EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

Quercus suber Network

Report of the first two meetings
1-3 December 1994 and 26-27 February 1995
Rome, Italy

E. Frison, M.C. Varela and J. Turok
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The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization operating under the aegis of the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI works in partnership with other organizations, undertaking research, training and the provision of scientific and technical advice and information, and has a particularly strong programme link with the Food and Agriculture Organization of the United Nations. Financial support for the agreed research agenda of IPGRI is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, Germany, India, Italy, Japan, the Republic of Korea, the Netherlands, Norway, Spain, Sweden, Switzerland, the UK and the USA, and by the Asian Development Bank, IDRC, UNDP and the World Bank.

The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe. It was established to implement Resolution 2 of the Strasbourg Ministerial Conference on the Protection of Forests in Europe. EUFORGEN is financed by participating countries and is coordinated by 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 analyze 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 workplan with their own resources as inputs in kind to the Programme. EUFORGEN is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries.

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First Network Meeting

Introduction

The first meeting of the *Quercus suber* Network was held at IPGRI's Headquarters in Rome, Italy from 1 to 3 December 1994. It was attended by ten participants and observers from six countries.

Participants were welcomed to IPGRI by Mr D. van Sloten on behalf of the Director General, Dr G. Hawtin, who was unfortunately not present in Rome. Mr van Sloten stressed the importance of international collaboration in the area of plant genetic resources and explained the involvement of IPGRI in forest genetic resources. He introduced Dr A. Ouédraogo, who joined IPGRI as forest genetic resources specialist at the beginning of 1993. He wished the meeting a success and apologized for not being able to attend the meeting.

Participants were also welcomed on behalf of FAO by Dr O. Souvannavong who explained the close collaboration of FAO and IPGRI in the development of the European Forest Genetic Resources Programme (EUFORGEN). He also expressed his wish to see this meeting initiate a successful collaboration among the four European countries in which *Q. suber* is indigenous. He apologized for not being able to attend the entire meeting, because of earlier commitments.

Dr E. Frison gave an introduction to the European Forest Genetic Resources Programme and explained the involvement of IPGRI in the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) which served as a model for the development of EUFORGEN. Dr Frison stressed the fact that the success of the species networks depended to a large extent on the enthusiasm and dedication of the participants. He also announced that Mr Jozef Turok had been selected as coordinator for EUFORGEN and would start working at IPGRI in January 1995. This is expected to considerably strengthen the Programme.

Dr M. Malagnoux introduced the activities of *Silva Mediterranea* to the meeting and highlighted the possible collaboration between this FAO programme and the *Q. suber* Network. He stressed the importance of establishing a close collaboration with North African countries where *Q. suber* is also important.

The origin and the past activities of the *Q. suber* Network were presented by Dr M.C. Varela. She reported among other things on the preparatory meeting which was held in Lisbon, Portugal in July 1993.

Dr M.C. Varela was unanimously elected as chairperson of the *Q. suber* Network.

Report

Country presentations

In order to give an overview of the situation in the different countries, each participant made a presentation of the activities taking place in the respective countries. The presentations are included in this report.

Objectives

Following these presentations, the group discussed the objectives of the Network. It was agreed to formulate an overall goal, a medium-term objective and a number of immediate objectives.

The overall goal of the Network is:

The sustainable management and conservation of *Q. suber* genetic resources.

The medium-term objective of the Network is:

The development of concrete strategies and methodologies for the management and conservation of *Q. suber* genetic resouces and recommendations for their implementation by member countries.

To achieve this objective, the Network will work closely together to jointly identify priorities and develop and implement workplans. The Network will also foster collaboration between institutions within Europe as well as with countries in North Africa.

The immediate objectives of the Network are:

To compile and distribute relevant literature and information;

To make an inventory of *Q. suber* genetic resources;

To identify research needs and to develop the knowledge base required to develop sound conservation strategies;

To develop methodologies and strategies for the conservation and sustainable management of *Q. suber* genetic resources;

To raise the awareness of decision-makers of the threats to *Q. suber* diversity.

Workplan

The meeting discussed each immediate objective and agreed on a workplan to achieve the objective.

Objective 1 **To compile and distribute relevant literature and information**

The Network was informed that Prof. Sardinha, Forest Research Institute, Portugal, is developing a bibliography on *Q. suber* within the framework of *Silva Mediterranea*. **It was agreed that** all participants will send references to Ms Varela by end of February 1995, and regularly thereafter. Ms Varela will link with Prof. Sardinha on this task.

It was recommended that the compilation of references relevant to *Q. suber* genetic resources activities be published as an attachment to the report of the third meeting of the Network. A draft compilation will be sent to all Network members by 1 August 1995; they will return comments by 1 October for incorporation before the next meeting of the Network.

Objective 2 To make an inventory of *Q. suber* genetic resources

Descriptor list

The first priority in this area is the development of a common language for the inventories. **It was agreed that** Prof. Schirone would take the lead for the development of descriptors for *Q. suber* stands and for individual trees.

Network members will send examples of descriptors already used in inventories in their respective countries to Prof. Schirone by 31 January 1995. Prof. Muhs will send a copy of the descriptors for forest stands used in Germany by the same date. For individual tree descriptors, IPGRI descriptors for other tree species will be used as a starting point. Network members should send all other relevant information to Prof. Schirone by end of January 1995.

Prof. Schirone will send a first draft to Network members by 31 March 1995. Comments and suggestions should be returned to Prof. Schirone by 31 May 1995.

Survey of distribution of *Q. suber*

The Network recommended that a more precise survey of the distribution of *Q. suber* be compiled. This information is available for Portugal, Spain and France, and partially for Italy (Sardinia and Apulia).

Prof. Schirone will contact the relevant forest services in Italy before 31 December 1994 requesting that this task be performed as a matter of priority.

It was recommended that Prof. Schirone assemble the results of inventories from all countries in order to produce an updated map of distribution for the species.

Detailed inventory of endangered and marginal population of *Q. suber*

Detailed inventory data of endangered and marginal population are partially available from Portugal, Spain and Italy, but not from France.

It is recommended that a more detailed inventory be carried out in the respective countries in order to cover all endangered and marginal populations. In France, **it is recommended that** *Q. suber* be added to the list of priority species.

European database for *Q. suber*

It was recommended that a European database be established assembling data on the results of inventories and on *ex situ* collections (including provenance and progeny trials and clonal banks).

Ms Varela agreed to initiate the establishment of a database after the descriptor lists are developed.

A project proposal will be developed by Ms Varela for submission to the EC Programme on genetic resources by early 1996.

Objective 3 To identify research needs and to develop the knowledge base required to develop sound conservation strategies

Study of the structure of genetic diversity within and between populations

The study of genetic structures within and between *Q. suber* populations will require the development of genetic markers as tools and the testing of adaptive traits.

Genetic markers

It will be necessary to compare the different markers available from a cost/efficiency point of view. Links with projects working on other oak species will be established by Dr Lumaret and Prof. Schirone to exchange information and possibly material.

Testing for adaptive traits
This will be carried out by provenance trials and progeny trials. The production of plants for the trials should be carried out in such a way as to maximize their uniformity. The provenance trials should provide reliable data from adaptive traits such as survival, growth, phenology and from other important traits such as form, cork quality and genotype-environment interactions. The development of early tests for important traits should be undertaken to speed up the evaluation. Early testing for cork quality would be a major breakthrough.

Study of the inheritance of economically important traits
This is a major objective which has to be addressed by progeny trials.

Study on the reproductive system
The study of reproductive system will be important to understand the genetic organization within the cork oak, its mating behaviour and the fundamental evolutionary processes in the species. Geneflow mechanisms need to be studied to determine the minimum variation required in a population for effective conservation and avoiding genetic drift.

Study of the causes of threats on the diversity within *Q. suber*
A first step in the study of the causes of threats on the diversity in *Q. suber* will be to assemble information on what are the major perceived causes of genetic erosion in each country.

A short report will be sent by each Network member to Dr Gil by 30 April 1995 for compilation. A draft summary will be circulated by Dr Gil by 30 June 1995 for comments and finalized by 30 September 1995.

Study of cryopreservation techniques for *Q. suber*
The Network recognized that cryopreservation could provide complementary conservation methods for safety duplication of particularly threatened populations. **It is recommended that** progress obtained in this area with other species be monitored in order to review the feasibility of applying this technique for *Q. suber*.

In order to address the research needs identified by the Network, a number of project proposals will be prepared for submission to different EC Programmes.

A proposal for concerted action will be developed by Ms Varela in collaboration with Prof. Muhs to put in place *Q. suber* evaluation trials using a selected set of provenances, on different sites in all member countries. The proposal will be submitted by 31 March 1995. The project will include collecting of seed from the different provenances, storing the seed, raising the plants, shipping of the plants to the different sites and establishment of evaluation plots. It will also include the initation of a database to assemble the data on the provenances and the trial sites.

A comprehensive research proposal will be developed by Prof. Schirone covering research on the structure of genetic diversity, the inheritance of economically important traits and the reproductive system of *Q. suber*. This will involve partners in the four member countries, Germany and Sweden. The proposal will be submitted by the deadline of the call for proposals, 31 March 1995.

Objective 4 **The development of conservation methodologies and strategies**
The Network agreed that immediate action to conserve endangered populations in Italy and Spain should be taken.

In Italy, seed will be collected from endangered populations in Apulia by Prof. Schirone who will carry out studies on genetic diversity found in these populations using molecular markers. Data obtained from this characterization will be used to submit proposals for *in situ* conservation measures to the relevant authorities.

In Spain, Dr Gil will collect twigs from the several relict populations in Central Spain during January/February 1995 and send them to Dr Lumaret for biochemical and molecular analysis. The results of these studies will be circulated to all Network members by end of June 1995 for comments. If appropriate, IPGRI will, upon request from Dr Gil, send a letter to the relevant authorities in Spain recommending *in situ* protection measures.

The Network welcomes and supports the initiative of the Estacao Forestal Nacional in cooperation with the Forest Service to establish Multiple Population Breeding System (MPB) for gene conservation and improvement of the species. The results from this initiative make an important contribution to the Network.

Objective 5 **Raise the public awareness of decision-makers of the threats to *Q. suber* diversity**

The Network should make appropriate recommendations to the relevant authorities, based on scientific results, for the implementation of conservation measures.

The press can play an important role but it should only be used when sufficiently solid knowledge has been gained.

Regional authorities can be made aware of the possibilities of measures within the framework of EC regulation 2080/92.

Local, national and international NGOs, such as WWF which is already involved in actions devoted to cork-oak protection in Apulia, should be contacted to request collaboration for the protection of endangered populations.

General discussion points

A number of other points were discussed by the group and agreement was reached as follows.

- It was agreed that links will be established with the private industry by members of the Network in order to seek support for some activities.
- Each member will establish a group of persons dealing with *Q. suber* in her/his country.
- Regarding the scope of the Network, it was agreed that the Network would focus only on *Q. suber* but that close links will be established with initiatives working on other *Quercus* species.
- The members of the Network recommend that support be provided to North African countries to conserve their diversity of *Q. suber*.
- The members of the Network agreed to develop a proposal for collaboration with North African countries.
- It was agreed that a brief meeting should be organized at the beginning of 1995 to allow the members of the Network to work together on the development of two project proposals to be submitted to the Commission of the European Communities.

Agenda

First meeting, 1-3 December 1994

Opening session
- Welcome address (D. van Sloten, IPGRI)
- Welcome address (O. Souvannavong, FAO)
- Introduction to EUFORGEN (E. Frison, IPGRI)
- Presentation of the activities of *Silva Mediterranea* (M. Malagnoux, FAO)
- Previous activities of the *Q. suber* Network (M.C. Varela, Portugal)

Presentation of the situation in the different countries
- France (R. Lumaret)
- Italy (B. Schirone)
- Portugal (M.C. Varela)
- Spain (L. Gil)

Conservation methodologies

Proposals for conservation activities

Discussion of research needs

Inventory of *Q. suber* genetic resources in Europe and establishment of a European database

Proposals for international cooperation projects

Development of a workplan

Future of the Network and discussion on the possible extension of the Network to other European *Quercus* species

Final session: discussion and approval of the report

Second Network Meeting

Report

The second meeting of the *Quercus suber* Network was held at IPGRI's Headquarters in Rome, Italy from 26 to 27 February 1995. It was attended by five participants and observers: Drs Varela (Portugal), Lumaret (France), Gil (Spain), Schirone (Italy) and Muhs (Germany). This follow-up meeting was intended mainly to give Network members an opportunity to discuss joint project proposals before their submission.

The meeting was opened by Dr E. Frison who welcomed the participants on behalf of IPGRI. He introduced Mr J. Turok, the EUFORGEN coordinator recently appointed by IPGRI. Mr Turok expressed his desire to facilitate close collaboration and further activities within the Network. Proposed agenda of the meeting was then approved. The first agenda item concerned brief information on the implementation of the workplan objectives[1] since the last meeting. The second, main item focused on development of two project proposals to be submitted to the Commission of the European Communities.

Dr M. Malagnoux (FAO) presented the most recent initiatives towards the conservation of genetic resources in Mediterranean countries. His offer on behalf of the *Silva Mediterranea* Network to serve as a link with North African countries was welcomed by the participants.

Objective 1

Since the last meeting progress has been made in the compilation of a bibliography. Dr Gil provided references on Spanish gene conservation in cork oak and the search of relevant literature in Italy added 40 titles to the list. It was agreed that Prof. Muhs would carry out a search for literature published in international journals and circulate the results until 15 April 1995. The deadline for sending draft compilation by Dr Varela to all Network members (1 August 1995) was confirmed.

Objective 2

Prof. Schirone informed participants about the progress made in developing the list of descriptors. Examples of descriptors used for stands and trees in other forest species were exchanged among Network members. Prof. Schirone will produce the draft list of descriptors for cork oak by 31 March 1995. Assembling information for an updated map of distribution has been started, but the Italian and French data were still not available. It was recommended for the next Network meeting to discuss and approve schedule for the database establishment.

Objective 3

Regarding the identification of research needs and the development of knowledge base required to develop sound conservation strategies, the first cork oak material (acorns and leaves) was exchanged within the Network. Genetic studies using the material from four countries were carried out in France (Montpellier), Italy (Viterbo) and Sweden (Uppsala). Part of the accessions originated from endangered populations. According to the Network workplan, draft project proposals were prepared by Dr Varela and Prof. Schirone.

[1] The Network objectives were identified in the Workplan (see p. 2).

The first of the two project proposals discussed at this meeting was a concerted action initiative to put in place *Q. suber* evaluation trials using a selected set of provenances on different sites in all countries. Under coordination from Portugal, the proposal includes the establishment of field trials using a broad range of genetic material from all the regions within cork oak distribution. Data generated will be assembled to initiate a database concerning the provenances and the trial sites. The session was chaired by Dr Varela from Portugal.

The second proposal covers research initiatives on the structure of diversity of *Q. suber* populations, since genetic knowledge of this species is both scarce and dispersed. This research proposal aims at assessing the genetic diversity in the species by use of biochemical and molecular markers. Traits of economic importance will also be studied, including phenology, drought resistance, photosynthetic efficiency and cork quality and a better knowledge of the reproductive processes of cork oak is also required to enable successful conservation. Prof. Schirone led the discussion on this second project proposal.

Participants approved both project proposals and agreed that the coordinators, Dr Varela and Prof. Schirone will send documents by deadline (31 March 1995) to the Commission of European Communities.

Objective 4

As was agreed at the last Network meeting, initial steps to conserve endangered populations in Italy and Spain were taken. The provision of areas for comparative studies in Apulia and Latium was assured by Italian authorities. Dr Gil collected twigs in several relict populations in Central Spain and sent them to Dr Lumaret for analysis. The meeting recommended circulating the results of these studies for comments within the Network by 30 June 1995.

Objective 5

WWF Italy was contacted by Prof. Schirone requesting collaboration for the protection of a relict stand of *Q. suber* in Apulia. No other activities on raising the public awareness of decision-makers about the threats to the species' diversity were reported.

Presentations

Conservation of genetic resources of *Quercus suber* in Portugal

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Quercus suber is a native forest tree species of significant economic importance to Portugal. It covers about 660 000 ha, which corresponds to 175 000 tons of cork. The value of the export of cork amounted to approximately US$ 200 million in 1990. These values give Portugal the world top position in cork production.

However, the species faces several threats, the main one being human impacts. Agroforestry with extremely low natural regeneration, substitution by faster-growing tree species and urban development are the major causes. All these conditions led to a considerable reduction of the total cork oak area. Extinction of some marginal populations of unique value certainly occurred, as can be inferred by historical documentation and toponymic references. *Quercus suber*, together with other species of the same genus, dominated the native forests in Portugal centuries ago.

Periodical surveys during the last 40 years did not show a decline of the total area, but rather significant reductions in stocking and marginal populations took place. Stands are often understocked with age distribution skewed to older age classes. Lack of juvenile stories is not due to intrinsic regeneration problems in the species but to overexploitation under agroforestry methods.

In the last two decades, the species has also shown an increased level of mortality with special incidence for stands of economical importance within the core of the geographical distribution of the species. Although Portugal is a small country, a strong environmental variation occurs to such an extent that *Q. suber* finds here the natural conditions close to its extreme limits.

In consequence of climatic oscillations originating in cooling due to glaciations in Europe and xerothermic interglacial periods, the species migrated. Some of the marginal populations are representative of such movements, revealing that the species has good abilities to cope with environmental changes.

Under the impulse of Resolution S2, an implementation of a national programme for conservation of genetic resources of *Q. suber* has been started. The concepts and selection procedures of this programme are developed according to principles and methods set out in Varela and Eriksson (1995).

The prime objective of the Portuguese programme in gene conservation of *Q. suber* is **the creation of good opportunities for future evolution** as the means to promote adaptability of the species upon environmental changes of unknown direction.

The strategy for gene conservation is strongly shaped by the absence of genetic knowledge. Together with financial scarcity these are constraints common to gene conservation of many species. Since the perspective of financial support for sophisticated breeding has not been achieved, a simple breeding methodology to be included in the gene conservation of *Q. suber* in Portugal is proposed in order to improve the amount of production of good cork. Besides, conservation of other species dependent on *Q. suber* was considered during sampling of the gene resource populations (GRPs). Genetically unique populations which may be endangered for various reasons were also included.

Methods

Small populations

Basics of population genetics state that the response to selection will be faster in a set of small populations than in one large population. The disadvantage of inbreeding in small populations was considered by restricting a population to at least 50 individuals.

Capture of environmental diversity

It is probable that natural selection has caused genetic differentiation of populations under different environmental conditions. Even if this adaptation is far from a maximum in fitness it is useful to exploit the adaptedness in gene conservation.

Classification criterion

The classification is in line with the need to identify functions of different gene resource populations (see Table 1).

Table 1. Methods for gene conservation based on the classification of gene resource populations with respect to population number, habitat diversity and genetic variation in the target species

Method	No.	Popula-tion(s)	Habitat diversity	Genetic variation: Kept	Genetic variation: Improved
Multiple populations					
MPBS[†], intentionally sampled to capture existing adaptation:					
Planted and managed	1	=20	+	+	++
Natural, with management	2	=20	+	+	+
Natural, without management	3	=20	+	+	
Provenance and progeny trials	4	few, large	+	- to +	
Seedling seed orchard	5	few, large	+	- to +	
Clonal archive, clonal seed orchard	6	few, large	-	- to +	
Seed bank	7	large	-	+	
Pollen bank	8	large	-	+	
Tissue culture bank	9	large	-	+	
Single populations					
Large natural populations managed to create maximum habitat diversity *	10	1	+	-	
Unmanaged natural population *	11	1	-	-	
Botanical gardens *	12	1	-	---	

† MPBS = Multiple Population Breeding System.

* Only potentials for improvement are indicated.

Sampling

Ecogeographic gradients were assumed to be one of the principal factors of genetic differentiation among populations. Climatic variables and soil types received special attention as criteria for the selection of stands for gene conservation. Summer drought is believed to be a key climatic variable. To present a neutral characterization of the GRPs selected, climatic and ecological references are shown in Table 2.

Management and isolation were also considered as important factors for genetic variability. Considerable variation of the species exists among populations receiving 500 to 2400 mm of annual rainfall. Minimum and maximum temperatures also result in wide variation in the populations, as does the combination of both factors. Soil variability is also a major aspect of the settlement of the species in Portugal.

Table 2. Climatic characterization of *Quercus suber* GRPs in Portugal

Meteorological point	P (mm)	M (°C)	N (°C)	Q_2	Ecological zone	Method
1 Gerês - Sobreiral da Ermida	2407.9	22.0[b]	2[b]	422.1	SA x AM	10
2 Sª de Bornes	1009.4	28.0[b]	2[b]	134.7	SA x SM	10
3 Alcobaça - MN Mestras	945.0	25.8	4.8	156.0	AM	2
4 MN Cabeção	640.4	31.0	4.4	82.8	SM	2
5 Mª Queluz	767.6	27.6	6.6	118.2	AM	11
6 Qª Serra	764.4	29.0	5.6	109.8	AM	2
7 Sª Arrábida	764.4	29.0	5.6	109.8	AM	2
8 H. Loureiro	719.4	26.5	7.2	128.5	AM x SM	2
9 Sob. do Tio Sales-Contenda	729.5	31.8	6.1	97.2	IM x SM	2
10 M.N. Conceição - Tavira	515.1	31.0	8.3	77.5	M	1
11 H da Parra-Silves	1076.9	30.5	7.5	160.0	SM	2

P = annual rainfall in millimeters; M (°C) = mean of the maximum temperatures of the hottest month; N (°C) = mean of the minimum temperature of the coldest month; Q_2 = climatic index of Emberger; ecological zones from Carta Ecológica de Portugal; [b] = estimations from the nearest meteorological point under the correction factor of 0.3°C/100 m.

Perspectives

The Portuguese programme for gene conservation of *Q. suber* has accomplished the initial phase of prospecting and selection of populations. Implementation of the programme under the methodologies proposed (Fig. 1) for each of the populations is essential to achieve results. In cooperation with the Forest Service, studies aiming at definition of regions of provenance and provenance trials will be launched during 1995. It is an important step for the knowledge of the genetic architecture of species and essential support for breeding and gene conservation activities.

Marginal gene resource populations need special attention. Some are on the threshold of extinction due to overgrazing. At the current stage lack of appropriate financial support is in general the main constraint to the implementation of the Portuguese programme of gene conservation of the species.

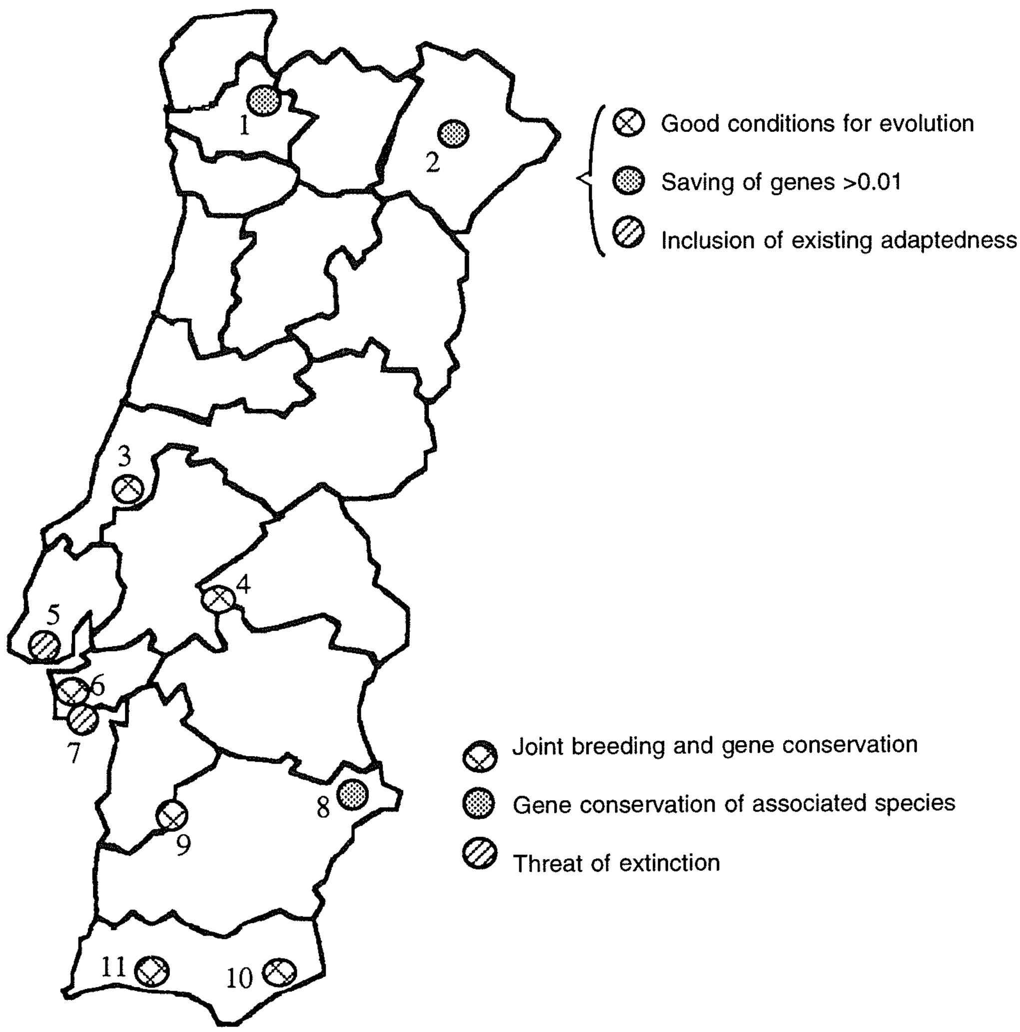

Fig. 1. Gene resource populations (GRP) in Portugal: location and main objective

Research needs

The past decade has shown severe patterns of drought which are considered one of the reasons for the unusual mortality of the species in some zones. Climatic studies to evaluate if it is a random oscillation within the pattern of the climate in southern Portugal or if it is a deterministic change included in global changes are urgently needed. Owing to the high economical and ecological value of cork oak for Portugal, conservation of genetic resources should be, as soon as possible, part of an intensive joint breeding and gene conservation programme under the *ex situ* Multiple Population Breeding

Strategy. This is the methodology that rationalizes the use of genetic variability under the financial constraints to support long-term sustained breeding and gene conservation.

A sound gene conservation strategy must rely on the knowledge of the genetic architecture of the species to conserve. It has repeatedly been shown that adaptive traits and markers (isoenzymes, DNA fragments) result in different architecture. Therefore, there is an urgent need to initiate studies on the genetic architecture of adaptive traits in *Q. suber*. Of primary concern is to start research on traits of adaptive significance, of which drought tolerance should be given priority.

The studies ought to be designed as long term combined among- and within-population studies with trials located along an ecological gradient. Studies of the mating system must be carried out to be able to improve the acorn production of superior trees in the future breeding programme. This is especially important for breeding that has to rely on acorn production in any form of seed orchards.

Artificial reforestation technique is needed for any intensive form of breeding. Therefore, studies covering the path from identification of phenotypically superior trees to planting must be continued. Knowledge of geneflow mechanisms is also absent for *Q. suber*. As an important role can be played by these capabilities in the success of the species in facing environmental uncertainties, studies for their comprehension should be performed.

Conclusions

For Portugal we have identified several objectives for gene conservation of *Q. suber*, besides the overall one of saving all genes at a frequency of 0.01 and higher. We have also included a breeding objective to improve the production of good cork. Saving unique populations from extinction is also an objective. Of great concern is also the gene conservation of associated species. Finally, preparation for a possible rapid change of climatic conditions is another objective. There is no single method that can match all these inter-related objectives. This conservation programme has been designed for Portugal but can easily be extended to other countries in which this species grows. If this is done, the gene conservation of this species will be well taken care of.

Reference

Varela, M.C. and G. Eriksson. 1995. Multipurpose gene conservation in *Q. suber* - a Portuguese example. Silvae Genetic 44:28-37.

Present state of *Quercus suber* in Spain: proposals for the conservation of marginal populations

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Considerations on the distribution of *Quercus suber* in Spain

The world area of *Q. suber* can be divided into two parts: one is formed by the Atlantic sides of the Iberian peninsula and Morocco, with the best stands of the species, and the other is the central Mediterranean Sea, and covers Catalonia, Southern France, Italy and the Tyrrhenian islands. This disjunction is due to Quaternary climatic changes, which divided the former Tertiary area of *Q. suber*.

Cork oak stands in Spain belong to both areas (Fig. 1). We can distinguish a northeastern centre in Catalonia, and another one in the western Duero basin, Extremadura and Western Andalucía. The environmental characteristics of these territories are rather uniform because of the precise ecological requirements of the species. *Quercus suber* grows almost exclusively in acid soils, and its occurrence on limestones is limited to leached soils under humid climates. Cork oak needs a high rainfall compared with other sclerophyllous oak species and it is also sensitive to cold winters and late frosts. It appears only scarcely in continental areas. With these restraints, *Q. suber* manifests in Spain its greatest ecological variability, occupying oceanic, arid, continental and mountain climates, as well as a wide variety of soils.

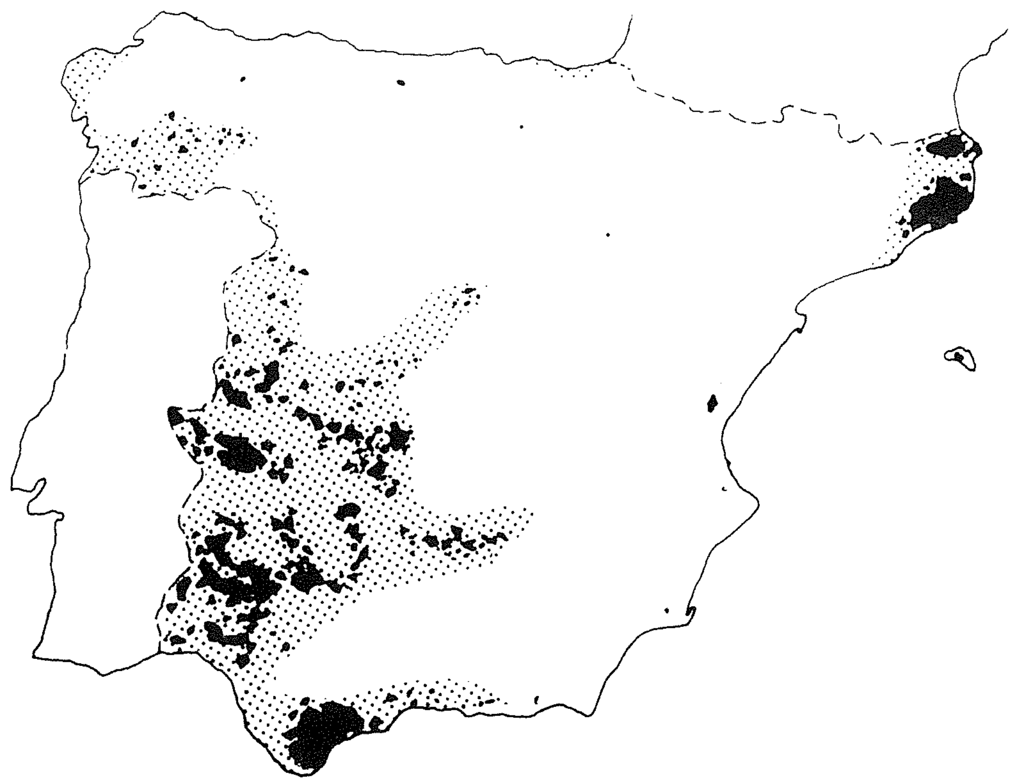

Fig. 1. Range of distribution of *Quercus suber* in Spain (main stands in solid black)

The distribution of *Q. suber* cannot be fully explained without considering the effects of human action on the species. This has defined not only the limits of its present area but also the structure and dynamics of its populations. The interest of cork oak for man has undergone important changes over time. Before the industrial use of cork in the late 18th century, *Q. suber* was a rather damaged species. The low value of its acorns compared with *Q. ilex* caused the latter to be favoured in mixed populations, to the detriment of cork oak. The scarce vigour of *Q. suber* coppices and its inability to produce root suckers also contributed to the recession of *Q. suber* faced with other *Quercus* species more resistant to the usual disturbances (grazing, firewood extraction, recurrent fires, etc.). Cork was used in a unorganized way; its extraction was made without respecting rotation, diameters or heights, since there was no need to keep the tree alive. As a consequence of such exploitation, a great number of cork oaks died, as happened in Valencia (García-Fayos 1991). The extraction of tanning barks was an even more damaging activity, being specially important in some times and regions; in Cádiz, for example, 1 300 000 trees were felled in the second half of the 19th century to sell their barks and firewood (Cerón 1879).

The situation changed with the rise of the cork industry. Mainly in Catalonia, where this industry was initiated, there was a period in which cork oak was promoted. *Quercus suber* plantations in farmlands were frequent, especially in former vineyards affected by *Phylloxera* (Zeller 1958; Vilar *et al.* 1992). During the 20th century this recovery continued, as is illustrated in the case of El Robledal and La Sauceda in Cortes de la Frontera (Málaga) (Montero *et al.* 1992). In 1894, when these forests began to be managed, they were described as seriously damaged and aged woods; in 1914, the forested area rose from 2054.6 ha to 3800.9 ha, and, in the last revision of 1990, the number of trees was nine times more in El Robledal, and five times more in La Sauceda.

The alienation processes in the 19th century were of crucial importance in the history of cork oak forests, since most of them passed into private hands. Private property looks for short-term returns, determining different management in each region. In Catalonia, certain circumstances meant that the greatest value of *Q. suber* forests is due to cork extraction, so they remain as dense and closed woods. In southwestern Spain, on the other hand, grazing and farming are the more profitable activities, cork extraction being only a complement. In this case, private owners do not promote the forest, but the pasture with cork oaks. The main problem of this management is the limitation of natural regeneration. In the forests, the shrublike species protect seedlings but when the scrubs are eliminated in pasture lands, the number of germinated acorns decreases and a great number of seedlings die because of predator attacks, frosts and droughts (Montero 1987). In the management of *Q. suber* "dehesas" (savanna-type forests) extreme prunings are usual, in order to improve fructification and to produce leaf fodder for the cattle when the pasture is faded. These activities cause weakening of the tree, so it is more easily affected by pests and diseases (Montero and Curras 1991). The mortality of cork oaks observed in recent years is higher in previously disturbed forests (Montoya 1992; Fernández and Montero 1993).

Finally, human action has led to an important loss of variability in cork oak forests, since it has only allowed survival of those trees capable of resisting the alteration of its natural conditions. Most of the current *Q. suber* forests are open woods, with numerous damaged trees; usually conditions are far from the optimum. Populations are aged and with regeneration problems, not only because of present uses but also because of their past management. Nevertheless, in recent years, some actions aimed at the recovery of stand variability have been carried out in several sites; either afforestation, or favouring of natural regeneration have given good results. This kind of programme should be generally applied in order to reverse the degradation trend of wide forest areas.

Isolated populations, their status and importance

Apart from the two main areas of *Q. suber* mentioned, there are in Spain isolated stands of the species, forming several small-sized populations. They usually survive in hostile regional contexts, protected by local environmental conditions. The number and dispersion of these stands are explained by the changes in *Q. suber* area correlated with Holocene climatic variations. During the Atlantic period (8000-4000 BP), the warm and humid conditions permited its expansion, as shown by different paleopolinic works (Pons and Reille 1988; Mateus and Queiroz 1993). The subsequent climatic worsening was the reason for its regression, linked to the human action in recent times.

These marginal populations grow under environmental restraints and are isolated from the main stands. The lasting and efficient isolation, selection pressures and small size could have determined their differentation, owing to the increased genetic drift. For example, adaptation to cold climates could have caused the occurrence of biennial cycles in fruit-setting (Elena-Roselló *et al.* 1993).

Conditions that could have marked more strongly the genetic diferentiation of the populations are the extreme climatic features (length of dry season, frost period) as well as edaphic characteristics (calcareous soils, swamping). It is difficult to estimate the area covered by these spots, since often *Q. suber* is not the main species; frequently they are mixed stands or scattered trees included in other types of forests.

The cork oak stands can be grouped according to the characteristics of their territories.

Cantabrian populations

They are the northernmost stands in Spain. They enjoy climates with very short summer drought. *Quercus suber* is rarely the main species, being more frequently isolated trees. Usually it is mixed with *Q. ilex*, forming mediterranean forests in dry sites. Three areas can be distinguished:

Galicia-El Bierzo

Small stands, mainly in the river Sil valley and its tributaries, where it appears together with other Mediterranean species (*Q. ilex, Q. pyrenaica, Pinus pinaster, Arbutus unedo*). There are also scattered trees in the most thermic of *Q. robur* forests. They grow on granitic lands, with steep slopes and little-evolved rocky soils. The main stands cover about 200 ha.

Valley of the river Navia

In the borders of Asturias and Galicia some groups of cork oak still remain, being more frequent in the valley of the river Navia. The warm climate of this site favours the occurrence of the species, which occupies the driest points where the regional Atlantic flora cannot grow. Cork oak seems to have been more abundant in the past, being replaced by vineyards. Nowadays, it remains in slopes and rocky soils, unsuitable for farming. *Quercus suber* forms mixed groves with *Castanea sativa, Q. robur* and, to a lesser extent, with *Q. pyrenaica* and *Q. ilex*. The species occupies 11 ha, although this area is probably higher, because of the frequent isolated trees which are hardly quantifiable.

La Liebana

This valley has a particular climate that allows the existence of Mediterranean vegetation in an Atlantic region. Together with *Q. suber, Q. ilex* and other termophyllous species appear. Probably cork oak forests were more extense, being displaced by pastures and farming. The area covered is 500 ha.

Sierra de Besantes

In the Upper Ebro valley there is a population with rather strange features, the oddest one being the dolomitic soils very unusual for the species. In the best sites *Q. suber* is mixed with *P. sylvestris, P. pinaster, Juniperus* spp. and other *Quercus*. About 5000 trees remain, in no more than 100 ha.

Continental populations

They occur in very contrasting temperatures. The long winters, minimum temperatures and the frequency of late frosts are the most adverse factors for the survival of the species. This forms little stands in humid and sunny slopes, together with some pines, *Juniperus* and other *Quercus*. Four populations are included:

Sayago-Tierra del Vino

The main forests occur in the Duero valley, near Portugal, and in the border between Zamora and Salamanca provinces. The populations in the frontier with Portugal are small groves in very warm slopes and canyons, while the rest are usually "dehesas" in the plain. Cork oak is mixed with *Q. ilex, Q. faginea* and *Q. pyrenaica*, sometimes with *Pinus pinea*. This population covers 450 ha.

Tiétar valley

In the southern side of Sierra de Gredos and in the valley of the river Tiétar the occurrence of stands and scattered trees of *Q. suber* is frequent. They appear in *Q. ilex* or *P. pinaster* forests. The figure given for this population is 680 ha.

Sierra de Guadarrama

It is the easternmost population in the river Tajo basin. There are small stands or dispersed trees in the southern side of Sierra de Guadarrama (Madrid). They grow on granitic and sandy soils, in sunny expositions with an extra water supply. Cork oak appears in *Q. ilex, P. pinea* or *P. pinaster* forests. The area covered is estimated at 19 ha.

Moncayo

In acid soils of Sistema Iberico mountains (Aragón) grows a small population of *Q. suber*, mixed with *Q. ilex* and with other oaks (*Q. faginea, Q. pyrenaica*). Cork oak is also mentioned in areas close by, in holm oak forests. These stands occupy about 200 ha.

Populations in coastal Mediterranean mountains

They occur on acid soils, within a region dominated by limestones. They survive in some of the driest climates of the peninsula, refuged in points with an extra moisture source (mists, orographic rains). Usually they form mixed woods with pines and holm oaks. Three populations have been found in this situation:

Alpujarras

In the south of Sierra Nevada (Granada). A couple of stands and some scattered trees probably represent the rest of a former Andalucian population that could be joined with the forests of Málaga and Cádiz. Nowadays, cork oak remains in warm and very humid areas because of the sea mists, but their soil is seriously eroded. *Quercus suber* has regeneration problems and *Q. ilex* is displacing it. The woods dominated by *Q. suber* cover 185 ha.

Sierra de Carrascoy

In the mountains of Murcia there are some small stands of *Q. suber*, very isolated by the aridity of the region and by the scarcely suitable soils. They appear linked to outcrops of quartzites and to microclimatic conditions (valley bottoms). *Quercus suber* is mixed with *Pinus halepensis* and termophyllous shrubs.

Sierra de Espadán

It is a population in the Castellón province, linked to triasic sandstones. It is formed by open woods of *P. pinaster* and, sometimes, *Q. ilex*. These cork oak forests are similar to those from Cataluña, although they are richer in termophyllous species. They are seriously damaged by forest fires, brush-clearing and inadequate management. It is the biggest of this type of marginal populations, with 5000 ha.

Quercus suber appears in other sites as scattered trees or very small groves. In spite of their reduced size, they are also of great interest as a sample of the species variability and as remnants of former areas of distribution. Some of these minor locations are: Sierra del Teleno (León), Aliste (Zamora), Basque country, Menorca, Pinet (Valencia), Sierra de Filabres (Almería) or Sierra Nevada.

All these isolated populations have similar problems for their conservation. The main one is posed by their size, which causes a greater sensibility to disasters such as fires, cuttings, etc. The problem is worsened by the restrictive environment which restrains their recovery ability. The low economic value of cork in these forests makes their conservation not profitable for private owners. Endogamy can also become a problem, mainly in the populations with a low number of oaks, or in those formed by scattered trees.

Gene conservation in Spain: present state and proposals

The first step in any conservation programme must be the study of the ecogeographic variation of the species, which will allow definition of ecologically uniform areas with presumably genetically similar populations. In this way, provenance regions of *Q. suber* in Spain have been defined (Fig. 2). Nine regions have been distinguished, seven of them in the southwestern area, and the other two in Catalonia. The marginal and isolated populations already mentioned have been classified as 'small area provenances', in order to distinguish them from the regions of the main area. It is intended to point out the peculiarity and importance of these forests, especially from the point of view of genetic resources conservation. Investigations of genetic structure represent, together with the ecogeographic study, an essential means for evaluating the current variability, and subsequently, to promote its conservation and guide the actions aimed to increase the diversity.

Some current programmes will allow determination of the variation between provenances. In 1993 a provenance test was established in La Almoraima (Cádiz), with seeds from Extremadura; there is a previous test planted 1989, with 12 provenances (8 from Spain, 1 from Portugal and 3 from Morocco) in three different sites, but we have no notice about its conditions at the present day.

Several actions whose objective is conservation have been initiated in different regions. Among the so-called *in situ* programmes, the Autonomic Administration of Extremadura has carried out the delimitation of selected stands, based on the cork quality. To date 20 stands have been defined, corresponding to the four provenance regions in which the cork oak forests of Extremadura are included.

The conservation of marginal populations deserves special attention. Because of the distance from the usual behaviour of the species, it is necessary to research the ecology of these populations, particularly their phenology and their reproductive characteristics, in order to design programmes aimed to recover a population, as well as to avoid incorrect actions. Another urgent action in these stands is the creation of *ex situ* plantations which permit preventing the risk of important losses of genetic resources due to fire or other menaces. These plantations, in addition, would facilitate the crossing of genotypes impeded by the present dispersion of parent trees, ensuring an appreciable genetic gain.

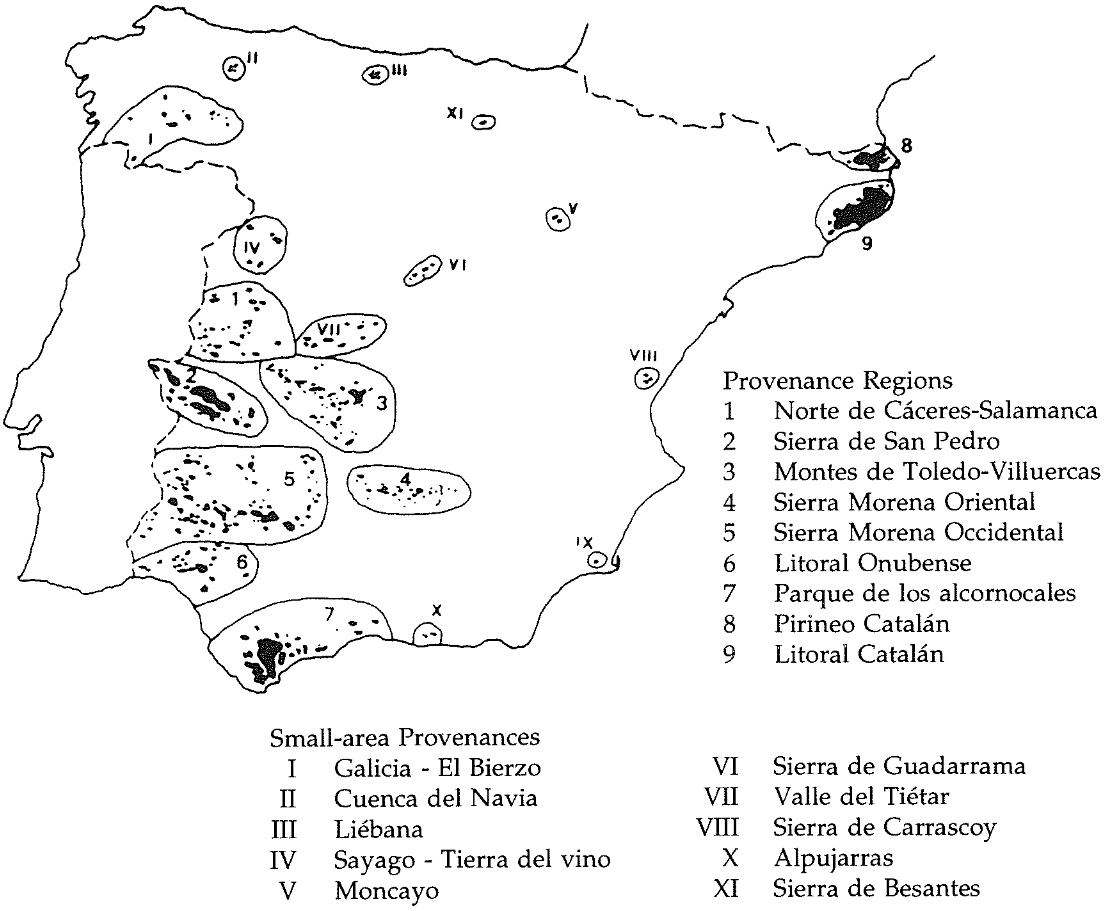

Fig. 2. Provenance regions of *Quercus suber* in Spain

In relation to this, the Authonomous Government of Valencia has began a conservation programme in several forest species, cork oak among them. In this region, the only remaining cork oak forests correspond to small populations enclosed in one of the highest fire-risk areas in Spain. In this programme, it is projected to undertake the ecological characterization of the stands, the mapping of the occurrence of the species and genotype conservation by means of *ex situ* plots. In general, the main problems posed by direct plantations of cork oaks derive from the low and irregular acorn production, the endogamy caused by a low number of trees, and from the difficulties in finding suitable lands for the species. Besides the edaphic characteristics, there are usually problems related to the ownership of the lands. It is intended to avoid these questions by trying the grafting of *Q. suber* on *Q. ilex*. In this way, a high number of genotypes would be joined together, leading to the production of seed of greater gene diversity in shorter times than on a normal plantation.

Final remarks

The maintenance of the genepool of *Q. suber* is a fundamental need for the conservation of healthy forests. Variability is the basis that makes possible a quick response to environmental changes. Populations with adaptable genetic structure will be able to respond more efficiently to disturbances than others with problems of endogamy.

Besides that, from an economic point of view, keeping the diversity of the species is a guarantee to obtain better results in selection and genetic improvement programmes.

After the delimitation of *Q. suber* provenance regions, which show ecological variation, it is time to begin the study of genetic structures, which will permit us to contrast and improve the present defined regions. It would be desirable to create a network of gene reserves, in which all the provenances and most ecological situations were represented; in this reserves natural regeneration would be favoured as a way to increase variability.

The peculiar characteristics of marginal populations implies that their maintenance needs particular actions for each one. In the first place, the protection and recovery of the stands must be promoted. Ecological and genetic studies are needed to estimate the species' actual state and for planning the most suitable programme. The more immediate actions must have as an objective increasing the genetic diversity of the species. The *ex situ* conservation of a wide sample of genotypes will decrease the risk of an irreversible (perhaps complete) loss of diversity due to fire or another cause.

The programmes of gene conservation must be viewed from a global view of the species, so they exceed the local, regional and even national scope. It is indispensable for greater coordination of the different administrations involved in the management of cork oak forests in order to ensure the success of the present and future programmes.

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Quercus suber genetic resources in France

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The decline of cork oak stands has been noticed in several countries of southern Europe, mainly in Spain, Portugal and France. In this last country, the areas occupied by cork oak have steadily regressed from 200 000 ha at the beginning of the 20th century to less than 100 000 ha today of which only 43 000 ha are more or less extensively managed for cork production (data from H. Oswald, pers. comm.). At the same time, the annual harvest of cork decreased to 3000 tonnes of which only 15% are suitable for bottle corks whereas the actual potential production has recently been estimated by the National Forest Inventory to be about 8000 tonnes per year. Owing to the decline of the cork market, there was no significant progress concerning cork oak silviculture and management during the last 30 years.

During the last decade, the French authorities became aware of the high environmental value of cork oak in the Mediterranean forest area. Cork oak can grow in varied (even difficult) environmental conditions in association with other Mediterranean oaks or conifers and can regenerate rapidly after fire. That species contributes efficiently to protect areas against soil erosion and to maintain forest vegetation which is necessary for development of tourism.

Recently, environmental constraints (fire, erosion, tourism), the negative commercial balance for cork and the more favourable economic evolution of the cork market, incited the French regional authorities to reactivate the regional cork industry and to take measures to preserve cork oak ecosystems and improve cork quality. Although cork has been used since antiquity, breeding programmes in cork oak have started very recently in several European countries but not yet in France. To be successful, such programmes should rely on a good knowledge of genetic variability in cork oak and more specifically on the genetic spatial organization in regard to environmental characteristics and human activity. In France, cork oak grows in four regions, namely in a part of the southwest (les Landes, near Dax), in the French Catalogne (near the Spanish border), in Provence and in Corsica. The first region has an Atlantic climate characterized by substantial humidity whereas the three other regions are Mediterranean with the occurrence of summer drought. In France, cork oak populations are usually native and show good regeneration. Such a geographic and climatic situation may favour genetic differentiation among the distinct regions.

Activities developed in France for inventory of cork oak genetic resources

Recently, the laboratory of Mediterranean Forest Research (INRA-Avignon; Director: Dr H. Oswald), which is in charge of forest research in the French Mediterranean region, has initiated a research project on cork oak in order to study the possibility of conservation and improvement of reference stands. These will be selected after preliminary analysis of all the data available from the National Forest Inventory. Experimental plots will be established in stands representative of the main cork production areas of France. Unfortunately, the project remains at a very preliminary stage (only four plots have been established) because of lack of financial support.

About 2 years ago, the study of genetic variation in French populations of cork oak (both managed and more natural plots) was initiated in the Centre Emberger in Montpellier (CNRS, Centre d'Ecologie Fonctionnelle et Evolutive, Department of Population Biology) in Dr R. Lumaret's Genetics group. Enzyme polymorphisms have been studied at 10 polymorphic loci and restriction fragment length polymorphism

(RFLP) of chloroplast DNA are used as nuclear and cytoplasmic genetic markers respectively. Intraspecific variability for these markers has been shown to occur in cork oak which is able to hybridize occasionally with other Mediterranean oak species (Elena-Rosselló *et al.* 1992). A method of chloroplast DNA extraction from chloroplast isolation, adapted to sclerophyllous tree species, was perfected in the Montpellier laboratory (Michaud 1993, and paper submitted) thanks to support from the EC programme Biotechnology. Moreover, a new method adapted from that already developed in A. Kremer's group (INRA, Pierroton, France) to study chloroplast DNA variation in deciduous oak species, will be applied very soon on the cork oak in the Montpellier laboratory. That method combines amplification of specific chloroplast DNA fragments using PCR with RFLP studies (B. Demesure, unpublished).

Regarding enzyme polymorphism, the present objective is to study at least six populations in each of the four French regions where cork oak grows naturally. Up to now, and because of lack of financial support for allozyme analyses, a maximum of two populations per region has been analyzed. Preliminary results already show substantial genetic differentiation of the Atlantic populations compared with the populations from the three Mediterranean regions. Variation among populations from distinct regions was also observed for chloroplast DNA.

Moreover, to be meaningful, the genetic data from the French populations must be compared with data obtained in similar conditions in cork oak populations from other countries. As genetic data on cork oak are not yet available in scientific literature, a few populations representative of the several countries should be analyzed by the laboratory of Montpellier or by other foreign laboratories to establish the necessary comparisons. Powerful molecular methods (for both nuclear and cytoplasmic DNA) are used in the laboratory of INRA-Pierroton on deciduous oak species. The methods can be adapted easily to evergreen oaks. Laboratory facilities and technical assistance, if necessary, can be provided by this laboratory.

A good knowledge of genetic variation in cork oak using several types of genetic markers (including morphological and physiological characters) is crucial for the establishment of any conservation strategy of the species. Preliminary results from the studies of genetic variation using enzyme and molecular markers in French populations of cork oak are promising. Therefore, further inventory of cork oak genetic resources in France as well as in the other countries of the western Mediterranean Basin should be encouraged.

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Some conditions of significance for forest tree gene conservation

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The essence of gene conservation can be summarized in the following way:

> The **methods** in gene conservation should ensure that the **objectives** in gene conservation are fulfilled while taking the **genetic structure** into consideration.

One of the greatest problems encountered in gene conservation and tree breeding is that both environmental conditions and economic value of the traits may change from one generation to the next. As an example, one type of wood may be highly appraised at one time but of low economic value at another time. The longer the rotation time the more pronounced is this conflict of present and future values. The change of the environmental conditions may be split into two groups, the first being beyond direct human control and the other due to development of new management regimes in silviculture. To cope with these uncertainties the prime objective of gene conservation ought to create good conditions for future evolution.

Two additional objectives can be identified, to save all genes at frequencies above 0.01 and to capture existing adaptedness. Saving of endangered populations may or may not fall under this last objective.

For the different traits it is important to determine the mode of inheritance, whether it is monohybrid, dihybrid or polygenic. For polygenically inherited traits it is important to estimate whether the gene action is additive or nonadditive. For nonadditive gene action the gene conservation is much more complicated since any geneflow would change the genetic constitution in a detrimental way.

For a genetic entry to respond to selection there is a need for some additive variance. In Figure 1 the relationship between gene frequency and additive variance is illustrated. This figure shows that genes in both high and low frequencies do not contribute much to the additive variance. To catch very rare alleles the gene resource population must be extremely large, tens of thousands of individuals. However, it must be remembered that the contribution to the additive variance will be practically nil and natural selection cannot act upon such alleles. Rare alleles may be rare for three reasons: they may be new mutations, they do not contribute to fitness or they may be new migrants.

As population sizes become larger the additive variance increases but at sizes of approximately 500 the increase is asymptotic towards a maximum value. Therefore there is no great merit in increasing the gene resources population above an N_e of 500 (N_e = effective population). It must be remembered that the sensus number may have to be a few times higher than N_e.

To capture existing adaptedness it is important to identify and know the role of the forces influencing among-population variation. They are schematically depicted in Figure 2.

Natural selection is the only force directly connected with adaptation (Brandon 1990). It ought to be stressed that natural selection mostly operates on the phenotype (cf. Mayr 1988).

This means that it rarely operates on individual genes. Nor does it directly operate on components of a trait.

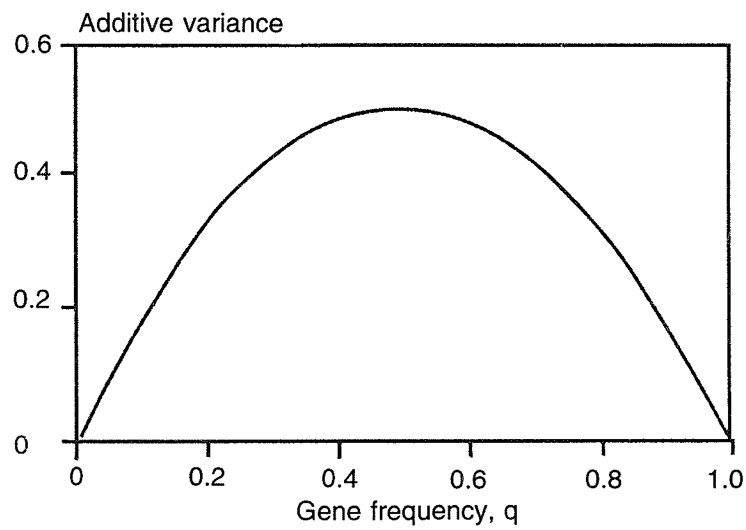

Fig. 1. The relationship between gene frequency and additive variance at total additive gene action of a polygenic trait

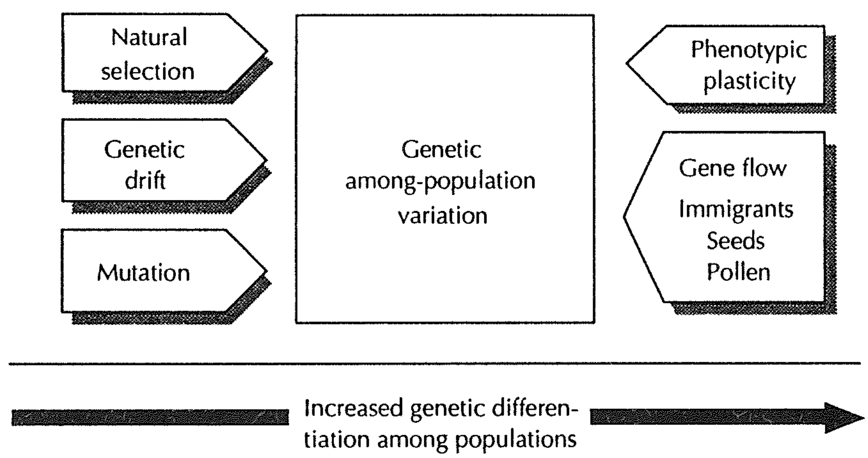

Fig. 2. Schematic illustration of the counteracting forces influencing the among-population genetic differentiation

Since the derivation of the selective effects on genes of varying fitness by Haldane (1932), selection in favour of homozygous recessives have been regarded as an extremely slow process owing to the low initial frequency of the recessive gene which in turn means that there are no homozygous recessives to select among. The response to selection was derived for large panmictic populations. However, in many cases in nature the intermating population may be much smaller. For small populations carrying a low-frequency fitness-improving allele the chances of obtaining homozygosity for such an allele increase considerably. In other words, in small populations fitness-increasing homozygotes for rare recessive alleles will reach selectable frequencies faster.

One of the most comprehensive reviews of the occurrence and strength of natural selection is to be found in the book Natural Selection in the Wild, by Endler (1986). Based on an analysis of hundreds of cases fulfilling certain criteria for being regarded as natural selection (directional, stabilizing as well as disruptive), he concluded that the belief that natural selection is usually weak is not true. Rather, natural selection can take any value, weak or strong, and moreover there are many cases in which the selection intensity is as strong as in breeding.

Random genetic drift will occur in populations with low N_es. Genetic drift causes a loss of additive variance with a rate of $1/2\ N_e$ per generation. Its effect on additive variance over ten generations for different population sizes is shown in Figure 3. From this figure, it may be seen that the loss is strongly dependent on populations below a size of 25. Genetic drift will in most cases lead to a reduction in fitness but may in some rare cases also give rise to improved fitness.

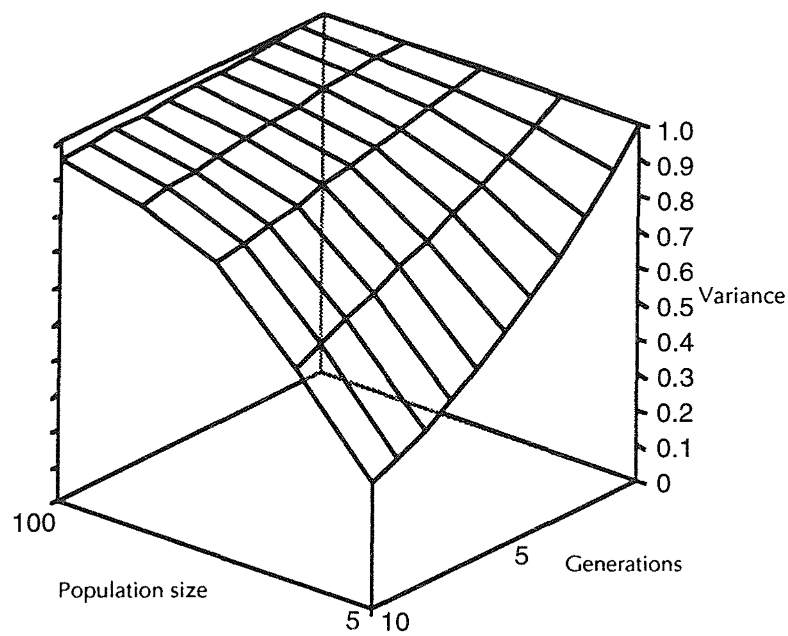

Fig. 3. The loss of additive genetic variance by random genetic drift over 10 generations at population sizes of 5-100 individuals

Mutations are assumed to be different in the different populations thereby causing differences among the populations. The impact is probably low owing to low mutation rates. Major gene mutation rates are assumed to amount to 10^{-5}-10^{-6}. The mutants contributing to additive variance of quantitative traits are of greater interest. They can obviously not be studied individually but estimates from studies in maize, mice and *Drosophila* suggest rates of 10^{-2}-10^{-3} (Lande and Barrowclough 1988). Unless their mutation rates are much higher than for major genes these figures suggest that genes in a large number of loci are involved in the regulation of the quantitative traits studied.

Phenotypic plasticity is defined as the amplitude of the phenotypic expression of a genetic entry studied under two or more environmental conditions. It has to be emphasized that phenotypic plasticity must be regarded as a genetically regulated trait (for a recent summary see Eriksson 1991). Phenotypic plasticity can be thought of as a disguising the genotype and thereby reducing the role of natural selection. When the ratio of the generation time over the selective fluctuations increases, the probability for evolution of phenotypic plasticity increases (Endler and MacLelland 1988). Species which during their lifetime are exposed to selective forces in a multitude of directions must be able to cope with that without a change of the genotype. Therefore, it is probable that genotypes with high phenotypic plasticity are favoured under such conditions.

Geneflow among populations occupying different sites reduces previously existing differences and counteracts differentiation. For mobile species geneflow can be accomplished by immigrants.

The mathematics of the process of gene dispersal is fairly complex and different formulae have been derived for different types of population structure: continuous distributions with isolation by distance, island types of structure, stepping stone models (Lande and Barrowclough 1988). In the absence of any other forces it suffices with an exchange of one individual between two populations per generation to prevent fixation of different neutral alleles in the same locus in the two populations.

Hardly ever are any of these five forces in operation alone; rather, one or more of them operate simultaneously. Theoretical derivations of formulae for the effects of these factors are available and some basic information will be given.

Loss of variance due to genetic drift can be compensated for by variance contribution by new mutants. If the figure for mutants regulating a quantitative trait amounts to 10^{-3} as mentioned above, the N_e must be 500 ($1/2\ N_e = 10^{-3}$) to compensate for the loss of additive variance caused by drift.

It is usually stated that genetic drift will reduce the additive variance of a population when the mutation rate is << than $1/4\ N_e$. The same is true for the coefficient of selection (S), defined as $S = (110-w)/100$, in which w is the fitness in percent of the fitness of the genotype with highest fitness. The formula indicates that extremely high mutation rates or selective forces are needed to overcome the effect of drift when N_e is low.

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Propagation by cuttings and identification by isozymes of some cork oak (*Quercus suber*) plus trees from Portugal

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Summary

To increase cork production, a tree improvement and germplasm conservation programme has been initiated in Portugal for which knowledge about the genetic architecture and the development of propagation methods is necessary. Progenies of 30 open-pollinated plus trees were raised in the greenhouse to supply tissues for the analysis of biochemical markers and cuttings for the rooting experiments.

The rooting of little lignified cuttings from young seedlings is possible. From some half-sib families up to 100% rooting was achieved. However, not all of the genotypes can be rooted easily, which is a prerequisite for tree improvement and gene conservation programmes. Thus, more effort into research and development has to be put into this method in order to be able to propagate all desirable genotypes.

The establishment of isozyme gene markers was successful for several isozyme gene loci. For a number of further loci the inheritance still has to be proved. To do this, it will be necessary to produce progenies by controlled pollination. Estimates of the gene variability of the species in comparison with other species were not possible at this early stage.

Introduction

Cork is a regenerative raw material with high technological qualities combined with positive ecological characteristics. The production of raw cork gives income to rural communities and the processing of cork and the export of cork products is a major source of income of some west-Mediterranean countries, especially Portugal. Because cork oak stands (montados) are becoming less productive because of over-aging and often get replaced by other tree species like eucalyptus, a tree improvement and germplasm conservation programme has been initiated in Portugal. Traditionally, farmers regenerate cork oak stands naturally, which means it is random. The farmers' main interest has been the raising of cattle. Cork production was secondary. Therefore this simple and cheap way of regeneration was welcome and there was no reason to study the genetics of cork oak or to establish tree improvement programmes. However, the demands are changing and the income from cattle growing is decreasing. On the other hand, there is increasing interest in cork as a unique regenerative raw material with multipurpose uses and ecological characteristics, as for instance easy waste disposal. Therefore, an EU-funded project was initiated to improve the quality and productivity of cork oak and its ecosystems. Several research institutions of different countries cooperated in this joint project. The Institute of Forest Genetics at Großhansdorf was involved with two tasks, the macrovegetative propagation by the rooting of cuttings and the characterization of plus trees by isozyme gene markers. In order to accomplish these tasks, progenies of open-pollinated trees were raised in the greenhouse at Großhansdorf to supply tissues for the analysis of biochemical markers and the cuttings for rooting experiments.

Macrovegetative propagation by the rooting of cuttings

Material and methods

The cuttings were taken from shoots of the present year. These shoots were up to 40 cm long. At the top, the shoots were still green and slightly lignified. The cuttings were about 10 cm long and divided according to their position along the shoot into apical, middle and basal cuttings. The cuttings from the middle and bottom were more lignified than the ones from the top.

The seedlings were from three different years: one set of four half-sib families was sown in the fall of 1990, another three families in the fall of 1991, and a third of 23 half-sib families in the fall of 1992. Thus, the seedlings were in their third, second, and first growing period at the time the cuttings were taken. The seedlings had flushed in April and the cuttings were taken in the fall at different periods between September and November 1993.

The cuttings were generally not treated by hormones and rooted in a peat/sand 2:1 VN medium plus 1 kg of lime per m^3 of medium. One set of cuttings (Set 4) was rooted in sand/perlite medium. Effects of the hormones IBA (3-indolyl-butyric acid) and NAA (naphthyl-acetic acid) were tested (Sets 2 and 4) by applying a commercially available rooting powder (trade names Seradix 3 and Wurzelfix respectively). In Set 4 a combination of IBA and NAA in aqueous solution was tested which was not commercially available. The basal end of the cuttings were submersed for one hour in darkness in the following solutions:

- 100 mg/L IBA + 20 mg/L NAA, pH 5.8
- 50 mg/L IBA + 5 mg/L NAA, pH 5.8
- 10 mg/L IBA + 1 mg/L NAA, pH 5.8
- plain water as control.

The rooting was done under plastic covering with the plastic sheets lying directly over the cuttings, giving a 100% relative humidity. The temperature in the greenhouse was kept at 18-22°C. Watering was done as necessary. In one set of cuttings (Set 4) the effect of watering with CO_2-enriched water was tested.

After 5-5.5 months of rooting time the cuttings were lifted in the spring between February and May and scored on the development of roots, callus and shoots. A number of the rooted cuttings were potted and kept to observe their further growth development.

Results

The rooting experiments show that about 3-8% of the cuttings root readily after a short period of about 1-2 months under mist or under plastic cover. Further cuttings root later, in some cases even if excessive callus has been formed which is, however, usually unfavourable for the initiation of roots. While scoring the cuttings, it was observed that some of the roots had developed just recently. Therefore it is important to keep the cuttings viable for a long time.

A number of effects on rooting were found. There is a strong genotypic (family) influence with some genotypes rooting more readily than others. Among the 1-year-old seedlings (families 1-23) rooting ranged between 35 and 100%.

The 2- and 3-year-old seedlings (families 24-26 and 27-30) have a lower rooting percentage which seems to be not only a family but also an age effect. Families 14 and 29 are identical but the acorns were harvested in different years. The 1-year-old seedlings give a rooting percentage of 43.48% while the 3-year-old seedlings give one of only 25%.

The position of the cutting in the shoot of the mother plant has only little influence. Cuttings from the top of a shoot do not root more readily than cuttings from lower positions. Thus, lignification was not found to affect rooting in this experiment.

The type of substrate has an influence, e.g. peat/sand is more favourable for rooting than sand/perlite. However, in the present experiment the difference found is only small.

Rooting hormones had nearly no effect in this experiment. In earlier experiments the hormone 3-indolyl-butyric acid (IBA) showed a positive effect whereas naphthyl-acetic acid (NAA) showed little effect.

Earlier experiments have shown that rooting percentages are higher on 10-cm-long cuttings (being used here) than on only 5-cm-long cuttings. The addition of CO_2 into the irrigation water has a slightly negative effect.

Discussion

The results of the rooting experiments show that macrovegetative propagation of physiologically young materials is generally possible. However, the goals aimed at may not always be reached with this method because there seem to be strong genetic effects and it may not always be possible to propagate all the genotypes selected. For tree improvement, macrovegetative propagation opens the possibility to make direct use of genotypic effects which is not possible by generative means of propagation (by seeds). The same is true for measures of genetic conservation, e.g. *ex situ* measures. Because it cannot be expected that easily rooting genotypes are the ones selected for tree improvement or conservation programmes, further research and development work may be necessary.

In the first year of the project a smaller study on rooting was carried out. The strong genetic effect was also found. Highest rooting reached 85% in one set of seedlings. In this study, in one family even 100% rooting was achieved. This high rooting percentage is possible only in very young materials. In this study, evidence for age effects between 1- and 3-year-old seedlings in rooting are shown. In the first study, effects of application of hormones were found and also distinct effects of the rooting medium.

The rooting hormones applied showed nearly no effect and the rooting in the peat/sand medium was only little better than in sand/perlite. An explanation for these differences might be that in the present experiment the cuttings were left undisturbed for almost half a year before scoring the rooting success. The medium and the hormones probably just accelerate rooting, which means that effects may have been found if the scoring had been done earlier. Thus, time of scoring may have an influence on the results found for the effects of rooting parameters.

Characterization of plus trees by isozyme gene markers

Material and methods

Root tip, bud and pollen tissues were analyzed to test if these tissues give interpretable isozyme banding patterns, and if these patterns are interchangeable with respect to a genetic interpretation.

Acorns of 29 selected plus trees were collected and germinated at Großhansdorf in the greenhouse. The progeny of between 6 and 30 seedlings per half-sib family and pollen of these and further number of selected plus trees were analyzed.

After prerequisites concerning the patterns of different tissues were fulfilled, seedlings were analyzed by their root tissue, the adult trees by their pollen. The isozymes were separated by common horizontal starch gel electrophoresis using two different gel buffer systems: (a) 0.06M NaOH-0.3M boric acid, pH 8.0 and (b) 0.14M tris-0.04M citric acid, pH 7.0.

A hypothesis of the genetic control of a variable locus in question was developed and verified by the observed frequency and genotype of the progeny resulting from open pollination. The goodness-of-fit of the observed frequencies to expected Hardy-Weinberg proportions was estimated by calculating X^2.

Results

Of the tissues tested, both pollen as well as root tips give interpretable zymograms. Buds also gave good zymograms but it is difficult and time-consuming to prepare the small buds of cork oak. The tissues used gave similar zymograms. Thus they can be interchanged without giving rise to errors in the genetic interpretation of the allozymes. Because either root tip or pollen tissue are available, the problem of which tissue to use seems to be solved.

Fifteen isozyme systems were assayed. No enzyme activity was found in three systems: aconitases (ACO), acid phosphatase (ACP) and peroxidase (PER). Enzyme activity but no variation was found in six enzymes: diaphorase (DIA), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), menadion reductase (MNR) and phosphoglucomutase (PGRM). The analysis of further material might give evidence of genetic variation in these isozymes which has not been found so far.

In five systems variation was found: alpha and beta esterase (αEST, βEST), glutamate oxaloacetate transaminase (GOT), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucose isomerase (PGI) and shikimate dehydrogenase (SKDH). In some of these systems, for certain zones, putatively the inheritance and genetic control of the alloxymes could be postulated (αEST, PGI and 6-PGDH). In other systems the zymograms are too complex to be able to postulate a putative gene locus (βEST, GOT, SKDH).

Discussion

Fifteen isozyme systems have been assayed up to now. The analysis of further isozymes is possible and might give under certain circumstances more information on the variability of cork oak or enable the identification of more individuals.

The relevant question concerning the overall variability of the species in Portugal is important for the improvement of programmes and conservation measures. From the material which was available consisting of a collection of plus trees selected in different montados, this question could not be answered. In order to study this question, populations at different places would have to be analyzed to estimate the variation within and between populations.

The study shows that there is definitely genetic variation among the individuals analyzed (von Wuehlisch and Nóbrega 1995); however, it can only be viewed as a first survey into this question. Therefore no conclusions are possible at this stage. From the related oak species which have been analyzed more thoroughly and from other European broad-leaved species like *Fagus sylvatica* the variation found is usually among the largest found in any animal or plant species (Michaud *et al.* 1995). In comparison, the results here indicate a smaller variability. This may be explained by the small area occupied by the species and the geographical limitation of the presently tested material. On the other hand the possibility of genetic erosion or reduction of genetic variability due to anthropogenic influences should also be considered. The present results do not answer this question. Future studies analyzing a set of different populations in different parts of the range of distribution may give appropriate data.

Acknowledgements
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Research on *Quercus suber* by *Silva Mediterranea*

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Introduction

Conscious of the value of the forests in the Mediterranean Basin, foresters meeting at the Ninth International Congress on Agriculture and Silviculture in Madrid in 1911 recommended the creation of a mechanism to facilitate cooperation in studies of problems facing the Mediterranean forests; *Silva Mediterranea* was established in 1922. One of the main tasks of *Silva Mediterranea,* which has been since 1948 a statutory body of FAO, is the coordination of forestry research in the Mediterranean through the establishment of coordinated research networks. Work is harmonized with that of other relevant FAO Statutory Bodies, including the FAO Panel of Experts on Forest Gene Resources, and is carried out in close cooperation with organizations such as the International Union of Forestry Research Organizations (IUFRO). Activities related to forest genetic resources form an important part of the research activities of *Silva Mediterranea,* and include its pioneering efforts in the exploration, exchange, evaluation and conservation of forest genetic resources.

Within the framework of the country-driven research networks established and coordinated by *Silva Mediterranea,* a number of activities are underway for the conservation and management of genetic resources of native as well as introduced forest tree species. The research networks in this field include, among others, the following:

- Selection of Stands of Mediterranean Conifers for the Production of Seeds for Reforestation Programmes
- Selection of Multipurpose Tree Species for Arid and Semi-Arid Zones
- Silviculture of Species: *Cedrus* spp.
- Silviculture of Species: *Pinus pinea*
- Silviculture of Species: *Quercus suber.*

The *Quercus suber* research Network of *Silva Mediterranea*

This research Network was established by the 15th session of *Silva Mediterranea* (Portugal, 1992) to meet a perceived need for a better information flow between researchers in Mediterranean countries, institutions and individuals active in cork oak forestry research. Prof. Dr Raul M.A. Sardinha, Director of the Forest Research Station of Portugal, was entrusted with its coordination. Focal points of the Network were nominated by concerned countries (Table 1). The following presentation is made from the Secretariat note which was addressed by Dr Sardinha to the Committee at its 16th Session, held in Larnaca (Cyprus).

It was recognized that advances in scientific knowledge depend largely on access to current information, ability to capitalize on past work and rapid dissemination of current research results. It was understood that cork oak research was rather lagging behind the other Mediterranean forest species, was long term and, by its nature, often costly and scattered. A further consideration was the view that cork oak ecosystems were important in the developing environmental debate which may justify an added importance to networking activities.

Table 1. Coordinator and focal points (at 30 November 1994) of the cork oak research Network *Silva Mediterranea* (* = country information predates the 16th Session)

Focal point	Address
Network Coordinator/Portugal **Raul Manuel Albuquerque Sardinha**	Director da Estação Florestal Nacional Rua do Borja 2, 1300 Lisboa, Portugal Tel: +351 1 60 16 61; Fax: +351 1 397 31 63
Algeria * **M. Messaoudene**	INRF BP 37 Cheraga, 42300 Algier Tel: +213 2 94 12 80; Fax: +213 2 78 32 11; Tlx: 61407 and 61300
Bulgaria **Spas Todorov**	17, rue Antim I, 1303 Sofia Tel: +359 2 88 04 15, +359 2 58 38 84; Fax: +359 2 87 32 35
Cyprus **Lucas Peonides**	Director, Forestry Department, Ministry of Agriculture and Natural Resources, Nicosia Tel: +357 2 30 22 61; Fax: +357 2 45 14 19
Spain * **Ramon Montoya**	Jefe Servicio Agentes Nocivos, ICONA Gran Vía de San Francisco 4, 28005 Madrid Fax: 1 26 58 379
France **Daniel Bourgoin**	Direction départementale de l'agriculture et de la forêt des Pyrénées Orientales 19 avenue de Grande Bretagne, 66025 Perpignan Cedex Tel: +33 68 51 95 00; Fax: +33 68 51 95 95
Italy **Carlo Forteleoni**	Directeur de l'Àzienda Foreste Demaniali Regione Sarda 44 via Trieste, 08100 Nuoro Tel: +39 784 35 730; Fax: +39 784 23 04 44
Jordan **Mahmoud Abu-Setta**	Head of Studies Section, Department of Forest Ministry of Agriculture, PO Box 2179, Amman Tel: +962 6 85 27 51; Fax: +962 6 83 402
Morocco **Houcine Ballis**	Direction des eaux et forêts et de la conservation des sols Ministère de l'agriculture et de la mise en valeur agricole Rabat Chellah Tel: +212 7 76 54 29; Fax: +212 7 76 44 46; Tlx: 36960 M
Tunisia **Habib Abid**	Chef de service des études et du suivi des aménagements Direction générale des forêts, Ministère de l'agriculture 30 rue Alain Savary, Tunis 1002 Tel: +216 1 89 11 41
Turkey * **Yusuf Cengiz**	Bati Akdeniz Ormancilik Arastirma Md. PO Box 264, 07002 Antalya Tel: +90 31 15 04 42; Fax: +90 31 15 14 56

It was decided to consult the focal points to ascertain a common interest on which to have networking flow of information and eventual exchange of experience and research results. It was pointed out that a very low level of ecosystem or silvicultural research was being carried out on this species compared with other species or the expression of research weaknesses did not allow increased commitments. Against this background a single proposition was put to all partners to share a common database on all research done so far. It was proposed that CDS-ISIS software be used, which could be exchanged and concentrated with marginal costs. Only two partners have shown interest in the activity, which could have been the starting point for the flow of information and the enhancement of synergy among institutions and researchers.

Among the European partners (Portugal, Spain, France and Italy) it was possible to link actions undertaken as a follow-up to Resolution 2, "Conservation of forest genetic resources", of the Ministerial Conference on Forest Protection in Europe (Strasbourg, 1990) in the context of the cork research Network and elect this species to be part of a common action. The Cork Oak Genetic Resources Conservation Scientific Advisory Group met in Lisbon from 8 to 10 July 1993. Unfortunately, it was not possible to have all focal points participate in this initiative.

Key points for the implementation of gene conservation were:

1. to develop the operational model of an *in situ* version of the Multiple Population Breeding System;
2. to define objectives and develop conditions to generate knowledge on genetic architecture of cork oak populations and their adaptive traits, needed to achieve a sound gene conservation;
3. to intensify or implement studies aimed at the study of long-term adaptive traits under provenance and progeny traits as well as on reproductive behaviour and population dynamics.

It is felt that a mobilizing project, able to attract some research funds, is needed as a catalyst for the Network. The coordinator mobilized some international teams for a cork oak project. This project involving teams and institutions from France, Germany, Morocco, Portugal and Tunisia was submitted to the European Union for financing under phase three of the Science and Technology for Development Programme (STD3). Its main objectives were:

1. evaluation of geographic, pedologic and climatic factors involved with cork oak decline;
2. establishment of relations between noxious agents and physiological parameters of the host;
3. understanding the spatial and temporal relations of insect/insect, insect/fungi, fungi/fungi related with the decline;
4. determination of optimum period for noxious agents to affect biological cycles of fungi and pests;
5. establishment of a database for integrated protection of cork oak ecosystems;
6. extent of variation in gene resources, populations and allelic diversity with interest for gene conservation;
7. implementation of provenance trials, reproductive biology, phylogenies and pedigree studies, aimed at the understanding of the systematic and random process on the evolution of the species;
8. technical-economic analysis of cork oak production systems;
9. implementation of a network all over the productive units to evaluate spatial and temporal alterations due to biotic agents.

Although the project was considered "an extremely important problem which deserves considerable attention and its multidisciplinary and international cooperation activities look very promising", its financing was not approved on the grounds that "the proposal does not provide for the enhancement of research capabilities in developing countries".

The main conclusion is that recognition of the interest in networking is not sufficient to avoid its pitfalls and enhance factors which ensure the best means of obtaining value for money from investments in them. It is considered that targeting the major objectives of a network, i.e. synergy and coordination, requires the elaboration of cost-effective strategic and perspective approaches to science and technology cooperation which may be better than or complementary to the FAO mechanisms available for this purpose. Outlining a vision for science and technology cooperation and looking at possible approaches to define its scope seems to be a good starting point to visualize the complementarity between research and development of partners and development policies, without which it is difficult to see the usefulness of networking.

It is important to keep in mind that the follow-up to Resolution 2 has chosen pilot species (i.e. cork oak) with the aim that such a research network activity could also potentially serve as an example and guide for action in regard to other oak and hardwood species.

Other activities of *Silva Mediterranea* in Forest Genetic Resources

Regarding other Mediterranean forest tree species, among the achievements of the *Silva Mediterranea* research networks in the field of genetic resources, the following can be highlighted:

1. Exploration of the natural ranges of distribution, and selection and documentation of seed stands of the following species: *Abies cephalonica, A. nordmanniana, A. pinsapo, Cedrus atlantica, C. libani, Cupressus sempervirens, Pinus brutia, P. halepensis, P. nigra, P. pinaster, P. pinea*. National research institutes in a total of ten countries in the Mediterranean cooperate in Network activities, which have continued in this field since 1963. Remnant populations of the endangered species, *Abies nebrodensis* (Italy) and *Cupressus dupreziana* (Algeria), have also been explored, documented and publicized by the Network, and reproductive materials have been collected from them for *ex situ* conservation measures aimed at complementing recommended *in situ* conservation strategies.

2. Establishment of species and provenance trials for *P. brutia* and *Pinus halepensis* in four countries in the Mediterranean Basin, and in Mediterranean climate zones in some countries outside of it (Australia, Chile, California-USA).

3. Organization within the framework of *Silva Mediterranea* of two Seminars on *Cedrus* spp. (Turkey, October 1990 and Morocco, June 1993). The meetings helped take stock of available knowledge on species of this important genus, including results from a large-scale programme established between 1972 and 1978 on five sites in France, in which 68 provenances of *C. atlantica, C. libani, C. deodara* and *C. brevifolia* were included. Following an inventory of cedar genetic resources and the identification of national seed stands, started in 1972 in Morocco and 1982 in Turkey and 1986 in Algeria, 46 provenances of *C. atlantica* and *C. libani* were distributed in 1992-93 to 10 countries in the Mediterranean for field testing within the framework of Network activities.

Concluding remarks

The *Silva Mediterranea* research networks have played an important role in fostering forestry research in the Mediterranean and have helped catalyze collaborative programmes in a number of fields. The association with IUFRO has been decisive in the success of activities undertaken. This mutually beneficial collaboration should continue, while action should also be closely coordinated with that of other international actors to ensure complementarity.

Work on genetic resources of Mediterranean forest tree species has continued over a number of years and, in many aspects, it has been of a pioneering nature. Some important results have been achieved which provide a sound basis for continued action towards the conservation and sustainable use of the main species concerned. Additional and strengthened efforts are, however, needed to generate further information on the distribution, distribution patterns, reproduction, genetics and silvics of priority species in order to better define and implement appropriate strategies for safeguarding these valuable resources. Special emphasis should be placed on strengthening cooperation between scientists from the countries north and south of the Mediterranean Basin, and on the exchange of know-how and experiences in the application of new technological tools to traditional conservation and tree breeding strategies. As an example, cooperative research in the use of genetic markers in investigating diversity and variation patterns in cedar species has already been started within the framework of *Silva Mediterranea*; this work could be most usefully extended also to Mediterranean oaks.

Previous activities of the *Quercus suber* Network[2]

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Estação Florestal Nacional, Lisboa, Portugal

The Ministerial Conference for the Protection of Forests in Europe, which was held in 1990 in Strasbourg, approved six Resolutions. Resolution 2 is devoted to the conservation of forest genetic resources. A follow-up group with representatives from France (M. Arbez, also the international coordinator of Resolution S2), Finland (V. Koski), Poland (J. Matras) and Portugal (M.C. Varela) were in charge of acting upon this Resolution. The main considerations resulting from the Resolution S2 were:

- genetic diversity is the raw material of evolution
- there are serious risks of the impoverishment of genetic diversity
- severe environmental changes are taking place specially in what concerns climate patterns
- owing to long generations, forest trees face high risks of environmental uncertainty
- measures should be undertaken without waiting for all the scientific answers
- simple and long-term methodologies should be privileged.

Given the strategy of conservation of forest genetic resources in Europe, Resolution S2 is aiming to act as an incentive for the coordination of various scattered and *ad hoc* actions. The synergistic effect of S2 in Portugal was noticeable because gene conservation in forestry was a distant concept. The Resolution S2 framework was developed through meetings in Warsaw in October 1991, Rome in April 1992 and Brussels in February 1993.

For implementation of European actions on conservation of forest genetic resources, the Follow-up Committee of S2 proposed four networks: *Picea abies*, Noble hardwoods, *Populus nigra* and *Quercus suber*.

Portugal volunteered to lead the *Q. suber* conservation Network. The initiation of the cork oak Network was facilitated by the nomination of a scientific advisory group composed of R. Lumaret (France), L. Gil (Spain), B. Schirone (Italy), M.C. Varela (Portugal), M. Malagnoux (FAO), H. Muhs (Germany) and G. Eriksson (Sweden). Initial links with the North African cork oak countries through the FAO Network *Silva Mediterranea* were also provided although with little response so far.

Beyond the international significance of the *Q. suber* Network, Resolution S2 was the incentive and will further constitute the backbone of the development of the Portuguese programme for gene conservation of the species. On behalf of S2 implementation Portugal proposed to host the first Meeting of the Scientific Advisory Group for Conservation of Genetic Resources of *Q. suber*. It took place in Lisbon from 8 to 10 July 1993 with the following agenda.

[2] Report from the preparatory meeting held in Lisbon, Portugal in July 1993.

Agenda

8 July
1. Synthetic description of the situation of *Q. suber* in each participating country with emphasis on key problems
2. Perspectives of the implementation of the Network for conservation of genetic resources (CGR) of *Q. suber* mainly focused on:
   2.1 Methodologies for gene conservation
   2.2 Medium- and long-term national programmes
   2.3 Main constraints for implementation of gene conservation in each country
   2.4 International cooperation among the institutions working with conservation: possibilities of regular contacts, by direct as well as by indirect means, in order to promote bibliography exchange, common research projects contributing to clarifying the genetic structure of the species and common research projects connected with sustainable management.

9 July
Visit to *Q. suber* gene reserve areas in Portugal:
1. Matinha de Queluz - Ecological reserve without any management. It is under the jurisdiction of the Parks and Reserves National Service
2. Alcobaça - Monoclonal archive under the responsibility of Estação Florestal Nacional
3. Mata Nacional das Mestras - natural regenerated stand managed for economical purposes; ownership by Forest Service.

10 July
1. Quinta da Serra - Private stand managed for economic purposes, under natural regeneration
2. Mata Nacional do Cabeção - Ownership by Forest Service
3. Final debate and elaboration of conclusions of the meeting.

Portugal, Spain, France and Italy made presentations about the situation of the status of PGR of *Q. suber* in the respective countries. A summary of the presentations of the four countries is presented below.

Spain
(L. Gil)
A description of the geographical distribution of the species in Spain was presented, focusing on its economical profile: 365 000 ha of pure stands and 121 000 ha of mixed stands form the actual area of the species in Spain. Economical returns of the species came from cork extraction and fruit production in a scenario of 93% private stands. Saving marginal threatened populations is considered to be the most urgent task for gene conservation of the species in Spain. The need for enhancement of genetic research in the species was stressed, with special reference to international cooperation. There is no specific programme for gene conservation of the species.

France
(R. Lumaret)
Cork oak is declining in France. From an area of 200 000 ha it is now restricted to 43 000 ha. The actual cork production reaches 8 000 t/year. There is no specific programme for gene conservation of the species.

Italy

A document written by R. Bellarossa (not present at the meeting) on "Report on the research status of Italian *Q. suber* L.: Characterization and strategy for its germplasm storage" was presented. The species occupies an area of 101 000 ha, corresponding to 2400 t/year of cork production. The species is found in Sardinia, Sicily and on the Tyrrhenian coast from Liguria to Calabria and Apulia. Studies using biochemical markers are underway. Saving marginal populations, especially the Apulian stands, is a major concern for Italy, although no specific programme for gene conservation of the species exists.

Portugal

(G. Guerreiro, M. Carolina Varela and R. Sardinha)

R. Sardinha presented a general view and the problems in cork oak. The species occupies an area of 660 000 ha with average production of 175 000 t of cork, which corresponds to US$ 200 million per year in value. The species faces decline in some areas with mortality up to 15%.

G. Guerreiro presented a general view of the Portuguese stands and focused specifically on the ecological significance of the species. Prospective measures for the improvement of management were mentioned.

M. Carolina Varela made a presentation about the Portuguese programme for conservation of the genetic resources of the species. The programme for *Q. suber* is guided by the principle that conservation of genetic resources exists for two major objectives, for scientific purposes and to promote the fitness of populations upon uncertain futures. Capture of genes with frequency above 0.01, existing adaptedness and creation of good conditions for future evolution are therefore the guidelines of the Portuguese programme for conservation of genetic resources of cork oak. For such objectives, the Multiple Population Breeding System was elected as appropriate methodology. MPBS combines intensive joint breeding and conservation objectives when applied *ex situ*. However, to fit existing material means (a common constraint in gene conservation with strong tendency to reinforcement as environmental problems grow), the MPBS has been applied through other methods beyond *ex situ*, some with less evolutionary merit. Under these circumstances, 11 populations were chosen through ecogeographical patterns and diversity, with the aim of capturing high levels of environmental diversity which may be linked to the genetic background.

Gösta Eriksson (Sweden) and Hans Muhs (Germany) were observers at the meeting. Gösta Eriksson stressed that the goal of conservation of genetic resources is to create conditions for evolution of the species for uncertain futures. The following principles were defended:

- rare genes are of minor importance for additive genetic variation and therefore for adaptive traits
- small populations evolve faster
- a sound genetic knowledge is necessary. Studies on mating system and on adaptive traits shall be the base for understanding of the genetic architecture of species
- Multiple Population Breeding System is the appropriate methodology for genetic conservation in forest trees.

Hans Muhs defended the following principles:

- the first need of gene conservation is to understand the genetic variation of a species
- conservation of genetic resources is not for static collecting but to face dynamic economical and ecological changes

- a representative collection of provenances in field tests is a basic requirement for this knowledge.

M. Malagnoux (FAO) presented the following issues:

- *Q. suber* was considered by FAO as a priority species for gene conservation
- an inventory of the research projects dealing with the species is a major need in order to rationalize human and materials means
- cooperation with North African cork oak countries is indispensable.

Perspectives

The Portuguese programme is in its first phase, when objectives are to be defined, methodologies elaborated and GRP (Gene Resource Populations) selected. Implementation of the programme through management of populations according to the proposed plan is urgent. Knowledge of the genetic architecture of the species is virtually nonexistent. Research on adaptive traits and reproductive system of the species is indispensable for further progress.

The meeting adopted the following document:

Recommendations by the Scientific Advisory Group for the Conservation of the Genetic Resources of Quercus suber *L. after the meeting in Portugal, 8-10 July 1993*

The Scientific Advisory Group for the Conservation of the Genetic Resources of *Quercus suber* L. met in Portugal, 8-10 July 1993. By consensus the key points for the implementation of gene conservation actions are:

1. The programme for gene conservation of cork oak (*Quercus suber* L.) in Portugal suggested by M.C. Varela was endorsed by the participants of the meeting. The concepts of this programme can be extended to other countries where cork oak is grown. The suggested programme contains an *in situ* version of the Multiple Population Breeding System developed by Prof. Gene Namkoong for joint breeding and gene conservation. The suggested programme will further be developed in order to incorporate simple breeding.
2. The three main components of gene conservation — **objectives, knowledge of genetic architecture and methods** — were discussed during the meeting. It was emphasized that identification of clear objective(s) for gene conservation must precede a decision on methods for conservation. It was agreed that **creating conditions for future evolution** is the most important objective of gene conservation in cork oak.
3. It is necessary to know the genetic architecture of cork oak populations and their adaptive traits in order to achieve effective gene conservation. Since such genetic knowledge of cork oak is lacking there is an urgent need to initiate research.
4. A long-term study of the development of adaptive traits under provenance and progeny trials must be established. Likewise, studies on reproductive behaviour and population dynamics are urgently needed. Long-term experiments should be complemented by studies on early identification of useful traits. Studies using biological markers should also be included among the tools to study the genetic structure of cork oak, particularly concerning mating systems, pedigree studies and geneflow mechanisms. It was recognized that there is also an urgent need for an inventory of current projects dealing with cork oak in different countries.

Since the ecological, social and economic importance of cork oak is unquestionable in the Mediterranean countries, policy-makers, governmental organizations and private institutions dealing with cork oak or its products should increase their support. The countries with indigenous cork oak forests should strengthen their cooperation in common projects. Cooperation with North African countries via the FAO Network *Silva Mediterranea* is an excellent means to achieve this goal.

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