage
Poland	Flushing, height, damage, quality traits
Scotland	Flushing, height, diameter
Sweden	Height, diameter, damage quality traits

Table 1 - **Measurements and data available from the 1964/68 IUFRO provenance**

2. Legal regulations

2.1 Assumptions

- Phenotypic selection may tell little about the true genetic value of progenies from the stand and their performance when planted at another site. The conclusions we can draw about performance of "selected" materials from one region of provenance will therefore not be much different from those of the category "source identified" reproductive material of the OECD Scheme.

- Based on domestic ecological conditions, individual countries will set their own requirements and will give general recommendations.

- To help the purchaser to choose among appropriate tested materials performance in some basic adaptational traits should be required in the seed certificate. Information of different traits will be required in different planting regions.

- Information about the performance of materials have be available within reasonable time. Long-term tests for yield and timber quality will not be practical both due to the time intervall and needed plot sizes. Testing av materials therefore has to be done by early or short term tests.

- Tested material will be subdivided into two classes;progeny tested and comparatively tested.

- The genetic structure of reproductive materials will have to be tested by genetic marker traits (isozymes, DNA-markers) (Hattemer 1987).

- Two classes of traits :
  - Performance traits characterizing - adaptation to the environment, e.g. flushing
    - yield, e.g. height growth, volume production
    - quality, e.g. wood density
    - resistance to pests and diseases.
  - Traits characterizing the genetic structure by genetic markers
    - isozymes
    - DNA.

2.2 Evaluation of materials

Evaluation of materials is necessary for several purposes : deciding on national requirements and recommendations, provide information to seed certificate, provide sufficient information for approval of tested material, legal control of approved material.

A better description of the performance of "source identified" material can be given by combining the information available from a large number of international trials. Identification can be made both of regions or provenances with a superior and stable and of those with a poor or unstable performance. Such information will be valuable for countries when making their local recommendations.

The norms of reaction of a material discribes its phenotypic performance under changing environmental conditions. It is determined both by the genetic constitution of the material, its phenotypic plasticity and possible interactions between genotype and environment. Joint evaluations of traits from the international trials may provide such information both for regions of provenance and for individual provenances.

Examples are shown i Figs. 1 and 2 based on data from 11 sites from the IUFRO 1964/68 Norway spruce experiment (Skrøppa et al. 1993).

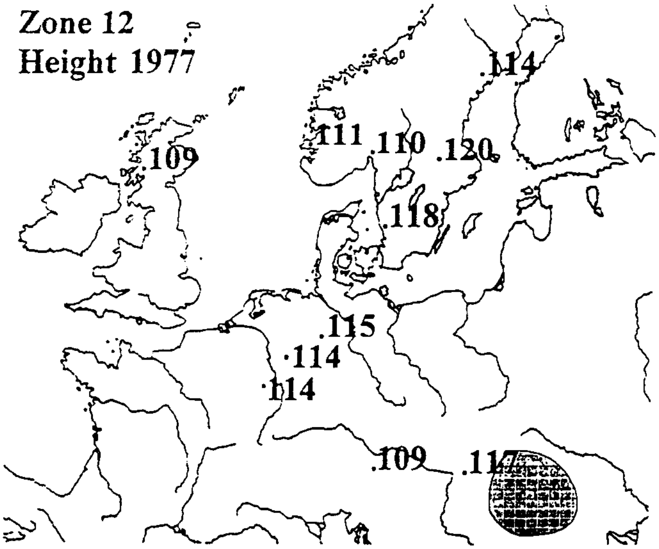

Figure 1 - **Mean heights in percent of the block means of zone 12 (Carpathian mountains). Measurements in 1977/79 at 11 sites in the IUFRO 1964/68 provenance trial with Norway spruce.**

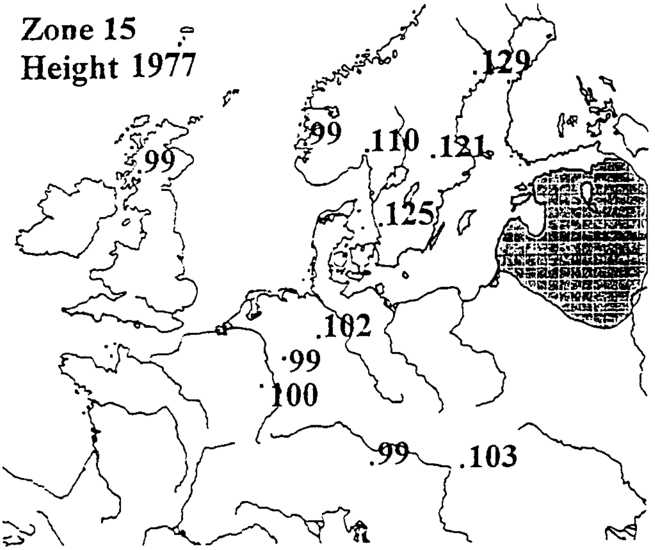

Figure 2 - **Mean heights in percent of the block means of zone 15 (Baltic countries, White Russia and Western Russia). Measurements in 1977/79 at 11 sites in the IUFRO 1964/68 provenance trial with Norway spruce.**

2.3 Early test results should be available internationally

Early testing of adaptational traits can often be done under controlled conditons. As an example, 45 commercial seedlots of Norway spruce, both provenances and seed orchard material, have been tested in freezing test after phytotron cultivation. A database of early test results would be valuable for countries that have common interest in the same materials. However, standardization of test conditions and further development of methods will be necessary. Actual traits under boreal conditions are: timing of flushing and bud-set and development of frost hardiness. Resistance to specific diseases are important for some species and can be tested by combining laboratory and field tests.

2.4 Combine early and field test data

Early tests are often made of adaptational traits, while short and long term field tests are for yield and quality traits. In some regions, i.e. under boreal conditions, information about adaptational traits are necessary before yield and quality can be evaluated.

Field tests for progeny or comparatively testing of materials will have to be done at least at one site in the intended region of planting.

Joint networks for testing of materials that are of common interest to several regions or countries ?

In a common testing program a lower number of test sites will be needed totally. Norms of reaction can be studied over a wider environmental range. A better coordination of experimental procedures will be required. This relates to:

- comparable experimental designs
- identical observational and measurement procedures
- time tables for observation and measurement
- data processing, analyses and reporting.

2.5 Long-term breeding tests should be done regionally

This is due to large variation in ecological conditions and different silvicultural priorities. Even if basic materials from the same region of provenance will be used, adaptation to the local conditions may soon occur. Exchange of bred material and test data will never the less be advantageous, in particular if conditions and priorities are similar. Concerns of keeping a high genetic diversity should favour local breeding populations.

Testing of genetic markers could be done under standardized conditions at central laboratories.

At present, no standard requirements can be given of genetic marker traits. It is difficult to relate this type of genetic variability to specific ecological conditions and use it to evaluate the adaptive potential of different materials. In the future such traits may be required also for the testing of performance and not only for identification.

2.6 Should international standards for testing be developed ?

Such standards have sometimes been used in international provenance trials. Several problems: different standards are needed in different regions, the maintenance of standards, are standards reproducible ?

3. Research and gene conservation

As mentioned above, comprehensive analyses of the performance of provenances across a number of test sites under variable environmental conditions will give valuable information for recommendations of the use of source identified material. Norms of reaction studies can be made both in space and time and for several traits simultaneously. Such analyses will have to be done as research projects and require databases containing data about provenances, field trials and measurements. There are several obstacles in such research: lack of ecological information about provenances and test sites, inadequate field test designs, variations in the quality of experimental data, lack of conformity of measured traits both across locations and in time.

The older experiments can be used to study the direction and rate of land race formations of non-indigenous material. As an example, progenies of selected trees from East-European provenances in the IUFRO 1938 Norway spruce experiment at Umeå, Sweden, did not retain the adaptational properties of their original provenance (Skrøppa et al. 1993). Similar studies could be done in parallel trials of the same or other experimental series.

Biochemical and molecular genetic analyses are often performed on materials whose quantitative traits are not characterized. The same materials, preferrably from the experimental networks, should be used in different types of genetic studies. By combining results of genetic structure and reaction norm studies, the genetics of the tree species can be better understood. The study by Lagercranzt and Rymann (1990) based on the 1964/68 IUFRO Norway spruce experiment is a good example.

Early test methods, standardization of techniques and the relevance of early tests need further development if results from such tests are to be generalized.

Early testing of off-spring from Norway spruce seed orchards have shown large differences in performance between off-spring from different years (Kohmann and Johnsen 1993). The extent of such effects and their implications both for legal control and gene conservation should be investigated by early testing, field trials and biochemical methods for different types of material.

The research tasks mentioned above are important both for legal control and for conservation of genetic resources. Methods for rapid assessment of biodiversity, for detecting and quantifying adaptive potentials of species and populations should have top priority in research. The long-term international experiments may provid evaluable materials for such research. These experiments are in addition reservoirs of genetic variability and should be maintained for the future.

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Marker-assisted selection for the evaluation of forest reproductive material

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Abstract

Molecular genetic markers may play a significant role in the reduction of breeding cycles and in the characterization of forest reproductive material. Their use has already produced valuable results in the study of plant taxonomy, population genetic structure, mating systems and genotype fingerprinting. The development of linkage maps has already started with some species, including poplars and pines providing the backbone for a more intensive use of molecular methods in tree breeding.

Prospectives and limitations of marker-assisted selection and of its special case represented by the detection of and selection for quantitative trait loci are reviewed and discussed. High levels of heterozigosity, inbreeding depression, linkage equilibrium and long reproductive cycles limit the potential of molecular techniques with respect to agricultural crops, but significant results have already been obtained in the field of disease resistance. Future achievements are guaranteed by the fast evolution of molecular biology, the increasing efforts in this field and well established scientific cooperations.

Introduction

The discovery that the DNA is responsible for the conservation, replication and transmission of genetic information, that the "genes" are sequences of nucleotide bases linearly arranged on chromosomes, gave new grounds to breeding theories, but, until recently, few fresh tools for breeders.

Most of the traits of economic value are polygenic and the effect of the single components are confused by the blurring effect of the environment. Sophisticated statistical tools (Baker, 1986; Falconer, 1989; Mather and Jinks, 1982; Namkoong, 1979; Narain, 1990) play a central role in the improvement of tree species, but do not remove the main drawback of tree improvement, that is, the long time necessary for results to be applicable on a commercial scale.

Indirect selection based on correlations among traits and juvenile selection that exploits age-age correlations are valuable aids towards shorter breeding cycles. A special kind of indirect selection is that based on genetic markers, characters that are easy, fast and cheap to identify and that can be used as clues for the detection of other characters to which they are associated but that cannot be observed directly or need big investments in terms of time and money.

A first class of genetic markers is represented by biochemical markers (isozymes, terpenes, phenols) that need relatively small amounts of material, can be assessed quickly, are often inexpensive in comparison with field tests but are usually influenced to some extent by the environment and/or by the developmental stage and type of tissue of the samples. Molecular genetic markers, on the other hand "read" the genome without confusing effects.

1. Molecular genetic markers

Although the linear arrangement of the genes on chromosomes was discovered some 80 years ago (Sturtevant, 1913) the major breakthrough towards a fine analysis of DNA was represented by the discovery of restriction endonucleases that degrade DNA by splitting both strands of the double helix at specific nucleotide sequences (Smith, 1979); more than 500 of them exist (Neale and Williams, 1991; Smith, 1979; Ayala and Kiger, 1984), but the most important ones, for the purposes of practical applications, are those that recognize palindromic sequences, specific sequences of nucleotides that have an axis of symmetry and read identically in both directions, and make a double strand cut at a fixed point within that sequence.

2. Restriction fragment length polymorphism

A first class of MGM is represented by Restriction Fragment Length Polymorphisms (RFLP) that are based on the isolation of DNA (Murray and Thompson, 1980; Dhillon, 1987; Weissenbach and Weissenbach, 1988; Schuler and Zielinski, 1989), its digestion by a Restriction Endonuclease, the separation of the fragments by electrophoresis and the "hybridization" to radio-labeled DNA probes: the position of the hybridized fragments is showed by autoradiography. Different fragment patterns correspond to Length Polymorphisms in the DNA surrounding the hybridization site and can be treated as alleles at a locus for the construction of genetic maps (Botstein *et al.*, 1980), the analysis of segregation (Young, 1992), and many other practically important uses.

Among the advantages of RFLPs as MGM one should mention (Tanksley *et al.*, 1989):

- **repeatability**: the same combination of endonuclease × probe produces the same pattern;
- virtually **illimited number of polymorphisms**;
- **co-dominant** behaviour that allows the distinction of heterozygotes from homozygotes (Rafalski *et al.*, 1991);
- high level of **allelic variation**;
- **phenotype-neutrality** and freedom of epistatic effects (Neale and Williams, 1991);
- coding, as well as **non-coding regions** of the genome can be studied;
- both **Mendelian and non-Mendelian** (chloroplasts and mitochondria) inheritance can be identified (Wagner, 1992).

3. Random amplified polymorphic (DNA)

DNA segments can be amplified (that is selectively copied) by means of the Polymerase Chain Reaction (PCR) in the presence of suitable primers (Saiki *et al.*, 1985). The RAPD technique (Welsh and McClelland, 1990; Williams *et al.*, 1990) makes use of arbitrarily constructed primers. The result of the PCR amplification is visible with ethidium bromide staining, after separation by length with electrophoresis on agarose gel (Kricka, 1992).

As with RFLP markers, the recognition sites can vary among individuals due to base substitutions, insertions or deletions and all lead to fragments of different size. With respect to RFLP markers, RAPD present many advantages (Taramino and Pé, 1992):

- **no** need for **radioactive probes**;

- **moderate requirements of DNA** (20 ng instead of 7-10 μg of RFLP);

- easy **automation** of the process;

- use of the **same primers** on a large variety of species, with no need to construct species-specific probes.

but they behave as dominant markers: absence of a band represents all possible alleles at that locus (Rafalski *et al.*, 1991) and therefore heterozygous loci cannot be singled out. As RFLP, also RAPD markers are virtually infinite in number as both the length and the sequence of the primers can be varied at will and even single base substitutions lead to different polymorphisms (Williams *et al.*, 1990).

On the other hand RAPD markers are strongly dependent on experimental conditions and require rigorously optimized reaction conditions in order to be repeatable (genomic DNA, primer, polymerase and Mg^{2+} concentrations, temperature, PCR equipment).

4. Other molecular techniques

Polymorphisms can also be detected as length variations of tandemly repeated short sequences of DNA bases (minisatellites). The repeated sequence itself is used as probe for DNA fragments obtained by digestion with restriction endonucleases. The limits in the application of minisatellites are the same as for RFLPs. The typical field of application is in forensic medicine and in taxonomy.

A recently developed technique, the AFLP (Amplified Fragment Length Polymorphism) represents a bridge between RFLP and PCR. It makes use of two different restriction endonucleases to split the DNA into fragments of different length and the ends are ligated to specific adaptors; PCR is then carried out using oligonucleotides designed on the adaptors as primers. The AFLP techniques detects a higher number of polymorphisms than RFLP and its use on forest trees ahs already started.

5. Marker-based linkage maps

A genomic map is the central organizing concept of almost all activities in the field of genetic analysis as it allows the integration of results from separate studies of the same species or genus (Stettler, 1993). A map composed of molecular genetic markers provides the framework for mapping other traits coded by genes segregating in a pedigree; when a detailed linkage map is available, selection can be based on a direct knowledge of the genotype instead of on its estimation through the phenotype (Cheliak and Rogers, 1990).

Genetic molecular mapping work started with humans in the early eighties (Botstein *et al.*, 1980; Donis-Keller *et al.*, 1987) and was soon followed by agricultural crops. In a non-exaustive list of molecular linkage maps of crop species Young (1992) mentions nine species as already mapped and six more with mapping projects under way. Among forest trees, genetic moderate-density maps have been constructed for *Populus* (Bradshaw, 1993; Bradshaw *et al.*, 1993; Bradshaw and Stettler, 1993; Stettler, 1993) and *Pinus taeda* L. (Neale *et al.*, 1992), but the genetic material is available for several other species, especially conifers (Neale and Williams, 1991). Mapping work will also take advantage of the fact that work on related or even distant species can be based on common DNA probes due to large homologies in DNA sequences (Ahuja *et al.*, 1992; Kriebel, 1993).

6. Marker-assisted selection (MAS)

Genes coding for economically important traits can be located on an marker-based map by analysis of linkage with Molecular Genetic Markers. The presence/absence of a particular gene in the individuals of a segregating population can then be inferred from the detection of one or more markers that are known to reside nearby (Tanksley *et al.*, 1989; Cheliak and Rogers, 1990). Selection can be made at the seedling stage (McCouch *et al.*, 1988), even though the trait is not apparent in the phenotype of juvenile material (e.g. sex for dioecious species such as Poplars).

Crop breeding depends, to a large extent, on the introgression of genes (typically, resistances) from wild relatives into commercial stock. However, undesirable genes are introduced together with the target one, a phenomenon called "linkage drag". The traditional approach involves repeated backcrossing to the commercial variety of those individuals that, at each generation, display the desired trait and are the most similar to the recurrent parent for the other relevant traits. However, after several cycles, a substantial part of the wild genome can still be inherited due to low recombination in the area around the target gene.

Time is of course the limiting factor. An RFLP-based map can be employed to reduce it. If two RFLPs flanking the target gene are singled out, a screening in a segregating population could reveal individuals that differ from the recurrent parent for the region where the target gene is located, being otherwise very close replicas. It has been estimated that introgression of a small region of 2 cM in tomato would need only 2 generations with the assistance of a very dense RFLP map, over 100 without (Young and Tanksley, 1989; Tanksley *et al.*, 1989).

Unfortunately, such striking results are guaranteed only for crops that make use of near-isogenic lines. With highly heterozygous species such as forest trees (Hiebert and Hamrick, 1983; Bousquet *et al.*, 1987), backcrossing may provoke undesirable effects in terms of inbreeding depression. One possibility would be to obtain interspecific hybrids and to backcross to an unrelated individual of one of the parent species; this strategy, that has already been adopted with poplars (V. Steenackers, pers. comm.) could exploit the ability of molecular genetic markers to detect those individuals of the B_1 progeny that have retained the largest share of the recurrent species, while, at the same time, harbouring the desired gene(s).

Significant results with MAS have been accomplished for many agricultural crops, typically in the field of genetic resistance to diseases (Young, 1992), that is often found to be controlled by single genes.

However, when it comes to forest tree species, MAS does not promise such dramatic results. The reasons for that are reviewed by Neale and Williams (1991) and by Neale *et al.* (1992) and will be briefly summarised here.

Forest tree breeding programmes are usually based on large, little domesticated, breeding populations that are near linkage equilibrium. In such a condition, it is impossible to predict if a useful gene and a marker are in linkage (cis) or in repulsion (trans) in any single member of the population. However, even if a single resistance gene was identified, vertical resistance would not be the first choice with forest trees.

Anyway, it would not be wise to drastically reduce the level of genetic variability of available populations, as heterozigosity is a pre-requisite of adaptive ability: it has been shown that the level of heterozigosity of mature populations is considerably higher than that of seed or seedlings from the same location, a demonstration of negative selection against homozygous individuals (Morgante *et al.*, 1991).

The majority of characters that are of economical importance for forest trees are certainly under polygenic control and would need multiple sets of markers. Furthermore, in case strong genotype × environment interactions existed, it would be necessary to develop different sets of markers for each environment (Paterson *et al.*, 1991).

Potential applications of MAS are far greater in case of breeding programmes making use of small elite breeding populations; a marker-based mapping of all the members of the population might be justified. Indeed, this could be the case with poplars, a genus on which considerable work is under way (Villar *et al.*, 1993; EC-funded project AIR1-CT92 00349).

7. Quantitative trait loci (QTL) mapping

Most of the economically important traits of forest trees are the result of the simultaneous action of many genes, each with a small effect: e.g. yield, stress tolerance, resistance to pests and diseases. The number of individual genes is generally unknown as well as each gene's contribution to the phenotype. Traditionally, these traits have been treated with a statistical approach without regard to the identification of the loci involved (Cheliak and Rogers, 1990).

Now that saturated marker-based genetic maps are being produced, the "genetic dissection" of complex traits into components seems feasible by studying the co-segregation of traits and markers located on the map at regular intervals of 10-20 cM throughout the genome (Paterson *et al.*, 1988; Tanksley *et al.*, 1989).

Traditional methods for the association of markers to QTLs involves comparing the mean and variance, for the trait in question, of subgroups in a segregating population differing for each marker in turn. Markers that correspond to significant differences are believed to be linked to at least one QTL (Young, 1992; Ottaviano *et al.*, 1991). However, if the procedure is correct for any single marker, when many markers are screened simultaneously, there is an increasing risk of false positives. Moreover, loose linkage to a QTL with large effect cannot be distinguished from tight linkage to a QTL with small effect.

Lander and Botstein (1989) proposed a new methodology, called "interval mapping" that consists of splitting the map into intervals defined by pairs of flanking markers. Each interval is then screened for the ratio of the likelihood that the observed data arise from the presence of a QTL versus the likelihood of its absence. Appropriate values of the Logarithm of the ODds (LOD) ratio can be chosen that correspond to a desired overall level of protection from type I errors (false positives). Interval mapping also presents the advantage that "selective genotyping" can be applied, that is, only the most divergent phenotypes of the segregating populations would be screened for markers, thus reducing the costly lab work (Lander and Botstein, 1989; Darvasi and Soller, 1992). With traditional ANOVA-based method this would bias the tests.

Using this technique Paterson *et al.* (1988) detected QTLs explaining considerable shares of total variance of several polygenic traits in tomato. Although it is still not possible to say if detected QTLs are really single major genes or rater combinations of tightly linked minor genes, this is not likely to be a serious drawback for practical applications. Some markers appear to be linked to more than one trait, thus explaining observed correlations; possible explanations, tight linkage or pleyotropic effects are discussed also in Paterson *et al.* (1990).

Among forest trees, QTLs for several dendrometrical and physiological indices have been detected for a poplar pedigree originating from *Populus deltoides* Bartr. and *P.trichocarpa* Torr. *et* Gray ancestors (Bradshaw *et al.*, 1993; Bradshaw, 1993); the variance explained by the QTLs varies between 25 and 95% of the total variance; evidence suggests that a surprisingly small number of QTLs is involved, that the hypothesis of "equal and small" individual contribution of components in the phenotypic expression of a quantitative trait may not hold and that pleiotropy more than linkage might explain observed correlations among relevant quantitative traits.

When the study was extended to sharply different environments, different QTLs were detected for the same characters, only a fraction of which appeared stable (Paterson *et al.* 1991).

The highest advantages from marker-assisted selection for QTLs are to be expected for traits of low heritability.

Statistical genetic models (Dudley, 1992; Jansen, 1992; Martinez and Curnow, 1992; Moreno-Gonzales, 1992b), experimental designs (Motro and Soller, 1993; Van der Beek and Van Arendonk, 1993), and methods of analysis (Moreno-Gonzales, 1992a; Hoeschele and VanRaden, 1992a; Hoeschele and VanRaden, 1992b) are being proposed and tested with real data or computer simulation so frequently, that the whole subject is certain to evolve significantly in a matter of months.

Typical co-segregation analysis involves the use of highly inbred lines that are not common in forest tree breeding programmes. However, if the F_1s in a three-generation pedigree are obtained from parents that differ sharply, the procedure would be probably successful.

Conclusions

Linkage equilibrium, wide breeding populations, inbreeding depression and genotype x environment interactions are the main obstacles to a massive use of molecular genetic markers in forest trees. However, several practical applications are already available, at least for those cases where a complete molecular characterization of a set of genotypes is feasible.

Molecular genetic markers can be employed for the detection of foreign pollen contribution in a seed orchard (Smith and Adams, 1983; Friedmann and Adams, 1985) or for the correction of sexual asymmetries among the parent trees included.

Breeding programmes that are based on a small number of highly selected individuals may base the selection of parents on the molecular characterization of their genome, especially in relation to disease resistance. Maps are already available for some forest trees and are likely to be saturated in a near future.

There are several reasons, analyzed by Lande and Thompson (1990) why molecular genetics can never replace traditional breeding methods and strategies. Breeding work will still require many crosses, careful selection and good luck; molecular genetics will make the process more deterministic and reduce chance components (Young, 1992).

Of course, more research is needed to validate results obtained in pedigrees, but a lot of work is being done at present throughout the world and appropriate integration of information and coordination of efforts ensures positive results in a very near future.

The freedom of marker-assisted selection from any real or feared negative environmental impact that makes other innovative techniques suspicious in the eyes of the public, e.g. genetic engineering, will be an additional factor of success.

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Age-age correlations for the evaluation of forest reproductive material

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Abstract

Age-age correlations represent a useful tool to reduce the long breeding/selection cycles of forest trees. Selection based on the juvenile expresson of a trait that will be of interest at harvest time is a special case of indirect selection: its efficiency is determined by heritabilities at the juvenile and mature age and by the degree of statistical correlation between juvenile and mature observations. Two classes of traits are treated separately in the paper: cumulative and non-cumulative. The first class includes wood characteristics (density, fiber length, chemical composition), resistance to adverse factors, phenological characters: observed correlations are usually sufficiently high to justify an early or very early selection, at least for those characters whose expression is weakly affected by the microclimatic factors that change with stand life. The second class comprises all size-related characteristics; due to the obvious fact that the size at age T includes the size at age t<T, part of the correlation between observations is due to the variance of the juvenile observations alone. The consequences are discussed in the paper with a review of existing literature and computer simulations.

During the process of evaluation of forest reproduction materials age-age correlations can be used with confidence, at least for the release of provisional authorizations or for selective thinnings in seed orchards. The same principles that suggest early selection in breeding operations are valid here: the time spared by making the results of breeding work available for operational use in a shorter time compensates the loss of information.

Introduction

With respect to agricultural crops, forest trees need a much longer time to reach harvest size. Although they are sometimes compared with long living fruit trees, their unique feature is that harvesting is usually done only once, the product being the tree itself.

Breeding and selection cycles can be reduced through the prediction of the future behaviour of trees based on observations made at a previous age (age-age correlation or juvenile-mature correlation): if predictions are reliable, then decisions about the value of the material available can be accelerated accordingly, be it for the selection of parents for a new generation, the establishment or the thinning of a seed-orchard, or the start of commercial operations with experimental material. Statistical correlation is the concept involved; causal relationships are not necessarily looked for.

The basic question is: considering that the behaviour of mature trees is only imperfectly related to juvenile performance, how much information can be sacrificed in order to save time?

1. Indirect selection

Juvenile selection is a form of indirect selection, that is, selection for a secondary (non target) trait in order to improve a more relevant trait to which the first one is correlated. The genetic gain (*GG*) due to direct selection on the target trait is $GG_T = i_T h_T^2 \sigma_{P_T}$ where the subscript *T* refers to the target (or mature) trait, *i* = selection intensity, h_T^2 = heritability ($h_T^2 = \sigma^2_{G_T} / \sigma^2_{P_T}$), $\sigma^2_{G_T}$ = genetic variance, $\sigma^2_{P_T}$ = phenotypic variance.

The genetic gain in the target trait after selection on a correlated (non target) trait is (Falconer, 1980)

$$GG_{T|t} = i_t h_T h_t r_{G_{T,t}} \sigma_{P_T} \qquad (1)$$

where the subscript *t* refers to the correlated (or juvenile) trait, $r_{G_{T,t}}$ = coefficient of genetic correlation between target and correlated trait and the other symbols have the same meaning as indicated above.

By solving the inequality $GG_{T|t} > GG_T$, and assuming equal selection intensities ($i_T = i_t$) one can find the conditions for indirect selection to be more efficient than direct selection, namely (Searle, 1965): $h_t^2 > h_T^2$; $r_{G_{T,t}} > h_T / h_t$.

However, even if these conditions are not satisfied, indirect selection may still be advantageous if selection on the target trait is impossible or too expensive, or when the time saved compensates the loss due to imperfect correlations. This is the typical case of selection made at a juvenile age: the comparison shifts from genetic gains *per se* to genetic gains per unit time. If we now denote by the subscripts *T* and *t* parameters at ages *T* and *t* (*t*<*T*), then the objective is to find the conditions under which $GG_{T|t} / t > GG_T / T$.

Some Authors (Squillace and Gansel, 1974; Magnussen, 1988) include a delay factor (*d*) in both denominators to allow for breeding operations to be carried out, so that a whole breeding and selection cycle would take *t*+*d* and *T*+*d* years with and without indirect selection on a juvenile trait.

In many cases, economic considerations have been introduced (Squillace and Gansel, 1974; Kang, 1985; McKeand, 1988; Newman and Williams, 1991) by calculating the present monetary value of future genetic gains on various but often disputable assumptions about discount rates, cost of breeding operations, price of roundwood, etc.

However, for clarity, the discussion here will be limited to genetic gain, that is, the shift in the mean of the population for a certain trait as a consequence of selection.

The key role is played by the coefficient of correlation (Pearson's *r*) between the performance (*Y*)of trees at different ages. Two definitions of *r* are relevant in this context: the phenotypic correlation coefficient $r_{P_{T,t}} = \frac{COV_P(Y_T, Y_t)}{\sqrt{VAR_P(Y_T) \cdot VAR_P(Y_t)}}$ (where the subscript *P* refers to phenotypic parameters, *COV* and *VAR* to covariance and variance, respectively) and the genetic correlation coefficient $r_{G_{T,t}} = \frac{COV_G(Y_T, Y_t)}{\sqrt{VAR_G(Y_T) \cdot VAR_G(Y_t)}}$ where the subscript *G* refers to genetic parameters; by $COV_G(Y_T, Y_t)$ one generally denotes the additive genetic covariance; when dealing with clones, however, $COV_G(Y_T, Y_t)$ can include non-additive effects and $r_{G_{T,t}}$ should be referred to as coefficient of genotypic correlation.

The ratio $CGP_{T|t} = \frac{COV_G(Y_T, Y_t)}{\sqrt{VAR_P(Y_T) \cdot VAR_P(Y_t)}}$ was defined by Baradat (1976) as coefficient of genetic prediction (*CGP*). It reduces equation (1) to $GG_{T|t} = i_t \cdot CGP_{T|t} \cdot \sigma_{P_T}$ and corresponds to the shift in standard deviations of the mature trait mean as a consequence of a selection intensity *i* applied in the juvenile phase.

In any case, it is important to separate the genetic and environmental components that combine into the phenotypic correlation (Franklin, 1979)

$$r_{P_{T,t}} = h_T h_t r_{G_{T,t}} + \sqrt{(1-h_T^2)(1-h_t^2)}\cdot r_{e_{T,t}} = CGP_{T|t} + \sqrt{(1-h_T^2)(1-h_t^2)}\cdot r_{e_{T,t}}$$

where $r_{e_{T,t}}$ = coefficient of environmental correlation and the other parameters have been defined above. In some cases genetic gains have been calculated on the basis of $r_{P_{T,t}}$: by rewriting (1) as $GG_{T|t} = i_t \cdot \left[r_{P_{T,t}} - \sqrt{(1-h_T^2)(1-h_t^2)}\cdot r_{e_{T,t}} \right] \cdot \sigma_{P_T}$ and assuming $\sqrt{(1-h_T^2)(1-h_t^2)}\cdot r_{e_{T,t}} = 0$, then: $GG_{T|t} = i_t r_{P_{T,t}} \sigma_{P_T}$.

However, some Authors (Namkoong and Conkle, 1976; Nepveu, 1980; Lambeth *et al.*, 1983; Loo *et al.*, 1984; Foster, 1986; Riemenschneider, 1988) pointed out that assuming null environmental correlation or $h^2 = 1$ is not realistic in most situations. Environmental correlations between observations made on juvenile and mature material are generally positive (which is only to be expected, at least for those traits, such as growth, where soil-related characteristics play an important role).

2. Juvenile selection for non-incremental traits

In the context of juvenile selection there are essentially two types of variables: in the first case the observed level of a variable at age T includes any value attained at age $t<T$, in the second case it does not. The first case is typical of size-related variables and will be discussed in more detail in the next section: the second one refers to all the other cases, such as resistance to diseases, wood quality, chemical contents and so on. The reason for separating the discussion of the two cases lies in the peculiar mathematical nature of the correlation coefficient in the first case, which must be properly understood for a correct application.

Pleiotropy (multiple effects of the same gene) or linkage (associated inheritance of two genes due to short distance of *loci* residing on the same chromosome) are commonly invoked to explain correlations among traits (Falconer, 1981). As far as age-age correlations of the same trait (most often quantitative in forest trees) are concerned, a likely explanation is that of a multiple set of genes affecting the expression, with some genes getting out of and others getting in the set as time goes (Kremer, 1992); it is also possible that interactions with other genes (epistatic effects) are the forces driving the evolution of correlations. However, the causal relationship between a predictor and a predicted variable is not the primary concern in the field of age-age correlation; its magnitude, whatever the causes, determines practical applications.

The analysis of literature makes it clear that traits which are likely to be the expression of relatively basic biochemical or physiological processes usually display a high juvenile-mature correlation. This is the case, for instance, of limonene contents in *Pinus pinaster* Ait. where a monogenic control has been demonstrated (Marpeau-Bezard *et al.*, 1983) with $r_G = 0.97$ ($CGP_{T|t} = 0.62$) or oleoresin yield in *Pinus elliottii* Engelm. which displays $r_G = 0.87$ between ages 10 and 18 (Squillace and Gansel, 1974).

A group of traits that was frequently studied in the context of forest trees is that related to wood characteristics, considering that the quality of the product determines the value of forest trees, at least as much as quantity.

Specific gravity in *Pinus taeda* L. was found to be strongly correlated between ages 2 and 25, with $r_G = 0.73$ to 0.96 ($CGP_{T|t} = 0.58$ to 0.77) (Loo *et al.*, 1984). High levels of r_G have been reported also for poplars: $r_G = 0.60$ to 0.72 between 1-year-old shoots and 10 to 12-year-old trees of *Populus nigra* L. (Nepveu *et al.*, 1978); $r_G = 0.65$ to 0.72 between 2-year-old nursery sets and mature trees of *P.trichocarpa* Torr. *et* Gray (Pichot, 1993); $r_G = 0.87$ (genotypic) between 2-year-old nursery sets and 10 to 12-year-old trees of *P.* × *euramericana* (Dode) Guinier (Scaramuzzi, 1973).

Fiber length of 2-year-old nursery sets of *P.* × *euramericana* was found to be as well correlated ($r_G = 0.74$) with fiber length of trees at harvest age as that of 8-year-old ones (Scaramuzzi and Ferrari, 1982). Nepveu *et al.* (1978) found rather weaker correlations in *P.nigra* ($r_G = 0.41$), but with even more juvenile material. In *P.taeda*, on the other hand, tracheid length seems to change drastically with age (Loo *et al.*, 1984).

Age-age correlations in *P.* × *euramericana* are weak for holocellulose content ($r_G = 0.22$), more substantial for α-cellulose ($r_G = 0.67$) (Scaramuzzi and Ferrari, 1982).

Much effort has also been devoted to the development of early tests of susceptibility to diseases and to the evaluation of their reliability. However, due to obvious difficulties in the quantitative expression of observations in this field, the degree of correlation is seldom translated into figures.

The reliability is often high when the organs affected by the disease are renewed every year (e.g. leaves), so that age and size of the tree are of secondary importance, and when the behaviour of the disease agent is relatively neutral with respect to those microclimatic factors that vary with the size of the trees. As far as Poplars are concerned, juvenile selection is very efficient for *Marssonina brunnea* (Ell. *et* Ev.) P.Magn.(Anselmi *et al.*, 1975) and *Xanthomonas populi* (Ridé) Ridé *et* Ridé (Ridé and Ridé, 1978; de Kam and Heisterkamp, 1987), dubious for *Melampsora larici-populina* Kleb. (Pichot, 1993). A better control of the environmental factors in the juvenile evaluation, especially *inoculum* pressure, certainly improves the efficiency of early tests; this is the case of lab tests that have been developed for *Melampsora* species (Pinon *et al.*, 1987).

High age-age correlations have been found also for crown architecture (Muhle Larsen, 1967) and phenological traits (Pichot, 1993).

3. Age-age correlation of tree size

The amount of literature on age-age correlations of size-related traits reflects the primary importance of growth in the value of forest trees, but also the ease of statistical treatment of quantitative (continuous) variables. A review of the literature reveals quite substantial positive age-age correlations from very juvenile years with regard to rotation age in many systematic groups.

Height growth in conifers, especially pines, received much attention, ease of retrospective studies based on whorl position being one of the reasons (Namkoong *et al.*, 1972). Phenotypic correlations with respect to rotation age (30 years) were found to vary between 0.67 at age 10 and 0.71 at age 15 in *Pinus elliottii* Engelm., *P.taeda*, *P.palustris* Mill. and *P.echinata* Mill. (Wakeley, 1971). Similar levels of r_P are reported by Squillace and Gansel (1974) for *P.elliottii*: $r_P = 0.47$ (age 8 *vs* 25), $r_P = 0.74$ (age 14 *vs* 25). Substantial phenotypic correlations are also reported by Namkoong and Conkle (1976), although most of them is to be attributed to environmental factors. In *Pseudotsuga menziesii* (Mirb.) Franco Namkoong *et al.* (1972) found phenotypic correlations to be 0.62 and 0.82 at 1/3 and 1/2 of the 53-year rotation.

Lambeth (1980) proposed an empirical equation that describes the evolution of age-age phenotypic correlation of height in several conifer species as a function of $\mathrm{LAR} = \ln(t / T)$, that is: $r_P = a + b \cdot \mathrm{LAR} = 1.02 + 0.308 \cdot \mathrm{LAR}$.

When genetic age-age correlations were estimated (Lambeth *et al.*, 1983; Loo *et al.*, 1984; Foster, 1986; McKeand, 1988; Riemenschneider, 1988), they were found to be invariably higher than phenotypic correlations. This implies that, if genetic gains are calculated using phenotypic instead of genetic correlations, optimum selection age is overestimated, that is, one would not run the risk of selecting too early.

In *P.banksiana* Lamb. Riemenschneider (1988) found that, when calculating a linear regression of genetic correlations on LAR, the slope was considerably lower than that of Lambeth ($b = 0.177$), while the intercept was very close to 1, as in Lambeth and in agreement with theoretical considerations (Kang, 1985, Burdon, 1989).

Age-age correlations for DBH and volume are generally higher than those for height (Wakeley, 1971; Squillace and Gansel, 1974; Lambeth *et al.*, 1983; Foster, 1986) which suggests that height could be replaced by or at least supplemented with diameters in practical applications.

Genotypic correlations can be calculated in experiments with clonal material: they are usually high or very high, be it for height growth of *Picea abies* Karst. (Roulund *et al.*, 1986), or circumference (Padró and Orensanz, 1984) or volume (Mohrdieck, 1979; Randall, 1977) of poplars. A word of caution, however, comes from Sekawin (1972) who found high positive correlations for *P.nigra* and *P.deltoides* Bartr. but very low ones for their interspecific hybrid.

The evolution of genetic and environmental variances during stand life also affects the genetic gain from juvenile selection; Namkoong *et al.* (1972) and Franklin (1979) divide stand life into three periods on the basis of the relative magnitude of variance components and suggest that selection during or at the end of the central one, which corresponds to the period of maximum height growth, maximizes genetic gains. However, the general value of this conclusion seems to have been reduced by subsequent experimental evidence (Lambeth *et al.*, 1983; Foster, 1986; Kremer, 1992).

4. Mathematical aspects

In order to understand the particular aspects of age-age correlations, the cumulative nature of the commonly used size variables must be properly understood from a mathematical point of view; by cumulative I mean the obvious fact that, say, height at age T (Y_T) includes height reached at age $t<T$ (Y_t); consequently, if we let $D_{T-t} = Y_T - Y_t$,

$$r_{T,t} = \frac{COV(Y_T, Y_t)}{\sqrt{VAR(Y_T) \cdot VAR(Y_t)}} = \frac{COV(Y_t + D_{T-t}, Y_t)}{\sqrt{VAR(Y_T) \cdot VAR(Y_t)}} = \frac{VAR(Y_t) + COV(Y_t + D_{T-t}, Y_t)}{\sqrt{VAR(Y_T) \cdot VAR(Y_t)}} \tag{2}$$

that is, correlation (be it phenotypic or genetic) is made of two components, the first one being proportional to the variance of size at the juvenile age alone, and the second to the covariance between juvenile size and subsequent increments.

Let $Y_T = \sum_{i=1}^{T} X_i$, where the X_i's are the annual increments, and let us assume that the X_i be normally and independently distributed, $\mathrm{NID}(\mu,\sigma^2)$; such an assumption is not necessarily realistic, but it is useful as an example; equation (2) is reduced to

$$r_{T,t} = \frac{t\sigma^2 + 0}{\sqrt{t\sigma^2 \cdot T\sigma^2}} = \sqrt{\frac{t}{T}} \qquad (3)$$

Given the above assumptions, $r_{T,t}$ for a given T increases continuously with age even though annual increments are totally random and do not reflect any genetic effects. The relationship (3) bears a striking resemblance to Lambeth's (1980) equation. When plotting $\sqrt{t/T}$ against LAR in the range -2 < LAR< -0.2 (i.e. 0.14 < *t*/*T*< 0.82), the relationship is not far from linear, with a slight upward concavity (figure 1). The least squares linear interpolation of $z = \sqrt{t/T}$ on LAR for points spaced 0.2 on the abscissa within the above-mentioned range is $\hat{z} = 0.927 + 0.296 \cdot \mathrm{LAR}$, with $r^2 = 0.985$. This suggests that Lambeth's equation actually describes the age-dependent variation of that part of the correlation coefficient that depends on $VAR\,(Y_t)$ only and might explain an upward concavity in real data observed by Lambeth (1980) himself.

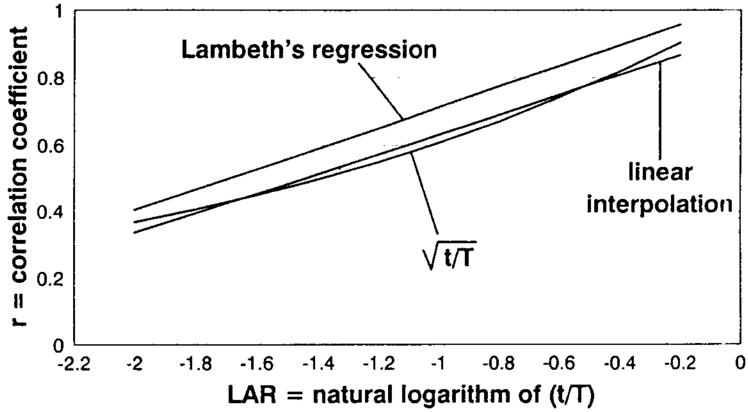

Figure 1 - **Plots of Lambeth's regression (r = 1.02+0.308·LAR) and of the square root of t/T within the range (-2.0 < LAR < -0.2). The linear interpolation of the square root is represented by the line** $\hat{z} = 0.927 + 0.296 \cdot \mathrm{LAR}$.

Phenotypic correlations for height reported by several Authors (Wakeley, 1971; Squillace and Gansel, 1974; Namkoong *et al.*, 1972) are surprisingly close to $\sqrt{t/T}$. This does not diminish the relevance of Lambeth's equation (which was meant by the Author to be a reference and not a "law", anyway), but I think that $\sqrt{t/T}$ would be a better reference for analysing height phenotypic $r_{T,t}$ as it would correspond to a purely mathematical relationship under simplistic but well defined assumptions.

Some more purely theoretical examples are now proposed in order to give a visual feeling of what happens on various assumptions about the distribution of annual increments.
Let $X_1, X_2, \ldots, X_t, \ldots$ represent a (Gaussian) Markov chain in which $\Pr(X_i < X | X_{i-1}, X_{i-2}, \ldots, X_1) = \Pr(X_i < X | X_{i-1})$, that is, the annual increment of year *i* depends on the increment of the previous year but not on that of the years before; let the X_i be $N(\mu, \sigma^2, \rho_{i,i-1})$ where $\rho_{i,i-1}$ is the coefficient of correlation between X_i and X_1. The following situations are considered over a period of 20 years: **a)** $\rho_{i,i-1} = 0$; **b)** $\rho_{i,i-1} = 0.5$; **c)** $\rho_{i,i-1} = -0.5$; **d)** $\rho_{i,i-1} = 0.9$ at age 2 and shifting to -0.9 at age 20 with -0.1 annual steps (non-stationary Markov chain); **e)** $\rho_{i,i-1} = -0.9$ at age 2 and shifting to 0.9 at age 20 with 0.1 annual steps.

Let us now assume that $\Pr(X_i < X | X_{i-1}, X_{i-2}, \ldots, X_1) = \Pr(X_i < X | X_1)$ that is, the annual increment of year *i* depends on the increment of year 1 but not on that of the years between 1 and *i*-1 (a crude way to simulate genetic effects); let the X_i be $N(\mu, \sigma^2, \rho_{i,1})$ with $\rho_{i,1}$ coefficient of correlation between X_i and X_1. The following situations are considered over a period of 20 years: **f)** $\rho_{i,1} = 0$; **g)** $\rho_{i,1} = 0.5$; **h)** $\rho_{i,1} = 0.9$ at age 2 and shifting to -0.9 at age 20 in -0.1 annual steps.

Situations **a** and **f** coincide with the first example: X_i $N(\mu, \sigma^2, \rho = 0)$; in case **b**, the more a tree grows one year, the more it is likely to grow during the next year; in case **c** the opposite is true; in case **d** the correlation between the annual growth of consecutive years shifts from strongly positive to strongly negative; in case **e** the reverse; in case **g** there is a persistent positive correlation of the annual growth with the growth of the first year; in case **h** the more a tree grows in juvenile years, the less it grows at a mature age.

Figure 2 shows the evolution of the correlations of each year with year 20, obtained by computer simulation, with 500 runs per case. The simulation was carried out by generating pairs of pseudo-random uniform deviates (U_1, U_2) in the range 0 to 1 (built-in RND function in Hewlett-Packard Basic 4.0 programming language) and deriving a normal deviate as $X_i = \sqrt{-2\ln U_1} \cdot \cos 2\pi U_2$ (Zelen and Severo, 1964). In cases **b** and **c**, that is small positive or negative lag 1 correlations between annual increments, there is an almost perfect coincidence with cases **a** and **f**, that is $r_{T,t} \approx \sqrt{t/T}$. Cases **d** and **e** indicate that a positive correlation between consecutive annual increments in the juvenile phase produces much higher correlations of juvenile *vs* mature size than does a similarly positive correlation in the second half of the rotation. When pseudo-genetic factors are considered, age-age correlations of size are very high in case **g** (constant positive correlations with first year's increment) but substantial also when juvenile and mature annual growht rates are inversely correlated. In all cases, except case **e**, correlations between year 20 and year 10 exceed 0.5. According to experimental evidence (Kremer, 1992) case **h** can be taken as a lower boundary for correlations.

An alternative to Lambeth's equation for the extrapolation of observed age-age correlations was proposed by Magnussen (1988); the model, which is independent of estimates of heritabilities, is applicable to normally distributed variables and is based on the relative proportion of correct (E_J) and incorrect selections made at year *t*<*T*. It assumes that rank stability is monotonically increasing with age and derives formulae that relate variations in E_J with observed age-dependent variations in the coefficient of correlation.

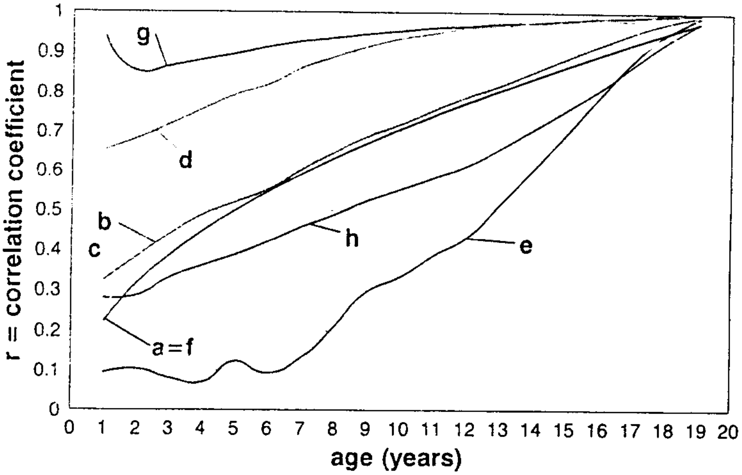

Figure 2 - **Plot of the correlations between ages from 1 to 19 vs age 20 under various assumptions about the correlation structure of annual increments generated by computer simulation.**

Variations of optimum selection age are analyzed by Kang (1985) as functions of variations of heritability $\frac{h_t}{h_T} = f\left(\frac{t}{T}\right)$ and age-age correlations $r_{T,t} = g\left(\frac{t}{T}\right)$ over time. Although entirely theoretical, the models say a word of caution about selections made before 1/3 of rotation age.

A growth model was also proposed by Magnussen (1989) as an aid for the interpretation of age-age correlations. The size ($Y_{t+\delta_t}$) of a tree at time $t+\delta_t$ is described as a function of its size at age *t* as follows: $Y_{t+\delta_t} = (1 + k_{\delta_t} \cdot d_{\delta_t}) \cdot Y_t$, where k_{δ_t} represents an ideal rate of free growth in the absence of any constraints (k_{δ_t}>0) and $1 - d_{\delta_t}$ is the fraction of k_{δ_t} that is lost due to any disturbing factors (0 < d_{δ_t} < 1). By calculating age-dependent variations of variances and covariances, the correlation between ages *t* and $t+\delta_t$, r_{δ_t} is defined as $r_{\delta_t} = (1 + k_{\delta_t} \cdot \bar{d}_{\delta_t})\sqrt{\frac{VAR\,(Y_t)}{VAR\,(Y_{t+\delta_t})}}$. For $\bar{d}_{\delta_t} = 0$, the growth is totally random and $r_{\delta_t} = \sqrt{\frac{VAR\,(Y_t)}{VAR\,(Y_{t+\delta_t})}}$ as expected from theoretical considerations (see above). For $\bar{d}_{\delta_t} = 1$, the growth is totally deterministic and $r_{\delta_t} = 1$. For practical applications, k_{δ_t} and $\bar{d}_{\delta_t}$ are derived from means and variances of at least two juvenile measurements and all subsequent r_{δ_t} are derived from these two parameters and observed variance ratios.

Kang (1991) analyzes the components (variances and covariances) of age-age correlations by describing growth as a size-dependent variable as: $S_i' = S_i + D_i = S_i + \delta_i S_i = S_i + (\alpha + \beta_i) S_i$, where S_i' is the standardized size of a tree at age *t'*, $S_i' = Y_i' / \sqrt{VAR\,(Y_i')}$, $VAR\,(Y_i')$ is the genetic additive variance of Y_i', S_i is the size of a tree at time *t* < *t'* standardized with $\sqrt{VAR\,(Y_i')}$, D_i is the increment in *S* between *t* an *t'*, $\delta_i = D_i / S_i = \alpha + \beta_i$, α is the population average of the δ_i and β_i is the individual tree deviation from α. The coefficient of correlation between *S* and *S'* is

$$r_{S',S} = \frac{COV(S',S)}{\sqrt{VAR\,(S) \cdot VAR\,(S')}} = \frac{VAR\,(S) + \alpha VAR\,(S) + COV(S,\beta S)}{\sqrt{VAR\,(S)}} = (1+\alpha)\sqrt{VAR\,(S)} + \frac{COV(S,\beta S)}{\sqrt{VAR\,(S)}}$$

When $COV(S,\beta S) = 0$, $r_{S',S} = (1+\alpha)\sqrt{VAR\,(S)}$; if the reason for null covariance is that $\beta_i = 0$ for every *i*, then

$VAR(S') = (1+\alpha)^2 \cdot VAR(S) = r^2_{S',S} = 1$ as one would expect from thoretical considerations. In the more realistic situation of $\beta_i \neq 0$ for some *i*, if $VAR(S') > (1+\alpha)^2 VAR(S)$, that is $r_{S',S} > (1+\alpha)\sqrt{VAR(S)}$, then $COV(S,\beta S) < 0$.

A necessary and sufficient condition for $COV(S,\beta S) < 0$ is $\sqrt{VAR(S)} > \frac{1}{1+\alpha}$; but $\frac{1}{1+\alpha} = \mu_h\left(\frac{S}{S'}\right)$, the harmonic average of the ratios of individual size at ages *t* and *t'*. One can write $\sqrt{VAR(S)} = \frac{1}{1+\alpha} + d$, where $-\frac{1}{1+\alpha} \leq d \leq 1 - \frac{1}{1+\alpha}$. The domain of *d* is always wide 1 but it shifts from the positive axis to the negative with decreasing α. Considering that α is high in juvenile years and tends to decrease, the domain of *d* shifts to the left as age increases. As a consequence $COV(S,\beta S)$ increases with age from initiallly negative values to positive ones.

The genetic gain from juvenile selection of expression (1) can be re-written $GG_{t'|t} = i_t h_S h_{S'} r_{S',S} \sqrt{VAR_P(S')}$. By substituting the expression derived for $r_{S',S}$ one obtains $GG_{t'|t} = GG_t \cdot \left[1+\alpha + \frac{COV(S,\beta S)}{\sqrt{VAR(S)}}\right]$, that is, a fraction proportional to 1+α depends on the juvenile gain itself and the rest is proportional to $COV(S,\beta S)$. If $COV(S,\beta S) = 0$ and $\alpha > 0$ then $GG_t = GG_{t'|t} = GG_{t'}$. In practice $GG_t < GG_{t'|t} < GG_{t'}$ because generally $COV(S,\beta S) < 0$. In case $COV(S,\beta S) > 0$, it can be demonstrated that $r_{S',S} > h_{S'} / h_S$, and juvenile selection is more efficient than selection at the mature age even without any consideration of time. Application to real data (Kang, 1991) showed that $\sqrt{VAR(S)} \approx \frac{1}{1+\alpha}$ and generally increases, sometimes irregularly, with age due to a decrease of α; $COV(S,\beta S)$ increases, starting from negative values. Considering that $COV(S,D) = \alpha \cdot VAR(S) + COV(S,\beta S)$, one can see that even the covariance between size at time *t* and subsequent growth is partly dependent on variance only: therefore $COV(S,D)$ or $r_{S,D}$ cannot replace $r_{S',S}$ as the criterion guiding juvenile selection and in most practical cases juvenile selection is either advantageous as indirect selection [$COV(S,D)$ » 0] or anyway desirable because most of the possible genetic gain is already incorporated in the young trees [$COV(S,D) \approx 0$ or $COV(S,D) < 0$].

An alternative to the direct analysis of age-age correlation of size (Y) is to study the structure of the growth process itself by considering annual increments (X_i) as the main variables (Kremer, 1992). If $r_{t',t}$ is the genetic (additive) correlation coefficient between two generic ages *t* and *t'>t* and CV_{X_t} is the genetic (additive) coefficient of variation of the X_t, then $COV(X_{t'}, X_t) = \overline{X}_{t'}\overline{X}_t \cdot CV_{X_{t'}} CV_{X_t} \cdot r_{t',t}$ and $COV(Y_{t'}, Y_t) = \sum_{i=1}^{t} \sum_{j=1}^{t'} COV(X_i, X_j)$, where $COV(X_i, X_i) = VAR(X_i)$. From this matrix of covariances one can calculate the coefficient of genetic correlation between sizes at any two ages. When a reliable model for growth is available, $r_{t',t}$ can be expressed as a function of age lag (*t'-t*) alone, and the evolution of CV_{X_t} can be predicted, one can easily calculate future genetic gains from juvenile selection. Real data of a large scale *P. pinaster* trial (Kremer, 1992) showed a serial correlation coefficient $r_{t'-t}$ decreasing with increasing age lag and stabilized around zero at age 12, while CV_{X_t} fluctuated irregularly around 4% without any appreciable upward or downward trend. When a growth model (Lemoine, 1991) was applied, the predicted age-age correlations of height were very close to those derived from Lambeth's equation in a normal Maritime pine environment; considerably higher with soil fertilization.

Conclusions

A fertile environment can considerably improve age-age correlations by accelerating juvenile growth and increasing $VAR(Y_t)$ which was seen to be the major component of $r_{T,t}$, although genotype × environment interactions may bias the estimates; a parallel check of environmental plasticity of tested genetic entries (St.Clair and Kleinschmit, 1986) would be a valuable aid to correct selections.

Also competition may be a disturbing factor; juvenile evaluations of conventional stands or of short-term narrow-spaced trials with small plots seldom recreate the same inter-tree competition as is found in mature stands. It was also demonstrated that tree reaction to competition is not necessarily correlated to growth rate (Cannell, 1982) and that age-age correlations for the same species vary with spacing (Magnussen, 1989); unfortunately, genetic tests with spacing as an experimental factor would be prohibitively expensive in most cases, the only exception being probably represented by the late stages of clonal selection in vegetatively propagated species, such as poplars (Panetsos, 1980).

The statistical nature of the correlation coefficient and heritabilities must be clearly appreciated: they being ratios of variances and covariances, any trick that increases the numerators and diminishes the denominators improves the gain. A sophisticated experimental design with an effective control of the environmental variations should be a primary concern (Kremer, 1992).

General recommendations about optimum evaluation age cannot be given for all species, environments, silvicultural systems, traits and economic backgrounds; specific experience, in many cases already available, is needed to orientate decisions. In the field of growth traits, however, both theoretical considerations and experimental evidence seem to point at the efficiency of early selection, say between one fourth and a half normal rotation. Kang (1991) suggests as optimum the age at which $\alpha / \sqrt{VAR\,(S)}$ is minimum.

Early evaluations could safely guide provisional registration of commercial varieties or a selective thinning of large seed orchards (Squillace and Gansel, 1974).

However, more research is still needed, especially in the field of size-related traits. The road opened by Kremer (1992) by shifting the attention from the size to its annual increments deserves further attention; the close relationship with research on growth modelling is clearly apparent and both aspects should be considered, from a mathematical-statistical point of view, within the framework of stochastic processes (Magnussen,1988) or time series; a fascinating research area in which the breeder, the silviculturist, the physiologist and the statistician should work in close cooperation.

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Evaluation of forest reproductive material

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There are increasing amounts of seed and plants moving in international trade which has been accelerated with the establishment of the single market in the European Union. It is vitally important that consumers are made fully aware of what they are buying. The consumer or forester must be protected from inferior quality planting stock and the control must be implemented through the inspection and registration of the basic material.

In the European Union the consumer is protected by means of the implementation of two Directives 66/404/EC and 71/161/EC. The first of these attempts to control the general genetic quality of the planting stock while the latter deals with the physical quality of the seed used to produce the planting stock. These Directives are implemented through National legislation and there are subtle differences in interpretation of the Directives by each Member State.

It is necessary to have uniformity in interpretation and application of the rules and three main topics will be considered:

- General implementation and management of the rules;
- Uniformity in comparative testing;
- Juvenile selection and marker aided selection.

1. Implementation and management of the directives

Due to the enlargement of the Community since the Directives were drafted and with the impending enlargement of the Community with the possible additions of Austria, Norway, Sweden and Finland it will be very difficult to have uniform application of the Directives. The newer Member States have vastly different ecological conditions to those of the original countries. Originally the Directives mainly applied to indigenous species but there has been increasing utilisation of non-indigenous species. In addition the main aim of the reproductive material was for wood production but now we must consider objectives other than production such as protection, conservation, amenity and recreation in short we are now dealing with multipurpose forests. It is necessary to identify which types of material we should control and the end use.

As already indicated the Directives were constructed almost 30 years ago with some amendments 20 years ago and in that time there has been a great deal of research carried out in the field of tree improvement (genetics/physiology/wood quality/propagation). This knowledge should be utilised to modify or establish new rules which can be more easily applied to the marketing of forest reproductive material.

On the question of uniformity of application it is difficult to see how this can be achieved under widely differing environmental conditions and objectives. It would appear that the Directives should state general principles and that the detailed implementation should be prescribed at National level.

2. Uniformity in comparative testing

Under the current Directives testing means the comparative testing of the reproductive material produced by the approved basic material. Due to the long-term nature of such testing relatively little reproductive material is marketed in the Tested Category and therefore there is little experience of the testing procedures. There is extensive experience of comparative testing in agriculture but here they are dealing with bred varieties which are reasonably stable. In forestry we are only at the early stages of domestication so we cannot use the agricultural systems at present. However there has been extensive testing undertaken of provenance and progeny material both at the National and International level. The oldest comparative testing on an International scale has been at the provenance level and these International trials should provide pointers to the possibilities of common protocols. The most important of these trials are probably those undertaken under the framework of the IUFRO series for a wide range of species. Many of the trials have tried to standardise on the number of seed sources, the experimental design, plot size and shape, measurement schedules and analysis of data. In the majority of cases it has not been possible to have common protocols for the entire duration of the experiments due to the varying ecological and economic conditions in the various participating countries. However in some instances common protocols have been applied at the nursery and early growth stages. For some of these trials databases have been established and the result obtained can be utilised to recommend rules for seed movement. The majority of the data in the databases relate to early measurements in the life of a tree crop and there is a need to have more data from older ages in order to establish juvenile/mature correlations.

Annex II of Directive 66/404 sets out some very minimum standards for carrying out comparative tests and this is as far as the Directives should go. It is up to individual Member States to set up the tests after due consideration of the prevailing ecological conditions under which the reproductive material is to be used. There is a need to have different levels and designs for testing depending on the traits being considered. All the testing should be undertaken to approved International Standards. The experimental design and the data should be published in an abbreviated form using a common protocol. Apart from comparative testing there is the question of what use can be made of the genetic information obtained from progeny and clonal trials which are commonly used in evaluation of breeding material.

3. Juvenile selection and marker aided selection

There is a great deal of pressure to make decisions about the possible quality and use of new material at as early a stage in the development as possible. In all cases it is a matter of determining the risk when making decisions on juvenile characters. Juvenile selection is used on the assumption that the higher rate of return on the investment will outweigh the shortcoming of early selection. The general 'rule of thumb' is that one should wait till at least one-third of rotation age before making a decision but this depends very much on the trait. There is a need to determine what is the minimum age for efficient selection.

The greatest amount of evidence regarding age-age correlations come from provenance experiments mainly with coniferous species. These indicate that for non-incremental traits such as wood quality, phenology and resistance there are good correlations between juvenile and mature stages. The evidence from progeny trials or genetic gain trials is more restricted and some of the conclusions are contradictory. The move to greater use of vegetative propagation programmes in forestry and the consequent rush to find superior genotypes for use in these programmes is leading to poor decisions from too early assessments of genetic superiority. Further research is required before selections can be made for certain traits during the juvenile phase of growth. Growth models must be constructed and tested using information from provenance experiments and especially from the networks which should be established.

Recent development in the field of biochemical markers suggest that in the future there may be possibilities of marker-assisted selection thus greatly reducing the time scale of the testing period. The techniques are still at the early stages of development but when one thinks of the rapid development in the field of identification, contamination and genetic diversity there is a definite hope that such a valuable technique will be available very soon.

Conclusions

It is very obvious that since Directives 66/404 and 71/161 were drafted there have been great advances in the fields of genetics and tree breeding. There has also been the expansion of the Community with an increase in the types of environments and ecological conditions not to mention species. The aims of 'forestry' have been modified to include 'sustainability', 'multipurpose use' and amenity and recreation. The time is therefore opportune to have a close look at the Directives to see if they are still applicable in a changing environment. The research which has been undertaken over the last 30 years would suggest that some changes should be made. Results from these projects should be synthesised and used to amend the Directives.

- Chapter 3 -

How to use forest material ?

Chairman : R. MORANDINI

Can blue label be independent from conditions of use (edaphic, sylviculture)?

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Introduction

Each country of European Community has in mind its own case of wrong choice of forest genetic material during the last century. Establishment of genetic transfer rules was decided not only to optimize economical gains thanks to maximum wood production, but also to protect tree-planters from such choice of a "less than most productive" genetic source. As a matter of fact, for many forest species, changes in the performance ranking of a given natural genetic source are observed when the latter is grown in different environments. The development of genetically improved forest material leads to ask the following questions to the scientific community : Must transfer rules be adapted to this new genetically improved forest material ?. Are Genotype by Environment (GxE) interaction problems different for this kind of material?

1. Magnitude of G xE interaction for forest material

We first have to define what can be considered as significant GxE interaction for a tree-planter. Responses of genotypes to varying environments can be completely different (figure 1). A type G4 response would be a low-yielding but stable genotype. The type G3 response is for a genotype that performs relatively well in poor environments and takes some advantage of improved environments in a linear manner. Stability measures such as stability variance or ecovalence would be optimal for such genotype. Although classified as unstable with these same parameters, the G1 type of response would be the desirable one for most tree-planters which practice intensive sylviculture in optimal environments. Consequently, it will be more precise to characterize genotypes by their **function of response over environments** rather than to estimate a **stability parameter** for them. With such functions, responses could be given to the following three questions.1- What is the maximum performance of the genotype? 2- In which environment this maximum value is reached? 3- What is the range of suitable environments for such a genotype?

Performance loss due to GXE interaction can be expressed as a gradual loss over an entire rotation and so can be caused by a general inadequacy of the genotype. It can also be explained by catastrophic loss at some different points in the rotation period and so corresponds to a specific inadequacy of the genotype, to extreme climatic events for example.

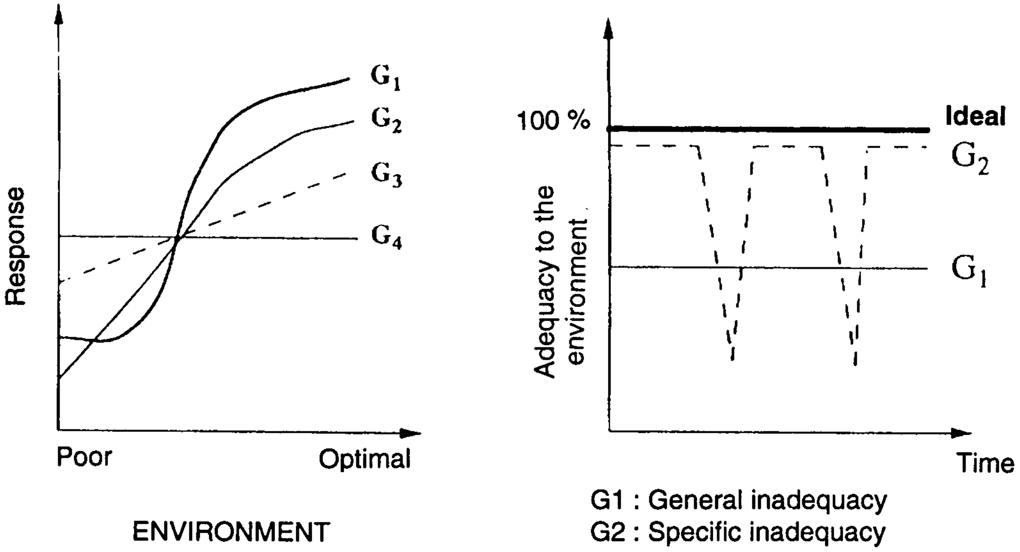

Figure 1 - **Magnitude of G x E interactions in forestry**

2. Characters affected by G x E interaction

Losses may be of three types :

- **death** due to different adaptative components (frost, drought, pest or insect resistance);
- **reduced growth** linked to a sub-optimal growth rhythm (bad synchronization of genotype's growth rhythm and seasonal rhythm for Scots pine in Sweden),or to low nutrient efficiency, or in the case of competition effects to bad allocation of resources between different organs;
- **quality aspects** are given little attention, but in some instances they can be more interactive than growth traits. It is especially the case for stem bending and several branching qualities such as

branch number and forking defects. Little is known about GxE interaction for wood quality traits.

3. Environmental causes of G x E interaction

Environmental causes to GxE interaction that are explainable are of two principal types :

- **predictable causes** which regroup environmental descriptors such as mean general climate, soil type, soil fertility and competition effects. Mean temperature, length of the growing season and rainfall are the most critical factors influencing forest species adaptation and productivity. They are often described by geographical descriptors such as latitude, longitude, altitude, distance from the sea because of the expensive price of precise climatic and edaphic data collection.
- **unpredictable causes** such as extreme natural events (extreme frosts or drougth).

Pest or insect attacks can be considered as predictible factors when the probability of attack is high in the range of environments concerned and as unpredictible factors when this probability is close to 0.

4. Magnitude of G x E interaction and the different genetic levels

Whether plasticity in forest tree species is a property of the genotype per se or one of the heterozygosity per se is today a matter of conjecture. When the magnitude of GxE interaction and its possible causes are compared for different genetic levels, provenance, progeny, clone, it seems that provenances are generally well buffered against temporally and spacially heterogeneous environments. Often, high selection pressures such as geographical gradients or pest susceptibility are responsible of the limited plasticity of some provenances.

High GxE interaction effects observed for narrow genotypes are mainly due to forest management. Fertilization or initial stand density greatly affects the environment and important genetic variability exists for the response to such factors. Some evidence exists for the existence of specific genes controlling plasticity, these genes being often independent from genes controlling performance itself. If plasticity over a wide range of sites and sylviculture is of major concerned, it has to be considered as a selection criterion in itself.

5. How to account for G x E interaction in defining source movement ?

In order to define movement options for the new genetically improved material provided by breeding programmes of different countries, prerequisite knowledge on G x E interaction are necessary.

5.1 Description of G x E interaction

For each species of concern, we must **identify** what environmental factors significantly affect both adaptation and productivity. These factors are then considered as **limiting factors** and responses of genotypes to these limiting factors must be analyzed.

To **characterize genotypes** as precisely as possible, biological traits that respond to these limiting factors have to be measured. Components of growth rhythm such as timing of bud burst, bud set, patterns of free and predetermined growth are often associated with risks due to cold and drought. Considerable efforts have to be done concerning the identification of **measurable biological traits** reflecting nutrient efficiency or reaction to competition effects. Some physiological tests can supplement growth tests in the field and provide indication of specific response.

Loss in terms of adaptation or productivity must be **quantified** in relation to the level of each limiting factor and, of course, in different test sites corresponding to different combinations of these limiting factors.

A good description of G x E interaction relies also on basic knowledge concerning potential environments for the new genetic entries. **Characterization of forest sites** using edaphic and climatic data is hard work and alternatives strategies are needed. Environments may also differ for sylvicultural practices which can greatly affect the response of genotypes. This approach will require long-term studies in forest as extensive data input.

5.2 The experience at provenance level

Creation of seed zones and breeding zones represents early attempts by the forest community to identify both areas with similar environmental regimes and genetic sources which perform optimally in these environments.

Decisions are generally based on genecological or multisite provenance studies. The natural genetic variation pattern is interpreted as an adaptative response to changes in site conditions and serves as cornerstone for seed transfer. The observed performance (adaptation or potential yield) is regressed against a set of environmental variables characterizing the range of environments tested. In the case of genecological studies, variables characterizing the native environments of the different populations tested are used.

These environmental descriptors are usually defined in terms of geographical factors (altitude, latitude, altitude, distance from the sea). For the provenance level, the latter reflect the major causes of G x E interaction. Then to maximise yield, the location of the optimum in the range of environments is determined for each provenance. Groups of provenances with a high yield over a wide range of environments are also defined.
Seed transfer rules for Scots Pine provenances are based on such an intensive evaluation of Provenance x site interaction over the target population of environments in Sweden.

5.3 What about more narrowly selected material ?

While the methods and results of this approach afford a means for providing source transfer guidelines on a seed and breeding zone level, it is questionable as to wether they can be directly applied to more selected material provided by a tree breeding programme.

Humans change the environment drastically when intensive forest management practices are employed. We can easily suppose that genetically improved material would be reserved to such intensive plantations. In this situation, soil types, nutrient availability and competition effects will play a major role in the modification of genotype responses. For less intensive forestry, plant stocks must be constituted by populations with a broad plasticity over a wide range of sites.
In conclusion, in order to define new rules, a target population of environments has to be defined for each species.

Another argument for reassessing new breeding zones for improved material is that selection process changes the response functions of natural populations. This modification may occur of course after classical selection procedures but also after gene transfer . Several examples of modification of adaptativeness have been demonstrated recently for genotypes selected only for their growth potential. Even in the case of multitrait and multisite selection, the evaluation of plasticity is limited on a restricted number of environments. Indeed, during the selection process, tree breeders rarely dispose of many plants per genetic entry. The number of test sites where one genetic entry is evaluated is rarely higher than 5. These sites can not be representative of all potential environments.

In conclusion, characterization of selected material is needed on a multitrait and multisite basis and in representative sylvicultural practices.

Conclusion

If for economical reasons, deployement of selected material provided by different breeding programmes must be maximum, new rules for this deployement must be defined. they must rely on :

- the characterization, on a biological basis, of the range of potential environments,
- the evaluation of the function of response of each genotype to limiting factors. Plasticity must be considered as a test criterion itself.
- the establishment of multisite tests and the long-term evaluation of multiple biological response (not only growth).

Is it better to regulate the commerce or the use of forest reproductive materials ?

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Abstract

Different elements are reviewed in order to try to answer to the above question.

Present regulations of the European Community (EC) including "compulsory species" and "optional species", scientific progress providing new improved genetic products, opening of borders between Member States, entry of new members into the EC, genetic conservation and increase of species for the sake of biodiversity, are discussed.

It seems difficult and even undesirable to restrict the free circulation of forest reproductive materials within EC by negative measures.

However, it seems advisable to confirm the possibility for Member States and Regions to enhance the use of what they consider as the best forest reproductive materials for their territory by positive measures as incentives for recommended provenances or varieties.

This implies however a more effective control.

Furthermore, a deepening of present regulations is necessary, mainly concerning vegetatively propagated clonal mixtures and family mixtures, progeny tested seed orchards, revision of some definitions and rules for seed orchards or other Basic Materials, ...

This work should be harmonised with the renewed OECD Scheme.

Keywords: *Regulations, Forest Reproductive Materials, EC, OECD, genetic conservation.*

Introduction

The question to debate is to known whether it is better to progress in the deepening and extension to new species of present EC regulations concerning the commerce of forest reproductive materials, or (and?) to begin to regulate the use of these materials in such or such country or region.

1. Present situation and discussion

The elements considered in this issue are summarised as follows.
Personal comments are separated and written in italics.

1.1 Present EC regulations and species

To date, present EC regulations (Directives 66/404/CEE and 75/445/CEE in: EC, 1966, 1975; ANON., 1976) organise the compulsory control for the commercialisation of forest reproductive materials moving within the Community for 13 + 1 main species (Art. 2.1):

a) Generative Reproductive Materials:

- *Abies alba* (Silver fir),
- *Fagus silvatica* (beech),
- *Larix decidua* (European larch),
- *Larix leptolepis* (Japanese larch),
- *Picea abies* (Norway spruce),
- *Picea sitkensis* (Sitka spruce),
- *Pinus nigra* (black pine),
- *Pinus silvestris* (Scot pine),
- *Pinus strobus* (Eastern white pine),
- *Pseudotsuga menziesii* (Douglas fir),
- *Quercus rubra* (Red oak),
- *Quercus robur* (Pedunculate oak),
- *Quercus petraea* (Sessile oak),

b) Vegetative Reproductive Materials:

- *Populus sp.* (Poplar);

To be commercialised, these materials must be controlled under:

- *either the category "**selected**" (**green** label)*
- *or the category "**tested**" (**blue** label).*

This system works satisfactorily and can be considered as a major progress as compared with the foregoing situation.

It has still to be up-dated to integrate new methods and products of Forest tree Breeding.

The strictness of the control should be also improved.

In case of shortage in seed production in the EC, some exceptions are however authorised annually for materials with "**less stringent requirements**" (Art. 15) coming generally from outside the community (e.g., douglas fir seed imported from USA).

> *Comments:*
>
> *Abuses are made of this measure and imports of less stringent requirements materials are made not because there is no flowering in EC, but because prices are lower, e.g., in eastern countries. The species, provenances and quantities of forest reproductive materials should be progressively reduced.*
>
> *Seed import of douglas fir and other conifers from Western North America are however still largely justified, especially when they are originating from recommendable regions (e.g., NANSON, 1978) and from stands selected by the EC mission in the recommendable regions (FLETCHER et al., 1993).*

Once they are duly controlled in their home country, all materials of these species may circulate freely, accompanied by their "**certificate of provenance**" (or of identity), through the territory of all Member States.

However, if a country is authorised to exert a right of genetic protection against certain specified materials (art. 13.2) according to the procedure set up in Art. 17, it may forbid this circulation on its territory.

> *Comments:*
>
> *This last measure is (fortunately?) not yet realised. It needs at least the publication of a Common Catalogue and further an agreement among Member States.*
>
> *It could represent a hidden custom barrier too.*
>
> *The opening of borders between Member States makes its practical realisation very difficult.*

Beside these 14 species, with **"compulsory control"** in case of commercialisation (called afterwards: **"Compulsory species"**), every country may exert an **"optional control"** on materials from other species (called afterwards: **"Optional species"**).

When this control is exerted, the requirements are usually presumed to be the same as for "compulsory" species; this however could differ from country to country.

In some of them, when the species is admitted to the control, all materials of the species have to be controlled.

In other countries, only the specific materials that are asked to be controlled (e.g., by seed merchants and nursery men) may be effectively controlled.

One can also imagine that in some countries, the optional control is less stringent than for Compulsory species.

To my mind, there is no restriction to the free circulation between Member States of Forest Reproductive Materials of these species, either controlled or not.

Furthermore, in some countries (e.g., Belgium) only the species set in an official national list may be controlled. As a consequence, a species that is not present on this list (e.g., *Pinus pinaster*[1]) is not liable to be controlled, even if the control has been perfectly made in its home country (e.g., France).

Comments:

Few people have clear ideas about the control of the "optional species": on what is exactly allowed or not, on what is effectively practised or not, in their home country as well as in the other Member States. A reflection about this confused situation should be made to try to standardise the system, perhaps through:

- *a common EC List of species liable to be controlled in every country (even if there is no basic material of this species in the country)* ?
- *a common harmonised system of control for optional species* ?

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1) Until recent time

1.2 Scientific progress

Present EC regulations though still largely valid have to be updated to match new situations arising mainly through scientific and genetic improvement progress (BONNIS and MUHS, 1992; MUHS, 1991, 1992; NANSON, 1992; NANSON and KRUTZSCH, 1992; TERRASSON and FLETCHER, 1992).

Comments:

For example, family mixtures and clonal mixtures ***vegetatively propagated*** *which represent now among the most performing breeds are not foreseen by the present Directives and paradoxally have to be commercialised without any control! This is a typical juridical gap to be rather urgently fulfilled.*

Similarly, some improvements have to be done to the present directives, as:

- *introduction of a category* ***"progeny tested"***,
- ***new types of seed orchards,***
- *adaptation of* ***some rules for seed orchards,***
- *revision of some wordings, etc.*

1.3 Opening of the borders between Member States

This control of Forest Reproductive Materials is abandoned at the borders since the 1st of January 1993.

Comments:

This has as a consequence that no member state can in fact forbid the access to its territory of such or such Forest Reproductive Material. The measures envisaged for genetic protection (art. 13) are therefore impracticable.

Control in general is more difficult to exert effectively and cheating is easier.

A reinforcement of the effectiveness of control is necessary.

1.4 Entry of new member states in the EC

The probable entry of new countries in the EC (Sweden, Norway, Finland, Austria, ...) raises new problems for Forest Reproductive Materials.

Materials selected in boreal zone (Scandinavia) or from higher altitude (Austria) are not adapted to the main forest regions of low elevation of Western and Central Europe and all the more to Mediterranean countries.

Comments:

The free circulation of this material can be therefore dangerous if the control is not very effective.

On another hand, necessity of trade may be that the raising of forest reproductive materials could be made in other countries than in that of cultivation. For example, seedlings are already raised in plugs under greenhouse in northern countries for plantation in Mediterranean countries. This could be a commercial argument for free circulation if control is really effective.

1.5 Genetic conservation of forest tree species

With the ecological pressure, wholly justified or not, there is an increasing interest to conserve forest genetic resources (ARBEZ *et al.*, 1987; PALMBERG, 1987; KLEINSCHMIT *et al.*, 1989; ANON, 1992, 1993; NANSON, 1993).

Comments:

This is a good thing in principle.

However, this concern put to its extremes by certain groups could lead to the interdiction of any Forest Reproductive Materials that is not quite indigenous to the spot of afforestation. Similarly, no new performing material, especially if it is vegetatively propagated might be used, according to these groups.

Furthermore, some countries could make pretext of this to forbid a lot of forest reproductive materials in order to protect in fact their state market (hidden customs' barriers).

To better manage forest genetic resources by taking trace of the genetic identity of plantations, one could imagine that any comercialised forest reproductive material should be accompanied by its certificate of provenance (identity) whatever its species (compulsory or optional) and its level of improvement.

In this respect, the more general OECD System (including "Identified Materials") could be contemplated at least for Optional species.

1.6 Biodiversity and a higher number of species to consider

Conferences of Strasbourg (1990), Rio de Janeiro (ANON., 1992) and Helsinki (ANON., 1993) have given a more ecological vision to forestry with more concern towards biodiversity: either within species, or between species, or between ecosystems. Therefore, more species should be considered: not only the major ones providing a rather large wood production (*Picea abies, Pseudotsuga menziesii, Fagus silvatica*, ...) but also minor ones in this respect (*Betula sp., Sorbus sp., Pyrus sp., Malus sp.*, etc.).

To better manage within species biodiversity, it is advisable to keep the trace in the forest of the provenance (identity) of every material, even for minor species.

Comments:

How to commercialise all these species?

As said above, perhaps the use of OECD "Identified Materials" (yellow tag) could be envisaged, at least for "optional species", if not for "compulsory species"?

However, this should not endanger the present system that has already brought appreciable progress.

2. Possible action

How to manage this situation with contradictory trends? That is the question.

From the chapters seen above, particularly the opening of borders, it seems now very difficult and even undesirable to rule the circulation of forest reproductive materials within EC by **negative measures** and to forbid it within certain States. This could be even somewhat contradictory to the spirit of the Rome Treaty ensuring the free circulation of goods, money and people.

However, it seems advisable to confirm that States or Regions might favour the use of the best materials for given regions by adequate **positive measures**. These ones could be a better diffusion of knowledge by publication of lists of recommendable provenances or varieties, and incentives for their use (e.g., subsidies at plantation for given species and provenances).

This however implies that the identity of every forest reproductive material could be reliably assured by an efficient control. This is surely the main challenge.

The stress should be therefore put on regulation of the trade and not of the use of forest reproductive materials.

2.1 Regulation of commerce

EC compulsory species (13+ 1 sp)

a) Deepening of present regulations

The most urgent action seems to continue with the deepening of present regulations by:

- *addition of:*
- vegetatively reproduced:
- Clonal mixtures
- Family mixtures
- Progeny tested Seed Orchards
- *new types of seed orchards,*
- *improvement of:*
- regulations on seed orchards,
- certificates and labels,
- wording, etc.

b) Harmonisation with OECD System

This deepening is already underway within the "Group of Experts" settled by OECD for the up-dating of its System (BONNIS and MUHS, 1992; MUHS, 1992).

A wish of this group is to harmonise as far as possible EC and OECD regulations that were still very near.

A question to debate is to which EC species (Compulsory, Optional ?) could OECD categories (Identified, Selected, from Untested seed orchards, Progeny tested, Tested, ...) be relevant ?

Optional species

A state of the art of control used for these species in the different Member States should be made.

a) Harmonised EC List of species liable to control

After examination of control used, a harmonised EC List of species liable to control (Optional species) should be established.

b) Use of the Harmonised OECD System

For these optional species, the use of the future Harmonised OECD System could be envisaged.

Reinforcement of the practical control

The effectiveness of present control should be questioned and improved.
A good starting point could be an assessment of how this control practically works in the different Member States:

- general organisation,
- delineation of regions of provenance,
- how selected stands are selected,
- mainly how the administrative follow up of forest reproductive materials is effectively done from the seed collection or import up to the delivery of plants to foresters,
- etc.

Field visits of a group of experts to the main Member States should be made for information and evaluation.

From this assessment, efforts for a better standardisation of control and chiefly for a more efficient and co-ordinated control between Member States should be developed.

Conclusions

It seems difficult and even undesirable to restrict the free circulation of forest reproductive materials within EC by negative measures.

However, it seems advisable to confirm the possibility for Member States and Regions to enhance the use of what they consider as the best forest reproductive materials for their territory by positive measures as incentives for recommended provenances or varieties of given species.

This implies however a more effective control.

Furthermore, a deepening of present regulations is necessary, mainly concerning vegetatively propagated clonal mixtures and families' mixtures, progeny tested seed orchards, revision of some definitions and rules for seed orchards or other Basic Materials, ...

This work should be harmonised with the renewed OECD Scheme.

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Utilization of forest material

The need for regulations

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Introduction

Since the implementation of the EEC directives on Forest Reproductive Material (66/404/EEC), considerable changes have occurred:

- The perception of "forestry" has developed from traditional production forestry to multipurpose forestry, where a number of different products or services are in demand.
- The amount of genetically improved reproductive material is increasing and many different types of reproductive material are available.
- The awareness among users of the importance of using proper planting material has improved.

Based on this development, considerations are made for the need of regulations for the production and trade of reproductive materials. The practicalities of applying such regulations are also considered.

1. The objectives for applying regulations

Major objectives are as follows:

1) User requirements: For the user, i.e. the single tree planter, the objectives are often to maximise the economic return from the plantings in the short term. The users main requirements are the following:

- Availability of a wide range of materials

- Well defined materials
- Correctly identified materials, i.e. "trueness to name"
- Appropriate and correct information about the reproductive material.

2) Additional National State requirements

National requirements for the development of forests are usually formulated in the national forest policies and forest legislation. In addition to the short term interests of the user, the long term stability of the forests is generally a primary objective. The state requirement may consequently be formulated as follows:

- Prevention or limitation of the use of reproductive material, i.e. planting material which in the long term will result in forests of low or poor stability.

2. Potential conflicts between the long and short term interests

To be able to obtain a short term gain it will in many cases to a compromise with the national long term interests. The question is therefore conse-quently how far will the state be prepared to go in accepting a higher degree of "user regulation" of the production and the trade with forest repro-ductive material.

3. Elements/phases to be regulated

To be able to ensure the best quality of plantings the following 3 main elements or phases are important:

1) The basic material; i.e. the genetic material that will be provided for the market (= the input). Under the present EEC-scheme the basic material is regulated through rather strict approval criteria of the material.

With a growing demand for more varied reproductive material to match multipurpose forestry it will be increasingly difficult to establish approval criteria which can be applied in all EEC-countries. There will most likely be a need for establishing a more "liberal" and "flexible" approval system. In the case of applying a wider range of approval criteria, it is most important that ***standardized documentation*** and ***genetic information*** is provided to the user of the reproductive material.

2) The ***production and trade*** of reproductive material (= the process). In the chain of processes from e.g. seed collection to buying of plants, it is essential to ensure efficient ***identity controls*** of each reproductive material.

3) The ***utilization*** of reproductive material (= the output). To be able to make the best choice and use of the material, it is important that the user (= the tree planter) knows what he has got (= well identified material, proper information about selection criteria and genetic quality) and can feel confident that the identity is correct.

Regulation of the use, through rules and controls (i.e. which specific material to plant on which specific locality) will only be possible with great difficulties. Instead proper information and guidelines/recommen-dation for the use of the material should be compiled and made available (i.e. through extension services) at the national level. Such information would not only cover national reproductive material but also relevant material from other countries.

3. Proposal for a regulation scheme

Regulations can be enhanced through **rules and control procedures** or through **information combined with advice/recommendations.** In the latter case much of the responsibilities for the regulation are placed decentrally in the hands of the producers and users of the reproductive material.

Proper documentation is very important and serves a dual purpose i.e. identification and information about the material.

It is important to develop a scheme having a healthy balance between the regulation measures of the three elements in chapter 4.

3.1 Basic material

As mentioned earlier the criteria for approval will most likely be widened in the future. It should therefore be considered limiting the central approval requirements to a ***few criteria*** of ***general and national interest***:

- effective population size (i.e. genetic diversity)
- health and resistance (i.e. stability)
- isolation from other pollen sources (i.e. identification) of the material.

Provided such general criteria are fulfilled, the countries could supplement **specific national approval criteria** such as "end-use" approval, e.g.

- volume production
- christmas tree production
- wind tolerance
- etc.

A further step would be to allow the producer/user to decide, quite freely, the specific characteristics of the basic material.

With a more flexible approval system as described, there is an increasing need for ***documentation and information***:

1) National lists with approved material. This list would include:

- Identification [ID-codes, species, location]
- Category of material are "level of information" [Selected, Progeny tested, Tested etc.]
- Selection/approval criteria, test criteria + results
- References to further information

2) Forest reproductive material should be accompanied by a document having the following content:

- identification
- type of material (seed, cuttings) and quantity
- category/"level of information"
- selection/approval criteria

3.2 Consequences and risks

A wider range of material will be available on the market, and the risk consequently that some of the material will not fulfil the fairly strict requirements for selection as in the present directives.

The "national interests" should, to a satisfactory degree, be met through the "general approval criteria".

There is a risk that a more "liberal" approval may result in a considerable increase in the number of approved prove-nances. More responsibility is placed on the shoulders of the producer and the user, and the decentral/liberal approach can only be carried out satisfactorily if the documen-tation and information is of high standard.

The question is to what extent the ***categories*** of the present EEC and OECD schemes should be maintained or whether the categories should be considered as standar-dized and well-structure information.

The risk in using categories (= different coloured labels) is that the different categories often are perceived as different levels of genetic quality, which is not always the case.

3.3 Production and trade

The identity controls through the process are crucial and there are probably only limited possibilities for applying a more liberal mechanism than is found in todays schemes. The controls can be administrative or hopefully in the future increasingly biotechnical.

3.4 Utilization of the material

From the user point of view it is important to have the best opportunities for the choice of reproductive material. It will be the responsibility of the countries, through distribution of information and extension services, to provide the users with proper advice and guidelines for choice of material.

As the information about the reproductive material will be considerable it will - although standardized - be difficult to "digest" and interpret for the single tree planter. It will therefore be necessary, at the national level, to develop appropriate and easily accessible information/extension material about the reproductive material available.

In some cases the countries may further wish to influence the use of material (primarily through incentives) to be able to meet the objectives of the national forest policy.

3.5 Consequences and risks

It is considered difficult and also generally undesirable to implement general rules and control mechanisms for the utilization of the material.

How to use forest reproductive material

State part regarding regulation and use of forest material

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Introduction

Forests mean much more today than in the past. The multifunctional importance of our forests becomes more and more obvious at the same time as their health, stability and perhaps even existence are increasingly undermined. It is of vital importance for the future to plant and recultivate our forests with material which meets the demands of today´s and tomorrow´s ecology, society and economy. Scientific concern and socio-political aspects seem to indicate that the use of forest reproductive material should be regulated by public guidelines.

1. Existing legal regulations in forestry

Whatever the reasons for the maintenance of forests may have been in the past, today´s demands on the multipurpose functions of forests are certainly much more diverse and ask for a still better care for the welfare of this natural resource. The general awareness of the value of our forests is great and they have become kind of public property, even if they are privately owned.

As a rule, the forest owner has to manage his forests for a good economic return. He may have ideas for the silvicultural treatment, choice of species, production goal, cultivation method , harvesting system etc., all highly efficient, but they may more or less severely collide with the concepts of other "public" users of his forest land. More and more, common public consideration, by means of laws or public pressure, interacts with the intentions or not-intentions of the land owner.

Most states in their Forestry Act prescribe as one of the most basic regulations: Harvested forests have to be reforested. So generally, the Public demands that forests must be maintained. At the same time, though rather unspecified, the Law dictates the use of at least suitable reproductive material.

2. The long-term importance of planting stock

In commercial forestry, rotation periods of more than 50 years are normal. In certain cases, as for high value timber of oak or Scots pine in the North, 150 to 200 years are realistic expectations of lifetime. So, the planting of forests is certainly the most long time investment we undertake.

It seems, that it has become more and more essential to plant a carefully chosen material, well adapted to the climatic and edaphic conditions of the very planting site, with good genotypic diversity, which will enable the population to have a reasonable number of "winners" in the end.

The expenses in the choice of an appropriate material are neglectable. Seed costs amount to a mere few per cent of the plant costs, plant costs again are a mere few per cent of the forest management costs. There is very little money saved in the careless choice of a cheap and perhaps less suitable stock.

3. What can go wrong in the choice of forest reproductive material ?

Here we must consider two, or possibly three, different levels:

3.1 At Species level

A great number of tree species is simply impossible for the particular climatic conditions of the new habitat. Others may be climatically suitable, but are threatened by pests and diseases, others may be of low or no value at all for cultivation. Some, self-reproducing, may run wild and turn out to be noxious weeds. Some may be alternative host plants or carriers themselves for pests or insects not existent in the new habitat. Some may be considered "foreign elements" disturbing or threatening the existing ecosystems.

A few might even be so successful that their use must be restricted in order to protect endemic species or varieties or to secure biological diversity locally as well as on a greater scale. Some might be so strange that amenity aspects speak against a large scale introduction or common use.

3.2 At Provenance level

Most of our forest species have a wide range of distribution and thus a wide racial variation for adaptive characters. Provenance research has shown this fact again and again, and indeed it is our main concern in this section of the meeting.

The key word of our concern is Genotype - Site - Interaction.

The suitability of a certain material must always be measured against the natural conditions of the planting site. A basic requirement of our planting stock is good adaptation, above all a good survival, frost hardiness in spring, autumn and winter, also drought tolerance may be considered, long time stability is essential, especially now, since air pollution causes modern forest calamities. Also tolerance against diseases is an important ingredient in the choice of new planting stock.

Expectations on productivity may vary widely, depending on the value of different products, and on the silvicultural costs which must be invested in addition to initial investments for the establishment of new forests.

On top of these biologically and economically oriented considerations, public aspects will have an impact on the choice of planting stock. I am not sure whether society, for example in Central Europe would tolerate large scale short rotation forests with Norway spruce with an expectation age of less than say 40 years. Thus, public opinion may interact with both species and silvicultural treatment.

At the provenance level also artificial, bred, forest reproductive materials must be taken into account. Probably, these advanced materials will be highly specialised. Their use will require good and specific knowledge of the particular Genotype-Site-Interaction connected with every single material. The normally valid rules for provenance transfer and use may be difficult to apply to "artificially obtained" stock.

Genetic diversity will have to be taken into account, when clones, clonal mixtures, half or full sib families become available on a large scale. The question will then be not only which material is suitable - with reference to fitness and value of product - but also on how much, on how large an area, and certainly in a longer perspective, to what extent material of limited genotypic diversity should be used in principle.

4. What is "wrong" material ?

Above it is pointed out in which way forest material can have different value. Certain advantages and disadvantages which might be specific for forest seed or forest planting stock are mentioned. All the characters considered have a genetical base: Fitness, hardiness, tolerance and last but not least the potential for a high and valuable yield. All these qualities can not be seen - neither in seed nor in plants. They must be proven under the conditions of the new habitat, before we can judge how good and valuable, alternatively how unsuitable or wrong the material is for reforestation.

In this evaluation, we are confronted with a new problem: What are reasonable threshold values for the forest owner and for the society? Is a 50 % survival (neglecting losses from careless planting etc.) a still tolerable value or should the limit be higher, at 75 % ? How much of frost damage is acceptable? How small or great is the tolerable degree of fungi infection? To what extent can drawbacks be accepted or compensated by other outstanding valuable properties? Certainly, the significance of different characters will vary with species and ecological conditions.

The question is relevant in the interpretation of Article 13 of the EEC directives. Member states may prohibit the marketing of reproductive material which has "adverse effect on the forestry". It will be very simple to point out really unsuitable material. Difficulties arise when it comes to half-good or half-bad material. Approved, it later on may turn out to be inferior, a potential threat to hybridise the local, perhaps autocthonous populations of the same species.

Another question concerns the minimum levels of genotypic variation. How much of mono clone planting is wise? Are 10 clone mixtures "safer" than 4 clone mixtures? Are clonal mixtures of 500 clones "better" than those of only 30?

Finally, how much of the annual reforestation area should be planted with new species or new material, and how well studied should these materials be before plantations at a large scale are considered? (Here the silvicultural option of one-species-stands versus mixed stands is omitted).

4.1 Why would the forest owner use "wrong" material ?

Lack of knowledge

The owner is unaware of the biological risks or consequences involved. He is therefore careless in his choice and probably goes for the cheapest possible buy. (The Law prescribes recultivation!). Here the transfer of all kinds of "surplus" plant stock from mild climates to harsh sites and conditions with a delayed planting season is an obvious risk. One has to keep in mind, that material for hard conditions by nature is always more expensive than average stock.

The forest owner´s special intentions

The owner may see some special advantages in the use of exotic, developed or even generally not accepted materials, perhaps with limited insight or understanding for the overall consequences.

False information or declaration

False information about the suitability and other characters of the plant material, will of course only work when the producer´s/seller´s counterpart has no or only limited insight. False declaration on the other hand is the case when only the name on the plant label is right, but not the attached plant stock. Unfortunately, both cases of fraud, reminding us of old times horse trading, seem to occur in the seed and plant business.

5. Responsibilities and consequences

Who are the actors in the procurement and use of forest reproductive material?

The forest owners: They have to obey the legal obligation to recultivate. They have to show good will to public considerations and wishes (environmental care, responsibility for the heritage of mankind, conservation of bio-diversity etc.). At the same time their work and their products are regarded as essential contributions to society´s economy.

Thus forest owners should possess the necessary knowledge for the choice of in many ways appropriate reforestation material.

The producers and suppliers of forest reproductive material: They should of course be honest and truthful in declaring their products. They should have good knowledge of their products and all its properties and even of the appropriate use of their products. Breeders in this way should have a special responsibility with regard to the composition and suitability of the new materials.

Should suppliers be obliged to give directions for the proper use of their material?

Should they be responsible that their clients are not supplied with unsuitable stock?

The State/Government should have the responsibility for "knowledge", i.e. research and dissemination of results etc. to everybody involved. Further, the Government should formulate the scientific and public considerations to be taken in the choice and use of forest reproductive material. The State may even issue regulations as guide-lines for the proper or acceptable use or transfer of forest reproductive material.

Should Governments shoulder responsibility for the use of forest reproductive material by monitoring its use in the field, in order to establish data for the assessment of its performance and its environmental impact?

It seems that the legislators, depending on the conditions of their parish should decide on measures to be taken. Here the importance of forests and of forestry, as well as the status of forest research, the diversity in forest ownership and the know-how of forest owners, must be taken into account. So, even if we all might feel, that something has to be "regulated" the regulations by no means have not to be uniform. The EEC principle of Subsidiarity applies well to our problem.

- Chapter 4 -

Conservation of forest genetic resources

Chairman : M.C. Varela

Conservation of forest genetic resources

Scientific and economical aspects

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New economical demands, aggravation of environmental problems and increased use of improved varieties will act in synergy challenging the genetic capabilities for evolution of forest species, i.e. the ability to use more efficiently the existing conditions and/or to face environmental oscillations.

Gene conservation, on the perspective of providing species opportunities for evolution, is one of the tools to face future pressures especially when accurate predictions of environmental changes are not possible (Roberts, 1987).

For species submitted to sustained breeding programs gene conservation is a natural part of them when the Multiple Population Breeding System is applied. Providing safe levels of inbreeding, allowing "stretch" of the genetic gains with the same initial population, the system provides the necessary basis for the evolution of the species (Namkong, 1984).

For species not included in breeding, gene conservation has to rely on *in situ* methods in spite of some inherent weakness.

In any case for genetic and economical reasons gene conservation should be based on populations of small size:

- Gene conservation must rely on the increase of the genetic variance that can respond to selection (Holsinger & Gottlieb, 1991; Eriksson et al, 1993).
- Maximum fitness does not exist (Gould & Lewontin, 1979).
- The present genetic structure of the populations is not necessarily the best neither the only possible (Namkoong, 1989; Eriksson et al, 1993).

- Various genetic structures may lead to the same level of fitness - multiple selective peaks - (Wright, 1982).
- The adaptation to new environments is more likely to result from future genetic variants rather than from the present-day low frequency ones (Holsinger & Gottlieb, 1991).
- Random differentiation of small and isolated populations provide species faster ways of adaptation than the maintenance of large few ones (Wright, 1988).
- Genetic variability shows an approximately linear relationship with log N_e rather than with N_e, which means that the gains of trapped variability become negligible after some critical levels of N_e. Values of Ne beyond the levels that prevent stochastic effects to surmount the genetic evolution are enough to capture the utilizable genetic variation needed for evolution. A sample of randomly chosen individuals with a N_e in the order of 50 -100 in many different sites is more efficient than collecting large samples at one site (Brown & Briggs, 1991).
- Additive genetic variance, i.e. the portion that can respond to selection, can increase as a result of population bottlenecks if there are significant interactions among loci in determining phenotypic characteristics (Holsinger & Gottlieb, 1991 quoting Goodnight 1987, 1988).
- Funds for gene conservation will always be limited and scarce (Eriksson, 1993). Besides the doubtful role of the importance of rare alleles, their preservation would demand heavy resources far beyond gene conservation will ever gather (Brown & Briggs, 1991).
- It is an imperative to avoid wasting resources on redundant sampling. The number of individuals or populations sampled can be estimated by appliance of the the law of diminishing marginal increments to gene conservation. Taking collection of genetic diversity as a "production" the benefit derived from an extra unit of input can be quantified by means of a marginal product curve which shows a negative slope in consequence of the overlap between additional samples (Falk,1991).

In gene conservation is fundamental to define the ***objectives*** and the ***genetic knowledge of the species***. These should guide for the decision upon ***methods***.

The Community should stress their clear statement on the programs. Genetic knowledge of most of forest trees is scarce even in some of the economical species. Furthermore, it is inexistent for the majority of the species which will require gene conservation actions in a short future, the non-economical, the minor species.

For the normative issue of what and how much to preserve knowledge on adpative traits is a basic requirement. Therefore, studies that can contribute to such knowledge need encouragement and support.

Biological markers are not the alternative to studies on quantitative traits. Long lasting field trials shall remain rather irreplaceable as the only way to a full appraisal of the reaction norm of the populations.

Molecular markers are most useful complementary tools that can contribute to the knowledge of the mating system, pedigree relationships, gene flow mechanisms,etc. However, fascination that laboratory studies may be the substitutes to field trials must be avoid, even if studies on hundreds of loci will become executable.

There is no simple relationship between a gene and a phenotypic character, (Mc Lannahan, H. 1993). Living beings, overall populations, are entities which reactions upon environmental are of dynamic complexity, away from single connections between spare parts.

Economical anxiety for safe but rapid results is a dangerous attitude in forestry. Unlike biochemistry laboratory studies, field trials are time consuming and carry an inevitable degree of physical contingency (due to uncontrolled events such as forest fires, late frosts, storms, etc.). However that is the way to have results of great level of confidence in what concerns appraisal of adaptive behavior. Therefore, adequate financial support and comprehension for results on longer time spans have to be claimed on forestry genetic research.

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Biodiversity Which part for intensive production and natural forests within the forest space ?

How to conciliate the use of improved forest varieties and the conservation of genetic resources?

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Introduction

The frame conditions for this topic are fixed in the resolutions of the conference of ministers for the protection of European forests, signed in Helsinki in June 1993 by 37 European states. Three of the four resolutions include guidelines for these questions :

1. General guidelines for sustained management of European forests
2. General guidelines for the conservation of biodiversity of European forests.
3. Strategy for long term adaptation of European forests to climatic change (not signed by France and Sweden).

The following principles can be summarized

1. Wood production will be an important aim of forestry in future too.
2. Multiple use forestry will be favoured.
3. Forest management should be based on ecological principles.
4. Genetic selection should favour adaptation characters in comparison to growth characters.
5. Indigenous provenances should be favoured.
6. Exotic species can be used if they have advantages as compared to indigenous species. At the same time actions for conservation of the natural flora and fauna should be conducted.
7. Sustained management has to conserve and improve biodiversity
8. Biodiversity includes diversity within species, between species and of ecosystems.

9. In respect of the evolutionary heritage we have to maintain genetic adaptability.
10. Genetic diversity is the base for genetic improvement.
11. Specific, practical, cost-efficient evaluation systems to measure biodiversity should be used.
12. Methods for the evaluation of biodiversity should be improved.
13. Conservation of genetic resources as well of major species as of minor or rare species has to be developed.
14. An interrelated ecological network of climax - and virgin forests should be created.
15. Knowledge of management of endangered and rare elements should be improved.

1. Biodiversity - which part for intensive production and natural forests within forest space ?

The above principles show clearly that also the forests of the future shall have different functions from purely protected "virgin" areas to mainly production areas. Biodiversity is the mainstay of stability and within species the precondition for evolutionary change (Table 1). The individual heterozygosity is an indication for adaptability and plasticity. The range of environmental conditions which can be handled by a single tree individual is much higher than the actual place. In the following parts we will discuss only within species and individual genetic variation and diversity.

Between ecosystem

Within ecosystems

Between species

Within species

- between populations
- within populations

- between families
- within families

- between individuals
- within individuals

Table 1 - **Levels of biodiversity**

For intensive production - e.g. in reforestation of abandoned farmland - the selected, economically most promising part of the diversity has to be used. Selection criteria have to be based on adaptability and production characteristics as well. This principle has a long tradition in forest tree breeding and of course adaptability will become more important in future due to environmental load and expected climatic change. The breeding populations can be managed as conservation populations at the same time even to increase diversity (ERIKSSON et al. 1993). On the other hand a considerable part of the forest area - more in public forests, less in private forests - will be managed due to ecological principles with mixed, unevenaged stands, natural regeneration and long rotation times. Here the conservation of biodiversity is guaranteed under the influence of natural selection and less intensive human interferences as it is in the natural reserves and "virgin" forests without human influence (figure 1).

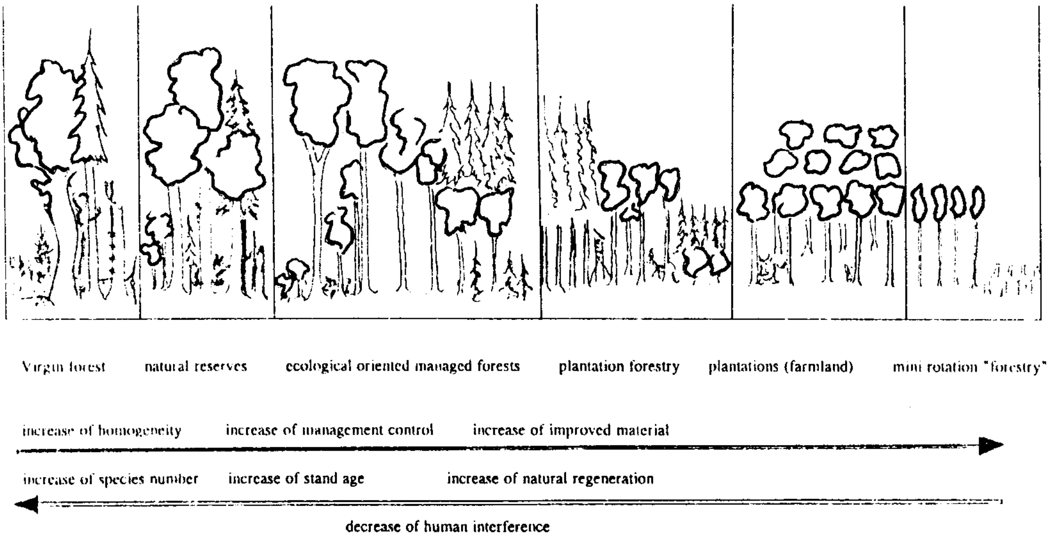

Figure 1 - **Maximal within species diversity ?**

In mixed stands with many species we may however meet a more isolated situation for the individuals of a species under consideration, which may reduce diversity or not (Table 2).

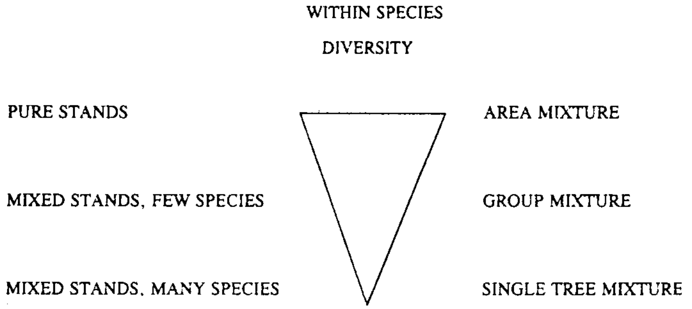

Table 2 - **Stand structure and biodiversity**

In between these extremes a gradient exists which allows to use the economically more interesting part of biodiversity (tested stands, selected stands, seed orchards e.g.) for increase of stability and production. An additional information about the diversity of these materials often would be necessary.

The transgression period from the present forest-situation to the ideal - if it can be achieved at all - will take more than a century. During this time a lot of material has to be planted to approach the goal of mixed forests often with a higher proportion of hardwoods. This is an unique possibility to establish highly adaptable and productive populations. This is true for abandoned farmland as well. Principles for biodiversity of improved forestry reproductive material have to consider the species, the knowledge of performance under variable ecological conditions, the rotation time, the spacing, the area to be planted, the duration of utilization, plantation in pure or mixed stands, just to mention the most important ones (Table 3). An extreme reduction of genetic diversity is only responsible in short rotation conditions with intensive management.

species (major or minor)
knowledge of performance
rotation time
area to be planted
spacing
duration of utilization
pure or mixed plantations

Table 3 - **Considerations for biodiversity of improved material**

For long rotation species under ecological oriented management a much higher part of biodiversity has to be maintained.

The methods to measure genetic diversity are available. Here is however an obvious lack of integrated approaches which take into consideration adaptability, morphological, physiological, biochemical and genetic characters together. Some characteristics are good measurements for genetic diversity but they do not give information about adaptability or performance.

At present there exist no regulations which restrict the unlimited utilization of a specific material as long as it is in agreement with the legal rules and no obligation to use broader material which may be available. There exist only legal precautions to restrict the genetic diversity too much in a specific material. There exist no regulations for a considerable number of tree species. Theoretically all cherry plantations in Europe can be planted with few selected clones.

2. How to conciliate the use of improved forest varieties and the conservation of genetic resources ?

Improved forest varieties from tested stands, seed orchards, controlled crosses or clones are of interest for wood production or other economical purposes. They can have a broad adaptability and superior production, which is of importance as well for the forest owner as for production of a valuable raw material which will be more important for future generations than for today. They can have a wide array of differences in diversity from a single clone, clonal mixtures, single families, family mixtures with different numbers to populations (Table 4).

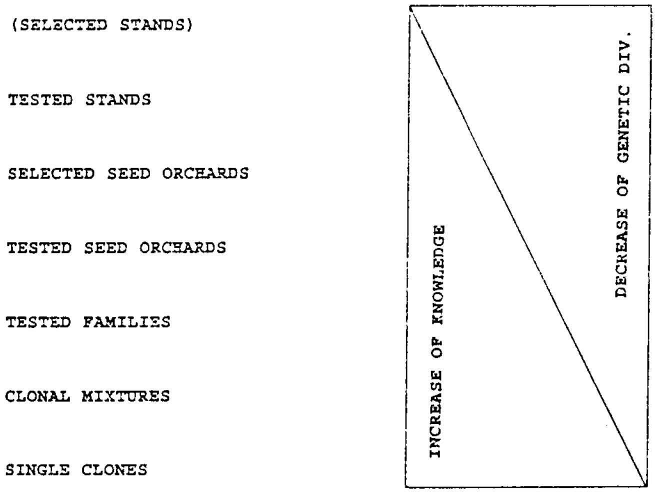

Table 4 - **Forest varieties**

The objective of conservation is to maintain or enhance evolutionary potential, so that genetic variation is available for species development in present and future ecological niches, as well as economic environments. Conservation cannot take the present state of the system as defining a norm. Conservation objectives must go beyond preserving all genes, individuals, populations, species, or ecosystems in the current places and sizes in order to conserve evolutionary potential. Diversity needs not to be decreased by human intervention, and it can possibly be increased (ERIKSSON et al. 1993).

Conservation has to be done for forest tree species mainly in situ for economic reasons. But ex situ conservation is necessary too for rare species, endangered populations, and as an additional security strategy, since catastrophes like fire, storm, ice - or snow break cannot be excluded. Ex situ conservation is a necessity for all economically important species which are included in breeding programs. Here the breeding populations can be developed into conservation populations as well, even with an increase of diversity as discussed by ERIKSSON et al. (1993).

The natural environment will change rapidly with expected global warming. But environments are also influenced by tree species which modify the climate. Most forest tree populations have considerable genetic variation and a considerably broader ecological potential than reflected by their present range as we know from plantation of exotics for example. Quite often competition of other species is more restricting than climate.

There exists an obvious conflict between the use of improved forest varieties and conservation at a first view. But on the one hand it is clear, that not all forests can be used for plantation of improved forest varieties, on the other hand not all forests can be put under protection. A compromise must be found on the same line as discussed before (Table 5).

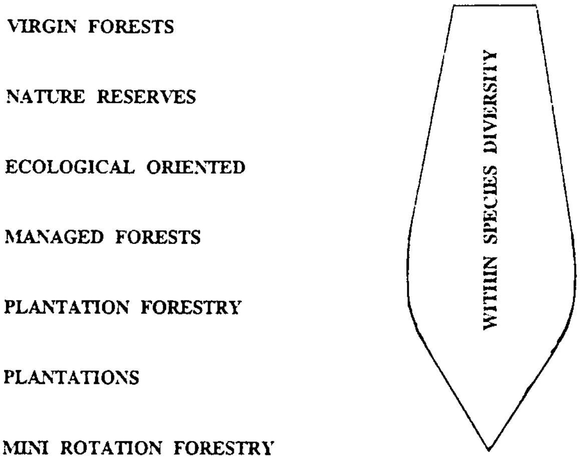

Table 5 - **Management system and genetic diversity**

The more diverse material is preferable under long rotation conditions, where less environmental and silvicultural control is possible, the genetically more homogeneous material under more controlled environment in intensively managed plantations with short rotation times. Optimally there should be a steady gradient from the one extreme to the other. However this is not practicable since no regulations exist which oblige the forest owner to use a specific material. Therefore regulations must include precautions and exclude the extremes as for example monoclonal varieties.

The use of improved varieties has the precondition that conservation is done as well.

This can be in special locations as natural reserves, conservation stands or in multiple population breeding systems. Optimal would be a combination of both, which would guarantee the maintainance of evolutionary potential as well under more controlled and improved conditions for economic utilization as under the regime of natural selection without human interference. An efficient in situ conservation however has the preconditions too, that a number of populations under different ecological conditions including the extremes are selected which have a sufficient size (as extended as possible) and a range of ages to exclude risks typical for specific age classes.

This will not be possible for any of the rare species and not for most of the minor species. For these ex situ measures are necessary as well, which should be combined with a certain minimum program of improvement and control of diversity. Such a procedure supports the reintroduction of those species into regular forestry management and extends at the same time the genetic variation by combining different relicts into genetically broader conservation/breeding populations. Here again a multiple population approach is preferable.

Under the view of climatic change international cooperation is of special importance, since in future Sweden may depend on German material and German on Southern France or Northern Italy.

With an integrated approach there does not seem to be a conflict between improved material and conservation of biodiversity. There exists however a number of obvious gaps which have to be closed :

- Lack of knowledge of the present range and frequency of most rare tree and shrub species,
- Lack of knowledge of the genetic structure of biodiversity for most species,
- Lack of activities with minor species in most countries,
- Lack of control of biodiversity in most applied programs especially in practical forestry,
- Lack of regulations for minimum requirements to ensure that biodiversity is really an element of sustained forestry.

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Which diversity (inter and intra) for forest varieties ? The risk for the forester and for society

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Abstract

Forest varieties can display a more or less genetic diversity from single clone up to different kinds of synthetic varieties. The advantages and disadvantages of genatically broad or narrow varieties are compared, both at the country level and for individual forester. It appears that, at least for long rotations which imply uncertainty both on ecological adaptation, pest resistance and evolution of the market, it is safe to maintain high genetic poymorphism at the stand level. This aim can be achieved both by mean of one variety with a broad genetic basis or by mixture of specialized variety. On the other hand, this approach can be conciliated with high genetic gains which may be concentrated on the different products obtained from successive thinnings and clearcutting.

Key words: *Genetic polymorphism, seed orchards, clones, breeding populations, varieties*

Introduction

It is well known that Modern tree breeding programs completetely separate both conceptually and physically two kinds of populations:

- Breeding population which is managed usually over several generations and must conciliate moderate genetic gains per generation on the main traits of practical importance (resistances, broad adaptability, growth, shape, wood quality...) and a great genetic diversity for further genetic gains (eventually with different economical and biological constraints). This breeding population may be managed into disconnected sublines selected into the same direction or may be made of initially different populations or divergent populations because selected for different traits. The second method is the multiple populations procedure (Burdon and Namkoong, 1983). Practically, initial differences may be obtained by starting the breeding program from a set of provenances with varying performances and ecological adaptation. Cautions necessary for long term breeding are reviewed by Kang and Nienstaedt (1987) who insist on the necessity to a prevailing effort on conservation of genetic variability and possibility to continuously satisfy the needs of society under changing environments and evolution of the market. So, long term breeding is not simply a reiteration of short term activities on several generations. The authors stress attention on population size, probability of allele fixation, gene flow among subpopulations and initial structuration.

- Varietal outputs, often resulting from intermediate "production populations" (seed orchards of different kinds) on which the breeder can realize great genetic gains with often a dramatic decrease of genetic diversity.

Forest improved varieties may be ranked in increasing order according to their genetic polymorphism (this list is not exhaustive):

- Unique clone (e.g.:poplars)
- Hybrid varieties with a unique female parent (e.g: larch)
- Bulk varieties, mixtures of crosses from selected parents (e.g.: maritime or radiata pine)
- Synthetic varieties from clonal seed orchards (e.g.: scotch pine, Douglas-fir)
- Synthetic varieties from seedling seed orchards (e.g.: black pine or maritime pine)
- F2 varieties from interspecific or infraspecific F1 seed orchars (e.g.:possibilities of new varietal outputs for Larch or maritime pine)

1. Advantantages and inconvenients of varieties with a narrow or large genetic basis

The classical main advantages of varieties with a narrow genetic basis are the following:

- clearly "calibrated " products which are easy to describe and to classify into categories of utilisation. On the otheir hand, the sylvicultural management is simplified.
- easy varietal identification of seeds, young plants or more or less mature stands.
- increased simplicity of cultural optimization, as for crop plants, because the limiting factors of growth (mineral nutrients, competition ...) are easier to quantify.

But these varieties present an increased risk relatively to adaptation for stress conditions or unforecasted diseases. The classical example of such a risk is the I 214 poplar clone which has revealed a great sensitivity to *Marssonina brunnea* (this fungus was introduced from USA after second Wold war) when it was propagated in large continuous zones (for instance in the Pô valley). Still now, array of poplars varieties used in France suffers from an unsufficient variablity: Three clones only represent 66 percent of the plants produced by the commercial nurseries (Villar *et al.*, 1992).

Mainly for monoclonal varieties, the minimum caution is to avoid propagation of the same variety in a great continuous area. The reasonable alternative is to propagate other varieties in the same area, at the same time or after a short time.

The principal advantages of varieties which present a large genetic basis are: listed below:

- They are well buffered populations (adaptation to unforecasted events)
- In the case of varieties from seed orchads, they are automatically generated mixtures (advantage for administrative definition of the variety)
- They can generate different categories of products (adaptation to evolution of wood market).

But their optimum silvicultural management is more difficult than for more uniform varieties. On the other hand, their identification is not easy and requires analyses of large samples. Lastly, at least classically, their increased polymorphism has the consequence to provide a reduced genetic gain.

Friedman (1992) reviews general means to take the best decisions in genetic resources management programs (GRM) taking in account risk and uncertainty in econometric models. These kinds of models are more useful in a case of varieties with a reduced genetic variability. The main procedures are:

- To test the sensitivity of the gain relatively to all the factors which can affect economic return. Lack of ecological adaptation to biotic or abiotic factors may be taken into account in that way.
- To minimize the effects of the worst decisions
- To weight the profit (positive or negative) by its probability

But this author gives in conclusion the opinion of Field (1988) which is that long term risk may be best handled by diversification of products. This aim, of course, transferred to varietal outputs , means that it is desirable to maintain a large variability, within or betwen varieties, except for very short rotations.

Risk of propagation of varieties with a narrow genetic basis may also be processed as in the theory of seed transfert zones. The possibilities of measurement of these risks, for instance based on discriminant analyses (probability of misclassification) are described by Westfall (1992). The notion of transfer risk from one seed zone to another one is similar to the risk to propagate within a given site a poorly adapted variety in place of a well adapted variety. The only difference lies on the lack of standard references for long term adaptation. In absence of such criterion, the choice of this kind of variety may be based on the basis of a limited genotype * environment and genotype * year interaction. For this purpose, use can be made for instance of ecovalence (Wricke, 1962) which was generalized for non-orthogonal data (Baradat, 1989, internal **INRA** document) or a method derived from general regression of genotype on environment (Finlay-Wilkinson, 1963) as stability parameters derived from this regression (Eberhart and Russell, 1966). On the genetical point of view, this practice has chance to propagate very heterozygous genotypes. So, lack of genetic variability between individuals is compensated by a strong diversity of allelic associations within the same invividual. Advantage of hetrozygosity for fitness in forest trees and, more generally, in outbreeding species, have been shown in many instances on isozymes data (Mitton and Grant, 1984, Ledig, 1986).

2. Possibility of combination of advantages of varieties with narrow or large genetic basis

The best way for the breeders as well as for practical foresters should be to combine the advantages of varieties with high and low genetic variability. This aim may be reached by several means closely dependant from the conception of the considered breeding program. So, it is not possible to give an exhaustive list of solutions.

However, two general approaches can be envisaged. There common point is to regenerate diversity by combination of populations which can realize the classical varietal outputs.

2.1 Increase of polymorphism by recombination of a set of highly selected individuals

Sexual recombination is the cause of genetic diversity of synthetic varieties from seed orchards. However, family seed orchards which provide the greatest variability suffer both from limited efficiency of combined selection used for selective thinnings and from reduced or null genetic gains on the parents of the progenies which constitute the base material of the orchard. At the opposite, elite clonal seed orchards authorize a great genetic gain due to progeny-test selection on the grafted material. But limitation of clone number may have heavy consequences on discrepancy from panmixia due to limited gametic contribution of some clones (Müller-Starck, 1982) or high selfing rate (Muona, 1989). The conception of clonal-equivalent seeed orchards (Baradat. 1987) realizes a synthesis of advantages of seedling and clonal seed orchards. Two cycles of sexual recombination increase genetic variability, as a slight super-selection of male parents which are represented in the pollen mixture increases the expected genetic gain compared to clonal seed orchards. So, both mean of additive genetic value and genetic variance of the synthetic variety can be increased. On the other hand, if varietal identification is based on allelic frequencies, it can be achieved by labelling only the limited number of parents involved in the polycross.

2.2 Creation of specialized varieties

Specialized varieties can afford a maximum genetic gain on one trait. In that cases, all the other traits display the role of predictors. Multitrait selection indices enable to reach this goal by setting to the corresponding estimates of additive or total genetic value a zero coefficient.The result is to cull the population perpendicularly to the axis of the forecasted genetic value. Use of these specialized varieties may be conceived into pure stands or mixed with other specialized varieties. In this last approach, the choice of economic weights is transferred from the breeder to the forester. The advantage is that the proportions of the different varieties can be continously adjusted to environmental constraints or to evolution of the wood market. Of course, intermediate solution is possible: selection for a limited number of traits which represent only a subset of the goals of users. We can remark that the concept of specialized varieties is the logical transposition to varietal outputs of the notion of multiple population breeding. The result is also a guarantee of flexibility when breeders and foresters have to face to unforecasted events. The main problem involved by the specialized varieties may be to provide adapted regulation to determine and control the different categories of mixtures which can be delivered to final users, because there is an infinity of possible proportions. Another difficulty is to base these regulations to a clear experimental basis as sylvicultural experiments can take into account only a limited set of combinations and proportions.

Conclusion

We have seen that the risk of varieties with narrow genetic basis may be balanced by creation at the same time or at short time intervals of different varieties with variable genetic backgrounds. These risks are both ecological and purely economic due to uncertainty of wood market. They of course increase with the rotation length. A good balance of the risks is obtained at a great scale (region or country).

On the other hand, very polymorphic varieties offer many advantages mainly from the point of view of individual sylviculturist who limits his risks on each stand established from improved variety. So, the global risk has a more uniform repartition and a silvicultural correction for imperfections of selection is easier.

These two approaches for varietal outputs cannot be counfounded with intensive or extensive forestry. In fact, the forester plays to the quality of the final products a role at least as important as the breeder. Heterogeneity of the products of a single stand due to genetic diversity may be a source of a more dynamic management continuously adapted to the new opportunities of the market and able to correct unforecasted damages.

The limit case of polymorphism is illustrated by disjuncting F2 varieties which could be realized at the interspecific level on larch (Pâques, 1992) or at the infraspecific level on maritime pine (Baradat and Pastuszka, 1992). It of course, requires an evaluation of the involved competition effects and feasability of a realistic succession of thinnings adapted to different hypotheses on the evolution of prices of industry and timber wood. It is important for the forester to be able to revaluate at each thinning the kinds of trees he has to cut to find the optimum dosage between the quality of final and intermediate products up to rotation age.

Finally, genetic poymorphism at the stand level has the advantage to give a lower general level of risk for the society but mainly to reduce the variability of this risk between the silvicultural units and then for the individual forester. On the other hand, it enables a more imaginative and efficient silviculture in the context of quickly evolving needs of industry. But, this approach requires for the sylviculturist more basic knowledge on the competition relationships between trees. In the extreme case of F2 disjuncting varieties, the minimum requirement for the tree breeder, is a good evaluation of the kind of segregation, in the F2 generation, of the original traits of the F0 parents.

The consequence is that such varieties can only be delivered after a set of experiments lasting up to the rotation age of the species. In the case of maritime pine, the choosen solution was to initiate such experiments (sylvicultural scenarios for heterogeneous varieties and F2 segregation) before starting the breeding program of recipprocal recurrent selection using corsican and landes provenances of the species.

The hybrid populations testing landes and corsican parents of maritime pine will be established into one-tree-plot designs. The F2 seeds resulting from intercrossing of these hybrids will be used as improved seeds only if the experiments described above demonstrate the sylvicultural interest of such varieties.

The general conclusion will be that long term risks in forest tree breeding is limited, as said in the introduction, by an adequate management of breeding population. Its sharing into multiple divergent populations according to the principle of multiple population breeding seems to be the best solution to allow the maximum flexibility. This strategy is no more than a dynamic gene conservation program according to the principles described by Gallais *et al.* (1992). By definition, varietal outputs are much more contingent and present a much greater level of risk for middle-term echeance.

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Use of genetically modified organisms in forestry

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Abstract

This paper outlines some of the opportunities, constraints and challenges for using genetically modified organisms in forestry. The following are discussed: some uses of coding sequences derived from plants, viruses or bacteria and the special context provided by transgenic trees in relation to the evolution of pests and pathogens; particularly viruses.

Keywords: *virus, Bacillus thuringiensis, plantibody, evolution, risk assessment.*

Introduction

A diverse array of tree genera (Allocasurina, Azadirachta, Betula, Carica, Citrus, Eucalyptus, Juglans, Larix, Malus, Picea, Populus, Prunus) have been regenerated after transformation. However, the technology is not yet robust enough to justify the comment that was made by Neale & Kinlaw (1991) in the preface to an issue of Forest Ecology and Management. They understated the problems of regeneration and gene delivery when they wrote that it "is a rather simple matter to insert a gene for resistance into a forest tree". However, they were correct to note that a "difficult aspect is to determine how to ensure that such a gene is expressed at the right time and place and in sufficient amount". Success depends on a numbers game and is proportional to the resources invested. Nevertheless, "obtained" trees, microorganisms and viruses all potentially provide opportunities for reconciling enhanced forest production with nature conservation: notably by lessening use of pesticides. Herbicide resistances have a somewhat negative popular image but provided convenient means to select transformed plants and were among the first successes to be reported (Shah et al.,1986).

Natural defences against pests/ pathogens (deriving from polyphenol or ethylene metabolism) are currently under investigation but derive from complex cascades which will be difficult to manipulate. In the meantime, a variety of single gene products have been assessed in genetically modified trees. Having regard to the differences in product utilisation (but also the fact that genetic manipulation has not had the damaging consequences which were earlier envisioned) it is probably appropriate to have different safety standards for fibre crops to those which are being developed "case by case" in the context of genetically manipulated constituents of human food. Nevertheless, transgenic genes in perennials may apply evolutionary pressures on pests and pathogens over a longer timescale. For this reason, forest ecosystems may be a special context for hazard assessment.

Forest trees are invisible - until they stand starkly leafless as durable tombstones on the skyline (following, for example, invasion by Ceratocystis ulmi) or as large brown patches in a contrasting curtain of green (as in the aftermath of insect or nematode attack) and, so far, none of the experiments involving GM trees have triggered critical debate. However, the opportunities will only be realised when substantial amounts of new information are available and this is unikely to be obtained unless both commercial and environmental interests are satisfied. Activities involving genetic engineering are a well established feature of modern science and the safety record with such work is good. Nevertheless, there are popular concerns which have not been allayed despite a generally cautious and open policy. A climate of trust must be maintained despite temptations to shortcircuit debates which are lengthy because of the need to balance unequal amounts of information. In some instances there are no data on which to base prudent judgement and in others only a little. Consequently, questions must be asked such as-does absence of data in the literature equate with the rarity of a phenomenon in the real world and does the subject merit reinvestigation? In the following, I have highlighted only a few opportunities and some of the areas of ignorance which seem important to me. I have not discussed the use in forestry of radiation-induced mutants even though this technique has a role in broadening the diversity of crops with difficult breeding systems and has a provided stable and useful variation for horticulture.

1. Exploiting genes from plants

Recombinant DNA technologies allow developmental processes to be addressed at the biochemical level and may accelerate the processes of breeding by identifying coding sequences linked to useful (and also undesirable) traits. It is now realistic to aim at tailoring wood properties through enzyme regulation. However, genetic engineering is a process that uses single genes with quantitative effects and, at present, no single coding sequence has been identified which directly influences a useful wood property in a predictable way.

The major components of wood are lignins, celluloses and hemicelluloses; each is a complex of chemicals and knowledge about biosynthesis is incomplete. Genetic determinants conferring the desirable pulp characteristics of interfibre bonding, fibre length or cell wall thickness are even less well understood. Nevertheless, approaches are being made to investigate the consequences of intervening in the processes which lead to lignin biosynthesis. Most of the lignin that packs the spaces between cellulose fibres is removed during pulping and the application of recombinant DNA technologies is largely justified with the aim of lessening the costs of this process. Two of the enzymes involved in the biosynthesis of lignin (cinnamyl alcohol dehydrogenase and phenylalanine ammonia lyase; Liang et al., 1989) have been characterised and it is likely that their expression will be modified by use of ribozymes (Rossi, 1992) or antisense sequences targeted on the appropriate mRNAs. Analysis of apple trees with rubbery wood disease suggests that the lignin content of wood can be lessened without greatly affecting tree stability. However, although the potential for lessening amounts of lignin is great, there is an important need to address the impact this changewill have on the reactions of the trees to pests and pathogens.

For maximum benefit, modifications in, for example, lignin biosynthesis must be restricted to developing wood and without interfering with induced lignification of nonvascular tissue that is thought to be a determinant of plant resistance (Vance et al., 1980). Such precision is not possible given the current ignorance about gene targeting, enzyme / substrate specificities or interactions in these complex biosynthetic webs.

2. Exploiting genes from non-plant sources

2.1 Use of genes from bacteria

Sequences coding for insect -specific toxins from Bacillus thuringiensis have been expressed in transgenic crops and, particularly after extensive modification that facilitated the expression of these procaryotic genes in a eucaryote (EPO 0385962), these plants limit the feeding damage due to lepidopterans or coleopterans (e.g. McCown et al. , 1991). I will only comment briefly on this bioinsecticide strategy because Strauss et al.(1991) extensively reviewed the opportunities for genetic engineering of insect resistance in forest trees and the hazards which might result.

There is a strong industry push towards widening usage of B. thuringiensis in agriculture. Nevertheless, there are concerns about rates of insect evolution for tolerance to specific toxins. Natural populations of B. thuringiensis are complex mixtures of toxins. Moreover, the bacteria kill insects not only by intoxication but also by infection/invasion and septicaemia. The impact of genetically pure toxins may not be equivalent to the natural situation-particularly having regard to the fact that proteolytic processing of one sequence can follow more than one path and receptor sites for any toxic moiety are unlikely to be constant within populations of target insects. Although it is technically feasible to transform plants so that they express more than one B. thuringiensis toxin (many distinguishable B. thuringiensis-derived toxins are known) or chimeras with neuropeptides, hormones or even viruses (Merryweather et al.,1990), I doubt whether the ringing of transgenic changes in perennials would keep pace with the emergence of tolerance in insect populations. The pessimism that I feel about the prospects for this biological control systems is restricted to its use in transgenic trees; the genetic engineering of biopesticides and their formulation for delivery either as sprays or when encapsidated in microorganisms is a faster process which is more likely to keep up with the evolution of multiple resistances in the target insects.

There are attractions about placing an insecticide within a tree where insects that bore wood or roll leaves can be intoxicated as they feed-traditional insecticides are ineffective against these targets. Systemic expression of insect pathogenic viruses should also be possible but, as with other biopesticide strategies, transgenic plants expressing an infectious viral genome will need to be rigorously safety-tested and the durability of pathogenicity will need to be judged in the context of counter evolution by insects. The development costs of such a system could be difficult to justify if use was restricted to a forest tree crop even if the stringency of testing wasless in a transgenic fibre crop than in a plant used for human food.

Since vertebrates as well as invertebrates damage trees, it is likely that other transgenic strategies for lessening feeding damage (to be set against fencing costs in nurseries and bioenergy plantations) will need to be developed. Plants produce a great many defences against herbivores including cyanogenic glycosides and neurotoxins. Not all such chemicals can be considered as equally realistic potentially protective transgenes but it is likely that distasteful or deterent products of one tree/shrub genus will be transferred into others of greater value. It is important to remember that the responses of plants challenged by pests, pathogens (or wounding in other ways) include the production of chitenases (Davis et al.,1991; Lamb et al.,1989), proteinase inhibitors (Bradshaw et al., 1989; 1991), terpenoids / phenylpropanoids (Hahlbrock & Scheel, 1989) and auxins which facilitate changes in cell wall thickness or otherwise deter feeding.

Transgenic resistances to fungal or bacterial pathogens are not as well advanced as are antiviral tactics. Nevertheless, there are several possibilities on the horizon. These include lytic cecropins (from insect larvae; Caseels et al.,1989) which lessened the severity of psuedomonad-associated disease, fungus-derived antibiotics which inhibit chiten sythetase (Bormann et al.,1989) and antifungal chiten binding lectins from stinging nettle rhizomes (Broekaert et al.,1989).

2.2 Use of virus-derived sequences

Trees are liable to be infected by a range of viruses (Cooper, 1993) and GM approaches are currently being developed against at least several of these (e.g. plum pox potyvirus in cherry, cherry leafroll nepovirus in walnut and poplar mosaic carlavirus in poplars). Three approaches are being assessed; the expression of satellite RNA, capsid-mediated protection or defective replication enzymes. With few known exceptions, each of the recognised methods for attaining pathogen-derived resistance / tolerance (Beachy et al.,1990; Wilson, 1993) protects against only a narrow range of closely similar viruses and superinfection of such transgenic plants with another virus sometimes has undesirable consequences. Each of the current virus resistance strategies can be envisioned in the context of some potential hazard; ribozymes might drive virus evolution by cleaving target RNA, satellite RNAs have unpredictable effects and are among the most mutable of virus-like agents and antisense RNAs may facilitate RNA recombination. At present, defective enzymes targeting viral replication seem to have fewest negative features. In assessing the hazards which might be associated with such tactics, there are many unknowns but there are strong reasons to accept that recombination between ssRNA genomes of viruses facilitates their evolution by copy choice. The fact that numerous viruses have short base sequences in common with plants may imply that recombination has occurred between virion and host sequences. There is unlikely to be any absolute barrier to gene flow between replicating viruses or between virus-derived transgenic messages and replicating viral sequences. It is unlikely that all viruses are the same; but which are really different?

Probably, translational and transcriptional controls are important factors in determining whether a recombinant sequence is perpetuated to act as a variable in evolution. Among the viruses with positive sense ssRNA genomes (c.75% of the viruses about which much is known), the following virus supergroups which have representatives recognised in association with diseases of trees differ substantially in how their genetic material is organised and expressed: picornaviruses (including potyviruses and nepoviruses), luteoviruses (including sobemoviruses,tombusviruses and carmoviruses) and alpha-like viruses (including cucumoviruses and tobraviruses). There is a need to experimentally address recombination frequencies and also consequences when representatives from each of these virus supergroups replicate together or when a virus replicates in transgenic cells expressing a component from a virus in one of the other supergroups.

Despite the importance of recombination for virus evolution, its underlying mechanisms are unknown. Furthermore, because the ways transgenic genes work to lessen the severity of virus diseases are unknown, rationalised (safer) designs cannot yet be made. By virtue of their longevity, trees probably provide more extreme microcosms for virus evolution than ephemeral plants. Unfortunately little is known about the viruses which infect trees or the significance to their ecology of plants in the viscinity of forests. There is no doubt that pests and pathogens can devastate forest trees and, in the better studied human or agricultural communities, catastrophic epidemics have often been sourced to new pathogens or new ways for old pathogens to spread into populations and communities that had not evolved to tolerate that challenge. In agriculture, viruses in food crops have usually been sourced to plants in the periagricutural communities where those pathogens are endemic (even though the direction of pathogen flow is rarely unambiguous). It is widely accepted that wild plants are tolerant of crop pathogens and are indeed used as sources of "natural " resistance genes in traditional and also GM applications. However, it is usually forgotten that, on first exposure, populations of plants which now are tolerant to specific pathogens were probably decimated: genes for tolerance take time to emerge and to be selected. The time taken is determined by the fecundity and variability of both the pathogen and the host populations. Trees evolve slower than many other plants and certainly slower than the viruses which infect them.

The most recently described virus control tactic requires a plant to express part of an antiviral antibody (a plantibody; Tavladoraki et al,1993). The mechanism underlying the effect is not yet known and there is a need to know whether the approach is appropriate against viruses other than the tombusvirus against which it was reportedly effective. Indeed, it is very early to assess whether such vaccination against viruses will be generally useful. Nevertheless, as an experimenter, I would like to see the plantibody technology applied against a tree virus (even though, if I was "a regulator", I would try to ensure that genetic pollution was prevented until more was known). It will be interesting to find out whether plantibodies provide durable protection against viruses and the longlevity of trees facilitates such prolonged study.

A convenient system might be poplar and poplar mosaic carlavirus because, in the context of a programme funded by EC under AIR, that viral genome has been almost completely characterised and a panel of monoclonal antibodies against the capsid protein of the virus has been made. I would, of course be delighted when transgenic poplars expressed the chosen plantibody: particularly so if there was an associated diminishing of damage caused by the virus. However, I would be no less interested to know whether and how quickly the viral target evolved to get around the problem created by the plantibody. Viruses of vertebrates have evolved a variety approaches to avoid or otherwise leapfrog over the constantly changing hurdles which are presented by a host's immune system and it will be interesting to see if a tree expressing a plantibody acts as a similarly powerful agent of virus selection - and, over what timescale.

The report (Taviadoraki etal., 1993) highlights a potential opportunity for foresters. The expression of part of an immune protein in food (particularly food available to humans) may, until proved otherwise, be considered threatening especially to hyperallergenic people. At least, such food would need to be clearly labelled and for this reason it may be tempting to express plantibodies in transgenic fibre crops. Although the plantibodies were targetted against a virus and a small antibody molecule was chosen for the purpose, larger plantibodies have been expressed in transgenic tobacco (e.g. Hiatt et al.,1989) and the use of trees as plantibody factories may provide a novel industrial feedstock. In many forestry systems, the "lop and top" is discarded and genetic engineering potentially provides opportunities for expressing useful chemicals in such tissue. A variety of useful compounds of plant origin are known but very few have been developed commercially because of low yield in their native state. Trees could be a starting point for the industrial production of some "fine chemicals" and, since so much of biotechnology is lead by the pharmaceutical industries of the world, it is appropriate to mention, as examples, the glycoside salicin (from poplars and willows) and taxol (from yew).

Conclusion

Current releases of genetically modified plants have been under restricted experimental conditions often designed to minimise transgenic gene escape to domesticated or wild relatives. In the future GM trees will be market commodities. In essence, GM trees do not differ from their traditionally bred counterparts but they do have features which it may be prudent to record (if only to broaden knowledge concerning their performance). Trees which are sourced from a genetically modified organism fall within the ambit of EC 90/220. There may be a need for centralised testing proceedures to help the end user to identify genera/ species /seed provenances most appropriate for planting in distinguishable conditions of altitude, soil, longitude etc. In this context, visual assessments of distinctness, uniformity and stability and the value for culture and use for any "obtained" variety may not be adequate.

The most modern discriminators are now removed from the field to laboratories where there is a need for correlations with field performance. Polymorphisms in protein patterns (isoenzymes) provide one handle on a genotype and the presence/ absence of specific products has taxonomic value even though it may reflect only a tiny part of the coding activities of any genome.

Polymorphisms in DNA sequences (after digestion with host restricting enzymes) provide a more direct measure of the genomic sequence but there is a need to know which genes code for economically important traits. Furthermore, there is a need to record and register a series of descriptors which may fall under the general requirement for free access to environmental information (EC 90/311). Questions of human safety will have been addressed before planting (under legislation effecting EC 90/220). However, a few questions on which answers may be helpful include; has the tree been sprayed directly or was it in the viscinity (how far away needs special consideration) of crops which were sprayed with a GMO, has the tree been exposed to B. thuringiensis toxins encapsulated within endophytes or a genetically engineered baculovirus, have tree seedlings been exposed to colonisation by GMOs with the aim of enhancing their growth rate in polluted environments, does the tree contain a virus-derived sequence capable of modifying vector relations of another virus?

The simplest way to insert foreign genes into plants depends on Agrobacterium spp. carrying plasmids which are chimeras containing coding sequences to facilitate selection (e.g. kanamycin resistance from bacteria) and also sequences to control the expression of transcribed RNA (either from viruses or from plants). These bacteria are common constituents of the rhizoplane and it is worth noting that they are not readily eliminated from transformed tissue. Different strains of A. tumefaciens have very different pathogenicities in, for example, Psueudotsuga menziesii (Morris & Morris, 1990) and there is a need to ensure that GM trees liable to be so contaminated are registered and identifiable so that they do not accidentally threaten other forest trees. In trade, there is a general need for labels and in the context of GM trees this seems particularly important. Having regard to the diversity of properties which should be recorded, computer-readable systems such as a bar code that might embrace essential features of the growth rings in the wood may be appropriate. As an additional marker, GM systems allow the incorportation of a novel and recognisable genetic sequence with neutral impact on performance. Such a trade mark could be used by tree breeders to reinforce the identity of their stock; to distinguish it , if the need arose, in the defence of plant breeder's rights (if applicable) or patent right. Since laboratory tests aiming at the finding of a specific genotype need to have appropriately specific molecular tools, the marker sequence and the chromasomal location will need to be registered too.

Integrated pest management systems involve a complex blend of genetics, sylviculture and pesticide usage that has been optimised during protracted field trials. "Obtained" trees must be compatible- sylviculturalists will take some time to come to grips with the use of genetically modified planting stock.

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European trends in conservation of forest genetic resources

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Abstract

Human influences can quickly modify and most often decrease the genetic variability between and within forest species (races, ecotypes). Then it is urgent to take efficient measures, able to save and to manage the existing genetic variability of the forest species, in view to increase their evolutionary potential.

Adaptation of the management rules to the different cases of forests (regarding their role in the conservation of the genetic diversity) makes compatible the continuation of the genetic improvement, the rational use of the improved varieties and the implementation of gene conservation programmes.

More researches remain necessary to improve the conservation strategies. We need efficient methods to optimaly sample the genetic units for conservation (core collection methods) and to further manage the genetic diversity (dynamic conservation).

For an improved efficiency, a maximum connection would have to be established between the two main existing projects in Europe = EUFORGEN and the European Community Project on Agriculture and Forestry genetic resources.

***KEY WORDS :** Forest gene conservation, regulation on forest reproductive materials, Picea abies, Quercus suber, Noble hardwoods, Populus nigra.*

Introduction

In spite of the global increase of the forest acreage in western Europe during the last half century, there are numerous threats concerning the forest genetic resources. Several factors are able to quickly modify and most often to decrease the genetic variability between and within the forest species (races, ecotypes) :

- increase of planted areas compared to naturally regenerated forests
- frequent geographic transfers of forest reproductive materials (seeds,seedlings,cuttings) outside their natural area.
- limited number of high yielding forest species and genetically improved varieties most commonly used in reforestation.
- consequently, land races and local ecotypes are threatened to disappear or to be hybridized by exotic populations.

Moreover, other anthropic factors are also responsible of this genetic erosion : river, road and urban managements, acid rains and perharps to morrow, global climatic changes.

That's why, a consensus happens between scientists, foresters and politicians to define and to urgently apply simple but efficient measures to conserve the forest genetic resources.

The major principle, often recalled, is to preserve the number of living forest species (biodiversity) and to maintain (or to increase) the **adaptability** (or evolutionary potential) of each of them.

During the Ministerial Conferences for the protection of forests in Europe, held in Strasbourg (1990) and in Helsinki (1993), more than 30 countries decided to apply practical measures to conserve biodiversity and genetic variability ; the first as a safeguard for future stability and the second to save further evolutionary changes within the forest species. To reach such objectives, technical recommandations have to be defined and applied in the different categories of forests.

1. Different categories of forests

We can distinguish different types of forests according to their role in the conservation of the genetic diversity.

1.1 Genetic reserve forests

They are strictly autochtonous and belong to a representative sample of the inter-racial genetic variability within a given species, chosen to maintain this variability *"in situ"*

1.2 Natural forests

They are not necessarily virgin forests, but only slightly modified by human influences, often naturally regenerated, still composed by several tree species with an understory of smaller trees and shrubs.

1.3 Multiple use forests

They derive from natural forests but are more artificialized, with a priority to the wood production of a target species. They can be almost monospecific and characterized by even aged stands, clear cuttings, natural regenerations occasionally completed by plantations using reproductive materials from the same initial species coming from the local selected seed stands, or even from adapted exotic species. In France large areas of oak and beech forests belong to this category.

1.4 Intensive wood production forests

The wood production is the main objective, due to excellent growth conditions, flat topography and possibilities of mecanization of the forest operations, closeness to the industries.

Such intensive production forests are monospecific, planted, even aged, composed of genetically improved high yielding varieties. Some of them are characterized by a high level of inputs : soil preparation, fertilizers, weed and pest control, eventually artificial pruning.

2. Adapted management rules for each category of forests

In the **genetic reserve forests**, all the precautions are taken to save the originality and the level of genetic variability of the populations during the long term. Such stands are identified and mapped in the management documents of the forest. As a measure of compromise, commercial thinnings are usually permitted to decrease the cost of the genetic conservation. In the case of social species, the area of one genetic reserve unit is around 100 ha and together they could represent no more than 1% of the total acreage of the forests of this given species.

The main constraint for the genetic conservation, is a forest regeneration using a strictly native reproductive material, preferably by a natural regeneration method but possibly by plantation when the natural regeneration cannot be obtained fast enough on the total area. Of course introduction of foreign reproductive material in, or close to, the conservation unit, is strictly forbiden

The **natural forests and the multiple use forests** must play a role in conservation of biodiversity and genetic resources to extend the effects of the forest genetic reserves. They allow to reach the "mass effect" : forest massives of more than 1000 ha or "corridors" necessary for migrations and survival of numerous plant and animal species, preservation of sufficiently large areas of forest to conserve rare non economic species in their usual ecosystems. They can be managed with "soft sylvicultures", long production cycles, natural regeneration, uneven aged structures and individual tree cuts when possible, preservation of a maximum number of plant and animal species.

When these recommandations cannot be observed, especially in private forests because of economical or technical reasons, it is clear that **use of local forest reproductive materials** remains wishable, to save the integrity of the naturally adapted native populations.

In **intensive production forests,** wood production is the main objective but health and stability of these forests, as well as **sustained management**, are also required.

Several recommandations could be made concerning their establishment and management ; particularly to avoid to establish such intensive wood production forests where natural forests still exist today. It means that a geographical specialization could exist between the long term preservation of ecologic and genetic resources and the pure short term economic logic. As a consequence, these highly intensive wood production forests would occupy **a limited amount of area, geographically located within a given country.**

Maintenance of a high gene diversity within the production forest is possible through different ways as reviewed by P. BARADAT, 1993 :

- high genetic variability within each variety, which was traditionally recommanded and achieved with **synthetic varieties** with large genetic basis.
- more **specialized varieties** coming from several recombinations of a limited set of highly selected and alike individuals.

Such varieties can combine good homeostasis and phenotypic homogeneity able to simplify sylviculture and wood use.

An additionnal variability can be brought in space and time by the simultaneous use of several specialized varieties, the limited life time of each of them, the limited size of the production plot planted with the same specialized variety (**patchwork genetic structure of the forest massive**). Such a solution is a safety, as well for the ecological stability of the forest faced to unpredictable adversities, as for the flexibility required because of the changes of the market concerning the quality and the different kinds of wood products. Such solutions are supposed to work well **to minimize the risk** at the forest massive scale. Theoretically, they could be more safe than the present solution consisting in the "blind" use of a limited number of genetically bad known selected seed stands.

In this framework, use of genetically improved material, in man made forests appears to be completely compatible with a conservation of the forest genetic variability.

3. The european forest genetic resources programme (EUFORGEN)

Following the adoption by the second Ministerial Conference of the protection of forests in Europe, held in Helsinki (June 1993), of the proposal concerning follow-up actions to the Strasbourg resolutions, the International Board for Plant Genetic Resources (IBPGR today IPGRI, International Plant Genetic Resources Institute) in collaboration with the Forestry Department of the Food and Agriculture Organization of the United Nations (FAO) has agreed to administer the European Forest Genetic Resources Programme (EUFORGEN) as a special project in implementation of resolution 2 of the Strasbourg Conference.

On the beginning, this project is based on four pilot gene conservation networks, each concerning a species or group of species representative of a typical situation regarding genetic and biology.

These first four pilot gene conservation networks are dealing with Norway Spruce (*Picea abies*) Cork Oak (*Quercus suber*), the Scaterred Noble Hardwoods (mainly *Rosacea*) and Black Poplar (*Populus nigra*)

3.1 Norway spruce (Picea abies)

This conifer species has a large repartition in northern and central Europe. It occurs in dense forest stands, at low elevation in the north, at high elevation in the south (alpin and carpathic zones). This is a monoïc, wind pollinated species. Regarding the large area covered, it is a major species for its ecological and economical importance. Important declines due to acid rains occur in central Europe, and genetic conservation measures must be taken urgently. Old international provenance experiments exist (established in 1938 and 1968), results from old progeny tests are also available in different countries, providing interesting data to scientifically base the conservation strategy.

3.2 Cork Oak (Quercus suber)

This is an evergreen hardwood with a mediterrannean repartition. The economic importance of the species is great for the corresponding areas (Sardinia, Southern Spain and Portugal, North-Western Maroco). The species is now submitted to a complex decay, extended to most of these regions. This is a monoïc wind pollinated species, with heavy seeds closely dispersed around the mother trees. Few data are now available about the structuration of the genetic diversity of this species, but urgent conservation measures would have to be taken anyway. Close connections with more advanced genetic studies performed on *Quercus ilex* and *Quercus petraea* are recommended.

3.3 Scattered noble harwoods

Several forest species are ordinary covered by this term, but *Rosaceas* would be favoured as an homogeneous biological group, characterized by scattered repartition and insect pollination. They are equally interesting for foresters (quality of the wood for furniture, general interest for forest biodiversity and wild animal foods) and fruit tree breeders. They are to some extend threatened by the evolution of forest economy and management since the second world war, favouring monospecific high yiedling forests, in place of the past mixed forests.

3.4 Black poplar (populus nigra)

The Black Poplar is found in Europe around the mediterranean sea and in Asia. It is considered endangered for two main reasons :

- Its riparian habitats are highly disturbed
- Its natural stands are often pollinated by close cultivated interspecific hybrids or by the ornemental cultivar “italica”.

It is considered as a model for other pioneer tree species. Dioecious and wind pollinated, *P. nigra* colonizes open areas in the river banks. Seeds are disseminated by wind and water, but dissemination also occurs through vegetative propagation. We also need **a model species of woody plant to work with as a tool for genetically modified organism (GMO).** Poplar seems a good model for such studies.

For each pilot species, **the main objectives of the network** were defined as follows :

- make an **inventory** of the situation regarding the genetic resources of the species
- develop a European **database**
- acquire informations from, and make it available to all countries participating in the programme
- promote the establishment in each country of **gene reserve forests**
- plan ***ex-situ*** **gene banks** where necessary
- develop appropriate **conservation strategies** for the species of concern.

For each network, an institution selected by the network participants on the basis of the technical expertise and the willingness of the country concerned to allocate the necessary resources to perform this role, will take the lead and maintain a European database for the species concerned.

It is proposed that the network participants meet annually during the first two years and that the frequency of the meetings be reviewed thereafter.

The coordinating structures will be composed of an **Executive Committee associating IPGRI and FAO**, and a **Steering Committee** formed with the national coordinators. Each State will hold to the programme on a voluntary basis. Several States are officially engaged to participate to EUFORGEN and to pay for its implementation.

Now, it is important that most of these financial participations are paid in 1994 to fasten the practical implementation of the European Programme.

4. CEC regulation on conservation, characterization and use of genetic resources in agriculture and forestry

The Council of the European Union has recently adopted a regulation on conservation, characterization and use of genetic resources in agriculture and forestry, 20 millions of Ecus would have to be devoted for the whole implementation of this regulation, on the next five years. Part of this project will concern the forest genetic resources.

The project excludes any research action but is mainly focussed on :

Permanent survey of *in situ and ex-situ* conserved genetic materials established on the territory of the European Union.

Coordination (through european networks) of programmes dealing with conservation, characterization and evaluation of plant genetic resources used in agriculture and forestry.

Funding of urgent programmes, complementary to those already undertaken by the State members dealing with safeguard and rationalization of existing collections of plant genetic material important for agriculture and forestry in Europe.

The objective is the promotion of increasingly adapted plants fitting with the new trends in agriculture and forestry, as well as increasing the level of biodiversity in agriculture and forest ecosystems.

Regarding the global coherence and the efficiency of the international cooperation, it is clear that **a maximum connection between EUFORGEN and this new European community programme** dealing with conservation of genetic resources, would be highly valuable.

5. New needs for researches in forest tree conservation strategies

Most of the existing programmes in forest gene conservation are based on traditional *in situ* conservation networks, or on *ex situ* collections more often established and managed in close connections with genetic and tree improvement programmes. More efficiency would need more researches in the field.

To rationalize our conservation strategies, researches would concern at least some important items like the following ones :

Methods of sampling of the genetic diversity to be conserved, able to combine informations from adaptive traits as well as from molecular markers. This adresses optimum sampling of populations for ***in situ* conservation, core collection,** and **reference system** as defined by KREMER, 1993.

Methods of dynamic conservation of the genetic diversity. Even if *in situ* conservation remains the cheapest, and in most of the cases, the safest method to maintain the adaptability of the forest genetic resources, we need to diversify the methods and especially to be able to combine more frequently *ex situ* conservation and dynamic conservation systems (like the **multiple population system** reviewed by ERIKSSON, NAMKOONG and ROBERDS, 1994).

This would be especially valuable to better prevent any steep genetic erosion or species disappearance caused by a global climatic change, or to better regard the case of the rare and scattered forest trees (including the *Rosaceas* in Europe).

Conclusions

The sustainable development of the forest resources is a key objective for european countries. A forest gene conservation strategy increases the adaptability of the species and is a good safety regarding future environmental changes. Going on the genetic improvement of the major forest species appears always wishable, when the genetically improved varieties are rationally used in the intensive wood production forests, and when simultaneously gene conservation programmes are developped in the natural forests and measures increasing the biodiversity favoured anywhere it is possible.

There was recent and fast progresses in international cooperation. A maximum connection would have to be established between the two main existing projects (EUFORGEN and the European Community Project on Agriculture and Forestry genetic resources).

More researches remain necessary to be completely efficient in long term conservation strategies. **Better methods of genetic sampling able to combine adaptive and molecular traits** are needed, as well for characterization of the populations (**reference populations**) as for their conservation (conservation populations = **core collections**). In the same time we have an urgent need of methods of **dynamic conservation** (as the multiple population system)

For immediate efficiency, most of the efforts were focused on few ecologically or economically important species. To maintain, and when possible, increase the biodiversity in the future european forests, we have also to imagine and to **manage a coordinated system of conservation of representative forest ecosystems**, able to conserve *in situ* the genetic resources of **non economic and rare forest species.**

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General synthesis

D. Terrasson

Synthesis

Looking at the genetic quality of forest material, there is basically two kinds of material : "wild material" cropped from natural stands and material obtained following breeding work. Each of these types raise specific problems for their characterization, and their use.

I - Wild material

1. Region of Provenance is the basic building bloc of our control system, and we have to carefully consider the value of the Region of Provenance and its definition. Different conceptions exist between Member States as to the meaning of Region of Provenance.

During the past 40 years a large number of international provenance trials have been established in Europe with both indigenous and exotic species. A lot of information has been obtained by individual countries from these trials but there is a need for more exchange and common analysis of the data. It is recommended that the information should be made available to help decisions on possible gene transfer, and to provide guidelines for extension services. In the framework of concerted action, the Commission should help to achieve this objective and to establish new networks for species not currently covered.

2. Biochemical markers have to be developed to a stage where they can assist the control system at different levels (identification but also validation for example of pollen contamination, etc...). We recommend that the three main types of genetic markers, used as identification and discrimination tools, should be applied to a common set of plant material, to give a clearer idea of how they can help rule-makers and controllers. This common set should include a set of widely distributed populations, and also bred varieties. This could serve both theoretical and practical applications. Moreover these genetical studies appear to be one of the pre-requisites for decisions on conservation of genetic resources.

II - Bred material

Genetically improved material raises two questions firstly identification and secondly evaluation. The question of identification is connected with what was already stated on biochemical markers. As far as evaluation or testing is concerned, the question is basically how can the work be speeded up. It is necessary to clearly distinguish between evaluation tests at the breeding level, and at the certification level.

1. Certain adaptive traits (pathological resistance, frost hardiness...) can sometimes be judged at an early stage. Regulation has to be applied at a selected trait level (by individual trait), and not globally. We recommend that the possibilities offered by fully established knowledge from genetic studies and juvenile/adult correlation are taken into account at the regulation level.

2. Currently, none of the biochemical markers can predict growth or essential traits of economic interest. However we recommend that research continues on this question, due to the strong interest in these methods to speed-up the breeding cycles and the early usage of improved material in commercial forestry.

3. We should not be afraid of in-vitro techniques since cultured tissues can be more stable that was first thought, except in the case of somatic embryogenesis. We need research concerning biochemical markers in order to check stability of the organisms created. The present importance is low for forest material, but the potential is large, and we need answers before it assumes greater importance.

4. Genetically Modified Organisms (GMO) require new research to look at the safety of the material and methods of release. It is nevertheless fundamental to stress the necessity of field-testing during the evaluation of GMOs. Research to produce gene-maps should be encouraged before any manipulation of any species or hybrid takes place.

III - Use of forest material

1. Before using the forest material it has to be marketed and this is organized through our Directives. There is an urgent need to revise these Directives and to attempt to harmonize OECD and EEC rules to make it easier for Member States in marketing forest material within and between countries. As previously indicated regulations have to consider the possiblity of marketing any new kinds of forest material obtained from breeding programmes, even if these methods are not already used at a commercial scale.

2. The EC Directives should be amended to take into consideration the end use of the reproductive material. The objectives of EC Directives should be to organize the control of the collection and production of forest material, and also to harmonize the minimum accompagnying commercial information.

3. The question must be addressed as to how we control all types of forest reproductive material. Currently this is done by registering the Basic Material and controlling the stages of production. With the increasing size of the Community and the changes in the interpretation of the meaning of "Forests" the use to be made of the reproductive material needs to be considered. Subsidiarity may be the key word, as decisions strongly depend on species, and ecological conditions.

4. If there is to be increasing control at the national level, there is a need for uniformity in the identification, control systems and dissemination of information. Standardisation is important and the amount of information to be provided by the supplier should increase with the amount of risk involved in the use of the reproductive material.

IV - Genetic conservation of Forest resources

1. First of all, we have to underline that there is a place for use of genetically improved material, and there is a place for conservation of genetic resources of forest species. There is no incompatibility, but there is a need for more information and education on both sides in breeders and conservationists.

2. Secondly we need genetic studies in order to determine the most efficient way to build up our strategies for conservation according to different situations created by the specific structure of each species (social species, scattered noble hard woods,...). The studies should concerned for example with the number of populations, minimum size of each population, sampling procedures... But we need also to start immediate pragmatic actions of conservation, and this should be done at an international level. The Commission should encourage these actions.

3. Things are moving and conservation is not something static ; conservation cannot be only preservation. We must have a diverse approach for the maintenance of our genetic resources, combining *ex-situ, in-situ* and dynamic methods.

4. We need a minimum coordination between the various initiatives taken at an international level for conservation of genetic resources of forest species.

5. EEC has probably to consider the need for Directives prescribing minimum requirements to ensure that biodiversity is really an element of any 'sustainable forestry' programme.

Résumés en français

- Session 1 -

Identification des matériels forestiers de reproduction

A. KREMER ***"Utilisation des marqueurs biochimiques et moléculaires pour l'identification du matériel forestier de reproduction"***

Cette contribution traite de l'applicabilité des marqueurs biochimiques et moléculaires pour l'identification des matériels forestiers de reproduction, issus de peuplements forestiers. Le matériel issu des vergers à graines n'est pas pris en compte. Les "implications" génétiques de l'utilisation des marqueurs à des fins d'identification sont d'abord discutées. Les différents marqueurs sont comparés quand au niveau de polymorphisme et de différenciation révélés. Les résultats actuels sont passés en revue, en ce qui concerne l'identification d'arbres individuels (i), de populations (ii) et de groupe de populations (iii). Enfin, une méthodologie générale est proposée, combinant l'information génétique de marqueurs biochimiques et moléculaires. Elle consiste en une procédure en deux étapes : définition d'unités régionales basées sur une analyse multiloci de marqueurs nucléaires, et identification des peuplements basée sur des marqueurs cytoplasmiques à hérédité maternelle.

H. MÜHS ***"Homogénéité ; identification et classement des provenances, régions de provenance, et vergers à graines"***

Les deux réglementations internationales sur la certification et la commercialisation du matériel forestier de reproduction (le système de l'OCDE et les directives de l'Union Européenne) permettent la certification de matériel issu d'une région de provenance. La question très complexe de la délimitation des régions de provenance pour une espèce, n'est pas résolue correctement dans de nombreux cas.

En conséquence cette contribution traite de la notion de région de provenance et des difficultés de délimitation et de classement des peuplements artificiels et des vergers à graines. La mise en évidence de la variabilité et de l'homogénéité dans l'espace et dans le temps sont également des critères importants pour la délimitation. Les caractéristiques génétiques et phénotypiques doivent être examinées de façon plus approfondie, et être pris en compte en priorité par rapport aux conditions écologiques du lieu de croissance des peuplements pour la définition des régions de provenance.

H. DORFLINGER *"Synthèse sur le problème d'identification des matériels forestiers de reproduction"*

Ce rapport tente de donner une réponse aux questions suivantes :

- la réglementation sur les matériels forestiers de reproduction doit-elle continuer à être fondée sur le suivi administratif des matériels ?
- quel statut pour les variétés hybrides ?
- que dire des critères de distinction, homogénéité et stabilité pour le matériel forestier de reproduction ?

Avec des méthodes biochimiques il est possible de régler beaucoup de problèmes d'identification. Ces méthodes ne sont pas suffisamment développées pour le contrôle, et pas encore complètement adaptées à ce rôle. Il y a peu de laboratoires spécialisés capables de faire ce travail. Le coût des identifications est élevé. Néanmoins ces méthodes doivent être utilisées plus souvent.

Il existe actuellement des variétés hybrides d'importance commerciale pour seulement deux genres botaniques. Les hybrides doivent être commercialisés uniquement dans la catégorie "contrôlée".

Pour pouvoir obtenir des droits d'obtention sur les variétés, celles-ci doivent être nouvelles, distincts, homogènes et stables. Ces critères sont indispensables pour l'identification. Pour l'usage forestier, il est important d'avoir des individus ou des populations hétérogènes pour réduire les risques sur des périodes de production longues. C'est la raison des difficultés d'identification.

- Session 2 -

Evaluation du matériel forestier de reproduction

T. SKROPPA *"Réseaux nationaux et internationaux d'expérimentation"*

L'auteur relate tout d'abord l'histoire des principaux efforts réalisés pour mettre en place des réseaux internationaux d'expérimentations forestières. Les réseaux de comparaison de provenances installés sous l'égide de l'IUFRO ont à ce titre une importance toute particulière. Après leur installation il se pose néanmoins des problèmes importants, pour l'homogénéité des protocoles de suivi, la constitution de bases de données internationales, l'exploitation des résultats, la description des conditions écologiques des sites expérimentaux. Au plan international il existe par contre encore peu de réseaux permettant la comparaison de clones et de familles.

L'auteur discute ensuite l'importance de ces réseaux pour l'évaluation réglementaire des matériels forestiers de reproduction, et les besoins de la recherche. Il peuvent notamment apporter des informations utiles sur les performances du matériel végétal classé dans la catégorie "source-indentifiée". Au niveau de la recherche, leur intérêt est renouvelé par l'urgence de disposer d'informations sur l'adaptation des provenances aux changements climatiques et sur le besoin de connaissances de base pour structurer les programmes de conservation des ressources génétiques.

Pour la connaissance du matériel forestier, il est judicieux de coupler des tests précoces dans des conditions normalisées, avec des tests à long terme répartis au niveau régional.

S. BISOFFI "Sélection assistée par marqueurs pour l'évaluation du matériel forestier de reproduction"

Les marqueurs génétiques moléculaires peuvent jouer un rôle significatif dans la réduction des cycles d'amélioration et la caractérisation des matériels forestiers de reproduction. Leur utilisation a déjà donné des résultats concluants dans l'étude de la taxonomie des plantes, la structure génétique des populations, les méthodes de croisement et les empreintes génétiques. Le développement de cartes des liaisons génétiques, a déjà commencé pour certaines espèces notamment les peupliers et les pins, en ouvrant la voie pour une utilisation accrue des méthodes moléculaires dans l'amélioration des arbres forestiers.

Les perspectives et les limites de l'utilisation de la sélection assistée par marqueurs, et en particulier la détection et de la sélection de loci indicateurs de caractères quantitatifs sont examinées et discutées. Un niveau élevé d'hétérozygotie, la dépression de consanguinité, les équilibres de liaison et la longueur des cycles de reproduction limitent le potentiel des techniques moléculaires par comparaison avec les plantes agricoles, mais des résultats significatifs ont cependant été obtenus dans le domaine de la résistance aux maladies.

Des résultats futurs sont garantis par l'évolution rapide de la biologie moléculaire, les efforts accrus dans ce domaine et des compétences scientifiques bien établies.

S.BISOFFI *"Corrélation âge/âge pour l'évaluation du matériel forestier de reproduction"*

Les corrélations âge/âge représentent un outil utile pour réduire la longueur des cycles de croisement / sélection chez les arbres forestiers.

La sélection basée sur l'expression juvénile d'un caractère intéressant à l'âge d'exploitabilité est un cas spécifique de sélection indirecte : son efficacité est déterminée par les héritabilités aux stades juvénile et adulte, et le niveau de corrélation statistique entre les observations à ces deux stades. Deux catégories de caractères sont examinés séparément dans cet article : cumulatifs et non-cumulatifs. La première catégorie comprend les caractéristiques du bois (densité, longueur de fibres, composition chimique), la résistance aux adversités, les caractères phénologiques : les corrélations observées sont souvent suffisamment élevées pour justifier une sélection juvénile ou très juvénile, au moins pour les caractères dont l'expression est peu affectée par les paramètres microclimatiques qui évoluent au cours de la vie du peuplement. La seconde catégorie comprend tous les caractères liés à la dimension de l'arbre ; conséquemment au fait indubitable que les dimensions à l'âge T incorporent les dimensions à l'âge $t < T$, une part des corrélations entre les observations est due à la variance des seules observations juvéniles. Les conséquences sont discutées dans l'article, à travers une revue de la littérature existante et des simulations informatiques.

Au cours du processus d'évaluation des matériels forestiers de reproduction, les corrélations âge/âge peuvent être utilisées en confiance, au moins pour la délivrance d'autorisation provisoire de commercialisation, ou l'éclaircie sélective dans les vergers à graines. Les mêmes principes qui conduisent à utiliser la sélection précoce dans les programmes d'amélioration sont également valables dans ce cas : le temps gagné pour rendre opérationnel plus rapidement les résultats de programmes d'amélioration, compense la perte d'information.

A. FLETCHER *"Evaluation des matériels forestiers de reproduction"*

Le développement des échanges commerciaux au sein de l'Union Européenne implique un besoin accru d'information et de contrôle sur les matériels forestiers.

Il existe des directives, mais une harmonisation entre les différents pays apparaît indispensable à 3 niveaux :

- les conditions d'application
- l'uniformité des tests de comparaison
- la sélection précoce

Les problèmes d'application sont accrus par l'apparition récente de matériel amélioré alors que les directives ont avant tout été conçues pour du matériel "sauvage". La question de l'uniformité des tests se pose principalement en raison de la durée des expérimentations forestières et du manque de recul dans ce domaine.

Doivent être considérées à la fois l'harmonisation des protocoles et la pertinence des tests précoces qui se développent rapidement sous la pression d'une demande forte pour accélérer la diffusion du matériel amélioré.

Ces trois questions auxquelles s'ajoutent l'évolution rapide des connaissances scientifiques, et l'évolution des objectifs demandés à la forêt vers des usages diversifiés, justifient un réexamen des directives européennes sur les matériels forestiers de reproduction qui datent de près de 30 années.

- Session 3 -

Comment utiliser le matériel forestier de reproduction ?

C. BASTIEN ***"Le label "bleu" peut-il être indépendant des conditions d'utilisation (conditions édaphiques ou sylvicoles) ? "***

Le développement du matériel forestier amélioré pose deux questions auquel l'auteur tente de répondre :

- les règles de transfert sont-elles différentes de celles envisagées pour du matériel issu de peuplements naturels ?
- les interactions génotype environnement sont-elles spécifiques .

L'auteur discute d'abord les différentes formes d'interaction génotype environnement, les caractères affectés, les causes environnementales et l'importance des interactions en fonction de l'unité génétique concernée. Il apparaît que la définition des règles de transfert dépend avant tout de la connaissance précise de ces interactions. Ceci implique une connaissance préalable au niveau de chaque espèce des facteurs limitant et une quantification de leur impact sur les caractères biologiques mesurables, ainsi que des méthodes pertinentes pour caractériser les stations forestières. Les réseaux de comparaison de provenance peuvent apporter des éléments déterminants pour approcher ces interactions. Néanmoins au niveau de l'utilisation du matériel amélioré, le processus de sélection peut changer la réponse du génotype à l'environnement, et il convient par ailleurs de prendre en compte la sylviculture cible qui peut s'accompagner de changements drastiques. La caractérisation du matériel amélioré est donc nécessaire sur une base multi-sites et multi-caractères.

A. NANSON ***"Est-il préférable de réglementer le commerce ou l'utilisation des matériels forestiers de reproduction ?"***

Différents aspects sont examinés afin d'essayer de répondre à la question ci-dessus.

Les réglementations actuelles de l'Union Européenne sur "les espèces obligatoires" et "les espèces facultatives", les progrès scientifiques fournissant de nouveaux produits améliorés génétiquement, l'ouverture des frontières entre Etats membres, l'entrée de nouveaux membres dans l'Union, la conservation des ressources génétiques et l'accroissement des espèces eu égard à la biodiversité, sont discutés.

Il semble difficile et même inopportun de réduire la libre circulation du matériel forestier de reproduction au sein de l'Union Européenne par des mesures restrictives.

Cependant, il semble judicieux de confirmer la possibilité donnée aux Etats membres et aux régions de favoriser l'utilisation du matériel qu'ils considèrent le mieux adapté à leur territoire, par des mesures incitatives (subventions, recommandations sur les provenances et variétés).

Ceci implique toutefois un contrôle plus efficace. De plus, un approfondissement des réglementations existantes est nécessaire, principalement pour ce qui concerne les variétés multiclonales, les familles, les vergers à graines testés sur descendances, et la révision de certaines définitions et règles relatives aux vergers à graines et autres matériels de base... Le travail doit être harmonisé avec la révision du système de l'OCDE.

B. DITLEVSEN *"Utilisation du matériel forestier, le besoin d'une réglementation"*

Depuis la mise en place des directives européennes des changements considérables se sont produits au niveau de la perception de la forêt, de la disponibilité en matériel amélioré et de la connaissance des utilisateurs. La demande des utilisateurs et les objectifs de l'Etat sont discutés ainsi que les conflits possibles entre intérêts à court terme et à long terme. Dans une approche plus libérale, il semble que les procédures d'inscription du matériel de base doivent être assouplies avec en parallèle la fourniture aux utilisateurs d'une information génétique standardisée. Le contrôle d'identité doit par contre être efficace avec une utilisation accrue des techniques biochimiques. Quand à l'utilisation, elle doit rester du domaine de la vulgarisation et non de la réglementation. Ce transfert de responsabilité de l'Etat vers l'utilisateur comporte des risques de mauvaise utilisation du matériel végétal qui impliquent un effort accru de vulgarisation et le cas échéant une politique incitative de l'Etat.

P. KRUTSCH *"Le rôle de l'Etat dans la réglementation et l'utilisation du matériel forestier"*

La forêt prend actuellement une importance accrue ce qui justifie des lignes directives publiques pour l'utilisation du matériel végétal. Le propriétaire est concerné avant tout par la productivité, alors que la demande publique s'exprime avant tout en terme de multifonctionnalité et de pérennité. La forêt représente par ailleurs un investissement à très long terme dans lequel les différences de coût en fonction de la qualité du matériel végétal sont négligeables. Les principales erreurs qui peuvent être faites à ce niveau concernent le choix de l'essence, de la provenance, et le cas échéant d'un matériel amélioré inadapté. Ces erreurs reposent sur des caractères génétiques qui ne se voient pas sur les plants et qui doivent donc être évalués dans les conditions du lieu d'utilisation. Dans cette évaluation nous sommes à nouveau confrontés avec les divergences possibles d'appréciation entre le propriétaire et la demande publique. L'utilisation d'un matériel inadapté par le propriétaire s'explique par un manque de connaissance, une volonté délibérée de celui-ci, ou une mauvaise information. Les responsabilités sont en fait partagées entre le propriétaire, le fournisseur du matériel végétal et l'Etat. Celui-ci a principalement la responsabilité de la diffusion de l'information, et la formulation des objectifs en matière de recherche et d'utilisation du matériel forestier. La règle de subsidiarité doit s'appliquer sur ce plan au sein de l'Union Européenne.

- Session 4 -

Conservation des ressources génétiques

M.C. VARELA ***"Conservation des ressources génétiques forestières ; aspects scientifiques et économiques"***

L'aggravation des problèmes environnementaux et le développement de l'utilisation de variétés améliorées concourent à justifier la nécessité de programmes de conservation des ressources génétiques. Pour les espèces concernées par des programmes d'amélioration génétique, la conservation est une partie même de ces programmes. Pour les autres espèces des programmes spécifiques doivent être engagés sur la base de conservation in-situ de populations de taille réduite. Les méthodes de conservation dépendent d'une définition claire des objectifs, et d'une bonne connaissance génétique de l'espèce. Les connaissances manquent notamment pour ce qui concerne les caractères adaptatifs et il serait dangereux de se reposer uniquement sur des travaux sur les marqueurs moléculaires même si ceux-ci restent des outils utiles. Des dispositifs comparatifs en forêt sont nécessaires pour des observations à long terme ce qui nécessite des financements appropriés.

J. KLEINSCHMIT ***"Biodiversité ; quelles proportions pour la production intensive et la forêt naturelle ? Comment concilier utilisation de variétés améliorées et conservation des ressources génétiques ?"***

Le cadre de ce thème est fixé par les résolutions de la conférence d'Helsinki. Sur cette base l'auteur en déduit les principes généraux pour la gestion forestière et les programmes de recherche génétique associés. Les variétés améliorées sont utiles pour les objectifs économiques, et la disponibilité d'un matériau renouvelable est importante pour les générations futures. Dans le processus de sélection de ces variétés l'accent doit être mis autant sur l'adaptabilité que la production, et les populations d'amélioration doivent être gérées en incluant un objectif de conservation des ressources génétiques. Une réduction forte de la variabilité génétique n'est acceptable que pour des productions intensives à courte révolution. Mais une part importante de la forêt, surtout publique doit être gérée avec des objectifs diversifiés pour lesquels les peuplements mélangés ont des atouts. Ces forêts doivent notamment contribuer à la conservation in-situ des ressources génétiques. Ce type de programme n'est cependant pas envisageable pour les espèces mineures et il faudra alors recourir à des méthodes ex-situ. La conciliation des objectifs de production et de conservation implique une amélioration des connaissances notamment sur la place et la génétique des espèces rares, mais aussi la mise en place de directives communautaires sur des exigences minimum en terme de gestion de la biodiversité.

P. BARADAT ***"Quelle diversité (inter et intra) pour les variétés forestières ? Le risque pour le forestier et la société"***

Les variétés forestières peuvent montrer une plus ou moins grande diversité génétique, depuis le seul clone jusqu'à différentes sortes de variétés synthétiques. Les avantages et désavantages de variétés génétiquement larges ou étroites sont comparés, à la fois à l'échelle du pays et du forestier individuel. Il apparaît qu'au moins pour des longues révolutions qui impliquent des aléas tant sur l'adaptation écologique, la résistance aux adversités et l'évolution du marché, il est sage de maintenir un haut niveau de polymorphisme génétique à l'échelle du peuplement. Cet objectif peut-être atteint ou bien par le moyen d'une variété à la base génétique large, ou par une combinaison de variétés spécialisées. Par ailleurs, cette approche peut être développée avec des gains génétiques élevés, concentrés sur les différents produits des récoltes successives lors des éclaircies et de la coupe définitive.

J.I. COOPER ***"L'utilisation d'organismes génétiquement modifiés en foresterie"***

Cet article expose quelques unes des possibilités, limitations et atouts de l'utilisation d'organismes génétiquement modifiés dans l'amélioration des forêts. L'auteur aborde les relations existantes entre les agents pathogènes, en particulier les virus et l'utilisation des séquences codantes des plantes, de virus ou de bactéries, ainsi que le contexte particulier créé par les arbres transgéniques.

M. ARBEZ ***"Les tendances européennes en terme de conservation des ressources génétiques forestières"***

Malgré l'accroissement des forêts en Europe des menaces pèsent sur celles-ci, et un consensus s'est dégagé pour conserver l'ensemble des espèces forestières et pour maintenir leur adaptabilité. Il existe différentes sortes de forêt (réserves génétiques, forêts naturelles, forêts de production), qui justifient des règles de gestion spécifiques. Sous cette réserve, l'utilisation de variétés améliorées est compatible avec la conservation des ressources génétiques. L'auteur présente le programme international EUFORGEN concentré sur quatre espèces pilotes. Celui-ci devra être coordonné avec les initiatives communautaires récentes. Ces programmes se heurtent à des lacunes importantes en terme de méthodologie de la conservation pour des arbres forestiers, dont les différents aspects sont discutés.

Conclusion générale

Pour ce qui concerne la qualité génétique du matériel forestier, il faut distinguer deux sortes fondamentales de matériel : le "matériel sauvage" récolté sur des peuplements forestiers, et le "matériel amélioré" issu des travaux d'amélioration génétique. Chacune de ces catégories soulève des problèmes spécifiques d'utilisation et de caractérisation.

I - Le matériel sauvage

1. La région de provenance est la pierre angulaire de notre système de contrôle, et nous devons examiner attentivement la validité des régions de provenance et de leurs limites. Des différences de conception sur la signification des régions de provenances, existent entre Etats membres.

   Pendant les 40 dernières années un grand nombre de comparaisons internationales de provenances ont été mises en place en Europe tant avec des espèces indigènes qu'exotiques. Beaucoup d'informations ont été extraites de ces tests par différents pays, mais il est nécessaire de développer les échanges pour une analyse en commun des données. Il est recommandé que cette information soit diffusée, afin d'aider les décisions en matière de transferts génétiques, et de fournir les éléments nécessaires pour la vulgarisation. Dans le cadre d'actions concertées, la Commission devrait aider à atteindre cet objectif, ainsi qu'à établir de nouveaux réseaux pour les espèces pas encore concernées.

2. Les marqueurs biochimiques doivent être développés jusqu'à un stade où ils pourront appuyer le système de contrôle à différents niveaux (identification, mais aussi validation par exemple en cas de pollution pollinique,...).

   Nous recommandons que les trois principaux types de marqueurs génétiques, utilisés comme outils d'identification et de discrimination soit appliqués sur un échantillon commun de matériel, afin de mieux cerner leur contribution potentielle dans l'assistance aux décideurs réglementaires et aux contrôleurs. Cet échantillon commun devra inclure un lot de populations largement réparties, et des variétés améliorées. Des applications tant pratiques que théoriques pourront en découler. De plus ces études génétiques apparaissent comme l'un des préalables pour bâtir des stratégies en matière de conservation des ressources génétiques.

II - Le matériel amélioré

Le matériel génétiquement amélioré soulève deux questions relatives d'une part à leur identification et d'autre part à leur évaluation. La question de l'identification est liée au constat préalable sur les marqueurs biochimiques. Que ce soit pour l'évaluation ou les tests, la question est fondamentalement de savoir comment le travail peut être accéléré. Néanmoins il est nécessaire de distinguer clairement les tests d'évaluation au niveau d'une part des processus d'amélioration et d'autre part de la certification.

1. Certains caractères adaptatifs (résistances pathologiques, résistance au froid...) peuvent parfois être estimés à un stade précoce. La réglementation doit être appliquée au niveau de chaque caractère sélectionné pris individuellement, et non de façon globale. Nous recommandons que les possibilités offertes par les connaissances complètement établies en matière d'études génétiques et de corrélations juvénile-adulte soient prises en compte dans l'application de la réglementation.

2. Actuellement, aucun marqueur biochimique ne peut prédire la croissance ou un des caractères essentiels d'intérêt économique. Cependant nous recommandons que la recherche soit poursuivie sur cette question, en raison de l'intérêt considérable que ces méthodes pourraient avoir pour accélérer les cycles d'amélioration, et la diffusion du matériel amélioré dans les circuits commerciaux de la foresterie.

3. Nous ne devons pas être effrayés par les techniques in-vitro alors que les cultures de tissus peuvent s'avérer beaucoup plus stables qu'on ne l'imaginait initialement, sauf pour ce qui concerne l'embryogenèse somatique. Nous avons besoin de recherches sur des marqueurs biochimiques susceptibles de vérifier la stabilité des organismes créés. L'importance actuelle est faible pour le matériel forestier, mais le potentiel est étendu, et nous avons besoin de réponses avant que des développements importants n'interviennent.

4. Les organismes génétiquement modifiés (OGM) nécessitent des recherches nouvelles concernant la sécurité de ce type de matériel, et les méthodes de diffusion. Il est néanmoins nécessaire de souligner la nécessité de test sur le terrain au cours de l'évaluation des OGM. Des recherches pour établir la carte génétique devraient être encouragées

avant qu'aucune manipulation n'intervienne quelque soit l'espèce ou l'hybride concerné.

III - Utilisation du matériel forestier

1. Avant que le matériel forestier ne soit utilisé, il doit être commercialisé dans les conditions prévues par nos directives. Il y a un besoin urgent de réviser celles-ci, et de tenter d'harmoniser les textes de l'OCDE et de l'Union Européenne afin de faciliter pour les Etats membres le commerce national ou international des matériels forestiers. Comme indiqué ci-dessus, les réglementations doivent envisager la possibilité de commercialiser n'importe quelle nouvelle sorte de matériel forestier obtenu dans le cadre des programmes d'amélioration génétique, même si les méthodes utilisées ne le sont pas encore à une échelle commerciale.

2. Les directives communautaires doivent être modifiées pour prendre en compte l'usage final du matériel de reproduction. Les objectifs de ces directives doivent être d'organiser le contrôle de la production et de la récolte du matériel forestier, et aussi d'harmoniser les informations minimum accompagnant les échanges commerciaux.

3. La question des modalités du contrôle de toutes les sortes de matériel forestier de reproduction doit être posée. Actuellement ceci est réalisé grâce à 'l'inscription du matériel de base et le contrôle de tous les stades de production. Avec la taille croissante de l'Union Européenne et les changements dans l'interprétation du sens de la "forêt", l'utilisation faite du matériel de reproduction doit être examinée. La subsidiarité peut être le principe clé dans ce domaine, alors que les décisions dépendent fortement de l'espèce et des conditions écologiques.

4. Si le contrôle doit s'intensifier au niveau national, il est nécessaire d'uniformiser l'identification, les systèmes de contrôle et la diffusion de l'information. La standardisation est importante, et la quantité d'informations devant être fournies par le fournisseur doit croître avec le niveau du risque encouru dans l'utilisation du matériel de reproduction.

IV. Conservation des ressources génétiques

1. En premier lieu, nous devons souligner qu'il y a une place pour l'utilisation de matériel génétiquement amélioré, et une place pour la conservation des ressources génétiques des essences forestières. Il n'y a pas d'incompatibilité, mais un besoin accru d'information et d'éducation, tant pour les améliorateurs que les conservateurs.

2. En second lieu, nous avons besoin d'études génétiques pour définir la manière la plus efficace de bâtir nos stratégies en fonction des situations différentes dues à la structure spécifique de chaque espèce (espèce sociale, feuillus précieux disséminés, ...). Les études devraient aborder, par exemple, le nombre de populations, la taille minimum de chaque population, les procédures d'échantillonnage, ... Mais nous devons également démarrer immédiatement des actions pragmatiques de conservation, et ceci devrait être réalisé dans un cadre international.

La commission devrait encourager ces actions.

3. Les choses évoluent et la conservation n'est pas un concept statique ; la conservation ne peut se résumer à de la préservation. Nous devons avoir des approches diversifiées pour la gestion de nos ressources génétiques, combinant des méthodes in-situ, ex-situ et dynamiques.

4. Une coordination minimum est nécessaire entre les différentes initiatives prises à l'échelle internationale pour la conservation des ressources génétiques des espèces forestières.

5. L'Union Européenne devrait probablement examiner la nécessité de directives prescrivant des exigences minimum pour que la biodiversité soit réellement un élément de tout programme de gestion durable des forêts.

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