Management of valuable broadleaved forests in Europe

3.2 Genetics and tree breeding
Jochen Kleinschmit and Jörg R.G. Kleinschmit

Abstract

The importance of genetic quality for timber production is outlined. Human influence on the present genetic structure of valuable broadleaved populations is discussed. The flower biology, natural range, and genetic studies for Acer pseudoplatanus, Fraxinus excelsior, and Prunus avium are mentioned. Results of provenance experiments for these species are presented. Methods for implementation of genetic knowledge like quality seed production in seed orchards or vegetative propagation of superior clones for further improvement are presented. Conservation activities for a range of valuable broadleaved species are summarized. The legal situation and recommendations for the use of reproductive material are outlined.

Key words: Genetic situation, provenance experiments, tree breeding, conservation, reproductive material

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Executive summary
3.2.1 Introduction

The genetic information and the environmental conditions influence the phenotype of a tree.

The genotype of a tree decides on its adaptability, growth potential and quality potential.

Environmental conditions – including climate, soil, coexisting organisms – modify the expression of this potential. Depending on the trait, the environmental modification of the phenotype varies (heritability). Phenological traits like flushing, bud set and length of vegetation period are under strong genetic control and can be influenced just little by manipulation of the environment. Whereas diameter growth e.g. is considerably modifiable by site conditions, spacing, competition, and thus can be influenced by silviculture. Stands with economically valuable phenotypes can only be grown, if both components genotype and environment are favourable.

In forestry the environment can barely be influenced. Climate, soil, and coexisting organisms are usually given and only the silvicultural treatment of the stand can be modified.

The aim of silviculture and tree breeding is to achieve phenotypes, which serve human needs, by influencing the environment and the genotypes. Silviculture mainly influences the environment of the trees. To a small extend – depending on the heritability of the traits of interest – the genetic composition of the stand is altered during thinning operations as well. Tree breeding on the other hand influences the genotypes of the trees by selection, controlled crosses and choice of propagation methods. The influence of the environment results indirectly by interaction of the selected genetic collectives with their environment. The approaches of tree breeding are:

- inventory of the genetic situation by historical studies, studies of genetic markers, provenance-, progeny- and clonal tests,
- the selection of genetic material desirable for economic or conservation purposes,
- and the propagation of selected material in seed stands, seed orchards – with controlled crosses or open pollination – or by vegetative propagation.

At the time of regeneration of the stand, the forest owner has the unique opportunity to introduce improved material into his forests, without considerably increasing the cost of regeneration. Using genetically qualified material for forest regeneration is a precondition for the silviculturist to be economically successful.

3.2.2. Genetic situation

In the course of the following text the terms “valuable broadleaved species”, “valuable hardwood species” and “noble hardwood species” are used synonymously. These are forest tree species which are not very abundant under natural conditions, weak competitors, and usually grow on rich sites in the lowlands or in the mountain regions. The timber of these species is valuable. Valuable broadleaved species usually occur in mixed hardwood forests, often in small disconnected populations. Some of the species have a high potential for vegetative propagation under natural conditions (Prunus avium, Sorbus torminalis).

Human interference during the past had drastic implications on the population structure of valuable broadleaved species: The habitats of these species were of special interest for agriculture. Clearing of forests for farming and settlement resulted in a destruction of tree populations, a reduction of population size, and the isolation of populations. Furthermore silviculture had low interest in these species as compared to oak, beech and conifers.
Therefore the abundance of valuable broadleaved species was reduced in the remaining forests as well. These conditions often resulted in a narrow genetic base in connection with inbreeding effects (=loss of vitality), genetic drift (=loss of genetic information), and decrease of adaptability.

Plantation of selected cultivars for fruit production or locally maladapted provenances resulted in introgression of undesirable characteristic traits for wood production into wild (fruit) tree stands.

The following part will focus on Acer pseudoplatanus (sycamore maple), Fraxinus excelsior (European ash) and Prunus avium (bird cherry). These are the most frequent valuable broadleaved species in Central Europe. They have a high economic value, and a high potential for the afforestation of marginal farmlands. Some aspects of other species, which are more rare, like Sorbus torminalis and Pyrus pyraster will be mentioned too.

Chromosome numbers are \( n = 26 \) in Acer pseudoplatanus (Rohmeder and Schönbach 1959, Spethmann and Namwar 1985), \( n = 23 \) in Fraxinus excelsior (Rohmeder and Schönbach 1959), and \( n = 8 \) in Prunus avium (Rohmeder and Schönbach 1959, Kuppers 1979). All three species are diploid.

Acer pseudoplatanus is insect pollinated and cosexual with functional male or female flowers (De Jong 1976, Spethmann and Namwar 1985).

The natural range of Acer Pseudoplatanus is concentrated in the Central European mountain range, however small populations can be found at the Baltic Sea. Acer pseudoplatanus extends in the mountains above the beech optimum in an elevational band of 600 – 800 m, going up to 2000 m as a maximum in Switzerland. (Figure 1). Today it is difficult to delineate the natural range of Acer pseudoplatanus since it has been extensively planted in the past. Thus the range extended into north and east direction and new genetic information was introduced into some natural populations within the natural range too.

Figure 1: Natural range of Acer pseudoplatanus (Meusel 1939, modified)
Fraxinus excelsior is wind pollinated and can be andromonoecious, gynodioecious, gynomonoecious and trioecious. All forms occur together with hermaphrodite flowers (Hegi 1927). According to Rohmender and Schönbach (1952) the majority of trees flowers either male (39 %) or hermaphrotite (41 %). Only female flowering trees are rare (4 %). Male flowering trees are on average superior in growth due to the fact that female flowers consume more assimilates than male flowers. The natural range of Fraxinus excelsior covers Europe in the north as far as Scotland, Denmark, Scandinavia up to the 64th degree northern latitude. The eastern border of its range is the Wolga river and the southern border is Anatolia, the Pindus mountains in Greece, southern Italy and northern Spain (Figure 2). It grows mostly in low elevations but goes up to 700 m in the Harz mountains and 1400 m in the Swiss Alps.

Figure 2: Natural range of Fraxinus excelsior (Meusel 1939, modified)

Prunus avium is insect pollinated, hermaphrodite and proterogynous (Hegi 1923). A self incompatibility system avoids inbreeding (Hilkenbäumer 1953). The natural range of Prunus avium covers Europe, Minor Asia and the most northern part of Africa (Krüssmann 1978). In the north of England and Scandinavia it goes up to the 61th degree northern latitude, but it is cultivated up to 67th degree northern latitude (Figure 3) The main altitudinal range is between 200 and 600 m, but it can be found in the Central Alps up to 1700 m. Prunus avium is regarded as the origin of cultivated forms of sweet cherry and it is extensively cross breeding with cultivars. This fact creates problems for forestry, since the selection criteria for fruit production are completely different from those for wood production (Kleinschmit et al. 2000).

Genetic marker directly allow the description of the genetic situation of a species. The marker phenotype permits direct conclusion on the underlying genotype. Genetic markers are
a valuable tool for the reconstruction of recolonisation routes, studies of gene flow, relatedness, conservation, and identification. The number of genetic markers is steadily increasing.

Isozyme gene markers were the first tools. They are easy to use and the biochemical function of the underlying genes is known. But the number of loci is limited and thus allows only the description of a part of the whole genome’s variation.

Theoretically the number of DNA markers is unlimited. But the costs for analyses are high and often the physiological function of the analysed DNA sequence is unknown. For DNA analysis there exist different techniques to analyse either known or anonymous DNA sequences of coding or non-coding regions. According to the aim of the study the DNA markers can be either biparentally inherited via the nucleus or uniparentally inherited via mitochondria and plastids (Gillet 1999). The aim to establish markers for the expression of quantitative traits (QTL) has not been reached yet for the species under consideration.

The number of studies using gene markers is higher for Prunus avium, which is of interest for horticulturists as well (e.g. Dosba et al. 2000, Kownatzki 2001, Mohanty et al. 2001, Struss et al. 2001, Dirlewanger et al. 2002, Zanetto et al. 2002), than for the other valuable broadleaved species. Populations of bird cherry are quite differentiated, often unrelated and vegetative propagation occurs regularly. Thus the number of genotypes in these populations is lower than the number of trees (Ducci and Santi 1997). Cultivars of Prunus can be genetically differentiated from wild populations by means of genetic markers (Badenes and Parfiitt 1995).
Isozyme, nuclear, and chloroplast DNA-markers are established for Fraxinus excelsior (Brachet et al. 1999, Lefort et al. 1999, Heuertz et al. 2000) and Acer (Bendixen 2000) but only limited information from marker studies is available. For Fraxinus an extensive study was started, combining quantitative and genetic traits as well as propagation methods and utilization aspects in a project supported by the European Union.

3.2.3. Tree Breeding

“Tree breeding is the activity of men to influence and improve forest trees and shrubs with the aim to satisfy human needs as good as possible” (Rohmeder and Schönbach 1959, translation of the authors). The existence of a broad variability of genetically controlled traits is the precondition for tree breeding and thus for tree improvement. As a consequence of adaptation to the different ecological conditions within the extended natural ranges of the species provenance differences are to be expected.

Tree breeding and tree improvement activities include provenance-, stand-, and single tree testing and selection, grafting and seed orchard establishment, controlled crossing, selection, testing and vegetative propagation of superior clones, and procurement of the material to the forest owner. The different levels of breeding activities allow different levels of improvement, which add about 20 % each above the level of the previous. The time span for reliable information from tests is 10 to 20 years as a minimum, for seed orchards from selection until production 10 years, and for a clonal program from the start of selection of superior clones to application 20 years. A clonal program needs as a base the existence of provenance-/progeny tests.

Provenance-, progeny-, and clonal tests are tools for the description of existing variation as well as for tree improvement, namely selection of well performing genetic collections. The tests are established under different ecological conditions to get information about the genetic control of the trait expression and the general adaptability of the genetic units studied. This information cannot be gained from marker studies. Important traits for adaptation are phenological traits, survival, vitality, health. These traits are also a prerequisite for adaptability to heterogeneous site conditions. Beyond this important traits for tree improvement are growth, quality, and wood characters.

Provenance experiments with valuable broadleaved species are comparatively young. The establishment of provenance experiments with these species needs different approaches compared to major tree species with extended stands. The collection of a limited number of single tree progenies per population is a compromise to get provenance as well as progeny information.

Provenance differences for Acer pseudoplatanus were reported already by Engler (1905) and Schwerin (1907). Provenances from southern Italy did not survive in Germany.

Stands from a limited region in Germany showed strong genetic differentiation especially in stem form in a 15 years old progeny test (Weiser 1981). In Belgium the study of 100 single trees progenies originating from 20 Belgium stands resulted in regional differences in seed size (Galoux and Falkenhagen 1965). Growth potential decreases with increasing elevation of the site of origin and increases with increasing temperature of the site of origin (Galoux 1969).

An Acer pseudoplatanus provenance experiment including 45 provenances, each represented by 5 single trees, from Germany, Switzerland, Austria, Chechoslovakia, Jugoslavia, Romania and Denmark was collected in 1981 by the Lower Saxony Forest Research Institute and planted on 10 field experiments in northern and western Germany. At age 16 of the field experiments there exist pronounced ecotypic patterns of variation. Some local provenances as well as some provenances from farther away show very good
performance at the test sites. A part of the variation in phenological characters can be explained by climatic differences of the site of origin (clinal variation). For other characters no clear geographic pattern can be found. In height growth and stem form the top performing provenances originate from Rosengarten (near Hamburg), Bodenmais in Bavaria, and Prachtice and Karlovice in Czech Republic. Provenances from Romania perform on an average. The best growing provenance has a height 30 % above average, the slowest growing provenance 15 % below average.

Since Fraxinus excelsior occurs on dry limestone sites as well as in alluvial plains, the existence of two races has been supposed by Münch and Dieterich (1925). But the studies of Weiser (1962, 1964, 1974, 1995) and Schönborn (1967) did not proof genetic differences between these types. This means, that sufficient gene exchange between these units occurred and/or that the selection pressure from soil conditions is not very strong as compared to the selection pressure due to climatic differences between geographic regions.

A Fraxinus excelsior provenance experiment including mainly German sources (43), but as well some from Switzerland (3), Austria (4) and Romania (2) each represented by 5 single trees was collected in 1982 and sown in 1985 by the Lower Saxony Forest Research Institute. 26 field experiments have been established in Germany (19), France (2), Belgium (2), The Netherlands (2), and Romania (1). The first results have been published by Kleinschmit et al. in 1996 and in 2001. Under German conditions Romanian provenances flush early, are damaged by late frost and therefore have the worst stem form. Phenological characters show a clinal pattern of variation. For height growth and stem form the pattern of variation had strong ecotypic components, indicating considerable differences between populations within short distance. Variation of trait expression within provenances was as high as variation between provenances.

For Prunus avium two limited provenance studies including German, French, Jugoslavian and Romanian material were established by the Lower Saxony Forest Research Institute in 1985. Variation within provenances is as high as between provenances at age 15. French provenances have similar growth as the German provenances. Those from Jugoslavia and Romania are inferior under German growing conditions. There exist drastic differences in stem form between progenies and between trees in progenies, offering an excellent potential for selection of superior clones for vegetative propagation.

The results of the provenance experiments show, that the pattern of variation is different for different traits. Phenological traits, which are under strict genetic control, show a clinal pattern of variation. This can be explained by a direct response to natural selection pressure. Growth traits on the other hand show more ecotypic patterns, indicating that already the selection of tested stands can give a considerable improvement. The results of provenance experiments stress the importance to choose carefully the origin of material for a specific plantation site.

Propagation by grafts is possible with Acer pseudoplatanus, Fraxinus excelsior, and Prunus avium. In Sycamore maple special wood structures occure “curly maple” and “bird’s eye maple”. Genotypes showing these trait expressions can be vegetatively propagated by grafts.

Only for Prunus avium methods of cutting propagation have a sufficient rooting success and methods of in vitro propagation are developed to commercial use (Meier-Dinkel 2000, 2001). For Prunus avium superior clones have been selected in different countries and reproduced vegetatively by in vitro propagation (Meier-Dinkel et al. 1997). Such clones can
be used as clonal mixtures of 30 or more clones in silviculture, offering an excellent base for the production of valuable timber.

For Fraxinus excelsior first results with in vitro propagation are available, but these have to be developed further (Hammatt 1996, Silveira and Cottignies 1993).

Grafted seed orchards have been established with selected superior adult trees for all the species on a regional base (Table 2). Seed orchards can overcome the problems of coancestry, inbreeding, and genetic drift, which are present in many of the small populations. At the same time they make use of some selection gain especially in stem form. Thus seed orchard seeds are the most interesting base material for silviculture with valuable broadleaved species. Seed orchards of Prunus avium are included into a testing program with different European countries (Germany, The Netherlands, Belgium) participating. When test results are available, the seed material from tested seed orchards should be preferred over seeds from untested sources.

3.2.4. Genetic conservation

The aim of genetic conservation in forest tree species is to maintain the natural patterns of variation and especially to maintain the adaptability of the species for future environmental changes. Today the natural patterns of variation rarely exist in the valuable broadleaved species. The long human impact on forest surface and composition has changed the natural pattern drastically. The implication of these changes are merely known.

In a long term view genetic conservation of forest tree species in a densely populated country like Germany can only be successful if the species is used in a regular forest management system. This approach is practicable with sycamore maple, European ash and bird cherry. In the past the integration of conservation and utilisation was however a problem with most of the minor hardwood species due to their rareness. Often no reproductive units existed any more. But even in sycamore maple, European ash and bird cherry the integration of conservation and utilization will be more successful, if the economic return of the silviculture with these species is of interest for the forest owner. This will be the case, if the value of the timber is high. Therefore tree breeding and conservation have an important link. All activities which improve the economic return will directly influence the participation of the species in artificial regeneration programs. The increasing timber prices for noble hardwood during the last decade had a direct influence on the request for plant material of noble hardwood species and therefore support the conservation of these species too.

In Europe the programs for conservation of forest tree species are coordinated by EUFORGEN (European Forest Genetic Resources Programme) which is located at the International Plant Genetic Resources Institute in Rome, Italy. There exists a special network for noble hardwood species, which was established in 1996 (Turok et al.). It is obvious, that most neighbouring countries have an interest in the same species. Since the ecological conditions do not necessarily respect political boundaries, it is meaningful to coordinate the genetic conservation programs on an European level.

Conservation can be done in situ with stands or single trees or ex situ by plantations, seed orchards, clonal archives, storage of seed, pollen, tissue, and even DNA sequences. In situ conservation stands have the advantage, that evolutionary processes are going on and that they can be integrated into normal forest management systems. A managment restriction is, that regeneration has to be done either naturally or with material from the same stand. In situ conservation by stands is only meaningful, if the populations are large enough. In situ conservation of single trees can only be an intermediate step, because they will die naturally sooner or later. Thus single trees have to be conserved ex situ.
Ex situ conservation in plantations, seed orchards and clonal archives is relatively easy. The choice of the ex situ conservation site should allow evolutionary adaptational processes to take place. Seed orchards for ex situ conservation facilitate the creation of new, genetically diverse populations of unrelated individuals. These seed orchards may represent a certain level of genetic improvement as well. If in situ conservation measures are taken, the improvement aspect of ex situ conservation activities can be stressed without the risk to lose important genetic information. This is the case with sycamore maple, European ash and bird cherry.

Seed orchards have the additional advantage, that reproductive material can be harvested soon. Storage of seed, pollen, tissue and DNA sequences must be done under controlled environmental conditions. These conservation measures are technically susceptible and from beginning to the end of the enumeration increasingly sophisticated.

Tree breeding includes a lot of conservation activities too. Therefore a broad overlap between tree breeding and conservation of forest genetic resources exists as long as breeding activities succeed in maintaining the important genetic diversity.

The general situation of noble hardwood species in Germany from a conservation point of view is summarized in Table 1.

Table 1: Common species of noble hardwoods in Germany
(number of stars indicate importance of aspect for species)

<table>
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<tr>
<th>Species</th>
<th>Importance</th>
<th>species under council directive 1999/105/EC</th>
<th>Reason of endangerment</th>
<th>Hybridisation with other species</th>
<th>Hybridisation with cultivars</th>
<th>Small population size</th>
<th>Inbreeding, genetic drift</th>
<th>Diseases</th>
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<td>Acer campestre</td>
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Acer pseudoplatanus and Fraxinus excelsior are species, which still have sufficiently large populations. The trade with reproductive material is controlled under the restriction of EC legislation (Council directive 1999). Therefore the conservation strategy can include an important in situ conservation component. This is not true to the same extent for Prunus avium, where small population sizes are frequent, where hybridization with cultivars occurs, and where up to now no control of the reproductive material was existing. Material originating from countries with different ecological conditions was used as well as material from jam factories. Problems with small population size and hybridization, which are typical for all wild fruit species, can partly be handled by ex situ conservation in seed orchards and clonal archives. The problems are much more pronounced with some of the other valuable broadleaved species, where in situ conservation possibilities are extremely restricted. Here the main emphasis has to be put on ex situ conservation. This is especially true for the other wild fruit species (Malus sylvestris and Pyrus pyraster). In Ulmus the problems with the Dutch elm disease restrict even ex situ conservation methods, since seed orchards will be attacked sooner or later by the disease too.

The main emphasis of conservation in Germany in forest trees is on the management of in situ conservation stands. With the valuable broadleaved species the activities shift more in direction of ex situ conservation. The status of activities in Germany up to the year 2000 is summarized as an example in Table 2. From this table it is obvious, that only few of the species – Acer pseudoplatanus, Alnus glutinosa, Fraxinus excelsior, and Tilia cordata - still have stands of reasonable size. For those, where stands are rare or very small, the main emphasis is on single trees in situ, which enter into clonal archives or seed orchards. For more details of other species and activities in other countries the publications of the Noble Hardwood Network (Turok et al. 1998, 1999) are a valuable source of information.
### Table 2: Conservation of valuable hardwood species in Germany up to the year 2000

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<tr>
<td>Sorbus torminalis</td>
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<td>2015</td>
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<td>Tilia cordata</td>
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<td>20</td>
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<td>159</td>
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<tr>
<td>Tilia platyphyllos</td>
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<td>599</td>
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<td>538</td>
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<tr>
<td>Ulmus glabra</td>
<td>123</td>
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<td>3574</td>
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<td>7</td>
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<tr>
<td>Ulmus laevis</td>
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<tr>
<td>Ulmus minor</td>
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<td>12</td>
<td>880</td>
<td>2</td>
<td>5</td>
<td>105</td>
<td>143</td>
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<tr>
<td><strong>Total</strong></td>
<td>1357</td>
<td>1165</td>
<td>21151</td>
<td>127</td>
<td>54</td>
<td>168</td>
<td>259</td>
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</table>

#### 3.2.5. Legal situation

Up to recently the trade of reproductive material of most valuable broadleaved species was not under legal control. Exceptions were Fraxinus excelsior and Acer pseudoplatanus in Germany. With the new EU – regulations (Council 1999) this situation will change and more species will be included in the national laws. The past situation favoured the silvicultural use of reproductive material originating from low wage countries for regeneration, irrespective of the ecological conditions at the site of origin. This can have implications for the existing populations, where an uncontrolled mixing of provenances occurred. The non adapted material represents a genetic load for the population.

Therefore in Germany the “Deutsche Kontrollvereinigung” (German Control Organisation) was established by private seed dealers, nursery men, forest owners, and public forest services, to guarantee the control of reproductive material of selected stands of high quality. This organisation circumvents at least part of the disadvantages linked to the legal, administrative control of seed transfer.

#### 3.2.6. Recommendations for the use of reproductive material

Natural regeneration of forest stands is the present preference in many countries. This should be critically checked under the aspects of genetic diversity and genetic quality of the
existing stands. Not every population is worthwhile to be naturally regenerated from an economic point of view. Criteria must be the population size, the growth and quality of the existing stand. Only if the population size is at least 20 individuals in reproductive age and if growth and quality are above average natural regeneration can be recommended. Otherwise enrichment planting with improved material or – in extreme situations – replacement of undesirable stands should be considered.

For practical seed and plant procurement there should be a clear ranking of the base material according to its status of genetic improvement.

1. Mixtures of tested clones; vegetatively propagated (available for Prunus avium);
2. Seed orchard material from tested seed orchards;
3. Seeds from tested stands (available for some valuable broadleaved species);
4. Seed orchard material (available for all valuable broadleaved species) and
5. Selected phenotypically superior stands (available for all species under legal control).

The forest owner should request the material from the nurseries in this sequence (1. being the highest priority) and never plant material, which is not genetically qualified above average.

The seed orchards established on a regional base in Germany can be seen in table 2. In Germany for Acer pseudoplatanus 17, for Fraxinus excelsior 11 and for Prunus avium 20 seed orchards exist. When these are in full production, they will produce sufficient seed to supply the majority of potential plantations in Germany.

As long as the seed orchards are not tested, the preference should be given to the seed orchards which correspond regionally to the plantation zone. However, due to the results of provenance testing, these zones must not be very fine grained. In Germany it seems to be sufficient for valuable broadleaved species to differentiate between northern lowlands, mountain region and alpine region. Eventually these regions can be subdivided into west and east.

Different European countries and the German states procure provenance recommendations, which basically follow our line of discussion (Baden-Württemberg, Ministerium ländlicher Raum 1997, Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten 1987, Centrum voor Genetische Bronnen, Nederland 2002, Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Forsten 1995, Sächsische Landesanstalt für Forsten 1996 e.g.).

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Executive summary
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