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Methods and progress in the conservation of elm genetic resources in Europe

E. Collin[1]*, M. Rusanen[2], L. Ackzell[3], J. Bohnens[4], A. de Aguiar[5], S. Diamandis[6], A. Franke[7], L. Gil[8], L. Harvengt[9], P. Hollingsworth[10], G. Jenkins[11], A. Meier-Dinkel[12], L. Mittempergher[13], B. Musch[14], L. Nagy[15], M. Pâques[9], J. Pinon[16], D. Piou[17], P. Rotach[18], A. Santini[13], A. Vanden Broeck[19] and H. Wolf[20]

[1] Cemagref, UR. Ressources Génétiques. D. des Barres. 45290 Nogent-sur-Vernisson. France
[2] Finnish Forest Research Institute. Box 18. 01301 Vantaa. Finland
[3] The National Board of Forestry (SKS). S-551 83 Jonköping. Sweden
[4] Hessen-Forst. FIV. Prof.-Oelkers-Str. 6. 34346 Hann-Münden. Germany
[5] Estaçao Florestal Nacional. Quinta do Marques. 2780 Oeiras. Portugal
[6] National Agric. Res. Foundation. Forest Res. Inst., 570 06 Vassilika-Thessaloniki. Greece
[7] Forstliche Versuchs-und Forschungsanstalt (FVA). Postfach 708., D-79007 Freiburg i.Br. Germany
[8] Univ. Politécnica de Madrid. ETSI de Montes. Paseo de las Moreras, s/n. 28040 Madrid. Spain
[9] Afocel Biotech. Domaine de l'Etançon. F-77370 Nangis. France
[10] Royal Bot. Garden. 20A Inverleith Row. Edinburgh EH3 5LR. Scotland. UK
[11] Univ. of Glasgow. Div. Biochemistry and Molec. Biology. Glasgow G12 8QQ. Scotland. UK
[12] Niedersächs. Forstl. Versuchsanstalt (NFV). Forstamtstr., 6. 34355 Staufenberg-Escherode. Germany
[13] Instituto per la Protezione delle Piante-CNR. Via Madonna del Piano. 50019 Sesto Fiorentino (FI). Italy
[14] Office Nat. des Forêts., Conserv. Génét. des Arbres Forest. Centre INRA. 45160 Ardon. France
[15] Forest Research Institute. Várkerület 30 / A. 9600 Sárvár. Hungary
[16] Inst. Nat. de la Rech. Agronom. (INRA). Recherches Forestières. 54280 Champenoux., France
[17] ENGREF-Arboretum des Barres. 45290 Nogent-sur-Vernisson. France
[18] ETH-Zentrum. Rämistrasse 101. 8092 Zürich. Switzerland
[19] Inst. voor Bosbouw en Wildbeheer (IBW). Gaverstraat 4. 9500 Geraardsbergen. Belgium
[20] Sächsische Landesanstalt für Forsten (LAF). Bonnewitzer Str. 34. 01796 Pirna. Germany

Abstract

The progress made in the conservation of European elm genetic resources since the 1st International Elm Conference is reviewed, and the complementarity of *in situ* and *ex situ* methods is discussed. The financial support of the European Union to RESGEN project CT96-78 has permitted to co-ordinate and rationalize the *ex situ* conservation of elms. The project, which involved 17 partner institutes in nine west European countries, aimed at a better evaluation, conservation and utilisation of the existing collections of native elm clones. Main achievements are: establishing a common database of about 2,000 clones; characterizing over 500 clones through RAPDs and chloroplast DNA PCR-RFLPs molecular markers; completing and rationalizing the existing collections; establishing a long-term core collection of 850 clones; cryo-preserving a subset of 444 clones; and identifying clones of interest for breeding and prudent use in the reconstruction of countryside hedges. The «Noble Hardwoods» network of the pan-European programme EUFORGEN groups members representative of 31 countries, and promotes the dynamic conservation of the genetic resources of several genera of broadleaf forest trees, including *Ulmus* spp. Strategies for the conservation of the adaptive potential of elm resources were defined and will be disseminated among foresters and conservationists through «Guidelines» leaflets. Some countries have already started implementing conservation measures for *U. laevis*, associating *in situ* preservation and the establishment of seed orchards. Others are undertaking inventories, or acquiring genetic knowledge on target populations.

Key words: vegetative propagation, international co-operation, *Ulmus*.

* Corresponding author: eric.collin@cemagref.fr
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1 and 3-14, 16, 17, 19, 20: participants in the RESGEN CT96-78 E.U. project «Conservation of Elm Genetic Resources» (Co-ordinator E. Collin).

2 and 15, 18: participants in the EUFORGEN «Noble Hardwoods» network (Chair-person M. Rusanen).

Resumen

Métodos y progreso de la conservación de los recursos genéticos de los olmos en Europa

Se discute el progreso realizado en la conservación de los olmos europeos desde la primera conferencia Internacional del Olmo y los métodos complementarios de conservación *in situ* y *ex situ*. El apoyo financiero de la Unión Europea al proyecto RESGEN CT96-78 ha permitido coordinar y racionalizar la conservación *ex situ* de los olmos. El proyecto, en el cual están involucrados 17 instituciones participantes en nueve países de Europa Occidental, tiene por objetivo una mejor evaluación, conservación y utilización de las colecciones actualmente existentes de clones nativos de olmo. Los principales logros son: el establecimiento de una base de datos común de aproximadamente 2.000 clones; la caracterización de más de 500 clones usando RAPD y marcadores moleculares PCR-RFLP de ADN cloroplástico; la finalización y racionalización de las colecciones existentes; el establecimiento a largo plazo de una colección central con 850 clones; la criopreservación de un conjunto de 444 clones; y la identificación de clones de interés para la mejora y para su uso en la restauración de setos en campo. La red «Noble Hardwoods» del programa pan-europeo EUFORGEN agrupa a miembros representantes de 31 países, y promueve la conservación dinámica de los recursos genéticos de varios géneros de árboles planifolios, incluido *Ulmus* spp.. Las estrategias para la conservación del potencial adaptativo de los recursos de los olmos se definieron y se dieron a conocer entre forestales y conservacionistas a través de folletos guía. Algunos países han comenzado ya a implementar medidas de conservación para *U. laevis* mediante el uso de la preservación *in situ* y el establecimiento de huertos semilleros. Otros están elaborando inventarios, o adquiriendo información genética de poblaciones de interés.

Palabras clave: propagación vegetativa, cooperación internacional, *Ulmus.*

Introduction

The enormous damage caused by the Dutch elm disease (DED) pandemics to the European elm populations (Brasier, 1996, 2000) has led to the development of diverse national initiatives to collect germplasm and identify conservation stands. At the end of the 1990s, two complementory international programmes were launched in order to co-ordinate the conservation of elm genetic resources in a European perspective (Collin *et al.*, 2000). One was the RESGEN 78 project, which was carried out over 5 years (1997-2001) and benefited from the financial support of the European Commission (EC). It involved 17 forest research teams in nine European Union Member States, and was focused on the *ex situ* conservation of elms. The other is the European Forest Genetic Resources (EUFORGEN) co-operative programme, which was established in 1994 to promote the dynamic conservation and sustainable utilisation of forest genetic resources in Europe, and which has defined conservation strategies for the European species of elms (Collin, 2002).

This article is a follow-up to the presentation (Collin *et al.*, 2000) on the same topic at the 1st International Elm Conference (Lisle, USA, Oct. 1998). In the first part, the objectives and methods for the conservation of elm genetic resources will be scrutinized. Then, the progress made since 1998 in the frame of the RESGEN 78 project and the EUFORGEN programme will be reported. Finally, perspectives for further research and conservation activities will be drawn. General information on the three native European elm species and their conservation requirements is available from other sources (Collin *et al.*, 2000; Collin, 2002), but background information will be briefly supplied when case studies are examined.

Objectives and methods for elm genetic conservation

Objectives

In each country, various groups of people are interested in the conservation of elm genetic resources, but their interests and objectives are quite different as summarized here:

1. «Elm enthusiasts» (either informal groups or NGOs) will take local initiatives for preserving and/or replanting local elm trees; they will care for remarkable old trees and try to restore elms in the landscapes, just as they were before the DED outbreak.
2. Breeders will make clonal copies of surviving old trees, test their susceptibilities to the fungus causing the disease, and build collections of tolerant clones of interest in a breeding programme.
3. Nature and biodiversity conservationists will protect elm habitats and stands, but may be not always fully aware of the genetic constraints and evolutionary

forces to take in consideration when defining stand management programmes.

4. Genetic resources conservationists will consider the situation in an evolutionary perspective, and aim at safeguarding the potential for adaption of the elm species in a changing environment (Eriksson, 2001) rather than preserving the present situation, or restoring the past.

The purpose here is not to oppose these different objectives and groups, which are all of interest and need to be connected to each other, but to stress the necessity to adopt conservation methods and techniques that match the objectives. In fact, as there may be several different objectives entangled in a same elm conservation programme, the methods adopted may not be fully adequate for each of them, and some clarification and re-definition of the work programme may be needed.

Methods for static conservation

Static conservation *ex situ* consists in holding seeds, pollen, parts of plants or whole plants in freezers, test tubes or field clonal banks. As the protected material kept in those collections is excluded from the interplay of genetic recombination and natural selection which creates new diversity, this type of conservation is described as «static». This method is adopted when a rare or valuable resource is endangered in its original site, and must be preserved in a protected environment, or when a collection is gathered for further research or breeding. Key issues for static conservation are sampling criteria, types of germplasm used for storage or propagation, and conservation techniques.

Sampling criteria for elm conservation will be considered at three levels: taxonomic, geographic and individual tree. Rarity and endangerment are the two major criteria used for setting conservation priorites at the taxonomic and geographic levels, whereas other kinds of criteria are used for sampling individual trees in a population.

Taxonomy. As shown in Fig. 1, rarity at species level is a complex notion associating constraints in terms of range size, habitat breadth and local population size. Several elm taxa belonging to the Field elm group (*Ulmus minor* Mill. *sensu latissimo*) have indeed a small range and small populations, and can be considered rare species. However, this kind of statement may be fallacious, as was the case with the Plot Elm,

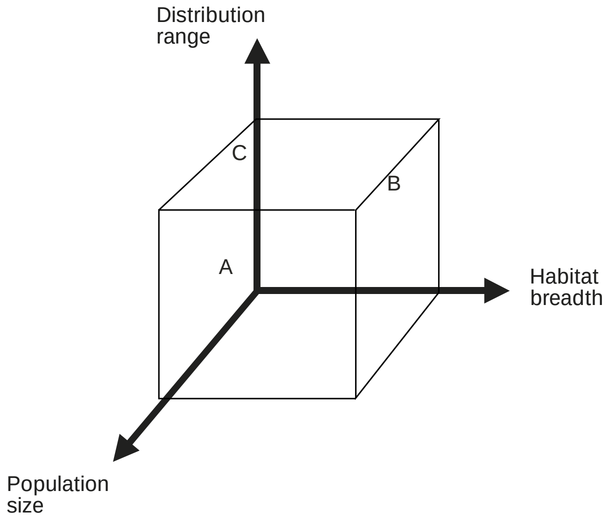

Figure 1. The different forms of rarity at species level. Each axis indicates an increasing abundance of a species at one of the three geographic or ecologic levels considered. Species (e.g. *U. plotii)* at position A are the rarest, whereas species (e.g. *U. minor)* at position B are very common; all other positions correspond to intermediate situations. Due to its narrow habitat and small population size, *U. laevis* would be in position C. Adapted from Eriksson (2001) and based on Rabinowitz (1981).

which some botanists (Melville, 1940) recognised as a true species under the binomial *U. plotii* Druce, and which was simply given a varietal rank by others (Richens, 1980, 1983). In fact, recent molecular work (Coleman *et al.*, 2000) showed that this red listed endemic species of the British Midlands was composed of a single widespread clone mixed with a number of similar but genetically unrelated entities, and that no large conservation effort needed to be allocated to this single genotype. Endangerment is also a complex notion associating constraints in terms of demographic and genetic stochasticity, and its appreciation may be difficult, especially when knowledge on the geographic partitioning of adaptive variation in the species is lacking. Emotional factors such as the consternation and alarm caused in the general public by the dramatic loss of adult trees of *U. minor* in the 1970s and 1980s are likely to blur our perception of effective threats in terms of loss of genotypes and erosion of genetic diversity. In fact, the priority given to *U. minor* in many national conservation programmes seems to have resulted more from such emotional factors than from a careful assessment of the respective situation of all elm species indigenous to the country.

Geography. Sampling at the geographic level can also be carried out specifically for the emergency safeguard of a rare ressource, but, generally, it is undertaken in the framework of a larger programme aiming at conserving a representative sample of the diversity at a regional, national or international scale. Even a species considered very common in the center of its distribution range will become rare at the margins of its repartition area, and thus become an important target for conservation activites. In some cases, such as the small populations of Wych elm (*U. glabra* Huds.) of Sicily, rarity can be associated with endangerment owing to losses caused by DED, and *ad hoc ex situ* conservation can be recommended. However, sampling at the geographic level is generally not strongly dependent from the risk criterion, but rather aimed at a good representation of the ecological diversity in the concerned territory. Despite the fact that they do not really reflect adaptive variation, neutral molecular markers are used to assess the amount of differentiation between populations and to better target the populations to sample.

Individual trees. The choice of the trees to sample for *ex situ* conservation is clearly dependent upon the objectives of the conservationist group:

— «Elm enthusiasts» will care for old trees which are remarkable for their old age, size and beauty, for having survived to the DED epidemics, and sometimes also for being associated with local history or legends.

— Breeders will make clonal copies of surviving old trees, regardless of their attractiveness or place in the local history and tradition; foresters will not only look at healthy trees, but also care for stem form.

— Genetic resources conservationists will adopt sampling protocols aiming at capturing as much genetic variation as possible in a limited number of samples. Such protocols are based on theoretical estimates of the number of trees per population needed to capture the major part of the adaptive variance in the population (Brown and Briggs, 1991). Final sampling may be carried out randomly or following an environmental gradient, and the phenotype of the tree will generally not be taken into consideration, except when revealing genetic variation (e.g. differences in leaf morphology, phenology, or sanitary status).

The kind of germplasm used depends on the species biology (its mating system, its ability for vegetative propagation), the population size and condition (e.g. the number of flowering trees, the risk of tree losses), the conservation objectives (propagating trees, safeguarding genotypes, preserving genes) and economic or practical constraints (collection time and practicability, facilities for propagation, maintenance facilities and costs).

Seeds are a cheap and comparatively easy way for sampling and preserving genetic ressources of the Wych elm (*U. glabra* Huds.) and the European White elm (*U. laevis* Pall.) which are found in natural populations reproducing sexually. Using this sort of germplasm is much more uncertain in the case of *U. minor* for two reasons: i) owing to natural root suckering, the genetic diversity in *U. minor* stands may be restricted to a small number of genets; ii) in some parts of its distribution range, *U. minor* does not often set seed, or seeds do not germinate. An advantage of seeds, as compared with grafts and cuttings, is that they are less likely to transmit diseases, and that they are safe from DED and Elm Yellows. For this reason they were used as basic material for the conservation plantations of continental Spain elm resources planted in the DED free Canary Islands. However, the possible risks of transmission of viral diseases like «Elm mottle» virus is still an impediment to the importation of elm seed from Europe into the USA (S. Wiegrefe, personal comunication).

Cuttings are the easiest method for duplicating and safeguarding an *U. minor* genotype; all kinds of cuttings (hardwood, softwood, roots) can be used with very good chances of success (Buron *et al.*, 2003) provided that the greenhouse temperature and hygrometry are perfectly controlled, which is not easy to accomplish. *U. laevis* can also easily be propagated using cuttings. Propagation success is much lower for *U. glabra*, not only in terms of average rooting percentage but —worse— in terms of duplication success (i.e. getting at least one plantlet of each sampled tree). In order to improve duplication success and rooting percentage in *U. glabra*, grafting can be used in a primary propagation step to acquire and re-juvenate the material before undertaking the secondary propagation using cuttings.

Grafting is an easy propagation technique for all European elm species but the drawback with *U glabra* is the high risk of scion death in field clonal archives —especially when they are kept as hedges— and the subsequent confusion with the rootstock genotype. Owing to rootstock influence, grafted plants are also not suitable for pathology tests where clonal susceptibility to *Ophiostoma novo-ulmi* Brasier (fungal agent of DED) is evaluated through inoculation of the pathogen in the plant stem.

Dormant buds or growing shoots can be used for two different reasons: either as simple alternatives to classic macropropagation techniques (cuttings, grafting), and leading to the same field maintenance prospects, or for the new possibilities they offer in terms of cryo-preservation. In the first case, in spite of the lesser sanitary risks procured by *in vitro* meristem culture as compared to classic techniques, one can doubt that micropropagation is really needed for the vegetative propagation of elm material. *In vitro* techniques are certainly more interesting for the massive multiplication of a limited number of genotypes than for the cloning in a few copies of a large number of non-juvenile trees, representing a broad spectrum of genotypes and possibly demanding different cultivation protocols. In addition, whereas *U. minor* and *U. laevis* can be micropropagated using the normal micro-cuttings technique, *U. glabra* necessitates a more sophisticated approach involving micrografting on *U. minor* micro-rootstock (Harvengt *et al.*, 2004). In the second case, *in vitro* techniques are not mere alternative tools for propagation but a major conservation strategic option, in which the maintenance of growing collections held in the field is abandoned in favour of dormant collections held in the laboratory, either as *in vitro* meristem cultures or as cryopreserved explants stored in liquid nitrogen (as described below).

Other types of germplasm can be collected in special cases: pollen for breeding purposes, seedlings or root suckers when available and when the nursery phase needs to be skipped for some reason. However, these possibilities are rarely used in practical conservation programmes.

Maintenance techniques can be grouped under two contrasted categories depending more on programme strategies than on technical options or economic constraints. The preservation of dormant elm material (seed, explants) can be viewed as a kind of Noah's Ark strategy to safeguard endangered resources, whereas growing material collections are built in a more active approach associated with research, breeding, use for plantings and dynamic conservation.

Conservation of dormant germplasm can be carried out on seeds when the conservation programme aims at preserving gene resources rather than genotypes of a particular interest, and when the collection of a number of seed lots sufficiently representative of the population or region sampled is easy. In all other cases, the cryopreservation of dormant buds can successfully be applied to elms. After proper treatment, elm seed can be maintained three years in cold storage without a significant decrease in its germination ability (Vincent, 1960), and recent progress in cryo-preservation techniques open perspectives for the safe long-term maintenance of seed lots. The vitality of dormant elm buds is not affected by storage at –196 °C, and this kind of treatment and maintenance is cheap and safe in comparison with classic field conservation. The more difficult and costly part of the process comes after cryo-preservation, when thawed buds need to be micropropagated in order to obtain at least a few vital plantlets of every clone, or at least of nearly every clone (Harvengt *et al.*, 2004).

Conservation of growing material is carried out in clone archives (or clone banks) which are generally kept on the land of research institutes and carefully tended to avoid loss of material and loss of clonal identity. To reduce their attractiveness to the bark beetles (*Scolytus* spp.) vectors of DED, and also to keep the material juvenile and accessible for sample collection, it is recommended to treat elm clone banks as low hedges, trimmed every year at about 1.5 m in height and laterally. Such clone archives are not to be confused with field tests where clonal behaviour can be judged on trees allowed to grow unprotected from pests and diseases hazards.

Methods for dynamic conservation

Dynamic conservation aims at preserving the species' adaptation potential over the long term. It can be carried out *in situ* in natural stands where natural regeneration will be promoted by silvicultural management and/or habitat restoration, and where trees will be submitted to natural selection in a changing environment. It can also be undertaken in artificial populations planted *ex situ*, holding a large genetic diversity owing to appropriate sampling, and managed in order to facilitate the mating of genetically diverse trees. Such artificial populations can be planted in a natural environment, forming reconstructed populations or managed in more controlled conditions in a seed-orchard where flowering and seed harvest can be facilitated. The seedlings appearing in reconstructed populations will be submitted to natural selection just as in the case of *in situ* conservation. The seedlings obtained from seed-orchard seed-lots will be assembled in genetically diverse batches of plant material for afforestation, contributing to the maintenance of the potential for adaptation of the considered species. Key issues for *dynamic* conservation are the sampling and management criteria of *in situ* units. The composition and design of the plantation plot for reconstructed populations and seed orchards need also to be discussed, and the need for the popularisation of dynamic management practices must be stressed.

Sampling criteria for *in situ* conservation units must be considered with as much genetic knowledge as possible on the genetic structure of the local resource. To fully understand the logic of genetic conservation it is important to note that losing one or two alleles during the reproduction is not a great loss to the objective of genetic conservation. As indicated by Brown and Briggs (1991), saving all alleles or allelic combinations that exist now or existed in the past is not feasible and is not necessary. The present state of a population is only partially a result of a deterministic force like selection, which moves the allele frequencies in a stand towards better adaptation. Besides selection, there are other evolutionary processes, namely mutation, genetic drift, gene flow and recombination, which act more randomly (Fig. 2). Mutation is the only factor leading to totally new variation in a population, but because of the low frequency of new viable mutations, it has no great significance within the time scale of gene conservation programmes. Genetic drift is of concern in that it is a stochastic process that changes the allele frequencies in small populations. In natural elm populations which are often small, genetic drift may cause a loss of genetic variation, thus leading to harmful inbreeding and reduced survival of the population in the future. Gene flow takes place through the movements of both pollen grains and seeds. Recombination is a mechanism by which diploid organisms rearrange combinations of alleles present in the previous generation. Mating pattern, e.g. flowering biology, breeding system and incompatibility mechanisms are the main determinants of recombination.

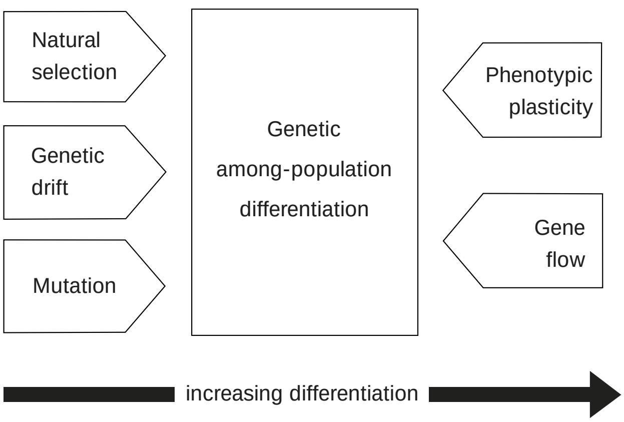

Figure 2. Evolutionary forces and their influence on among-population differentiation. When the pooled effects of the forces to the left outweight the forces to the right, the among-population differentiation will increase. Adapted from Eriksson (1996).

Lefèvre (2001) lists three criteria for selecting *in situ* conservation units: i), sampling for adaptive diversity (use data from provenance tests, cover the range of ecological context where the species is found, capture specific adaptations at the margin of the distribution range, sample crossroads of re-colonisation paths); ii), long-term survival likelihood (including stand size and number of potential seed trees); and iii), possibility for administrative classification as a special conservation area.

Management criteria for *in situ* conservation units must promote natural regeneration to facilitate recombination and adaptation. A sufficient number of seed-bearing trees must be involved in the natural regeneration process, and their contributions must be balanced. As a rule, efforts should be made to limit or buffer the influx of external sources of seed and pollen (preclude the plantation of exogenous material in the vicinity of the conservation unit). However, the point is not to eliminate the phenomenon of gene flow, «which can have a positive impact by importing new diversity and slowing the increase of inbreeding», but to «limit its potential negative impact» (Lefevre, 2001), especially when the conserved population is of a limited size.

Management criteria for *ex situ* dynamic conservation units must be based on a careful design of the plantation plot, ensuring a good repartition of origins and genotypes over the conservation plantation or seed orchard. Such a repartition will facilitate mating between many different parents, procuring a large array of diversity in the next generation. For seed-orchards, spacing should be at least 5 × 5 m, with a possibility to enlarge it later to 5 × 10 m (J. Kleinschmit, personal communication).

Promoting dynamic conservation by silvicultural management is of major importance because conservation is not a matter of gene reserves only, but also of good silviculture practices in commercial forests. The Swiss forest service has published a booklet aiming at the promotion of rare species (Barengo *et al.*, 2001) which not only can raise awareness among foresters for the conservation of genetic resources, but also gives recommendations for the management of a species like *U. laevis* at the national and local levels.

Complementarity between methods

It is possible to shift from static to dynamic *ex situ* conservation: e.g. use clonal material to reconstruct a population or create a seed orchard, make diverse «local» material available for the reconstruction of countryside hedges or prudent re-introduction in some forest sectors. It is also possible to shift from growing *ex situ* (clonal banks = work collections) to dormant *ex situ* (cryo-preservation = long term conservation) when no research programme and conservation funds are available any more.

Progress made through European programmes for elm genetic conservation

The RESGEN 78 EU Project

In the 1980s and 1990s, several European countries separately established national initiatives to collect elm clones in a combined effort to save their native elm resource and to gather collections for evaluation and further utilisation. However, for biological and economic reasons, an international initiative was needed to co-ordinate the conservation, evaluation and use of the hundreds of elm clones held by a large number of institutes across Europe. This was made possible when a proposal devoted to elm resources and co-ordinated by Cemagref (France) was accepted for funding by the European Commission in the frame of a programme (EC Regulation number 1467/94) in favour of the conservation and utilisation of genetic resources in agriculture. The 5-year «Conservation of Elm Genetic Resources» EU project (RESGEN number CT96-78) started in January 1997 and came to its administrative ending in December 2001. It united 17 partner institutes in nine western European states representing the geographic range of the EU (Fig. 3). It was based on an existing core of *ex situ* collections in several countries and complemented them with material originating from other EU countries where conservation actions had not yet begun. It was built upon a diverse group of scientists (pathologists, geneticists) and foresters, each of whom provided expertise and tested

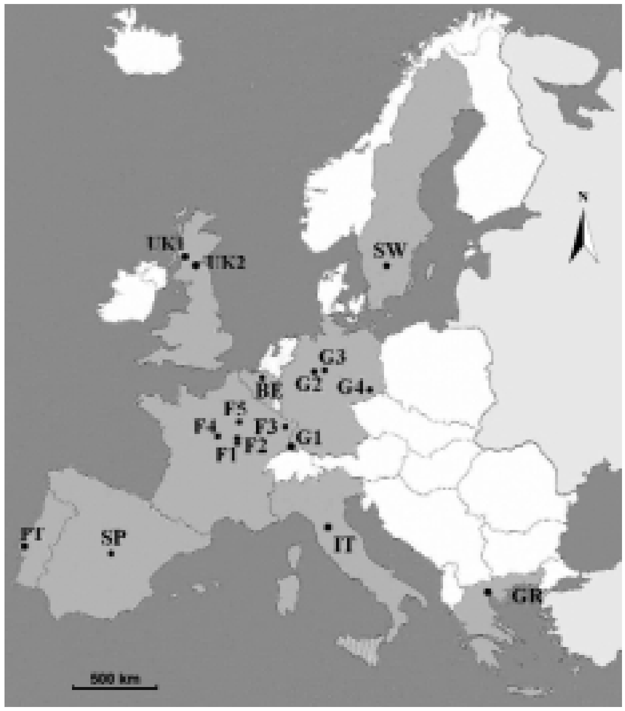

Figure 3. Institutes participating in the RESGEN 78 EU project. SW: SKS (Skogsstyrelsen); UK1: University of Glasgow; UK2: Royal Botanic Garden Edinburgh; G1: FVA (Forstliche. Versurchsanstalt Baden-Württemberg); G2: NFV (Niedersächsische. Forstliche. Versuchsanstalt); G3: FIV (Hessen-Forst, Forsteinrichtung, Information); G4: LAF (Sächsische Landesanstalt für Forsten); BE: IBW (Instituut voor Bosbouw en Wildbeheer); F1: Cemagref; F2: ENGREF; F3: INRA Nancy; F4: ONF Conservatoire Génétique (Orléans); F5: AFOCEL Nangis; GR: FRI (Forest Research Institute); IT: CNR (Consiglio Nazionale delle Ricerche); SP: UPM (Universitad Politecnica de Madrid); PT: EFN (Estacao Florestal Nacional).

methodologies in the different research fields. The project enabled the satisfactory completion of the six following tasks.

1. A **common database** was built to list and describe the ca. 2,000 clones held by project participants; it proved particularly helpful for the selection of the priority-conservation clones to exchange between partners and conserve in different places.

2. The **molecular characterisation** of a large subsample of the total collection was carried out using nuclear DNA markers (RAPDs and ISSRs on over 500 clones) and chloroplast DNA markers (PCR-RFLPs on over 700 clones); this permitted to clarify the taxonomy of elms (and particularly the status of narrow varieties of *U. minor*), to assess the extent of hybridisation, and to gain information on the routes followed by the elms when recolonising Europe after the Ice-Age.

3. The **evaluation for desirable traits** was facilitated by sharing expertise and adopting common protocols for experimentations and notations; a strong variability in bud burst period (see Santini *et al*, in this volume) and an interesting variability in tolerance to DED were found; knowledge on elm attractiveness to bark beetles was increased.

4. The **rationalisation of the European elm collection** was achieved through the selection of the priority-conservation clones to conserve in a restricted «core-collection» and the identification of geographic zones where complementary sampling was urgently needed; criteria for core-collection were taxonomy, geography, ecology and cpDNA diversity; genetic diversity and adaptive traits were not sufficiently taken into consideration due to the short duration of the project.

5. The **long term conservation** of the 850 core-collection clones was ensured by their duplication for conservation in low hedges (unattractive for the bark beetles) at two or more different Institutes, and by the cryo-preservation of buds of a 444 clone subsample in liquid nitrogen.

6. The **dissemination of project results** is being carried out to a variety of audiences: scientists, professional foresters and arborists, and the general public. Outputs are expected for amenity planting, afforestation and the reconstruction of hedges. Because it will provide methodological support for the implementation of the «Noble Hardwoods» network's strategy, the project will also contribute to the identification and sustainable conservation of valuable elm genetic resources throughout Europe.

However, the rationalisation and complementation of the core collection must be continued.

The EUFORGEN «Noble Hardwoods» network

The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilisation of forest genetic resources in Europe. It was established in 1994 to implement Resolution 2 of the Strasbourg Ministerial Conference on the Protection of Forests in Europe. EUFORGEN is financed by the participating countries and is co-ordinated by the International Plant Genetic Resources Institute (IPGRI) in collaboration with the Forestry Department of FAO. It facilitates the dissemination of information and various collaborative initiatives. The Programme operates through networks in which forest geneticists and other forestry specialists work together to analyse needs, exchange experiences and develop conservation objectives and methods for selected species. The networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from participating countries carry out an agreed work-plan with their own resources as «in-kind» contributions to the Programme.

As a result of the needs identified by 30 European countries, *Ulmus* is one of the genera included in the scope of activities of the «Noble Hardwoods» network. The gene conservation strategy for the European elm species (Collin, 2002) aims at creating good conditions for the future adaptation of elm species in a changing environment. It recommends the dynamic management of gene resources in order to maximize the genetic diversity among the conservation populations. This can be achieved by appropriate sampling methods, intensive management of *ex situ* populations of diverse origins and the identification of many small *in situ* conservation stands representing a broad array of site conditions (Eriksson *et al.*, 1993; Eriksson, 1996) and also populations in which hybridization is known to occur naturally. The strategy developed by the network provides guidance for the further development of national activities and encourages measures to be taken in each country.

Present status of elm conservation in Euforgen participating countries

In order to have a general overview on the status of elm genetic resources in Europe, a questionnaire was sent to the members of the Euforgen «Noble Hardwoods» network. Based on information available from 25 countries, we can conclude that elms have been given special attention. Only eight countries reported that there have been no inventories covering elms whereas 11 countries have included elms in national forest inventories and six countries have carried out special inventories on one or more elm species, sometimes focusing on an important or problematic region within the country. Almost all countries identify DED as a threat to elms and about half of the answers mention loss of habitats or change in land use. Species hybridisation seems to be a concern only regionally (*U. minor* vs. *U. pumila* L.). There is a lot of research on elms going on, partly within the RESGEN 78 EU project, but also as national projects, which include both field trials and molecular or isozyme studies. National gene conservation programmes vary from strict species level protection by law to extensive conservation of genotypes. As a preliminary measure, some countries have integrated genetic conservation with nature reserves. More dynamic *in situ* methods include establishment of gene reserve forests, which is applicable where the species form big enough stands, and guidelines for silvicultural management in regions where species have silvicultural value. *Ex situ* conservation is applied in several countries, either through seed orchards or through collections which are established to conservation. As an illustration of different approaches for elm gene conservation it is interesting to compare the status of *Ulmus laevis* in the centre of its distribution, in Hungary, and in the northern margin of distribution in Finland.

Status of U. laevis *in Hungary*

All the native European elm species can be found in Hungary, and *U. laevis* is probably the least threatened of them. Although its net distribution area is only about 530 ha, it is abundant along the major rivers, commonly found in plains and hills and absent only from the sub-alpine regions. Biotic stress including DED and competition with other tree species is very low for *U. laevis* if compared to the other native elms. However, *U. laevis* suffers from the change in land use and loss of suitable habitats. One of the main factors is widespread cultivation of hybrid poplars along the rivers. Inventories at a species level started in 1991 and reliable detailed distribution maps are available as a basis for conservation (Fig. 4). The total distribution area of elms in Hungary is about 2,000 ha which is about 0.1% of the total forest land (1.69 million ha). Comparison with a new inventory made in 2001 provides also information on the change in net distribution area (Table 1). In the period 1991-2001, there has been a 17 % decrease for *U. glabra*, a slight increase for *U. minor* (+13%) and a notable increase (+52%) for *U. laevis*. The guidelines for elm conservation have been set in 1997 and, because of the low number and limited size of eligible elm stands for *in situ* conservation, *ex situ* measures were prioritised. However, elms will be jointly conserved with other native species in larger stands appropriate for *in situ* conservation. Owing to its fairly good status compared with

Figure 4. Distribution of *Ulmus laevis* in Hungary. Adapted from Bartha and Matyas, 1995.

Table 1. Changes in net distribution area of elms in Hungary (ha) (State Forest Service, 2002). The total forest area is 1.69 million ha

	U. minor	*U. glabra*	*U. laevis*
1991	1,430.1	91.7	352.7
2001	1,614.0	76.5	534.9

other elm species, *U. laevis* has not been given high priority in the conservation programme, but endangered population fragments are subject to *ex situ* conservation.

Status of U. laevis in Finland

The flora of Finland includes *U. glabra* and *U. laevis*. The second species is considered to be more endangered because of its special requirement for riparian habitats. About 3,000 trees grow along a lake and river system in southern Finland (Fig. 5), distributed within an area of 20×100 km. In addition, two populations and some individual trees are found outside this main area. The populations are threatened by habitat change, as shores are no longer flooded regularly due to drying and water level regulation. As a consequence, Norway spruce may compete elms out. The longevity of the species, however, may act as a buffer against short-term changes. The genetic structure of 13 Finnish and of one Estonian *U. laevis* populations has been studied with allozymes using 15 enzyme systems (20 loci) (P. Vakkari, M. Rusanen, L. Yrjänä, unpublished). Finnish populations of *U. laevis* have low genetic diversity (H_e) compared with estimates of Finnish *Quercus robur*, North-European *Acer platanoides* and Scandinavian *Betula pendula*. Also, population differentiation (F_{st}) in the elm stands is highest in this comparison. These observations are putatively explained by the ecological characteristics of *U. laevis*, which is a 'specialist' and the most uncommon of these species. The distribution of variability within *U. laevis* presents a challenge for gene conservation. The level of genetic diversity varies notably from one stand to another, the range in expected heterozygosity being from 0.054 to 0.140. Thus, successful targeting of conservation activities is not possible if only ecological survey data is available. *U. laevis* in Finland is protected by law as an endangered species (Nature Conservation Act). However, the protection of a species does not extend to the

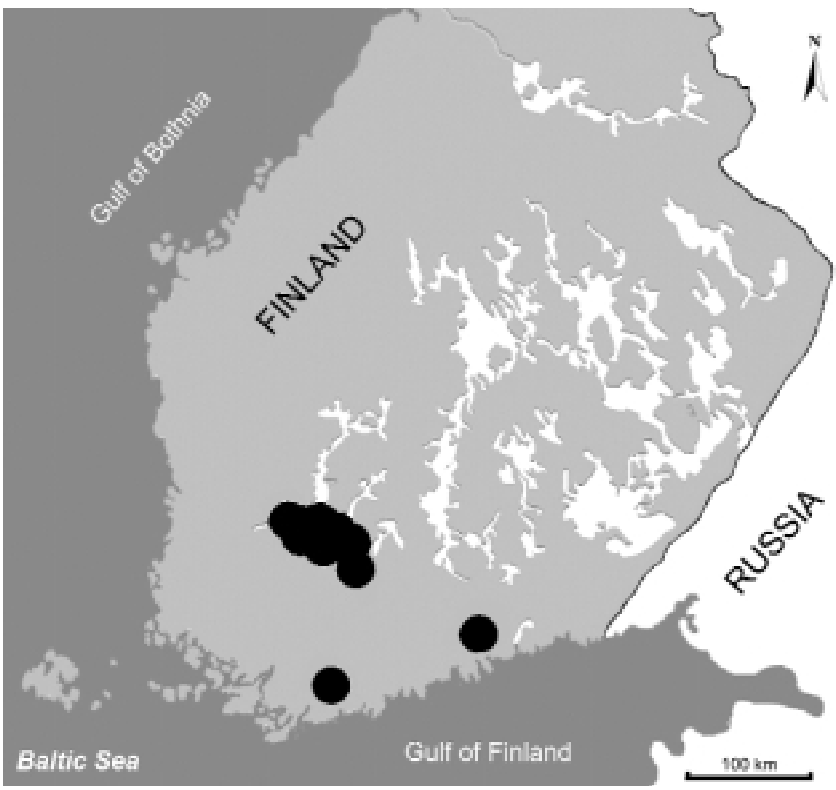

Figure 5. Distribution of *Ulmus laevis* in Finland. Adapted from Vakkari *et al.*, 2002.

habitats and even today stands along urban areas may be destroyed by construction of houses, roads and power lines. In natural forest, however, the habitats are protected. Forest Act lists certain special environments, where forest management is not allowed to alter the particular characteristics of that area. Natural stands of elms are given the protected status. In addition, some elm stands are part of larger nature conservation areas, which give them complete protection. However, full protection is not necessarily the best strategy for genetic conservation, which often needs active measures to promote regeneration and to guarantee wide genetic variability. In addition to *in situ* protection, the dynamic gene conservation strategy adopted for *U. laevis* is to establish *ex situ* graft collections. The collections are designed to serve both conservation and seed production. Presently, the collection includes 83 clones from 19 stands. This will be replicated in due course and planted on separate locations as a precaution against an unpredictable major threat.

Conclusions and perspectives

Static conservation methods can be applied to safeguard remarkable genotypes, whereas dynamic methods are needed to safeguard the potential for adaptation of the elm species in a changing environment. The static conservation of elms is generally carried out in field clonal banks where observations and collection of plant material can easily be performed, but the cryopreservation of dormant buds can be used for long term preservation. Dynamic conservation must be implemented in a network of natural conservation populations covering the ecological range of the species, and possibly complemented with artificial populations such as reconstructed populations (in a natural environment) and conservation seed-orchards (on cultivated land). In addition, it is important to raise awareness of elm genetic resources conservation among forest managers, and to incorporate elm dynamic genetic conservation methods into habitat and biodiversity conservation approaches. Similarly, possibilities for joint *in situ* conservation with other species (e.g. *Populus nigra*, *Alnus sp.*, *Fraxinus sp.*, *Acer sp.*) should be encouraged.

The E.U. project «Conservation of Elm Genetic Resources» and the EUFORGEN «Noble Hardwoods» network have prompted significant progress in the conservation of elm genetic resources at the European scale in the recent years. However, co-ordination is still needed for research (e.g. to develop molecular markers linked with adaptive traits) and for the implementation of an *in situ* pan-European conservation network. In particular, the possibility for combining research and conservation in «Intensely Studied Plots» should be explored.

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