Quercus suber Network

EUFORGEN

Report of the third and fourth meetings
9-12 June 1996, Sassari, Sardinia, Italy
20-22 February 1997, Almoraima, Spain

J. Turok, M.C. Varela *and* **C. Hansen,** *compilers*

International Plant Genetic Resources Institute
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EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

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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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Contents

Sassari meeting (Third Network meeting)

Organized jointly with Concerted Action EU/FAIR 1-CT 95-0202 "European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies"

1. Introduction

The meeting was held from 9 to 12 June 1996 in Sassari, Sardinia, Italy. Participants from seven countries attended the meeting (see Participants). The meeting provided a framework essentially to focus on preparing and discussing implementation of the Concerted Action project approved by the European Commission (EU/FAIR 1-CT 95-0202 "European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies").

A new research project proposal to be submitted for funding to the EU was another major point on the agenda. Most Network members are involved in either or both projects. Therefore, this joint meeting with broader attendance was organized as an occasion to combine the different efforts aimed at the conservation and better use of genetic resources of cork oak. Maria Carolina Varela, chair of the Network and coordinator of the Concerted Action, welcomed all participants at the meeting and thanked the organizers for their efforts. She expressed her satisfaction with the fact that urgent activities on genetic resources of cork oak were undertaken in a collaborative way in the Mediterranean region and that attendance at this meeting was very representative. M.C. Varela then briefly introduced the outline of the Concerted Action project and emphasized the main topics to be discussed and agreed upon during the meeting. The Network countries prepared the project proposal during 1994 and 1995 and it was approved under the EU/FAIR programme in January 1996. The main objective is to establish simultaneous provenance and progeny trials for the evaluation of genetic variation and heritability of economic traits, mainly cork quality.

EUFORGEN will continue to act as the catalyst of collaborative activities and projects in the different Networks. The EUFORGEN coordinator, Jozef Turok, welcomed all participants and informed them about the progress being made in the other EUFORGEN Networks. He wished the participants to maintain their enthusiasm and a collaborative spirit also for future activities.

The cooperation and support of FAO and particularly *Silva Mediterranea* to the Network was acknowledged by all participants. FAO's Forest Resources Development Service organized and supported the participation for the first time of a representative of Morocco and initiated contacts with specialists on the genetic resources of cork oak in Algeria and Tunisia. Christian Hansen (FORM/FAO) stressed the importance of maintaining close links in the future.

All the participants then introduced themselves and their ongoing work in the area of genetic resources of cork oak. The agenda of the meeting was approved, starting with a full-day discussion of the preparation of provenance/progeny field trials and their experimental design (see Agenda).

Working sessions of the meeting were kindly hosted for two days by the Associazione degli Industriali della Provincia di Sassari at Villa Mimosa in Sassari. The participants of the meeting welcomed several scientists of the Associazione, the Università di Sassari and the Istituto Sperimentale per il Sughero, to attend the working sessions and to contribute to the discussions.

2. Workplan update

Objective 1: To compile and distribute relevant literature and information
The participants decided to complete and publish the list of selected bibliography. It was agreed that Portugal (M.C. Varela), France (R. Lumaret in consultation with M. Bariteau) and Morocco (M. Benzyane) will send references in electronic form to J. Turok at IPGRI **by 15 July 1996**. Italy (B. Schirone) will update the list of references submitted last year. Christian Hansen will carry out a search in FAO documentation sources for relevant internationally published literature and will forward the results to J. Turok by 15 July 1996. The following information should be provided for each reference: author, year of publication, title, source, original language of publication, and/or summary. Titles of publications should be given in French, Spanish, Italian, Portuguese or English, and, if translated, put in square brackets. The entries should be separated by tabs. The list of selected bibliography will be published by IPGRI along with the Report of the meeting (see this volume) and updated by the Network regularly afterwards.

The need for a comprehensive monograph on *Quercus suber* was expressed by all participants. It was suggested that an existing international mechanism take the initiative to prepare such an important information resource. The possible facilitating role of *Silva Mediterranea* was specifically mentioned. The participants of the Network offered their contribution on chapter(s) related to genetic diversity and genetic resources issues.

Objective 2: To make an inventory of *Quercus suber* genetic resources
Further to the agreed workplan, a draft list of descriptors for *Q. suber* populations and individual trees was prepared by B. Schirone and discussed at the meeting. Examples of descriptors currently used in Spain and France were presented. B. Schirone will incorporate the comments provided by the Network and circulate an updated version **before 20 June 1996**. All participants will receive the list of descriptors for their comments and send them back to B. Schirone **before 1 August 1996**. J. Turok will interact with experts working at IPGRI on the development of descriptors for agricultural crops and will also send comments to B. Schirone **before 1 August 1996**. B. Schirone will forward the final version to M.C. Varela and to J. Turok **before 15 August 1996**. The developed descriptor list will serve as a basis from which a smaller number of the most relevant descriptors should be chosen for the passport form needed in the Concerted Action project. After receiving the final version from B. Schirone, M.C. Varela will propose the passport form decriptors and will circulate them **before 31 August** in order to use them for the collecting of acorns (see Passport data in this volume).

It was emphasized by the participants that, as a second step to achieving this Objective, a precise survey of the distribution of cork oak in all countries should be carried out by the Network. It will be necessary to apply results of digital inventory systems and devote sufficient time for this task. However, it was agreed that this issue be discussed in more detail at the next meeting and that the Concerted Action and joint research into genetic diversity be priorities for this meeting and for the coming year. B. Schirone offered to prepare an example of a similar survey carried out in the Italian region of Latium (Lazio) and to suggest concrete steps that should be taken at and after the next Network meeting. Participation of the North African countries in this activity should be facilitated. The suggestion will be circulated to all participants as a brief outline document **before the end of 1996**.

After discussing the development of the *Q. suber* database, which should assemble results of inventories and mainly include data from the field trials of the Concerted Action at this stage, it was agreed that M.C. Varela should prepare an outline of the objectives, format and structure of the database. It should be circulated to all participants **by the end of 1996.**

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

Both the Concerted Action and the research project proposal contribute principally towards achieving this Objective and the concrete steps and activities are described below in more detail.

Objective 4: To develop conservation strategies and methodologies

Luís Gil presented a paper describing results of studies on endangered and relic populations of cork oak in Spain. This paper entitled "Characterization of marginal populations of *Quercus suber* in Spain", partly a result of the previous Network activities, is published in this volume.

Objective 5: To raise public awareness of the threats to *Quercus suber* diversity

The follow-up to this important Objective of raising public awareness was not discussed specifically at the meeting. Several presentations of the participants, however, illustrated its importance with examples of activities contributing to the public awareness about cork oak genetic resources and their conservation at a national level.

3. Presentations of the participants on country activities

The ongoing activities in Portugal, Spain, France and Italy, as presented by the participants of the first two Network meetings, were published in the previous Report. At the Sassari meeting, representatives of all four countries provided updated information on practical activities in the field, research and further perspectives. Portugal presented a paper on the identification of Regions of Provenance and described a progeny test established in 1994. Besides the presentation on marginal natural populations of *Q. suber* in Spain (see Objective 4 of the workplan), the first phase of an extensive genetic improvement programme initiated in the country was described. Preliminary results from analyses of genetic variation among and within populations of cork oak in France, and comparisons with populations from other parts of the distribution area using isozyme polymorphisms, were also presented.

Genetic studies at DNA level are being carried out in Italy and a brief description of these studies followed the country report at the meeting. An interesting overview of the methods of commercial evaluation of cork, developed in Sardinia, was also presented. The Italian participants announced their intention to establish an interdisciplinary and country-wide programme on *Q. suber*, with genetic resources being an important component.

An introductory country report was given by M. Benzyane from Morocco. Absence of regeneration and overgrazing were emphasized as very serious threats to the reproduction of genetic diversity in cork oak in Morocco.

Advanced genetic studies on cork oak have recently been carried out at laboratories in Sweden and Germany.

The presented contributions with direct relevance to the conservation and use of cork oak genetic resources in the participating countries are published in this volume.

4. Concerted Action

4.1 Preparation of provenance/ progeny field trials

Seed lots

The number of seed lots to be included in the field trials, and the number of seed lots to be contributed from each of the participating countries, have been intensively discussed. The need for a balance between good representation of the entire ecogeographic range of cork oak and the size of field trials for sound statistical inference, as well as the costs involved, were considered. The number of stands (provenances) was discussed next. Countries decide themselves whether they wish to include one stand per provenance or to have certain provenances represented by more than one stand.

It was decided that the number of stands (provenances) in the international series should not exceed 40, in order to keep the trials manageable and effective while at the same time making a sample representative of the species' variability. The number of stands (provenances) to be included from each country was discussed and the following scheme was suggested:

Country	No. of provenances	Notes
Italy	5	3 from the continent + 2 from Sardinia
Portugal	8	from the Regions of Provenance III, IV,V and VI
Spain	8	6 from main production regions and 2 from marginal populations
France	4	–
Morocco	6	–
Tunisia	2	–
Algeria	1	–

Seed storage

Seed storage was another important issue to be agreed upon prior to the preparation of the field experiments. Seed storage in cork oak is generally considered a high-risk operation and it was therefore decided by the participants to do the experiments without it and to proceed with direct sowing.

Seed collection

Several options were discussed. As seed storage (even one year and more) was not considered applicable, and owing to the irregular flowering and seed crops in the case of several provenances, the question of timing and integration of the seed collection, nursery production and trial establishment becomes crucial. If seed collection is carried out over more than one year, the provenance trials could either be established with material collected during more years, or nursery production prolonged for the provenances collected in the first year. In both cases, the interpretation of data obtained from the trials is more difficult and uncertain, owing to the different age of the provenances included in the trials.

The forecast for the 1996 seed collecting season looked promising. The participants reported observations of unusually good flowering in all cork oak countries. It thus seems possible to collect seeds from the majority of provenances in this year.

It was agreed to collect as many stands (provenances) as possible during 1996. The need for additional seed collecting and the question of trial establishment over more than one year or prolonged nursery production will be discussed at the next meeting, when the results of the seed collections in 1996 are known.

Nursery and establishment of field trials

If acorn production follows the patterns now visible for flowering, it will be possible to collect all seed lots during Autumn 1996, sowing will be carried out in February 1997 and trials will be established during Autumn 1997. As the Concerted Action does not include any financial support for the establishment of field trials, the participants will seek additional financial resources as soon as possible.

4.2 Discussion of experimental design of field trials

Countries offered to host the following numbers of trials: Portugal (3), Spain (2), Italy (2), France (2) and Morocco (1). The involvement of North African countries in the Concerted Action project, financed by the EU in general, and the problematic support for the establishment of field trials in particular, were discussed. All participants expressed their wish for a stronger and closer collaboration.

The discussion at the meeting first focused on the establishment itself of the trials because it is not easy to have areas of required size (up to 24 ha) suitable for growing cork oak and with an acceptable level of environmental homogeneity. A reliable appraisal of genetic parameters might then become questionable.

The final spacing of the trials was unanimously accepted to be 5 x 5 m. The decision was based on taking into consideration that, in many countries, cork oak stands are managed in densities of 300 to 400 trees/ha (corresponding to 5.8 x 5.8 m and 5 x 5 m, respectively), and that in new afforestations there is a tendency for closer spacing.

Size of the trials was, therefore, reduced by four times. The possibilities of finding areas of around 6 ha and diminishing block variability are clearly higher. Consequently, the statistical power of the experimental results can be improved.

Several proposals for the experimental design of the field trials were discussed and should be used as background information for the preparation of the experiments in individual countries. The choice of any trial design depends on the possibly different objectives of the scheme in each country. The differences between trial sites and between the objectives in individual countries should be fully taken into account.

The decision on objectives of the field experiments and on details of the experimental design will thus be made under the responsibility of each participating country. Two proposals of the experimental design were initially discussed at the meeting and are briefly summarized below. Proposal A (H. Muhs) is relevant whenever high priority is given to the progeny testing part, and Proposal B (G. Eriksson) if the main objective is provenance testing.

Proposal A

Provenance and progeny trials:

- 34 provenances x 20 mother trees = 680 mother trees
- Initial spacing 2.5 x 5 m=12.5 m^2/tree (one thinning procedure after 10/15 years)
- 2 tree plots (within row)
- 680 mother trees x 2 trees/plot x 4 replications = 5440 trees/trial
- Seed requirements: 2 trees x 4 replications x 10 trials = 80 seedlings/mother tree.

Proposal B

Provenance trials:

- 34 provenances x 20 mother trees = 680 mother trees
- Initial spacing 2.5 x 5 m = 12.5 m^2/tree (one thinning procedure after 10/15 years)
- Code for provenances: 01, 02, .. , 34
- Code for progenies: 01 (till 34) - 01 (till 20).

Progeny trials:

To evaluate genetic parameters, only three provenances are included in progeny tests; the selection of provenances to be included varies from test site to test site.

- The chosen provenances should be tested for within-population variation
- 3 provenances x 20 mother trees x 60 seedlings/mother tree (x 6.25 m^2)= 2.25 ha
- 20 open-pollinated (OP) families
- 60 seedlings/OP family
- 15 replications with 4 seedling plots
- At final spacing (5 x 5 m) there will be 15 trees per OP family.

4.3 Development of database

The database is one of the main components of the Concerted Action. The goal is to compile an outstanding information resource on the genetic resources of cork oak.

The coordinator M.C. Varela will find a specialist (university-educated technician) as previewed and budgeted in the Concerted Action project.

A basic outline of the objectives, format and structure of the common database will be developed and circulated by M.C. Varela **by the end of 1996** (see workplan update of the Network).

4.4 Conclusions

Based on the approved original project proposal and on the discussions during the meeting, it was agreed that:

- The main objective of the Concerted Action is to assess genetic variation at a provenance level in the species' distribution area, to estimate within-provenance variation and G x E interaction, and to establish a basis for and to initiate tree improvement activities.
- Number of populations (provenances) contributed by each country to the field experiments will be as follows:

Italy	5
Portugal	8
Spain	8
France	4
Morocco	6
Tunisia	2
Algeria	1
Total	34

- Each participant will send a map location of all stands (provenances) selected for seed collection in the respective country to M.C. Varela **no later than 31 October 1996.**

- Number of trials to be established in each country will be as follows:

Portugal	3	
Spain	2	
Italy	2	
France	2	
Morocco	1	
Total	10	
Final spacing of the trials		5 x 5 m
Initial spacing of the trials		2.5 x 5 m

- Experimental design: It was agreed that each country will promote internal discussion on the proposals or present new ones. Further discussion should take place and agreements reached during the next meeting **in February 1997**.
- Number of mother trees:
  * at least 20 mother trees should be sampled per population
  * 50-100 m (preferably 100 m) should be the distance between the mother trees
  * several age classes should be represented
  * average phenotypic performance of trees and availability of abundant seed crop will be two main criteria for selecting the trees in stands.
- Number of acorns/tree:
  * 300-500 (at least 300 acorns should be sampled from each tree)
  * exception: one stand to be selected and sampled jointly by Portugal and Spain where 2000 acorns/tree have been agreed upon. This common stand will be selected and assessed by Portuguese and Spanish colleagues before 30 September 1996.
- Around 300 000 acorns will be sown in nursery. The number of seedlings at the end of the nursery phase is expected to be at least 102 000. The time schedule foresees that acorns be sown in a nursery in Portugal **before 31 January 1997.**
- Passport forms (descriptors): In accordance with the workplan update, the coordinator M.C. Varela will elaborate proposals based on the longer version of descriptors which was submitted by B. Schirone. M.C. Varela will send the final version to all participants of the Concerted Action by 31 August 1996. All seed lots must have a common passport label.
- Seed lots must always be sent by Express mail, preferably by fast land transport to avoid the below-zero temperatures in cargo compartments in aeroplanes. Containers made of plastic should be avoided. All participants will send a proposed budget for seed collecting (including cost of transportation) to M.C. Varela before **31 July 1996**.
- Seed storage: It was decided that there would be no seed storage because of the heavy risks. Efforts must be made in order to achieve one single seed collection **during Autumn 1996** and sowing **up to February 1997**.

5. Discussion of a new research project proposal

Network participants congratulated B. Schirone for the excellent score (B+) of the shared costs research proposal aimed at studies of genetic diversity and reproductive system by genetic markers he coordinated for the EU/FAIR call of March 1995. He was unanimously asked to coordinate the new shared costs research proposals. The observations of the Commission referees were briefly discussed and should be taken into account during the preparation of the new proposal.

It was agreed that B. Schirone prepare the final version of the new proposal **by 31 January 1997**. Each participant concerned will send suggestions and modifications to B. Schirone **by 1 November 1996.**

6. Future scope and activities of the Network

With reference to the recommendations of the first Steering Committee meeting of EUFORGEN National Coordinators (held in Sopron, Hungary, in November 1995), the broadening of the Network's scope was included on the agenda of the meeting. It was felt that more broadly conceived and comprehensive Networks dealing, on a problem-oriented basis, with a number of similar species could add flexibility to the ongoing international collaboration within EUFORGEN. The wish was also expressed by several Network members to extend the *Q. suber* Network towards other evergreen oaks of the Mediterranean region.

The extension of the scope of the Networks should also be seen from the perspective of interaction with regions that share the same species and/or face similar problems on the conservation of genetic resources. Increased collaboration with regions geographically adjacent to Europe and interested in certain common species, particularly in the south Mediterranean region, would be very important. Based on this consideration, it was decided to invite, for the first time, representatives from North African countries to the Sassari meeting.

The species-based approach was considered by the participants to be most appropriate for the networking activities related to the conservation and use of genetic resources in cork oak and in general. As major differences exist in regard to distribution area, biology, genetic structure and silvicultural practices between Mediterranean oaks, it was decided, in order not to make the scope and work of the Network too dispersed and general, to start the process of expanding the Network with caution and in several steps. It should take into consideration the specific needs and problems that individual oak species have in the Mediterranean region. *Quercus suber* should be the main species for further activities, not only in the framework of the Concerted Action and the research project proposal. However, genetic diversity and genetic resources of the three most closely related species, which can not be separated from *Q. suber*, will also be of concern in the future. *Quercus suber*, *Q. ilex*, *Q. coccifera* and *Q. alnifolia* represent a complex of closely related species with natural hybridization occurring between *Q. suber* and *Q. ilex*, as well as between *Q. ilex* and *Q. coccifera*. This group of Mediterranean evergreen oaks being within the scope of the Network, also provides a basis for inviting scientists and representatives from a wider range of Mediterranean countries to become involved in the Network activities in the future. Every effort should be made to encourage the participation of representatives from concerned countries outside Europe to the Network meetings.

The next meeting, which will focus primarily on the monitoring of progress and further planning of the Concerted Action project on *Q. suber*, will be held in Spain, 20-21 February 1997.

7. Field trip

The field trip included visits to cork oak stands and to a cork factory.

Cork oak stands in Sardinia are of medium to high densities. Management is characterized by mixed type composed of high forest and coppice. Reforestation is done by planting 2-year-old seedlings. Fencing of the reforested area is not used as it would raise the risk of intentional fires. An experiment of putting up iron nets of about 2 m high for individual protection of young trees against damage by grazing appears to have been successful as shepherds retain their free access to the land. Even so, some cases of destruction of the nets have been observed. The major drawback with this method is the high price.

Streaping coefficients are between 2 and 3. With a 10-12 year period of streaping, it is common that cork is sold on site. Some stands show sanitary problems but catastrophic situations are rare. Attacks of defoliating insects *Tortrix viridana* and of *Malacosoma neustria* were intensive during the spring of 1996.

A visit to a private cork factory in Tempio Pausania was another part of the field trip. The region has a very intense cork industry. A whole range of products from cork was processed in the factory, e.g. stoppers, isolation panels and ornamental goods. The management remarked that there was a growing market for cork products, including coarse-texture particle boards.

Agenda

1. Introduction

2. Progress in the implementation of the Network workplan and country activities (reports of participants)

3. Scope of the *Quercus suber* Network in the European and Mediterranean perspective

4. First meeting of the Concerted Action (EU/FAIR 1-CT 95-0202) "European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies":
   - Preparation of provenance/progeny field trials
   - Discussion of experimental design for field trials
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5. Discussion of a new research project proposal to be submitted to EU/FAIR

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6. Conclusions

Almoraima meeting (Fourth Network meeting)

Organized jointly with Concerted Action EU/FAIR 1-CT 95-0202 "European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies"

1. Introduction

The joint meeting took place from 20 to 22 February 1997 in Almoraima, Spain. It was attended by participants from eight countries (see Participants).

Director of the Centro de Investigaciones Forestales CIFOR-INIA, Madrid, Spain, Dr Gabriel Catalan, welcomed all participants on behalf of the organizers and agreed to chair the meeting. Maria Carolina Varela, coordinator of the Concerted Action and chair of the EUFORGEN Network, welcomed the participants to the meeting and thanked the organizers for their efforts. All sessions of the meeting were kindly hosted by CIFOR.

The agenda of the meeting focused on further development of the field experiments within the Concerted Action (CA) project. Another important issue proposed for discussion, as part of both the CA and the Network activities, included the compilation of a bibliography on *Quercus suber* genetic resources and common descriptors (see Passport data in this volume). After having approved the agenda of the meeting (see Agenda), participants started with discussions on the current status of the CA project.

2. Progress of the Concerted Action

2.1 Acorn collection (Table 1)

Algeria: One provenance sampled in lots representing 25 mother trees was included.

France: M. Bariteau explained the process of acorn collection in France and stressed the reasons for French provenances not being identified by mother trees. The first seed crop could not be collected because of the poor quality of seeds, as is common in *Q. suber*. The second seed production (with good reproductive quality) could not be used due to the great density of the French stands, and to the occurrence of strong winds which led to a sudden fall and mixture of acorns from the various mother trees. The acorns collected (bulked seed lots) were stored at 2-5°C while waiting for transport.

Italy: No major problems have been reported by Italy, except those inherent to the geography of the country, i.e. the long distance between the collecting sites. During seed collection, adequate sites for the installation of two trials were found.

Morocco: All requirements agreed during the previous meeting were met without major problems. Acorns from 6 provenances arrived in good condition.

Portugal: Problems were encountered in tracing seed collection by mother trees in one provenance, Romeo, in the northeast of the country. To keep the agreed numbers, one additional provenance with seed collection by individual mother trees was taken. In any case, the provenance Romeo was to be included in the collection due to the ecological importance that the provenance holds for the Portuguese cork oak distribution.

Spain: Owing to poor seed crops, two marginal provenances planned originally have been replaced by Sierra de Guadarrama and Alpujarras.

Tunisia: Two provenances sampled in lots representing 25 mother trees each were successfully included.

Table 1. Project status: corrected list of provenances and number of mother trees where acorn collection took place in the various countries

Country	No.	Provenance	No. of mother trees
France	1	FRI - Les Maures	11 (< 300 acorns)
	2	FR II - Le Pyrenées	One single seed lot
	3	FR III - Soustons (Atlantique)	One single seed lot
	4	FR IV - Sartene (Corse)	One single seed lot
Spain	5	Montes de Toledo - Cañamero (V)	25
	6	Sierra Morena Oriental - Fuencaliente (CR)	25
	7	Sierra Morena Occidental - Jerez de los Caballeros (J)	25
	8	Parque de los Alcornacoles - La Almoraina (ALM)	25
	9	Cataluña Litoral - Santa Cloma de Farmes (GE)	25
	10	Sierra de Guadarrama - El Pardo (PAR)	27
	11	J. Alpujarras - Haza de Lino (H)	25
Italy	12	Lazio - Tuscania	26
	13	Puglia - Brindisi	25
	14	Sicilia - Catania	25
	15	Sardegna - Cagliari	25
	16	Sardegna - Sassari	25
Portugal	17	Chamusca	25
	18	H. Palma	25
	19	Q. da Serra	25
	20	Ponte de Sor	25
	21	S. Brás Alportel	25
	22	Évora/Azaruja	25
	23	Santiago do Cacém	25
	24	Romeo	One single seed lot
Portugal + Spain	25	Besteiros + Sierra de San Pedro-La Tojera (A)	15+15†
Morocco	26	Bousafi	35
	27	Ain Rami	30
	28	Canton A, B	20
	29	Canton D - Ain Johra	38
	30	Oulmés - parcelle 65	30
	31	Bab Azhar - parcelle 202	30
Tunisia	32	A - Meckna	25
	33	B - Fernana	25
Algeria	34	Guerbès	25
Total	34	34 (29+5)	

† Stand common to Portugal and Spain.

Notes: The original table presented by the coordinator was subject to some corrections during the meeting: French provenances FR II (Le Rimbaut Provence) and FR III (Soustons) were renamed FR II (Le Pyrenées) and FR III (Soustons (Atlantique), respectively. The provenance FR V (Sartene, Corse) was renumbered FR IV (Sartene, Corse). Provenance FR V mentioned in the coordinator's document did not exist, making the total number of French provenances 4, and not 5. The total number of provenances from Tunisia was also corrected to 2 provenances instead of 3 (Meckna and Fernana). After these corrections, the total number of provenances is 34, and not 36 as suggested before. Therefore, the number of provenances with individual sampling by mother trees is 29, which implies the total number of mother trees, after correction, is 610= (29x20)+(1x30).

2.2 Description of nursery techniques

By the time of the meeting, the sowing process was ending. It started on 2 January 1997, then took several weeks because of the control required to ensure good progress. The sowing varied according to the number of involved persons available.

The coordinator made a presentation of the nursery techniques used, and described the background that led to the decisions taken. The research line developed by Dr G. Falconnet's team (École national du génie rural, des eaux et des forêts, France - ENGREF) was proposed and accepted by the participants as a guideline on the nursery operations The following items were discussed in detail:

- type of container
- substrate
- water supply control
- fertilization.

Plant production relies on the use of plastic containers with 400 cm^3 volume, chosen according to Falconnet's criteria. Substrate represents a mixture of soil, pine fibre and pine bark. The water supply system used is modern and works well.

To ensure performance above common commercial standards, the raising of the plants requires that special measures be taken. Therefore, the following additional technical measures should be introduced into nursery operations:

- Maintenance of moisture levels at pF-value of about 1.5 as an average of the minimum and maximum limits of 1-2.7[1], in order to assure good shape and development of the root system. Leaching of nutrients must be prevented at the same time. Irregular watering and subsequent drought lead to inverse root growth and curling. The pF-values cannot be measured accurately in the field, yet a practical way was recommended by Falconnet using a fixed course that allows the weight of 4-6 trays of containers to be monitored simultaneously. Previous calibration allows a scale of weight to be determined whose limits indicate watering requirements. The device is made up of a dynamometer that holds the set of trays to be monitored. For the total area of the project seedlings (about 190 x 12 m), at least three such devices are required.
- Seedlings should be lightly watered various times a day rather than less frequent, heavier watering, because of the problem of nutrient leaching due to excessive watering.
- With regard to the nutrient levels, their supplies should be monitored through conductivity tests, which must fall within the limits of 250-400 mS.
- There has been no initial fertilization ('osmocote' is usually applied in nurseries); therefore, to achieve good progress in plant production, the nursery systems require systematic visits by staff to ensure the following:
  * control of water quality: measurements of pH
  * control of watering levels and frequency: appraisal of pF-values
  * control of nutrient levels: conductivity.

[1] The value 1.0 indicates an excess of water, therefore high leaching of nutrients; 2.7 is on the threshold of causing death from drought.

3. Experimental design of field trials

Experimental design is the cornerstone of the project, and therefore particular attention is being paid to discussing different options during the implementation process. Incorrect experimental design may diminish the results of any future research based on the field trials.

The first step consisted of sending circular letter to all participants prior to the meeting, outlining the difficulties previously encountered with this item. The circular letter asked participants to reflect on the former two proposals for experimental design (see Report of the Sassari meeting). No replies were, however, received before the meeting. The Portuguese participants, A. Aguiar and M.C. Varela, prepared a new proposal which was presented for discussion at the meeting. G. Eriksson prepared a short note on some conceptual aspects that should prevail in the proposals (see below).

Some of the proposals were criticized by the participants because they implied large areas where the environmental heterogeneity was too large, and therefore the appraisal of genetic parameters would become questionable. In addition to the original proposal from the last meeting by G. Eriksson, one modified proposal from H. Muhs and the new proposal from Portuguese participants were presented and/or discussed at the meeting.

3.1 Proposal by A. Aguiar and M.C. Varela

Provenance trial

Genetic entries
Number of provenances: 34
Number of mixed seedlings/
provenance per trial: (9x12)= 108

Design
Randomized Complete Blocks (RCB)
Number of replications: 12
Number of plants/plot: 9

Number of plants
Provenance/plot: 9
Provenance/replication: 9
Provenance/site: 108
Replication: 306
Site: 3672

Acreage
Initial spacing: 16 x 1.5 m
Plant: 9 m^2
Plot: 81 m^2
Replication: 2754 m^2
Site: ~3.3 ha
Final spacing: 6 x 6 m
Plant: 36 m^2
Number of plants/site: 917= (277/ha x 3.3 ha)
Total number of plants for final evaluation/provenance: 27

Progeny trials

A non-schematic model for progeny trials was also mentioned based on the following assumptions:

- 610 mother trees is too large a number of genetic entries to be tested in cork oak distribution range. Usefulness of such a huge number of trees for evaluation of genetic parameters for further breeding is also questionable. Therefore, it has been proposed that countries interested in progeny trials would install a set of five provenances (100 mother trees) plus a chosen provenance, common to all progeny trials to be installed.
- Each trial would then be made up of 100+20* mother trees. Countries interested in family and individual selection could even install two progeny trials (if there are sufficient plants), leading to 100+20* plus 100+20*.

Compared with the model suggested by G. Eriksson for progeny trials (see Report of the Sassari meeting), this proposal argues for more trees in the test.

3.2 Proposal by H. Muhs

A key observation was made of particular importance to the entire philosophy of the project in that the results of this CA offer a rare opportunity in cork oak genetic research. The participating countries have succeeded in developing a seed collection of 34 provenances traced on labelled seed lots of 20 mother trees and leading to a total of 610 genetic entries. Therefore, every effort shall be made to obtain the maximum amount of information from this unique collection. This collection of basic material will give maximum benefit because it combines field trials, and related genetic research. Other fields may eventually benefit from the tracing of mother trees.

Therefore, H. Muhs proposed that all the plants to be delivered for field trials, be labelled individually. This proposal was supported by all participants and was considered feasible as long as financial investment for material and labour are available. Financially it raises no problems because it falls within the total budget of the project.

The actual proposal (modified from last year) is as follows:

Simultaneous provenance/progeny trials

Genetic entries

Number of provenances with individual sampling by mother trees (m.t.): 29
Number of mixed provenances (m.t. unknown): 4
(1 provenance Les Maures with acorns collected from only 11 m.t. also taken as a mixed provenance)
Total number of provenances: 34
Number of m.t./provenance: 20

Design

Randomized Complete Blocks (RCB)
Number of blocks/trial: 2 (or 3)[1]
Initial number of plants/plot per m.t.: 4 (in line)
Initial number of plants/m.t.: 4

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[1] All calculations are based on 2 blocks. However, if means are available, the experiment can be extended to 3 blocks.

Number of plants
m.t./provenance per plot: 4
m.t./provenance per site: 8
Provenance/site: 8 x 20= 160
Total number of plants/block: (20 x 4 x 29)= (5 x 80)= 2720
Site (2 blocks): 5440

Acreage
Initial spacing: 5 x 1.5 m
Initial number of plants/ha: 1334
Plant: 7.5 m^2
Plot: 30 m^2
Block: 20 400 m^2 = 2.04 ha
Site: (2 blocks)= 4.08 ha
Final spacing: 5 x 6 m
Plant: 30 m^2
Number of plants/site: 334 x 4.08 ha= 1 360
Acreage/trial (if 3 blocks) = 6.12 ha

Total number of plants for final evaluation/provenance/trial: 40
Total number of plants for final evaluation/m.t./trial: 2

3.3 General note on the design of the trials (by G. Eriksson)

The design of the field trials aiming at study of genetic variation within and between populations of *Q. suber* depends on the objectives and the statistical considerations.

As to the objectives, it is clearly of interest to obtain information on the variation among populations. In my opinion, for economically important tree species, as cork oak is in Mediterranean countries, there should be more information on the genetic variation within populations as well. There have been limited breeding efforts even though *Q. suber* is of great economic importance for at least some of the represented countries. The field trials could thus also be converted into seed production units in the future. This adds another objective to a total of three pertinent objectives:

- study of genetic variation among populations
- study of genetic variation both among and within populations
- study of genetic variation and breeding.

Objective 1 is best fulfilled by establishment of a classical provenance test. Objective 2 may be carried out as a combined provenance and within-provenance study. Each open-pollinated progeny must be represented by at least 24 trees at the age of evaluation. The problem arises when there are many populations, each represented by a relatively large number of progenies. In the project there are as many as 34 populations and, for most of them, single-tree progenies are available. Even if all populations were not represented by all 20 progenies, a combined within- and among-population study would not be feasible for practical reasons. The question then arises whether the number of progenies per tree at each site can be reduced, as suggested in the above proposal (by H. Muhs; see section 3.2). That proposal is based on the idea to pool information obtained from all test sites for studying the variation within populations. This would be a problem if there was a strong genotype x environment interaction. We do not have any information on the G x E interaction for quantitative traits. However, results of the studies of geneti information at isozyme loci (see presentation by R. Lumaret, this volume) show th classical pattern expected for wind-pollinated species, i.e. a limited genetic differentiation among populations. This in turn suggests high levels of geneflow among populations. From an evolutionary point of view, there is a low probability for specific adaptations to particular habitats; the genetic variation of adaptive significance is probably clinal and not ecotypic. The G x E interaction can be expected to be of minor importance. Therefore, the proposal presented by H. Muhs should certainly be followed for objectives 1 and 2 (study of genetic variation within and among populations).

As for objective 3, the possibility to achieve meaningful selection gains among and within families should be considered. As ruled out under objective 2, all progenies could not be fully tested at each test site. If Portugal, for instance, would like to include a breeding objective in the test plantation near Lisbon, I suggest that the test is set up with 3-4 populations that include individual testing of open-pollinated progenies represented by some 30 trees each at the time of evaluation. For the trial in southern Spain, a similar breeding objective might be fulfilled by choosing the 3-4 most suitable populations. The choice of populations will depend on the knowledge of climatic conditions at the sites and the information about stands where the material was collected. At each test site the populations are planted separately and the focus is on selection of the best-performing trees within best families of a population. I would recommend the design of plots with 12 trees in 5 replications in order to allow for the thinning to the desired number of 30 trees. This will give good statistical resolution among families within a population and a fair chance to achieve effective selection within a family. Those participants who decide to include a breeding objective in their field tests should consider planting in parallel 3-4 breeding populations at a site as outlined above.

3.4 Discussion

M. Bariteau disagreed with some aspects of the proposals, in particular with the possibly large size of the blocks. He also questioned the problem of simultaneous provenance/progeny trials, arguing in favour of separation.

There was an agreement regarding the huge variability of the Mediterranean environment. It is a particular type of climate where the coincidence of the dry season with the summer raises synergistic effects of drought stress, high temperatures and intense sun radiation. Very high heterogeneity within sites results from such factors. Therefore, it was agreed by the participants that attention should be paid to controlling the environmental component of the trials, if genetic parameters are to be assessed accurately.

The coordinator M.C. Varela stressed that limited time availability and the need for further reflection upon the issues discussed made a final decision regarding the coordination of the experimental design impossible to reach during the meeting. In view of the indecision on the various items, M.C. Varela proposed to study, circulate and consult the proposals by correspondence. Each document will require an answer within the given period of time and will be based on full new proposals.

It was decided that the coordinator would again circulate proposals which participants would have 30 days to reply to. Time constraints mean that responses regarding the decision to be taken in each country on the experimental design must be constructive and must in any case lead to a new fully documented proposal (see Recommendations of the meeting).

4. Plant delivery

All efforts shall be made to label the delivered plants so that future research can get the most from the seed collection made available by this CA project.

Plants will be moved to plastic containers as mentioned and shall be delivered by truck at a temperature of about +8°C and with approximately 75% relative humidity. Both wooden boxes and plants will be subject to phytosanitary treatment with fungicides.

To avoid transport complications, Portugal will send the plants as a donation for scientific purposes. The compulsory certificates necessary for plant transport will be provided by Portugal.

Participants proposed a provisional timetable for the lifting and supply of plants:

- Morocco: mid-October or December
- Tunisia: mid-October (it is important that transplanting takes place before early autumn frosts in order to allow for the development of a sufficient root system before stress factors related to frosts occur)
- Italy, France, Portugal and Spain: November.

5. Databases

Three different databases are currently under preparation: a bibliography on cork oak genetic resources, data resulting from the field experiments, and information about institutions involved in activities related to cork oak genetic resources. The information available in the databases is increasing as replies to previous contacts are being received.

A first list of selected bibliography was presented at the meeting (Word for Windows file with 300 references; this volume). It will be entered into a database software later. The compilation of this bibliography results from the interactions among the participants and Network members during the period after the meeting in Sassari (see Report of the meeting in this volume). The list will be updated regularly. Most recent entries made to update the list include bibliographic references from Portugal (about 650), France (35), Spain (1) and Italy (1). The references deal with issues directly linked to the conservation and use of cork oak genetic resources, but in many cases also cover broader relevant subjects such as the distribution of the species, its evolutionary history, ecology, physiology and silviculture. Great care must be taken in keeping the selection criteria of the bibliographic material sent. In fact, the material being received for updates includes references that go beyond the scope and subject of this database on cork oak genetic resources. This implies a careful selection of the references by each participant contributing to this database. Requests should be sent to the host institute, Estação Florestal Nacional in Oeiras, Portugal. The database will be available on the Internet.

Passport data from Spain, France and Italy were received and form the basis of the CA project's database. Improvement of the database structure and design is being looked into. For this purpose, M. Bariteau kindly offered to provide a short course on database implementation to the person in charge from Portugal.

Further contacts with both public and private institutions are being carried out to obtain information about projects on cork oak in which the institution had been involved. The requested information takes into consideration the following aspects:

- Project title
- Coordinating institution and scientific coordinator
- Objectives
- Duration

- Funding source
- Partnership both within institutions in the same country and between countries
- Information on the availability of the final report where available
- Perspectives of the project.

Institutions in a number of countries have been contacted. Some replies have been received so far from France (1 project), Spain (15 projects) and Portugal (2 projects).

6. Descriptors and passport data

The passport data form, developed by M.C. Varela, was used to document the selected stands (provenances) and single trees for the CA experimental trials. This form is simply and rapidly completed, sufficiently covering the needs of all participants (see Passport data, this volume). It was agreed that a similar form be developed and used for site descriptions of the field trials.

A more elaborate proposal of descriptors for cork oak was presented by B. Schirone. It was discussed and unanimously considered to be too demanding for the descriptions of sampled genetic resources populations. It was agreed to restructure the descriptor list according to a clear outline of the main objective. The latter will be a voluntary descriptor list.

7. Field trip

The first part of the field trip went to Almoraima, a large state-owned property of about 14 000 ha. This property has large, well-managed cork oak stands of good quality where plus tree selection took place. The second part of the field trip was focused on the region of Hoyo de las Naves de S. Luís, where forest associations of *Q. suber*, *Pinus halepensis*, *Q. ilex* and *Abies pinsapo* prevail at the altitude of 1000 m. In Sierra de las Nieves a relic *Quercus faginea* stand on the southern sunny slope and a grove of *A. pinsapo* on the northern slope, Sierra Vermelha, were observed. The field trial provided the opportunity for a parallel discussion on the objectives, methodologies and strategies to be used in the conservation of genetic resources of rare, economically less important species with a small population structure, such as is the case of *Abies pinsapo* in Spain and minor *Quercus* species along the Mediterranean Basin.

8. Recommendations

The following is a list of recommendations agreed upon at the meeting:

- Nursery plants should be raised in good, uniform conditions in order to reach good levels of germination among and within seed lots. Therefore, the methodologies available and best suitable, according to the present level of knowledge, shall be applied (see above).
- The nursery process should be based on a uniform methodology according to specific criteria. No research shall be performed on these seed lots.
- Plants to be sent for the establishment of field trials should be individually labelled so that the maximum genetic information is available for future evaluation of the field trials. The labelling applied to all plants is a measure that further strengthens the results of the project because it allows mother trees to be traced throughout the duration of the trials.

- The (four) outside edges of all sets of containers that constitute the production area shall be protected with isolating white boards to protect the outside layer of plants from being damaged by the intense heat due to direct sunlight.
- Plants should be moved to no-return plastic containers for delivery.
- The coordinator will circulate all proposals for experimental design of the trials; the participants will reply within 30 days after receiving the circular letter.
- An extraordinary meeting should be held, attended by one participant per country, for final discussions on the experimental design. There are two main reasons for this extraordinary meeting. Firstly, because the available number of seedlings per provenance and per mother tree is essential to plan the experimental design and for the establishment of field trials, and this figure will not be accurate before July 1997. Secondly, agreement on the coordination of experimental designs chosen by the individual countries was not reached during the Almoraima meeting. As experimental design is a key factor that can determine decades of future research, discussions on this matter must be as exhaustive as possible.
- All concerned participants will provide information on the details of the acorn collection stands in each country, according to the passport data form. An overview, including a map of all stands, will be compiled.
- The 1998 joint meeting (CA + Network) will be held in March or April, in France. It will provide an opportunity for discussing issues related to the enlargement of the Network.
- Practical guidelines for the management of genetic resources of cork oak will be developed.
- The Concerted Action project is being developed very rapidly and will, ultimately, lead to a considerable collection of material which forms a unique and strong basis for genetic research on cork oak in the future. The participants recommended that possible research frameworks be identified at this stage that would allow for the continuation of work. This is a very timely consideration, in particular with regard to the full participation of North African countries. Without appropriate, harmonized sources of funding, the trials and all the related genetic research undertaken may be at risk of not being adequately continued in the future. The outcomes of this CA should spread to the interested community, far beyond the circle of the actual participants.

Agenda

20 February 1997
Status of the Concerted Action project: Acorn collection, Description of nursery techniques used, Nursery timetable and observations
Experimental design for field trials

21 February 1997
Field trip

22 February 1997
Passport data used for the establishment of the field trials; descriptors
Plant delivery (timetable, transport, etc.)
Databases

Passport data

Passport data for stands of *Quercus suber*

1. Region of provenance

1.1 Reference of the collecting site/stand: name, code, etc.

1.2 Location on a map (1:25 000 or 1:50 000) - to be attached

2. Site descriptors

2.1 Location of collecting site: country of origin, province, nearest town or village

2.2 Longitude (degrees and minutes followed by N- North)

2.3 Latitude (degrees and minutes followed by E- East or W- West)

2.4 Elevation of collecting site (m above sea level)

3. Environment descriptors

3.1 Climate: should be assessed at nearest meteorological station

- 3.1.1 Minimum, maximum and mean monthly temperature (°C)
  (JAN, FEB, MAR, APR, MAY, JUN, JUL, AUG, SEP, OCT, NOV, DEC)
- 3.1.2 Mean monthly rainfall (mm)
  (JAN, FEB, MAR, APR, MAY, JUN, JUL, AUG, SEP, OCT, NOV, DEC)

3.2 Topography

- Plain
- Sunny slope
- Shadow slope

3.3 Rock type

3.4 Soil parent material

- Eruptive
- Sedimentary of silica type
- Sedimentary of limestone type
- Alluvium
- Clay
- Schist
- Metamorphic
- Other

4. Stand structure and composition

4.1 Composition pure or mixed: if mixed which species

4.2 Density: classification based on the average distance among trees

- high (up to 25 m)
- medium (about 25 m)
- high (more than 25 m)

5. Management

5.1 Natural regeneration

- Natural regeneration utilized
- Without natural regeneration

Passport data for trees of *Quercus suber*

1. Tree size
1.1 Total height (m)
1.2 Stem height (m)
1.3 Stem perimeter (cm)

Notes:
Number of trees per stand: 25 trees
Distance between trees: minimum distance 50 m; 100 m whenever possible
Number of acorns per tree: 300-500; at least 400 if possible.

Presentations

Characterization and conservation of *Quercus suber* germplasm in Italy

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Introduction

In Italy, cork oak covers an area of around 90 000 ha and the total cork production amounts to 6000 t/year. The species is present in Sardinia, Sicily, along the Tyrrhenian coast from Liguria to Calabria and in Apulia.

Up to now, no broad studies have been carried out on the genetic variability of Italian cork oak. Recently, characterization of several Italian provenances of *Quercus suber* L. has been started to define the most appropriate strategies for genetic conservation (Bellarosa *et al.* 1993, 1996).

Moreover, investigations on *Quercus crenata* Lam., a supposed natural hybrid between *Q. suber* and *Quercus cerris* L., have been initiated. Part of the results of these analyses, which were supported by an EEC-FOREST project, are reported here.

Material

Quercus suber material was sampled in the following sites (Fig. 1):

- Viterbo and Circeo (Latium)
- Elba island and Suvereto (Tuscany)
- Bultei, Pattada and Berchidda (Sardinia)
- Lucci, Paticchi, Cerrito and Pignicella (Apulia).

Quercus crenata was collected in three sites (Fig. 1):

- Viterbo and Castelporziano (Latium)
- Follonica (Tuscany).

Comparative analyses were carried out using seed storage proteins and rDNA genes as markers.

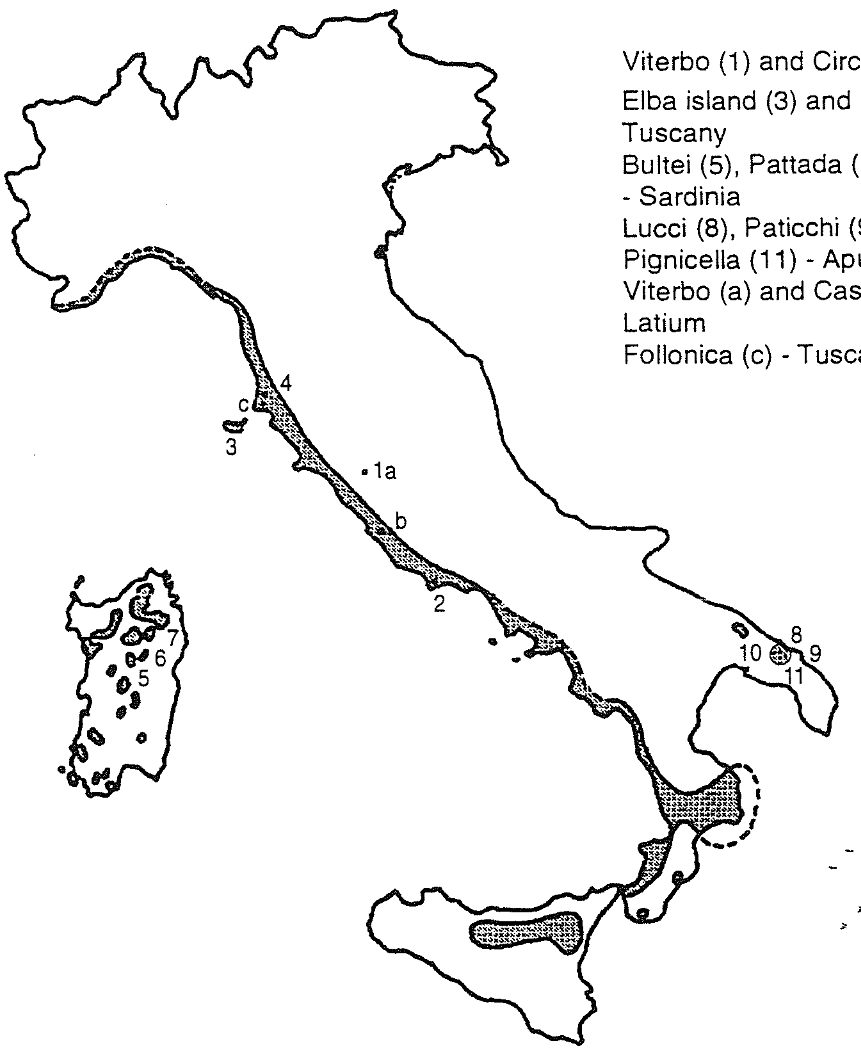

Fig. 1. Distribution of *Q. suber* and *Q. crenata* in Italy and collecting sites.

Experimental methods

Analyses of seed storage proteins

Protein extraction: A sample of at least 60 mature and viable seeds, randomly collected from different trees, was used for each species. The acorns were dried and ground to flour without teguments. The *Q. suber* seed lots came from Elba island, Viterbo, Circeo, Bultei, Pattada, Paticchi and Pignicella.

Total seed proteins were extracted following Payne *et al.* (1981) with modifications. After 12 hours, the extracts were centrifuged at 13 000 rpm for 10 minutes and the supernatant containing total proteins was collected.

Extraction and separation into soluble and insoluble fractions were performed as described in Gifford *et al.* (1982) with modifications. This method was applied because it permits the separation of globulins into two majors types: salt-soluble globulins (e.g. legumin-like) from those, insoluble globulins, requiring urea or SDS in the extraction buffer for their complete solubility (e.g. cristalloid proteins) (Gifford and Bewely 1983).

Protein electrophoresis: One-dimensional SDS polyacrylamide gel electrophoresis (SDS-PAGE) of the soluble and insoluble proteins under non-reducing (without 2-mercaptoethanol) and reducing conditions was carried out according to the method proposed by Laemmli (1970). The 1.5-mm slab gels consisted of an acrylamide separation (12.4% T; 3.2% C) and an acrylamide stacking gel (3.5% T, 1.6% C), in the presence of 0.1% SDS. After the electrophoresis, the gels were stained with 0.05% (w/v) Coomassie Brillant blue R-250 in 12% (w/v) trichloroacetic acid. Once destained, the gels were photographed with T-MAX 100 film. Protein molecular weight was estimated by using the Low Molecular Weight Calibration Kit (Pharmacia). The standards employed were: phosphorylase b, 94.0 kDa; albumin, 67.0 kDa; carbonic anhydrase, 30.0 kDa; trypsin inhibitor, 20.1 kDa; a-lactoalbumin, 14.4 kDa.

Analyses of ribosomal DNA genes

DNA extraction and purification: A pool of 1000 individuals (root tips of both germinated acorns and seedlings and immature catkins of adult trees) was used for the extraction of total genomic DNA of Viterbo provenances of *Q. cerris* and *Q. suber*. The other *Q. suber* provenances were: Elba island, Suvereto and Circeo (Tyrrhenian group), Bultei and Pattada (Sardinian group), Paticchi and Pignicella (Apulian group). The extraction was made as reported in Bellarosa *et al.* (1990). The DNA of *Q. crenata* was also extracted from shoot tips of seedlings and mature trees following the method reported by Dellaporta *et al.* (1983).

Restriction enzymes, electrophoresis and Southern blot hybridization: The DNA material from *Q. suber*, *Q. cerris* and *Q. crenata* was digested by using the restriction enzymes *Eco* RI, *Bam* HI and *Sac* I, and respective double digestions according to the manufacturer's instructions (Boehringer-Mannheim). To ensure complete digestions, at least 10 units of enzyme were used per 1 mg of DNA for more than 12 hours. Restriction fragments were separated by electrophoresis in 1% agarose gels. The fractionated DNA was blotted onto a nitrocellulose membrane (S&S) following the procedure reported by Southern (1975), after HCl depurination (Wahl *et al.* 1979). To better separate the restriction fragments corresponding to the short and long rDNA genes, *Eco* RI digested genomic DNA of *Q. suber*, *Q. crenata* and *Q. cerris* was submitted to a longer electrophoresis run of about 48 hours. Genomic DNA, extracted from *Q. suber* provenances, was submitted to a single *Eco* RI digestion.

DNA hybridization and detection: Cloned fragments containing only 18S (*Xba-Sac*, 1000 bp long) or 25S (*Eco* RV- *Eco* RI, 3000 bp long) rDNA from *Populus deltoides* (D'Ovidio *et al.* 1991) were used as probes to design the maps. The labelling, hybridization and detection of the hybridized membranes were performed according to the instructions supplied with the digoxigenin-kit (Boehringer-Mannheim).

Results

Analyses of seed storage proteins

Seed proteins, analyzed with electrophoretic techniques, have proved to be a very successful marker in plant systematics (Ladizinsky and Hymowitz 1979; Miege 1982; Aliaga-Morell *et al.* 1987; Collada *et al.* 1988; Jensen and Lixue 1991; Schirone *et* *al.* 1991). They can be used for the identification of the geographic origin of different taxa, the determination of parents of hybrid species, the characterization of polyploids and amphyploids, the establishment of similarities between species and the formulation of hypotheses on their phylogeny (Miege 1982).

The screening of the total seed proteins of several oak species demonstrated the possibility of recognizing different species of *Quercus* (*Q. robur*, *Q. pubescens*, *Q. frainetto*, *Q. trojana*, *Q. macrolepis*, *Q. cerris*, *Q. suber*, *Q. crenata*, *Q. ilex* and *Q. coccifera*) and of setting reliable taxonomic models (Bellarosa *et al.* 1996).

The detailed study on the total seed proteins of *Q. suber* provenances showed no great differences among the electrophoretic patterns. However, the total protein profiles from Latium provenances showed a higher number of bands when compared with those from Apulia.

Further confirmation of the differences detected through the analysis of the total proteins was obtained using monodimensional electrophoresis of various protein fractions separated on the basis of their solubility characteristics in different extraction buffers. Seed proteins, which are soluble only in the presence of SDS, were extracted both with and without the introduction of a reducing agent (2-mercaptoethanol, ME) that has the function of breaking the sulphur bridges of proteins. Analyses of the electrophoretic patterns showed that both with and without ME the Apulian provenances can be distinguished from the others (Bellarosa *et al.* 1993).

Furthermore, analyses were carried out to compare *Q. crenata*, a supposed hybrid between *Q. cerris* and *Q. suber*. The analyses suggest that the main *Q. crenata* protein patterns come from the addition of the profile of the two other species (Bellarosa *et al.* 1996).

Analyses of ribosomal DNA genes

DNA genes have been extensively studied in plant and animal species. Each transcription unit is composed of the coding regions for 18S, 5.8S and 25S DNA and a non-coding region named intergenic spacer (IGS). The coding sequences of the DNA units are conserved, whereas the IGS is highly variable in both sequence and length (cf. Polans *et al.* 1986; Rogers and Bendich 1987). These variations are useful in taxonomy, molecular evolutions and plant breeding studies (cf. Appels and Dvorak 1982; Maggini *et al.* 1988; Molnar *et al.* 1989).

According to Bellarosa *et al.* (1990), the restriction sites of the transcription unit 18S and 25S are very similar in ribosomal genes of *Q. cerris*, *Q. suber* and *Q. crenata* on the basis of both single *Eco* RI, *Bam* HI and *Sac* I and their respective double digestions endonuclease cleavage. Interspecific and intraspecific differences were mainly related to the intergenic spacer length variations.

In fact, after *Eco* RI digestion, both *Q. suber* and *Q. cerris* showed individual restriction patterns of ribosomal genes. Furthermore, the rDNA genes of both species appeared to be inherited codominantly in *Q. crenata*. Moreover, the restriction patterns of *Q. suber* provenances allowed their separation into three groups: Tyrrhenia, Apulia and Sardinia (Bellarosa *et al.* 1996).

The Tyrrhenian group appears to be the most homogeneous one because the corresponding three provenances showed identical patterns. The other two groups are more heterogeneous than the first one; the three examined Apulian sites, even if they are situated close to each other, seem to be most genetically heterogeneous.

Table 1 gives an overview of the lengths of the intergenic *Eco* RI fragments of the three examined taxa.

Table 1. *Eco* RI (25S) - *Eco* RI (18S) segment lengths of the *Quercus* taxa examined

		Tyrrhenian coast				Apulia			Sardinia	
Q.ce †	*Q.cr*	Viterbo	Circeo	Elba	Suver.	Pignic.	Lucci	Cerrito	Pattada	Berchid.
8.6	8.6	8.6	8.6	8.6	8.6	8.6	–	–	–	–
–	–	–	–	–	–	–	8.3	–	–	–
8.1	8.1	–	–	–	–	–	–	–	–	–
7.6	7.6	7.6	–	–	–	–	–	7.6	7.6	7.6
7.3	7.3	7.3	7.3	7.3	7.3	7.3	–	7.3	7.3	7.3
–	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	–	7.0
–	–	–	–	–	–	–	6.8	6.8	–	–
–	4.6	4.6	4.6	4.6	4.6	–	4.6	4.6	–	–
4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45
4.3	4.3	4.3	4.3	4.3	4.3	4.3	–	4.3	4.3	4.3
–	–	–	–	–	–	–	4.25	–	–	–
4.2	4.2	–	–	–	–	–	–	–	–	–

† *Q.ce* = *Quercus cerris*; *Q.cr* = *Quercus crenata*; all others are *Quercus suber.*

Discussion

The results seem to demonstrate genetic variability in both seed proteins and rDNA of *Q. suber.* The discrimination into three groups of provenances, derived from the study of ribosomal DNA genes, is confirmed by the distribution patterns of cork oak in Italy. Seed storage proteins supply less evident data, and this technique should be further developed.

Results obtained by the analyses of proteins and rDNA correspond to the separation of the Apulian provenances from the others. The Apulian populations of *Q. suber* are forest nuclei of a few hectares limited to the province of Brindisi, growing on red decalcified soil with intermediate levels of water table. In the past, these small cork oak areas attracted scientific interest (De Philippis 1937; Crivellari 1950; Corti 1955; Francini-Corti 1967) because they are separated from the main distribution area of the species and the sites apparently do not correspond to the ecological requirements of cork oak. Moreover, the trees in Apulian stands have particular reproductive cycle characteristics with the presence of acorns with both annual and two-year ripening and recurrent flowering (Corti 1955; Scarascia Mugnozza and Schirone 1983).

Research based on paleogeographic and phytogeographic evidence and on the biology of populations provided a model for studying the evolution of Apulian vegetation with particular reference to oak species (Schirone and Spada 1995). These studies suggest that Apulian populations of *Q. trojana, Q. macrolepis* and *Q. suber* are the result of migrations of the first two species eastwards and of cork oak westwards following a climate change with a peak about 20 000-17 000 years ago (Last Glacial Maximum).

Phytohistoric evidence (Amigues 1991) can confirm this hypothesis by demonstrating the presence of cork oak in Greece, even during ancient times. Even the presence of cork oak in Albania and Dalmatia (Giacomini and Fenaroli 1957) must be borne in mind, as well as the recognized presence in those regions of *Q. crenata* (probably a natural hybrid between *Q. suber* and *Q. cerris*). Therefore, the reduction of the distribution range of cork oak or local extinction in some areas can be due to anthropogenic pressure which seems to have acted on these ecologically rather unstable coenoses, notwithstanding a possible territorial recovery subsequent to a climatic improvement which took place about 6000 years ago. The movement of cork oak westward probably only left traces in southern Apulia (area not affected by glacial episodes) where micro-environmental characteristics of some sites allowed the survival of more ancient biotypes.

In the genus *Quercus*, the comparison between species is not easy owing to the difficulty in recognizing hybrids. Thus, the study of a species assumed to be a hybrid may contribute towards defining the criteria for discriminating the pure species. *Quercus cerris* and *Q. suber*, though belonging to the same group *Cerris*, are generally different, even at a morphological level. Hybridization between them is rather rare. When it occurs, the hybridization product is represented by *Q. crenata*, a sub-evergreen oak widespread in the Mediterranean region from France to Greece. Indeed, some authors believe that this taxon is the natural hybrid between *Q. suber* and *Q. cerris* (cf. Pignatti 1982), whereas other authors argue that it is a microspecies or an intermediate species (cf. Barbero *et al.* 1972) on the basis of the presence of *Q. crenata* in regions where *Q. suber* is absent (Fed. Rep. of Yugoslavia, Albania, Greece).

To define the origin and the taxonomic status of the species, morphometric analyses have been carried out on leaves and wood samples collected from several trees of *Q. crenata*, *Q. cerris* and *Q. suber* in Italy.

Wood samples have been studied at the macroscopic and microscopic levels and the observed results have been statistically analyzed. Macroscopically, hardwood, sapwood and tree rings are not easily distinguishable in *Q. crenata* and *Q. suber*. Broad rays are very evident and grain is strongly twisted. Microscopically, the vessels of *Q. crenata* wood appear located in long radial chains. Their diameter ranges from 20 to 40 mm in the early wood and from 100 to 150 mm in the late wood. Perforations are simple, tyloses abundant. The rays are uniseriate and multiseriate, the latter 25-30 cells wide. The statistical analyses of those data showed that in its anatomy, the wood of *Q. crenata* is intermediate between *Q. cerris* and *Q. suber* (Angelaccio *et al.* 1990).

Analyses of leaf characteristics have been carried out on 1000 leaves for each species (*Q. cerris*, *Q. suber* and *Q. crenata*). The characteristics considered were area, perimeter, maximum length, maximum width, length/width ratio, perimeter/length ratio. Analysis of variance and Duncan's test referring to single characters permit discrimination between *Q. cerris* and *Q. crenata*, but not between *Q. crenata*, and *Q. suber*, while PCA allows for clear distinction between the three taxa (Schirone *et al.* 1989).

Furthermore, based on its ecology, *Q. crenata* seems to be intermediate between cork oak and turkey oak (Mercurio 1985).

Results of the molecular study show that the main *Q. crenata* total protein patterns come from the addition of the *Q. suber* and *Q. cerris* profiles and that the rDNA genes of both *Q. suber* and *Q. cerris* are codominantly inherited in *Q. crenata*. This latter result is in agreement with studies of other authors who observed codominance of parental genes in hybrids of *Populus* and *Castanea* spp. (cf. D'Ovidio *et al.* 1990, 1991; Benedettelli *et al.* 1992).

On this basis, *Q. crenata* can be considered a hybrid between *Q. cerris* and *Q. suber*. This would explain the distribution and ecology of this species which is rather sporadic, with isolated individuals in areas having high natural or anthropogenic disturbance. However, to clearly define the origin of this taxon, further studies on its reproductive biology, complementary with our initial controlled crossbreeding trials, must be carried out. In fact, the antithesis period for *Q. crenata* is relatively close to that of *Q. suber*, but not to that of *Q. cerris* and other deciduous oaks having similar distribution. Therefore, its reproduction must be almost exclusively autogamic, in contrast with the data of several authors regarding the genus *Quercus* (Nason *et al.* 1992). One could also assume that *Q. crenata* is a hybri-species (*sensu* Nardi 1988: a fertile species ancestrally originated by hybridization), created during a more favourable climatic period than the current one, during which the cork oak occupied a larger area in the east and was characterized by a phenological cycle rather similar to that of *Q. cerris* (Palmarev 1989). This could explain its presence in the Balkan region. At the same time, the hybrid origin could explain the low capacity of *Q. crenata* to compete with parents and, thus, its scattered and occasional occurrence in oak stands.

Further deepening of these investigations may contribute toward clarifying the differentiation itineraries in the *Cerris* group and, more generally, explain the evolutionary role of hybrids (cf. Hewitt 1988; Schemske and Morgan 1990).

Conclusions

Several activities were started to develop a programme for *Q. suber* germplasm conservation. For example, a collection of the Italian provenances of cork oak has been established in the nursery of Tuscia University with 1-, 2- or 3-year-old seedlings from ten different areas.

Regarding a possible recovery of germplasm of the Apulian populations, a programme of micropropagation was developed using vegetative apices collected from 3-year-old plants. The culture medium used contained both the macro- and microelements of mineral medium WPM (Woody Plant Medium, modified) and the organic components of the medium named LP, enriched with BAP (2.5 mg/L) and IAB (0.01 mg/L). The neo-formed shoots developed a good root apparatus.

Finally, the inventory of the relic stands of cork oak in the province of Brindisi (Apulia) has been concluded. These stands will be protected by the Apulian Regional Forest Service.

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Research on the provenances of *Quercus suber* in Italy

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Genetic characterization

Research aimed at the genetic characterization of several Italian *Quercus suber* provenances and at the distribution of the species was carried out during 1995.

Nuclear ribosomal rDNA genes were extensively used in both phylogenetic and taxonomic studies at inter- and intraspecific levels, for identification of hybrids and for investigation of natural populations of species (see Appels and Dvorak 1982; Bellarosa *et al.* 1990, 1996; Bobola *et al.* 1992).

Previous studies carried out on the ribosomal rDNA genes of *Q. suber* provenances (Bellarosa 1996) showed that the intergenic spacer (IGS) can provide useful information with regard to the intraspecific genetic variation. Therefore, it seemed opportune to extend the ongoing research to include the ITS (Internal Transcribed Spacer) as well (see Fig. 1).

As already reported (Baldwin *et al.* 1995), the ITS region can be useful in providing a valuable set of characters for phylogenetic reconstruction in plants, in resolving conflicts between data sets and in supporting evidence about evolution. With the recent appearance of the polymerase chain reaction (PCR) technology, the entire ITS region and the two ITS-1/ITS-2 spacers can be easily amplified and sequenced by means of specific primers.

The ITS-1 and ITS-2 amplifications and sequencing of *Q. macrolepis*, *Q. pubescens*, *Q. cerris* and four Italian *Q. suber* provenances were carried out in the laboratory of the Dip. di Scienze dell'Ambiente Forestale e delle sue Risorse, and in collaboration with F. Maggini and R. Morocco.

The most interesting data obtained from these preliminary studies concern cork oak. Among the angiosperms sequenced to date, cork oak is the first species that shows two types of ITS-1: a short one of 181-183 bp and a long one of 212 bp. It is also interesting that the ITS-1 (181-183 bp) is the shortest intragenic spacer reported so far for angiosperm taxa. Moreover, the four cork oak provenances seem to differentiate among themselves at the short ITS-1 nucleotide level; while the long ITS-1 shows an identical nucleotide sequence in all provenances. The two types of ITS-1 show sequence homology of 64.85%. The other examined *Quercus* species show only one type of ITS-1 (212-214 bp) that is more related to the long ITS-1 observed in cork oak than to the short one.

The variations observed in the nucleotide sequences of ITS-1 appear to be useful to discriminate among the species and to draft a preliminary dendrogram of relationships between taxa. What emerges from these results is the different

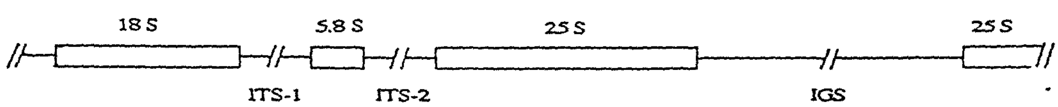

Fig. 1. Organization of a ribosomal DNA gene. ITS-1/5.8S/ITS-2 belong to the IT region.

position of *Q. suber* compared with other *Quercus* species. This evidence is indirectly confirmed by the results of recent karyological investigations obtained in collaboration with D'Emerico *et al.* (1995; pers. comm.). Based on Romero Zarco's asymmetry index (1986), the comparison between the karyotypic features of *Q. suber* and those of the other *Quercus* species shows that cork oak is not close to its own subgenus *Cerris*, as already suggested by Natividade (1954).

Distribution of *Q. suber* in Italy

Many investigations were carried out on the occurrence of cork oak in several Italian regions, with special reference to relic stands. In this way it was possible to locate an isolated cork oak stand in Basilicata that seems to be the most inland stand of the southern Apennines.

The data collected on cork oak distribution will be further evaluated in accordance with the European standard Corine Land Cover and the first cartography showing Latium region will be available next autumn.

The inventory of south Apulia (Brindisi province) relic stands has already been concluded (Fig. 2). Based on 1:25 000 scale, a number of maps indicating the precise position of each tree and their main dendrometric data have been constructed for each stand. Example of a transect and dendrometric data compilation is given in Figure 3 and Table 1 for Torre Pozzella stand. Moreover, the topographic map also

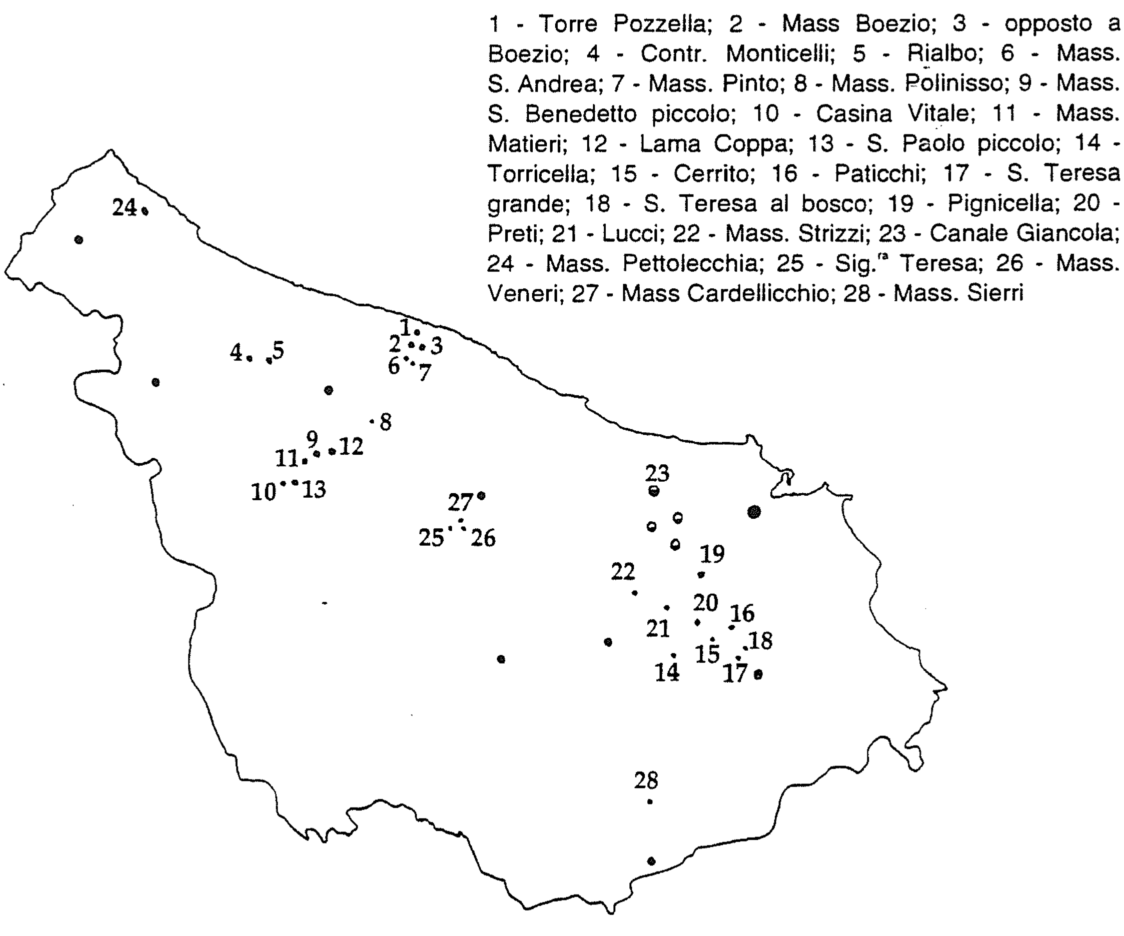

Fig. 2. Inventory of south Apulia (Brindisi Province) relic stands. The empty dots represent the stands already lost.

Table 1. Principal dendrometric data of the Torre Pozzella stand

Quercus suber	D diameter (cm)	G basal area (m^2)	HT total height (m)
plant 1	18.40	0.03	3.50
plant 2	31.80	0.08	5.00
plant 3	15.90	0.02	4.50
plant 4	14.30	0.02	4.00
plant 5	11.15	0.01	4.00
plant 6	19.70	0.03	5.00
plant 7	19.10	0.03	3.50
plant 8	–	–	4.00
shoot 8A	5.00	0.00	–
shoot 8B	4.00	0.00	–
plant 9	–	–	4.00
shoot 9A	19.10	0.03	–
shoot 9B	16.50	0.02	–
plant 10	13.40	0.01	4.00
plant 11	19.10	0.03	5.00
plant 12	25.50	0.05	6.00
plant 13	16.00	0.02	5.50
plant 14	29.30	0.07	7.00
plant 15	20.70	0.03	8.00
plant 16	–	–	9.00
shoot 16A	19.10	0.03	–
shoot 16B	24.50	0.05	–
shoot 16C	21.70	0.04	–
plant 17	–	–	5.00
shoot 17A	15.00	0.02	–
shoot 17B	12.00	0.01	–
plant 18	–	–	8.00
shoot 18A	28.70	0.06	–
shoot 18B	38.20	0.11	–
plant 19	25.50	0.05	6.00
plant 20	25.50	0.05	6.50
average	19.58	0.03	5.38
standard deviation	7.79	0.03	1.61

reports climatic and soil data for all studied stands. It was finally possible to verify that (1) Apulian *Q. suber* covers an area of only 45 ha, which is 230 ha less than in 1958, (2) cork oak in this area is characteristic with acorns ripening in 1 or 2 years, and (3) cork oak is able to grow on alkaline soil but its occurrence is related to higher availability of water compared with the surrounding area.

These investigations contributed to facilitating the decision of the regional forest authority to launch a programme for the protection and re-establishment of the species.

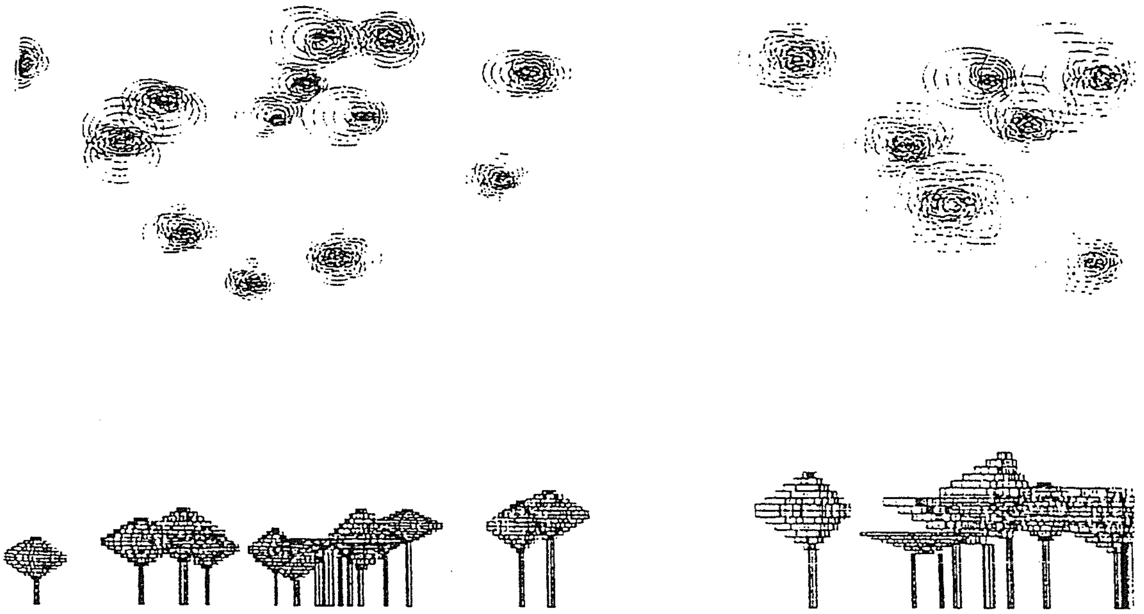

Fig. 3. Example of a transect in the Torre Pozzella stand.

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Analysis of genetic variation in French populations of cork oak using enzyme polymorphism: preliminary results

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In France, cork oak grows in four separate geographic areas: in Corsica, Provence and Catalogna, which are under the influence of Mediterranean climate, and in the Landes region along the Atlantic coast. In general terms, cork oak management is rather low (in Corsica and Catalogna) or quite absent (in Provence and the Landes region).

A good knowledge of genetic variation in cork oak using several types of genetic markers is crucial for the establishment of any conservation strategy of the species. In France a critical point is to assess the genetic differentiation occurring among the four distribution regions. With that purpose, a preliminary study of allozyme variation at eight polymorphic loci was carried out in six French populations of cork oak (two populations from the Landes region, two from Provence, one from Catalogna and one from Corsica). The genetic data obtained from French populations were compared with data from similar studies in 27 cork oak populations from other countries (Spain, Portugal, Morocco, Tunisia and Italy).

Based on the patterns of genetic variation at the eight polymorphic enzyme loci, two sets of populations were constituted. The French populations from the Landes region and from Catalogna belonged to the first set together with the populations from Spain and Portugal. Populations of this set were characterized by higher allelic richness and genetic diversity than the populations of the second set. Portugal and Spain might therefore be considered as the original area of cork oak. The French populations from Corsica and Provence were associated with the populations from North Africa and Italy (including Sicily) in the second set. This was characterized by overall lower allelic richness and genetic diversity. Moreover, alleles which were observed at low frequencies in the first set were found to occur at high frequencies in the second set of populations. These results suggest that North Africa and Italy may be areas of secondary extension of the species. Cork oak may have been introduced into those countries during ancient times. One more striking result is the occurrence of heterozygotes excess at several loci and in most cork oak populations. Because of lack of regeneration, these populations are mostly constituted of very old trees which are often more heterozygous than younger trees, as observed in several other tree species.

Results of the preliminary study of cork oak genetic resources in France and in the other countries of the western Mediterranean Basin are promising. However, more populations and more genetic markers should be studied in order to obtain a representative knowledge of the genetic organization in the species.

Regions of Provenance for cork oak in Portugal

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Background

The importance of artificial forest regeneration through sowing or planting has been increasing in cork oak management in Portugal. Because of direct interest of forest owners, or incentives from EU programmes to set aside farmland for slow-growing forest species, several thousand hectares of cork oak are reforested every year. Therefore, seed procurement is becoming a very important step, both in quantity and quality.

In contrast to previous periods when natural regeneration was the common practice, or seed for reforestations was collected from neighbouring stands, significant movement of acorns is now to be faced, often without a proper reference to the original place of collection.

Transfer of seeds can have either negative or positive effects on the genetic resources of forests. Lacking knowledge of norms of reaction of transferred genetic material means a high degree of uncertainty and, even if positive cases exist, they cannot provide justification. Serious economical consequences may follow. Undoubtedly, there is an urgent need for solid and reliable knowledge about the seed sources used for large afforestations. Well-known experiences were made in the case of *Pinus pinaster* and *Eucalyptus* sp. in France during 1985.

Provenance tests, the ultimate basis for delimiting Regions of Provenance and consequently seed transfer, do not exist for cork oak in Portugal yet. First steps are now being undertaken in the framework of the Concerted Action project *EU/FAIR 1-CT 95-0202* funded by the European Union.

Yet the management of the species, including artificial afforestations can not afford to wait for all scientific answers from provenance tests. Delimitation of Regions of Provenance based on an 'educated guess' and good knowledge of environmental parameters is the first step to be carried out (Varela and Eriksson 1995).

The delimitation of Regions of Provenance provides outputs at various levels. Immediately, the designation confirms that a Region has some practical use with regard to genetic information. It means a basis for characterization of the respective region useful to both authorities and users. At a scientific level it is the basis for evaluating the provenance tests. It is also a fundamental reference for breeding and gene conservation strategies (Varela and Eriksson 1995).

Cork oak may be considered the typical forest tree species of the humid western Mediterranean region and is widespread in Portugal. Dominant in large areas, codominant in others and scattered in some, its occurrence covers more or less the entire territory of the country. According to Natividade (1950), it is a 'plastic' species which can colonize almost all types of soils except for those with high active calcium contents.

Heavy frosts and severe summer drought are the limiting factors with regard to climate. The temperature limit allows the species to grow only rarely over 700 m asl. Cork oak stands grow well under yearly rainfalls from 400 to 2500 mm, as long as summers are sufficiently dry. Beyond ecological factors, human activities have a strong limiting influence on the species (Varela 1997).

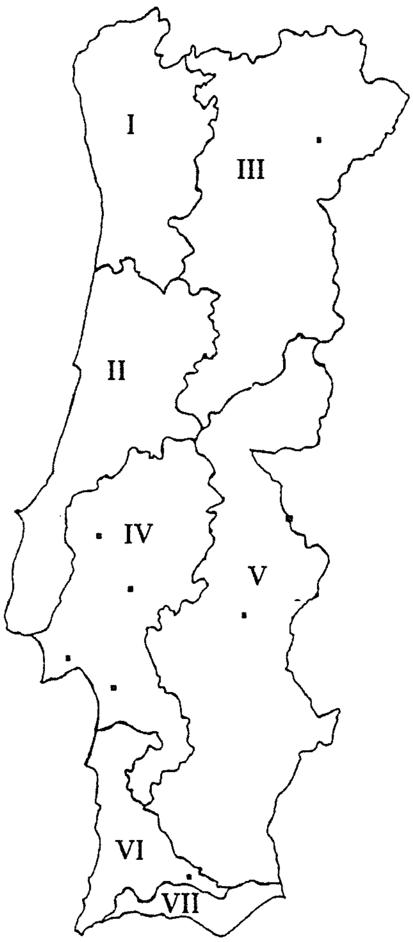
Delimitation of Regions of Provenance

Climate, soil and other environmental parameters play an essential role in the population dynamics of a species (Stern and Roche 1974; Anonymous 1991).

Based on these considerations, it was decided to cover the entire territory of the country for delimiting Regions of Provenance (Fig. 1). The species' distribution patterns in Portugal do not show any significant continuous zone where the species would be completely absent. A detailed splitting of the Regions of Provenance based on each individual, small occurrence of the species would not be a pragmatic approach to efficiently manage and control the transfer of forest reproductive material. It would also cause an even higher administrative load.

The decision was facilitated by a broad view of the future use of Regions of Provenance, beyond the economically important trade with seeds. Protection of the environment, conservation of genetic resources and specifically preservation of small marginal populations with low commercial value are cases that are associated with this methodology.

Regions of Provenance is a concept currently used in forestry practice to encompass several aspects of the management of genetic resources. The aim is to sample populations from geographical zones with correspondingly similar major relevant ecological parameters. Various authors have discussed the definition of Regions of Provenance (e.g. Burley and Wood 1976; Zobel and Talbert 1984) and it constitutes the basic terms of reference in the OECD Scheme (OECD 1974).



Region I	Noroeste Português
Region II	Centro e Oeste
Region III	Trás-os-Montes e Beira Interior
Region IV	Vale do Tejo e Sado
Region V	Alentejo e Beira Baixa
Region VI	Sudoeste
Region VII	Barrocal Algarvio

Fig. 1. Regions of Provenance for cork oak in Portugal. ■ = stands of acorn collection for the international provenance/progeny trial under the Concerted Action *EU/FAIR 1CT 95-0202.*

The work initiated on the delimitation of Regions of Provenance for cork oak in Portugal follows several main criteria. The ecological variability shall be low within Regions while it must be high between individual Regions. There are no rules prescribing the total number of Regions of Provenance. Individual conditions of the species must be taken into consideration for such a decision within the country. Each Region of Provenance must have a size that guarantees the seed supply with reproductive material under genetically sustained production. Boundaries should allow easy identification and practical operations.

Regions of Provenance based on environmental parameters are likely to be modified in the future, especially in view of the expectations related to genetic information from provenance testing. The main characteristics of the delimited Regions of Provenance for cork oak in Portugal are given in the Annex.

The described delimitation of Regions of Provenance for cork oak in Portugal was based on the following principal references:

- for climatic data and indexes: Albuquerque 1954, Emberger 1971, INMG 1991
- for climatic and geographical characteristics: Girão 1942; Emberger 1971; Teixeira and Pais 1976; Daveau 1985; Ribeiro *et al.* 1988
- for characterization of cork oak: Natividade 1950
- for genetic considerations: Stern and Roche 1974; Zobel and Talbert 1984; Anonymous 1991; Eriksson *et al.* 1993; Varela and Eriksson 1995.

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Annex

Region I Noroeste Português

Brief characterization

Lithology: It is a region dominated by the formation of sedimentary and metamorphic type rock and eruptive in a rather homogeneous configuration.
Climate: Wet summers and mild winters.
Management: The species shows a reduced area compared with its potential due to agriculture, strong replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative of Region I

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_s/M)	Frost (days/yr)
Monção/Valinha	42°04'	8°23'	80	182.3	2.8	12.7
Ermida do Gerês	41°42'	8°07'	525	422.1	8.2	–
Braga/posto agrário	41°33'	8°24'	190	231	4.3	28.7
Cabeceiras de Basto	41°32'	8°00'	280	–	–	–
Paços de Ferreira	41°16'	8°23'	320	254	4.8	64.5

Region II Centro e Oeste

Brief characterization

Lithology: It is a heterogeneous region.
Climate: Summers of medium drought and mild winters. Fog is a climatic factor that contributes considerably to differentiation of Region II from Region IV.
Management: The species shows a reduced area compared with its potential due agriculture, strong replacement by faster-growing forest species and urban activities. Presently, it is of almost no economical interest in this region.

Climate indexes for localities representative of Region II

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_s/M)	Frost (days/yr)
Nelas	40°31'	7°51'	440	160	3	26.4
Alcobaça	39°32'	8°58'	75	156	1.8	36.7
Paiã/escola agrícola	38°47'	9°12'	50	118	1	9.5

Region III Trás-os-Montes e Beira interior

This region deserves particular attention. Cork oak is characterized here by one outstanding zone (Terra Quente do Douro), with only scattered occurrence elsewhere. This is due to the orography profile of the region where altitude (translated in cold winters) banished the species in considerable areas. However, the species is present in several valleys where microclimate conditions occur. On the whole, the fact is that where cork oak is present in this region, it is always subjected to the same key ecological factor, the coldness of the winters.

The plan for this region still considers that changes in forest and agricultural policy may release potential zones for cork oak of which the ecological profile cannot be fitted in any other region.

Brief characterization

Lithology: It is a heterogeneous region.

Climate: Dry summers and cold winters.

Management: The species has two major zones: the Terra Quente where it is economically managed, and the rest where it is scattered because of climate adversities present on high mountains and reduced area compared with its potential due to agriculture, strong replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative for Region III:

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_e/M)	Frost (days/yr)
Chaves	41°45'	7°28'	348	89	2.6	55.5
Mirandela	41°31'	7°12'	250	60.6	1.5	59.4
Bornes	41°28'	7°01'	700	134.7	3.3	–
Figª Castelo Rodrigo	40°52'	6°54'	635	71	2.3	60.6

Region IV Vale do Tejo e Sado

Brief characterization

Lithology: It is a very homogeneous region of sedimentary deposits from the Tejo and Sado river beds.

Climate: Dry summers and mild winters.

Management: The species is intensively managed for economic purposes for cork production and for grazing. Yet it has suffered from replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative for Region IV

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_e/M)	Frost (days/yr)
Tancos/base aérea	39°29'	8°26'	78	110	1.5	35
Mora	38°56'	8°10'	110	82.8	1.2	33
Setúbal	38°31'	8°54'	35	109.8	0.9	8.2
Alvalade do Sado	37°57'	8°24'	61	69.8	0.6	36.8

Region V Alentejo e Beira Baixa

Brief characterization

Lithology: It is a heterogeneous region constituted by the formation of sedimentary and metamorphic type rock. Eruptive spots also appear.

Climate: Very dry summers and average winters.

Management: The species is intensively managed for economic purposes for cork production and for grazing. Yet it has suffered from replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative for Region V

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_s/M)	Frost (days/yr)
Castelo Branco	39°49'	7°29'	380	104.8	1.5	4.6
Évora/Mitra	38°32'	8°01'	200	92.7	1.2	21.2
Contenda	38°03'	7°04'	450	97.2	1.5	7.9
Ameixial	38°01'	7°52'	246	58.6	0.9	36.5

Region VI Sudoeste

Brief characterization

Lithology: It is a rather homogeneous region based on formations of sedimentary and metamorphic type.

Climate: Dry summers and mild winters.

Management: The species is intensively managed for economical purposes for cork production and for grazing. Yet it has suffered from replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative for Region VI

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_s/M)	Frost (days/yr)
Sant° do Cacém	38°01'	8°42'	228	128.5	0.9	0.7
Relíquias	37°42'	8°29'	270	111.7	0.4	0.2
Caldas Monchique	37°17'	8°33'	203	160.2	⁻1	0.2
S. Barnabé	37°21'	8°10'	250	106	0.4	–

Region VII Barrocal Algarvio

Brief characterization

The region is very specific in the context of Portugal (Girão 1942; Ribeiro *et al.* 1998).

Lithology: It is a region dominated by sedimentary formations.

Climate: Very dry summers and very mild winters.

Management: The species is a secondary species of the forest profile of the region. Yet it has suffered from replacement by faster-growing forest species and urban activities.

Climate indexes for localities representative for Region S. Bart. Messines VII

Locality	Lat. (N)	Long. (W)	Alt. (m)	Emberger coeff. (Q_2)	Summer index (P_s/M)	Frost (days/yr)
Tavira	37°07'	7°39'	25	89.8	0.8	2.3
S. Bráz Alportel	37°10'	7°54'	240	90.0	0.7	1
S. Bart. Messines	37°16'	8°18'	96	103.7	0.7	1

Open-pollinated progeny test of cork oak in Portugal

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Introduction

With the main objective of evaluating genetic control of some economic characteristics of cork oak such as cork quality, height and stem form, a progeny trial comprising 60 open-pollinated progenies was established in Portugal during the years 1993/94.

Materials and methods

It was originally planned to collect seeds from 70 trees representative of the major cork oak producing area. Owing to irregular fructification (Varela 1996), this objective could not be achieved. Acceptable levels of seed production occurred only in 62 trees. The following traits were initially used for the characterization of acorns from the various mother trees: 100-seed weight, seed length (100 seeds randomly sampled from each lot), seed width (100 seeds randomly sampled from each lot).

Nursery establishment of the 62 open-pollinated progenies took place in January 1993. The seedlings were raised in containers according to a randomized complete block design with 4 blocks. The characteristics assessed during the nursery phase were as follows:

- weekly observations on the seedlings' emergence
- germination capacity
- pre-planting height recorded on 40 seedlings for each progeny (10 seedlings/block).

The seedlings were planted out during autumn/winter 1993/94 at three sites.

Sites	Latitude (N)	Longitude (W)
Lamarossa- Herdade da Machuqueira do Grou	39°03'	8°44'
Sta. Margarida do Sado- Herdade de Miranda	38°10'	8°18'
Santiago do Cacém	37°58'	8°32'

The experimental design was randomized complete blocks: 60 progenies, 6 blocks, 60 plots/block.

No. of plants:		Spacing	8 x 4 m
per plot	8	per plant	32 m²
per block	480	per plot	256 m²
per site	2880	per block	15 360 m²
Total plants	8640	per site	92 160 m²
No. of progeny:		per site	9.22 ha
per plot	8	Total (3 sites)	27.66 ha
per block	8		
per site	48		
Total progeny	144		

During November 1994, observations on survival rate were also performed on all plantation sites.

Results

Seed harvest

Insufficient seed production was obviously the major limiting factor for the establishment of the progeny test. From 104 trees previously monitored for flowering production, only 25 bore seeds. This means a rate of 24%. Scarce rainfall, causing poor pasturages, led grazing animals to feed on high quantities of acorns, further diminishing the already low seed availability.

The selection of new trees under the criterion of fruit abundance took place during the short period of seed ripening (see Aguiar 1994). Altogether 37 trees were selected in this way. This selection presumably represents sampling of the entire potential variability of the populations. The appraisal of heritability values is thus more reliable than if based only on the distorted sampling of a set of plus trees.

Nursery stage

Highly significant differences ($P<0.0001$) were observed among the 62 candidate trees for seed length and seed width. Among the progenies, greatest differences were found for nursery germination and pre-planting height.

Large variation is also evident for 100-seed weight, the lowest value being 297 g in the seed lot of tree F16 and the highest 909 g (F33); with a mean value of 572.27 g and standard deviation of 140 g.

The nursery germination capacity of the 62 seed lots was also evaluated. Values of up to 70% were recorded for 50 progenies, despite the fact that one-third of the seeds in all seed lots showed signs of *Balaninus* sp. damage. The best result for nursery germination capacity was obtained from seeds collected from candidate tree F62 (94.75%), and the lowest value was recorded in the seed lot from tree F49 (39%).

Candidate trees F40 and F49 were excluded from the trials because of overall poor seed germination. However, they were studied during the entire nursery stage. Figure 1 shows the germination curves for 5 families with high values for nursery germination capacity and 5 families with poor behaviour for the same characteristics. The seedlings' emergence was observed weekly and it was noted that the third month was the most important. During this period, the proportion of germinated seedlings increases rapidly for all progenies. At the end of the fourth month, the germination period was generally finished. There were clearly no differences in germination patterns, despite the differences obtained among the different seed lots with regard to the nursery germination capacity. Germination in the nursery during the first 40 days only accounts for a small proportion of the germinated seedlings.

The mean values for pre-planting height revealed high variability ranging from 36.3 cm (progeny F15) to 22.2 cm (F29), with an average value of 29.2 cm and a standard deviation of 9.7 cm.

Field trials

The scarce rainfall after planting of the seedlings in the field caused very high mortality in all progenies of the Herdade de Miranda field trial. Consequently, the survival rate was only evaluated for the other two planting sites. The progeny trial of Herdade de Miranda was later replaced with stock plants from the nursery.

Average values of the survival rate, assessed in November 1994 in the two field trials, were 60% (Machuqueira do Grou) and 87.5% (Santiago). The data performed through analysis of variance showed no significant differences among families in both sites. Correlation coefficients for all studied characteristics in the nursery and in the field were calculated. The most important results are related to the pre-planting height, which revealed a significant correlation with 100-seed weight (r=0.581**), seed length (r=0.463**) and seed width (r=0.484**). The nursery germination capacity did not show any significant correlation with other traits. The results from this analysis are shown for nursery germination and 100-seed weight in Figure 2.

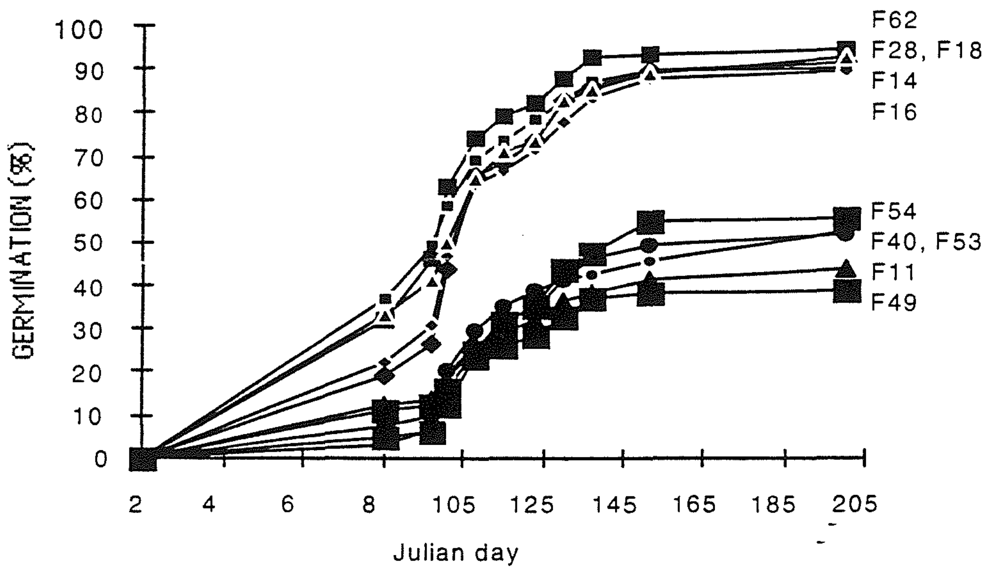

Fig. 1. Germination curves for five families with high values of nursery germination capacity and five families with poor behaviour.

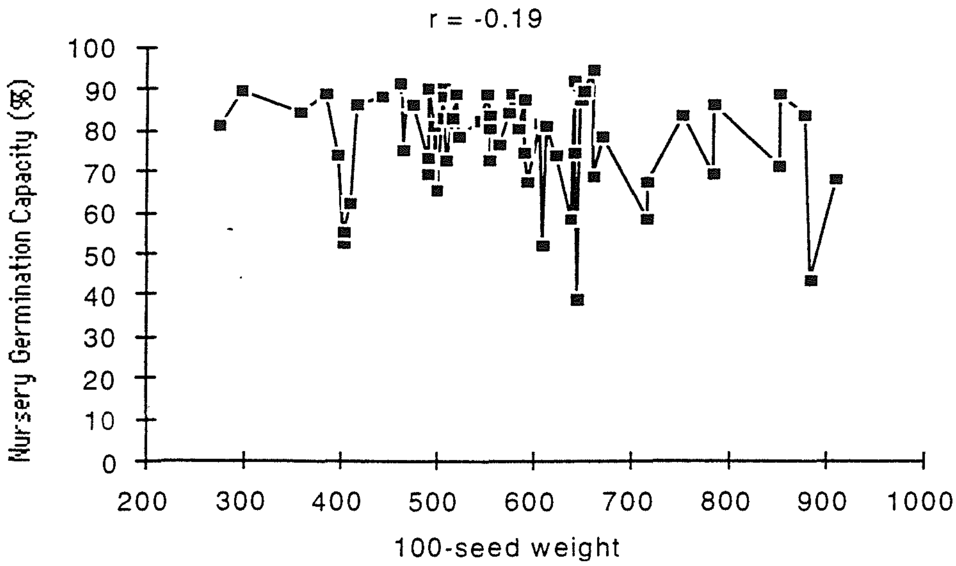

Fig. 2. Relationship between 100-seed weight and nursery germination capacity.

Discussion

The high discrepancy between flowering and fructification of trees, as observed in the major cork oak producing area, must be taken into account when planning future breeding strategies (see Aguiar 1994). This is particularly important with regard to the establishment and management of seed orchards. The ongoing studies of the reproductive behaviour of *Q. suber* should contribute knowledge to these observations.

Highly significant differences ($P<0.0001$) were observed among the 62 candidate trees for seed length and seed width. High variation for 100-seed weight was also evident. Significant differences for pre-planting height were observed among progenies. These results might be indicative of a good level of genetic variability.

Weekly observations of the seedlings' emergence during the nursery stage revealed that the germination rate was highest (germination peak) during the third month. The pre-planting height presented significant positive correlations with all the other characteristics under study, except nursery germination. These correlations suggest that the initial vigour of seedlings is probably better with the increasing 100-seed weight and the seed size.

The survival rate of progenies was observed at two planting sites. The first site, Santiago, with 87.5% rate represents excellent results regarding the behaviour of the species. No significant differences were obtained for the survival rate among progenies. However, observations in October 1996 showed low mortality. Research based on field trials carried out over several decades should certainly not rely on good climatic conditions.

The *Q. suber* trials established from seeds are associated with various factors that are hard to predict. An irregular acorn production may result in loss of a trial which may eventually be re-established only after a sufficient new mast year. This represents considerable losses in terms of time and financial resources.

Therefore, all efforts should be made to control factors which can improve the survival of the plants. Taking into account the unpredictable climate of Mediterranean countries, watering during the first two (or even three) years after the planting shall be an obligatory measure in all trials.

Acknowledgement
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Characterization of marginal populations of *Quercus suber* in Spain[1]

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Introduction

The definition of Regions of Provenance for *Q. suber* made a revision of the Spanish distribution area of the species necessary (Díaz-Fernández *et al.* 1995). The survey showed the occurrence of a particular type of stands, so called "marginal populations". Characteristics defining these cork oak forests are: small size, geographic isolation from the main distribution area of the species and environmental conditions generally far from the species' optimum. These traits make the marginal populations interesting from the point of view of genetic resources, since particular genetic structures may have developed, as considered in other proposals for the conservation of genetic resources in cork oak (Varela and Eriksson 1995). However, at the same time, these populations are very fragile; their small size and the restrictive environment cause a poor response ability against disturbance and a great risk of disappearance.

Little and disproportionate information existed about the characteristics of these stands, their extension and their conservation problems (Gil 1995). A broader comparison was not possible, thus it was difficult to provide guidelines for the conservation and management of these genetic resources. It was decided to make a detailed study on their present status (see also Report of the first two *Q. suber* Network meetings, IPGRI 1995).

Objectives

The main objectives of this study are:

- to gather the information needed to quantify the relative importance of each population as genetic resources
- to know the conservation constraints and problems of each population
- to identify subjects which need additional research
- to propose suitable actions for their conservation and management.

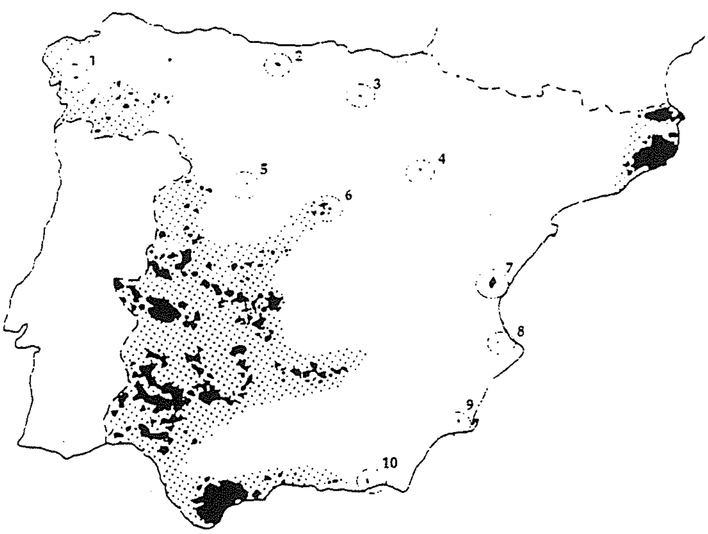
Methodology

The studied populations (Fig. 1) were chosen covering three main different ecological conditions where marginal cork oak forests appear: thermic northern sites, continental sites and coastal Mediterranean sites. Within each group, stands with sufficient size and growing in extreme environments were preferred.

In each population, a preliminary description was undertaken, gathering information on the location, access, ownership, surface, characteristics of the stand and previous management. Forest structure and dynamics were evaluated in the field.

Within each population, sampling was aimed at the most representative cork oak stands. Circular plots (7 to 10) with 10 m radius were defined. This number of plots and the subjectively chosen stands do not allow for extension of the results to

[1] This work has been supported by the Service of Genetic Material of the Spanish Dirección General de Conservación de la Naturaleza (Ministry of Agriculture).



1. Cuenca del río Ulla (C/PO)
2. Potes (S)
3. Bozoo (BU)
4. Sestrica (Z)
5. Foncastin (VA)
6. Hoyo de Manzanares (M)
7. Sierra Calderona (CS)
8. Pinet (V)
9. Sierra de Carrascoy (MU)
10. Haza de Lino (GR)

Fig. 1. Location of the studied marginal populations of cork oak. The map also shows the distribution area of the species in Spain. Main stands are in black.

the whole forest area. This objective should be fulfilled with complete inventories. However, the approach chosen provides fully sufficient knowledge of structural and dynamic features.

In each plot, the following data were recorded:

- number of trees present in the plot
- height and diameter at breast height (dbh) measured on each tree
- regeneration of tree species: all individuals below 1 m height.

Another parameter considered was the floristic diversity, expressed as Margalef index H (Margalef 1980), being:

$$H = \log_2 \frac{N!}{N_1! \cdot N_2! \cdot \ldots \cdot N_s!}$$

where N is the total number of trees of all species, and N_1, $N_2 \ldots$, N_s is the respective number of trees of each species. This index has been chosen because it takes into account the population size, not only the relative proportion between species. It has been calculated with densities of mature trees, without considering seedlings.

Results

In all studied populations, cork oak trees could be grouped into a few diameter classes. In all cases, 70% of adult trees were included in three consecutive classes. In some populations, this concentration of diameter classes is even more obvious. Around 80% of all trees in Sierra Calderona had diameters under 20 cm (diameter classes I and II); in Bozoo and Sestrica the same classes included 84% and 92%, respectively, of the total number of oaks. Moreover, in the three mentioned populations the origin of the trees was mostly vegetative – more than 70% from sprouts, while in the rest of the populations this proportion was up to 40%.

These features are caused by the past management, mainly charcoal and firewood extraction, which gave rise to nearly even-aged stands. In the three mentioned populations (Sierra Calderona, Bozoo and Sestrica) the forest was

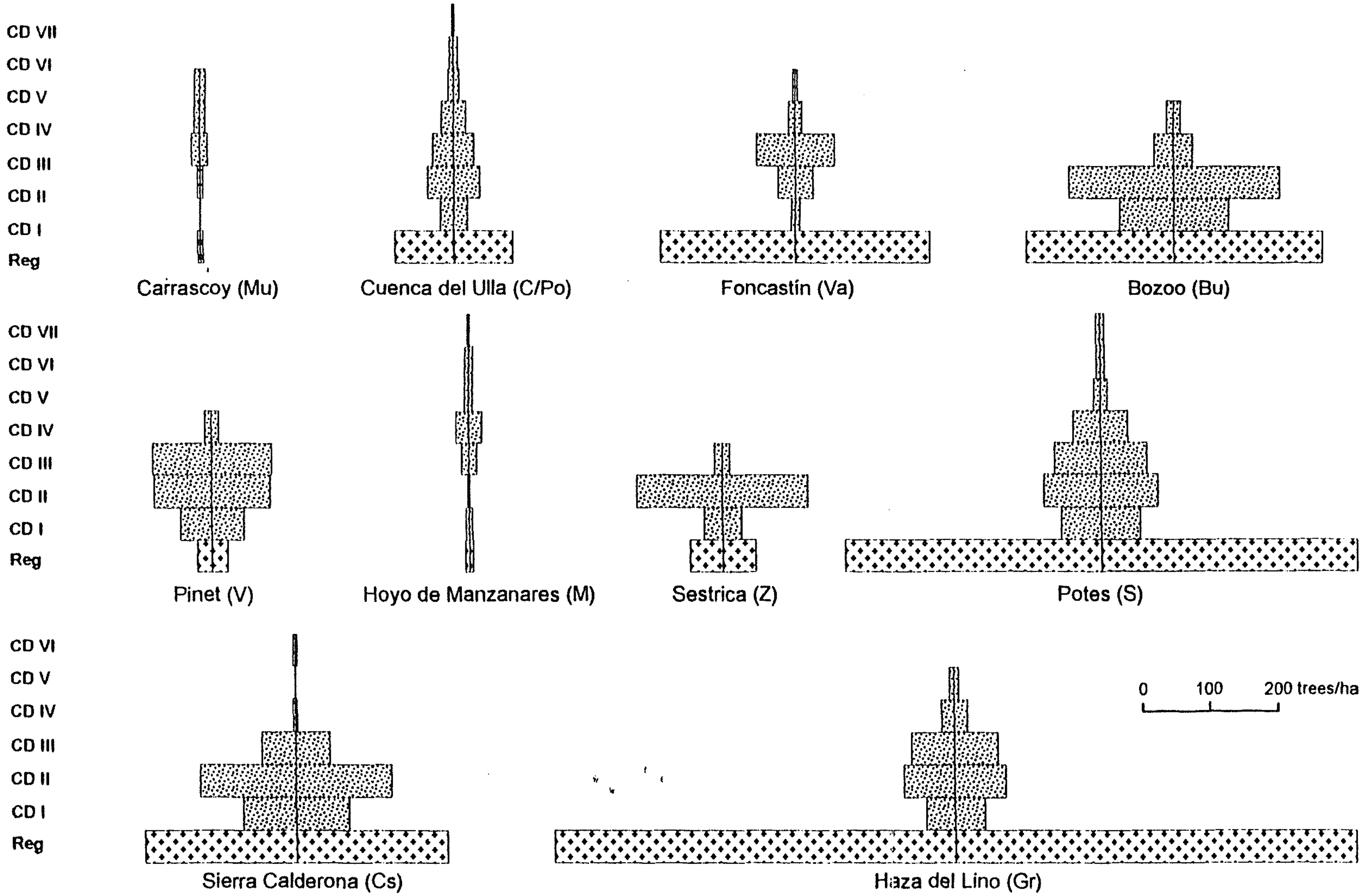


Fig. 2. Density expressed as (trees/ha) in each population. Reg = regeneration; CD I - CD VII = diameter classes.

completely felled after the civil war. Populations with older trees - Cuenca del río Ulla, Potes and Hoyo de Manzanares - are forests in which firewood extraction was less intense or more ancient. Other circumstances, such as cork exploitation or inaccessibility, also allow for the preservation of old trees.

Figure 2 represents the demographic structure of each stand. Without considering regeneration, all of them show a trend to ageing, with the density of diameter class I being lower than the density of older trees. In some populations, such as Haza del Lino, Foncastín and Potes, this trend is counteracted by a good regeneration of cork oak. An analysis of survival dynamics would be needed. In other cases, the situation is worsened by very low reproduction rates - Sierra de Carrascoy, Hoyo de Manzanares and Pinet. In these populations the density of cork oak regeneration is lower than the density of trees in the lower diameter classes, and thus the replacement of adult trees is not assured. Absolute data show the situation more clearly: in the stand Pinet, 11 young cork oaks were found (4 seedlings and 7 sprouts) on a total sampled surface of 2512 m^2. In Hoyo de Manzanares, 3 seedlings and 1 sprout appeared on 3140 m^2. In Carrascoy, only one young cork oak individual was found. Besides, our data generally overestimate the number of cork oaks, since the sampling was made around the *Q. suber* stands, where seedlings are more abundant than in plots without the species.

Different reasons explain the low reproduction rate of cork oak in each population. In Hoyo de Manzanares, adult trees were healthy and had good fructification, but germination was limited by the edaphic conditions: granites and sands, with steep slopes where soil evolution is difficult. In Pinet, oaks are rather short (less than 10 m), and have suffered from recent fires (the last one in 1991); trees showed a great number of new shoots, but acorn production is very scarce. In Carrascoy, where only 10 cork oaks appeared, all the trees were affected by drought, with tip decay and symptoms of a *Hypoxylon mediterraneum* attack. Small quantities of acorns are produced but, even if some of them germinate, they can not resist the drought.

In Table 1, all studied populations are classified according to the rate density of regeneration/density of mature trees.

Degree of dominance of cork oak (Table 2) is explained on the basis of different factors. In general, when cork is exploited, the species is favoured. When cork oak is dominated by other species, it can be due to a lack of interest in management (a low number of oaks, for example), or to limiting environmental conditions. Recent fires lead to the dominance of *Q. suber* because of its resistance to this event while the rest of the trees are burned.

The percentage of *Q. suber* seedlings compared with other species showed a trend in the decrease of cork oak dominance in all populations, with this trend being most pronounced in the populations Sierra de Carrascoy and Hoyo de Manzanares, where the ratio is about 2% (Table 2). Percentage of decrease also points to these two populations as the most regressive ones.

Table 3 shows the tree species in each population with regard to diversity. The proportions of the number of trees of each species are presented in Figure 3. In this figure, values of Margalef index for diversity are also indicated. In general, high floristic diversity was found, since environmental conditions allow not only the existence of cork oak, but also the presence of other species, some of them having marginal character: for example, *Q. pyrenaica* and *Q. ilex* in Potes, *Q. faginea* in Sestrica, or *Pinus pinaster* in Bozoo and Pinet.

Table 1. Density of regeneration of cork oak (Dr), density of mature cork oaks (Da) and ratio between them (R) (for abbreviations relating to the stands studied, see Fig. 1)

	GR	VA	S	CS	BU	C/PO	Z	M	MU	V
Dr	1175.16	391.7	748.41	436.31	429.94	171.18	95.54	12.73	7.96	43.79
Da	420.38	226.1	545.38	544.59	553.34	242.83	328	101.9	63.69	453.82
R	2.79	1.73	1.37	0.8	0.77	0.7	0.29	0.12	0.12	0.096

Table 2. A: Percentage of mature cork oak trees with respect to the number of mature trees of all species in the plot. B: percentage of juvenile cork oak trees with respect to regeneration of all species in the plot. (A-B): difference of the % rates. P: loss of dominance in % expressed as: [(A-B)/A] × 100 (for abbreviations relating to the stands studied, see Fig. 1)

	GR	S	VA	BU	V	Z	C/PO	CS	MU	M
A	100	42	47.7	36.4	85.7	67.6	38.4	89.5	24.2	28.3
B	73.7	30	26.4	17.6	38	29.2	13.4	30.5	2	2.1
A-B	26.3	12	21.3	18.8	47.7	38.4	25	59	22.2	26.2
P	26.3	28.5	44.6	51.6	55.6	56.8	65.1	65.9	91.7	92.5

Table 3. Tree species present in the plots of each population, according to their dominance

Bozoo (BU)	*Q. suber* (QS), *Pinus sylvestris* (PS), *P. nigra* (PN), *Q. ilex* (QI), *P. pinaster* (PR), *Arbutus unedo* (AU), *Q. pyrenaica* (QPy), *Q. faginea* (QF), *Q. petraea* (QP)
Cuenca del Ulla (C/PO)	*Q. robur* (QR), *Q. suber* (QS), *P. pinaster* (PR), *Q. pyrenaica* (QPy), *Arbutus unedo* (AU), *Pyrus cordata* (PC), *Castanea sativa* (CS)
Potes (S)	*Q. suber* (QS), *Arbutus unedo* (AU), *Q. ilex* (QI), *Prunus avium* (PA), *Ilex aquifolium* (IA)
S. Carrascoy (MU)	*Q. ilex* (QI), *Q. suber* (QS), *Pinus halepensis* (PH)
Foncastín (VA)	*P. pinea* (PP), *Q. suber* (QS), *Q. ilex* (QI)
Sestrica (Z)	*Q. suber* (QS), *Q. ilex* (QI), *Q. faginea* (QF), *P. pinaster* (PR)
Hoyo de Manzanares (M)	*Q. ilex* (QI), *Q. suber* (QS), *Juniperus oxycedrus* (JO)
Pinet (V)	*Q. suber* (QS), *P. pinaster* (PR), *Q. ilex* (QI), *Arbutus unedo* (AU)
S. Calderona (CS)	*Q. suber* (QS), *P. pinaster* (PR), *Arbutus unedo* (AU), *Q. ilex* (QI)
Haza del Lino (GR)	*Q. suber* (QS)

The degree of tree diversity in each stand is explained by climatic conditions and by management of the forest. Northern populations – Bozoo, Cuenca del Ulla, Potes – were most diverse, because they gather Mediterranean species together with deciduous trees. Besides, two of them (Bozoo and Cuenca del Ulla) were afforested with pines in the past: *P. pinaster* in Cuenca del Ulla and *P. nigra* and *P. sylvestris* (already present before afforestation) in Bozoo. In both populations, the highest number of species was found in plots with these pines. The effect of reforestation results in an increase of other trees, either due to the establishment of suitable conditions for their growth, or to the measures aimed at protecting the plantation (mainly from cattle grazing). The latter measures also favoured spontaneous vegetation in the observed plots.

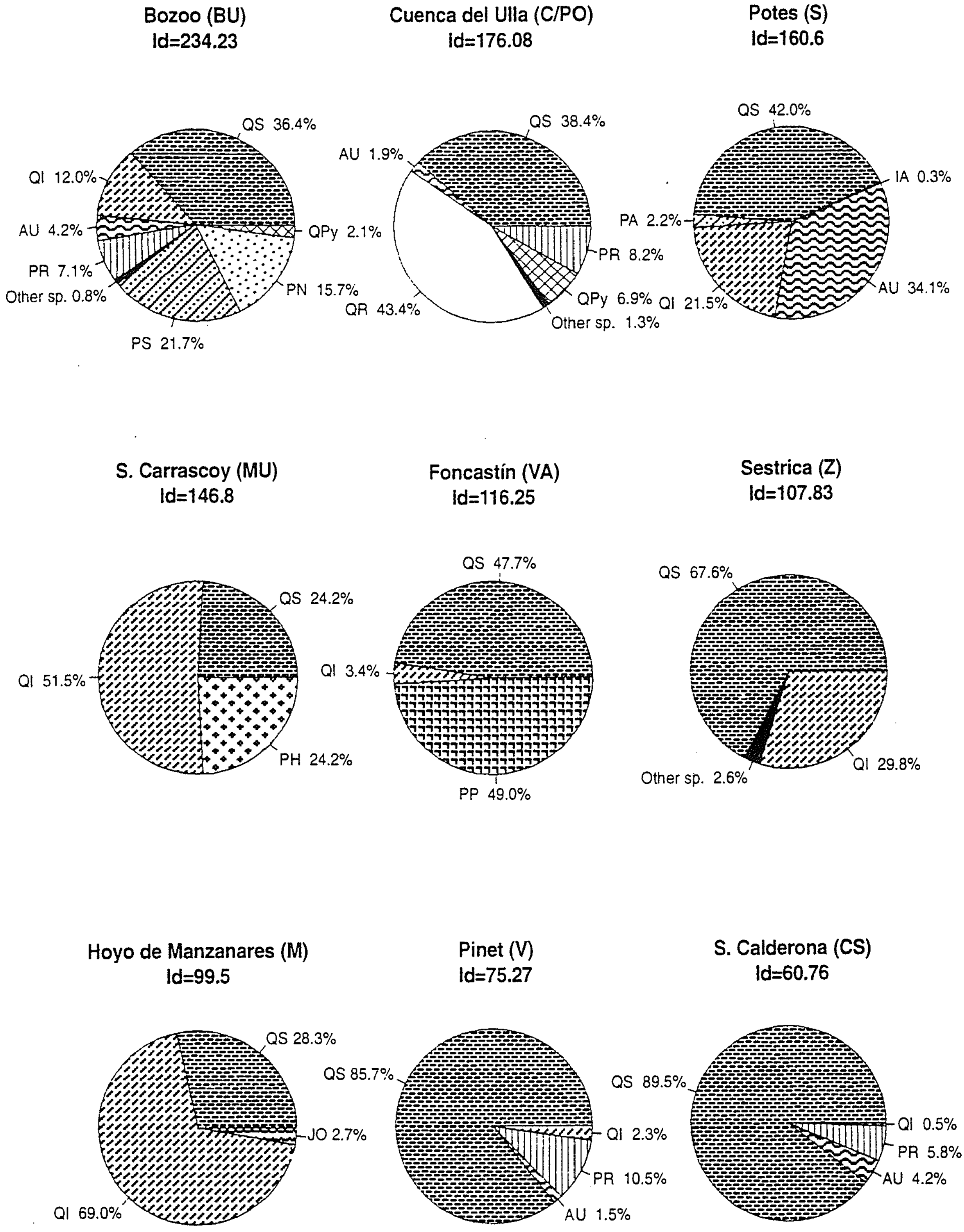


Fig. 3. Percentage of trees in each population and Margalef diversity index (Id), calculated with the densities of mature trees (trees/ha). AU=*Arbutus unedo;* IA=*Ilex aquifolium;* JO=*Juniperus oxycedrus;* PA=*Prunus avium;* PH=*Pinus halepensis;* PN=*Pinus nigra;* PP=*Pinus pinea;* PR=*Pinus pinaster;* PS=*Pinus sylvestris;* QI=*Quercus ilex*; QPy=*Quercus pyrenaica;* QS=*Quercus suber;* QU=*Quercus robur.*

Medium values of diversity were found in Sierra de Carrascoy, Foncastín, Sestrica and Hoyo de Manzanares. The low diversity of Pinet, Sierra Calderona and Haza del Lino is due to historic events: charcoal and firewood extraction was intense in the three stands, which also have suffered from recent fires. In Haza del Lino, cork oak is the only tree species, since the forest is regularly brushed and selective cuttings are made to favour cork exploitation.

Conclusions

The present status of *Q. suber* marginal populations is influenced mainly by the uses and management of the forest. Cork exploitation favours the species while, in the past, charcoal and firewood extraction gave rise to even-aged forests where vegetative reproduction dominates. Environmental conditions determine the biological diversity and the ability of populations to response to disturbances.

Two trends are shared by all populations: low number of young trees and poor regeneration, and loss of dominance. Except for Haza del Lino, the regeneration rate is higher for other tree species than for cork oak.

The results obtained allow the classification of the populations according to their greater or lesser decline risk. Criteria taken into account for this classification include the density of adult trees, density of regeneration, percentage of loss of dominance, surface of the populations and presence of other *Q. suber* stands in the neighbourhood. Haza del Lino has been qualified as '0', with no current risk of disappearance. Potes, Sierra Calderona, Foncastin, Sestrica, Cuenca del río and Bozoo are at slight risk of decline, and Pinet and Hoyo de Manzanares at greater risk. On the other hand, Carrascoy can be considered almost extinct.

The preliminary results obtained have mainly a qualitative character. In future, it would be desirable to carry out more exhaustive inventories, as well as precise studies on the phenology and mating system and an assessment of the regeneration patterns in each population. This information would allow us to establish priorities and management programmes for the conservation and increase of genetic diversity in the populations.

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Quercus suber genetic improvement programme in Spain: plus trees selection[1]

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Introduction

In 1987, a programme on *Q. suber* genetic improvement was initiated by the Genetic Material Service. The first stage of this programme was the selection of plus trees, finished in 1995. Selection was carried out in La Almoraima, a public estate in the province of Cádiz. It contains about 15 000 ha of cork oak forest are with nearly 800 000 trees of the species. Climatic factors of the region are highly suitable for cork oak growth so, in general, trees show good performance and a high cork production, but of poor quality. Regarding cork exploitation, the estate is divided into 9 plots (or 'dehesas'), corresponding to the number of years of cork rotation.

This selection provides the necessary basic step of an improvement programme: a group of individuals with the desired character (in this case, a high-quality cork), from which new superior trees can be obtained.

Methodology

The first step was the preselection of trees in the field (Fig. 1). Two factors were considered: the characteristics of bark, some of which are related to cork quality, and the overall performance of a tree (vigour, health, growth, morphology). Different numbers of trees were chosen in each dehesa, ranging from 21 to 92. For each selected tree, three 'control' trees with similar characteristics were taken among those closest to the main tree. These three control trees were submitted to the same analysis as the candidate cork oak. Altogether 1780 cork oaks, including the control trees, were analyzed during the period 1987-93.

Field data were taken simultaneously with the selection of these groups of four trees each. Parameters for cork evaluation were assessed after the extraction and boiling of the cork.

Information about each selected tree and its controls was recorded on specific forms, which included all measured values and the location of the trees. A database was established to facilitate the availability of information. Main data were accompanied with a photograph of the tree and a sketch showing its position.

The data available were grouped into three sections:

- Phenotypic data: height, coefficient of trunk surface, bark, etc.
- Productivity and quality of acorns. These data were, however, not taken into account as a criterion for selecting the trees to collect acorns in the whole forest and in the same year, and to avoid the influence of different conditions with regard to fructification.
- Productivity and quality of cork: weight per tree and proportion of each quality class expressed in percentage.

[1] This work has been supported by the Service of Genetic Material of the Spanish Dirección General de Conservación de la Naturaleza (Ministry of Agriculture).

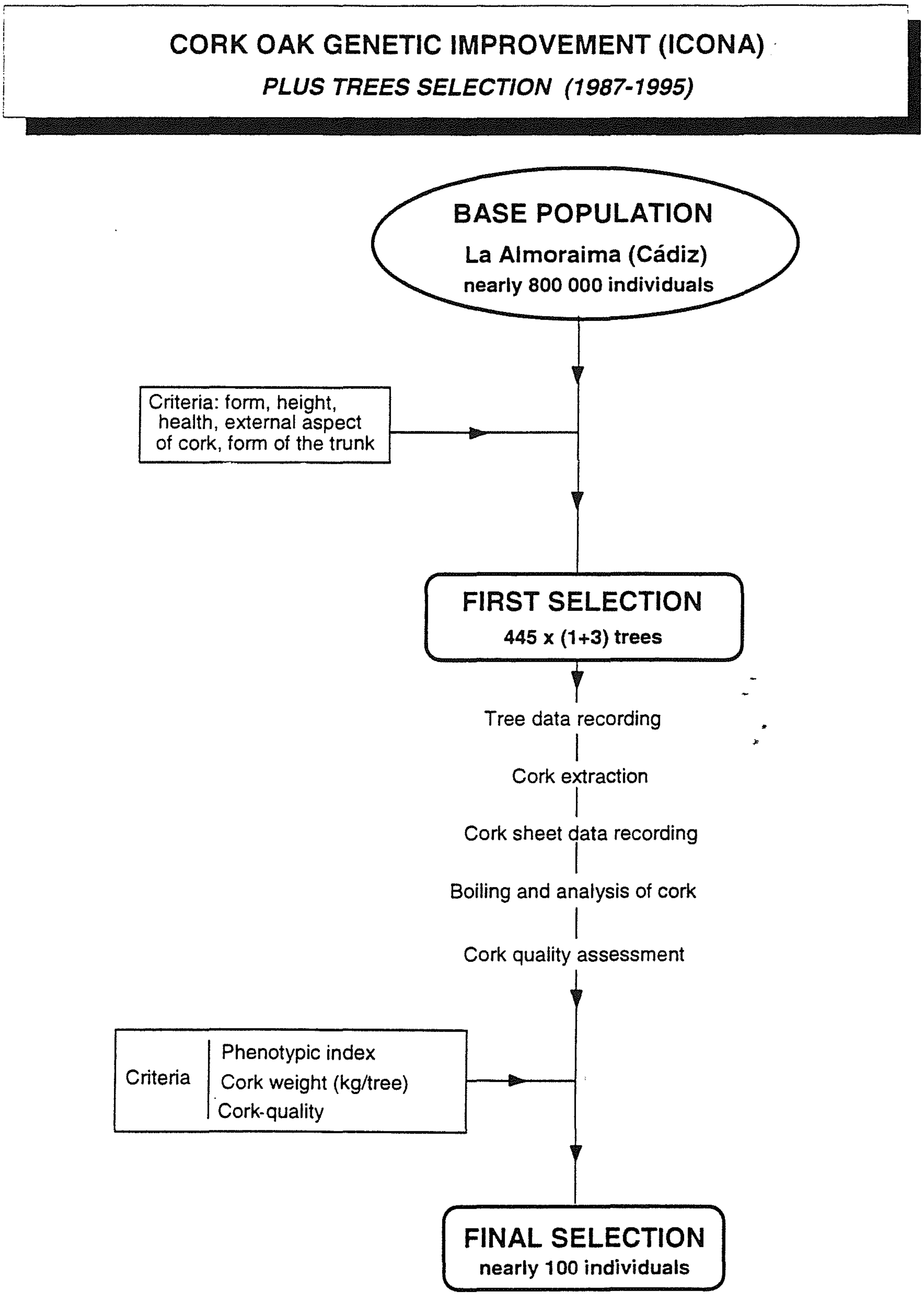

Fig. 1. The process of plus trees selection in La Almoraima, Spain.

The measured values were translated into points which represent the quality of the tree. The final score is a sum of points for phenotypic performance, acorn production and cork. Points for the cork characteristics are obtained from values of cork weight (maximum of 10 points when the tree produces 200 kg or more), plus an index of quality. This index gives the additional points for each quality-thickness class, based on the weight percentage of each class multiplied by a factor relative to the monetary value of that class. A 'perfect' tree would reach 100 points: 50 corresponding to section A, 10 to B and 40 to C. As group B was not considered at all, the total possible sum was 90 in this case.

Once labels were marked for all plus trees in a plot, the next step was to make the final selection, taking into account the data maintained in the forms.

For each tree of a group, candidate or control, the following values were considered: cork weight, points in A group and points for cork quality. Lower limits were established for these parameters; at first, the limits were 100 kg of cork, 22 points for the phenotype and 10 points for quality (Fig. 2). Trees that reached these three limits were directly selected. When a control tree was better than the candidate, the former replaced the candidate.

A second revision was then carried out, analyzing each tree separately. When necessary, less strict limits were considered, provided that the selected tree was superior to the average of the preselection in the year. Revision was repeated after 10 plus trees were sampled.

Different analyses were performed with the data, e.g. comparisons between dehesas, superiority of the cork quality of the selected trees compared with their control trees, and correlations between production and quality.

Results

Between 1987 and 1993, 73 trees were selected. The final selection for 1994 and 1995 has not yet been made, owing to the period in which cork sheets must be dried before cork analysis (one year). With these individuals, we expect to reach a total number of 100 plus trees, thus fulfilling the initial objective of the programme.

The mean values of the selected group were:

- phenotypic index (points of group A) – 33.7
- weight of cork per tree – 121.2 kg
- cork quality index – 14.6
- total sum – 52.5 out of 90.

Great differences have been found between the different plots, mainly with regard to the phenotypic and quality index (Fig. 3). The analyzed data show a negative correlation between cork weight per tree and its quality, thus confirming the previous understanding of the differences between quality of habitat and quality of cork.

The results of the analyses regarding superiority in cork quality were surprising: the mean superiority was assigned 307%, but some values were close to 3500% (Figs. 4 and 5). Such variability in trees standing very close to each other, thus growing under identical silvicultural and ecological conditions, support the assumption of a strong genetic control of cork quality. As a consequence of this assumption, cork oak improvement should be aimed at individuals rather than stands. Nevertheless, the analysis of the geographic distribution pattern of plus trees allowed a selected seed stand to be defined, characterized by higher mean quality than the rest of the plots (see Table 1).

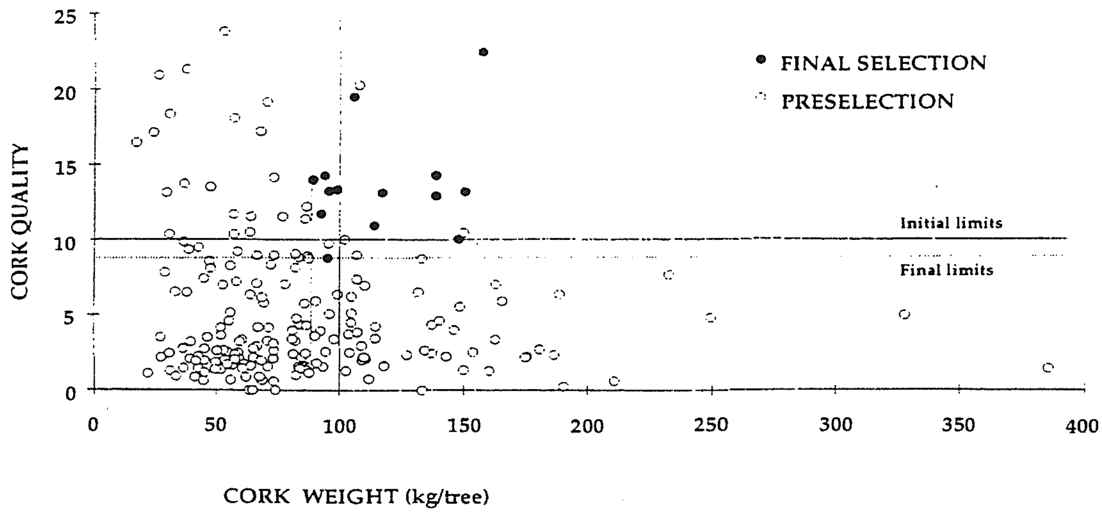

Fig. 2. Limits for the preselection and final selection of trees based on parameters related to cork quality and cork weight.

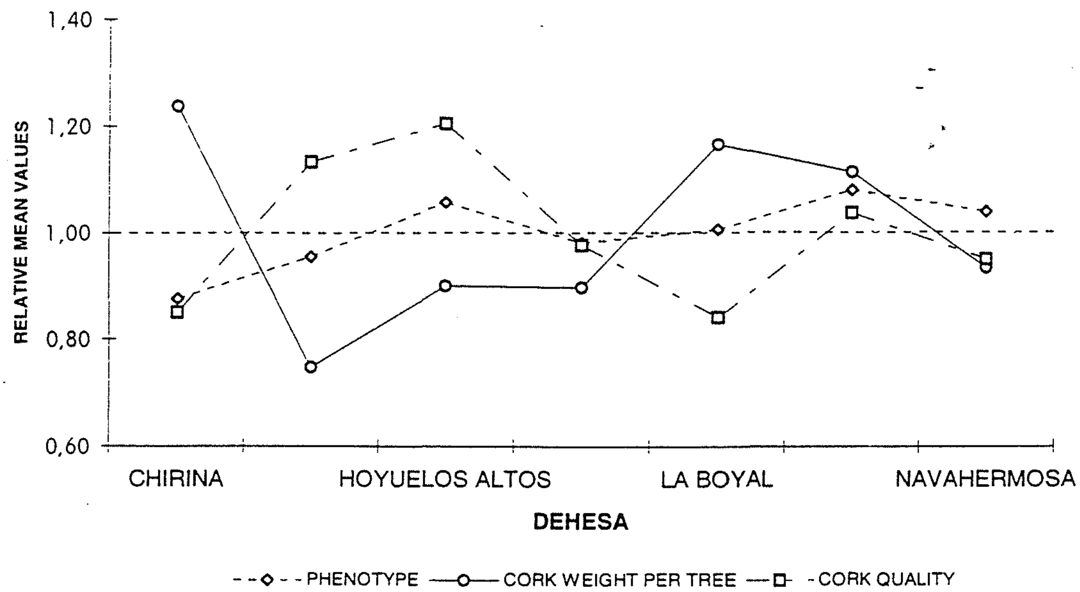

Fig. 3. Relative mean values of the three basic variables of the final selection in each dehesa.

Future actions

The cork extraction in summer 1996 will take place in the dehesa where the first selection was made. A new analysis of the cork from all selected trees will need to be carried out, to seek confirmation that quality does not change with time. This investigation will be repeated in the coming years.

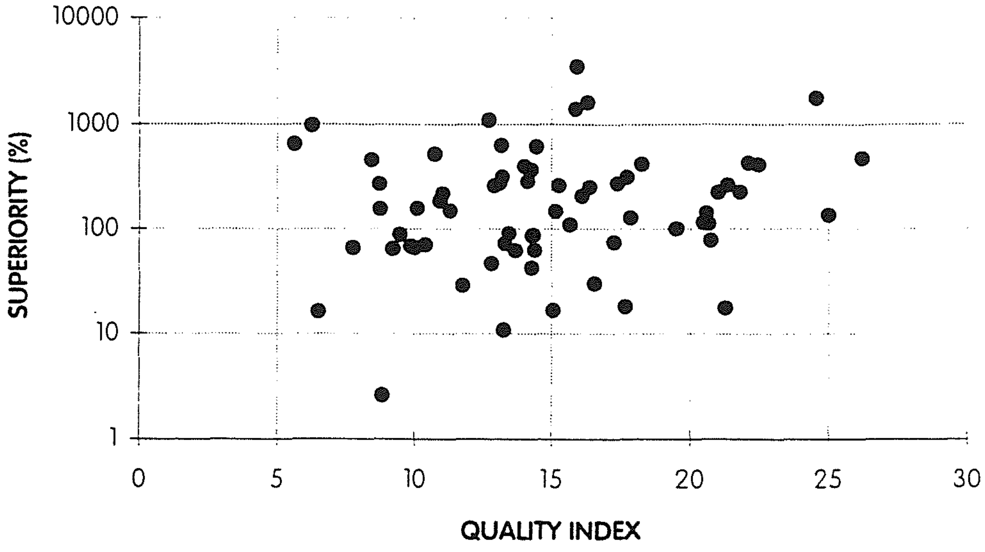

Fig. 4. Proportion (in %) of superiority in cork quality of the candidate trees with respect to control trees. Mean superiority of the selected group: >300%.

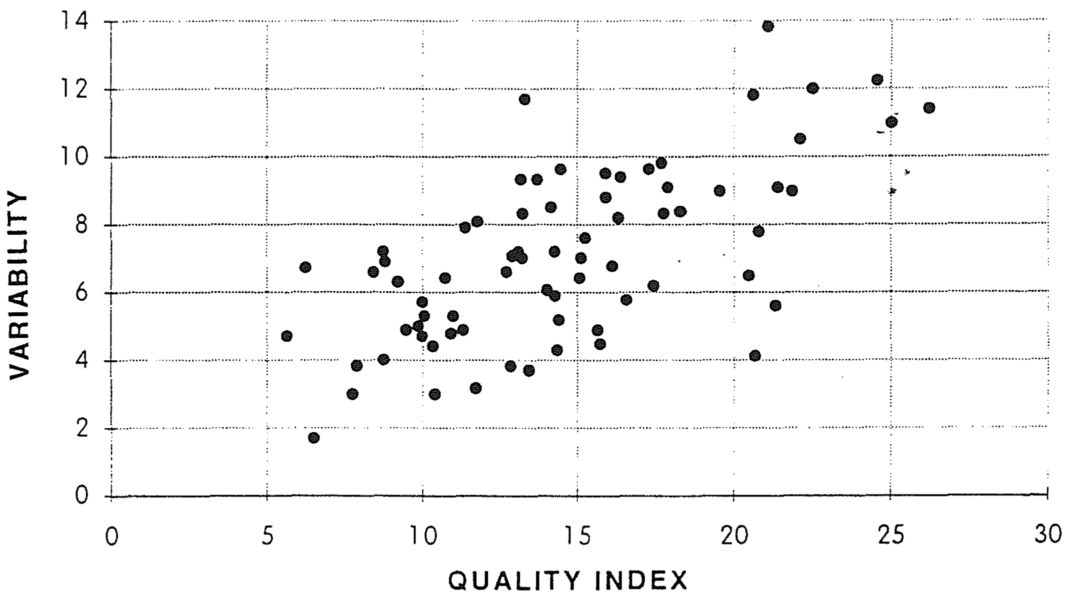

Fig. 5. Variability expressed as standard deviation of the quality index for four trees in each selected group. Mean variability of the population: >50%.

It is also necessary to start the study of acorn quality. Before that, a suitable method of collecting has to be developed. At first, it will focus on the selected plus trees but, in the future, a specific programme on acorn improvement may be convenient.

In conclusion it can be said that the first stage of the improvement programme – the selection of plus trees in La Almoraima – is nearly complete. The next step will be to sample seeds or vegetative material from the selected trees in order to propagate them. This work must be accompanied with studies on heritability of

Table 1. Proposal for the delimitation of selected stands according to cork quality

Stands	H ‡	Ks	R	A	Kf	If	Ic	No.	P1	P2	P3	P4
Casa de Tablas †	16.8	4.4	1.7	2.3	4.5	22.4	14.1	53	22.6	26.4	22.6	28.3
Loma del Tizón	15.6	4.3	1.4	2.7	5.0	23	14.5	28	17.9	25	25	32.1
Cueva del Acebuche	11.7	1.8	1.7	1.9	1.9	16.6	7.4	30	10	10	0	80
Cerro Botijero	15.3	4.0	2.3	1.8	6.2	23.9	8.7	28	6.2	10.4	14.6	68.7
Los Teatinos (testigo)	13.1	4.2	2.3	1.9	1.4	15.3	8.2	44	13.6	2.3	13.6	65.9

† The selected stand.

‡ Variables:
- H: mean height (m)
- Ks: surface coefficient
- R: 'rugosity' of the trunk
- A: cracking of the bark
- Kf: shape coefficient
- If: phenotypic index
- Ic: cork quality index
- No.: number of trees analyzed
- P1: percentage of trees with Ic > 20
- P2: percentage of trees with 20 > Ic > 15
- P3: percentage of trees with 15 > Ic > 10
- P4: percentage of trees with 10 > Ic

relevant characters aimed at its ultimate use in seed orchards or in controlled crossings. On the other hand, techniques of vegetative propagation must be improved. The well-known difficulties of this kind of propagation are even worse in our case, because mainly older trees were selected. It is also important to develop a method for characterization of cork quality by means of histological parameters. This would make it unnecessary to wait for cork extraction and new selections and tests would be easier, earlier and faster, not to mention that heritability could be assessed more directly.

Improvement strategy of forest tree species in Morocco: the case of cork oak[1]

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Introduction

In Morocco the forest area managed by the State Forestry Department covers almost 9 million ha composed of deciduous tree species (41%), Esparto grass (35%), conifers (13%) and various other species (11%). These stands are found mostly in arid, semi-arid and subhumid bioclimatic conditions. Up to 93% of the national territory is located in semi-arid and arid bioclimatic zones.

Forests play a significant role in the life of the rural population (50% of the Moroccan population). Since the beginning of the century, the impact of humans and their pastoral activities on the fragile natural forest ecosystems have become noticeable. This anthropogenic pressure has become stronger with the increasing needs of the population affected by an explosive demography, and the extent of the degradation requires that urgent and concrete measures be taken for the conservation and improvement of forest genetic resources. Cork oak, an important element of this wealth, whose economic, ecological and social roles are undeniable, is also submitted to this increasing pressure.

The place of cork oak in the Moroccan economy

Moroccan cork oak stands cover an area of 350 000 ha representing 15% of the world total cork oak area, estimated at 2 280 000 ha, or close to 38% of its area in North Africa.

In Morocco, cork oak spreads across subhumid (stands of the Rif, of the Middle Atlas, Atlantic plain) to semi-arid (Gharb plain) bioclimatic zones. The distribution area of cork oak covers the northwestern region from the coastal plains to the central Rif and Middle Atlas. Plain forests represent about 51% of the area and mountain forests 49%. All cork oak forests play a predominant socioeconomic, ecological and recreational role.

The average cork production in Morocco is 15 000 t/year, from which over 90% of its cork and cork-derived products are exported. The average production, including all categories, is 155 000 m^3/year, so that the sale of cork products accounts for at least 30% of the Moroccan forest economy.

Cork oak stands play an essential social role for the populations using forage products, sweet acorns for human consumption (5000 t/year in the Maâmora), mushrooms, lichens, honey, medicinal plants, etc.

Regarding labour, 1 m^3 of harvested cork represents 1.5 working days; 1 m^3 of harvested wood, 1 working day. The total annual production of wood and cork represents at least 375 000 working days.

Problems of cork oak stands in Morocco

Cork oak used to occupy considerable parts in Morocco. Natividade (1950) mentioned that a large forest area (5 million ha) existed 4000 years ago, spreading

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[1] Translated from French by Elinor Lipman, IPGRI.

from the Atlantic coast to the foothills of Atlas south of Marrakech, as is also testified by the numerous relic stands inventoried by Sauvage (1961).

At the beginning of the century, the Maâmora (plain) forest, with its 130 000 ha, was considered the largest in the world and now only covers 70 000 ha. Besides drought, the major cause of degradation, many other factors threaten the cork oak stands. The most important ones are listed below.

Overgrazing

It threatens natural regeneration but also affects standing trees because of pollarding and pruning practices, which have unfortunately become ever more frequent, particularly during the last dry years.

Acorn harvesting

The harvesting of acorns for cattle or human consumption favours the development of parasite fungi through wounds on the trees and strongly reduces the possibilities of natural regeneration. Such is the case for the stand of the Maâmora whose sweet acorns are highly appreciated by the local population.

Lack of regeneration

Since the beginning of the century, cork oak stands were regenerated only by coppicing. However, given the existence of an age limit for coppicing capacity and the stump loss after each cutting, this technique is no longer recommended.

Bad harvesting of cork

Cork harvesting by non-specialized enterprises leads to wounding of the trees and therefore predisposes them to parasitic attacks. A bad harvest leads to a tree decline of 3% per year.

Cork oak decline

Worrying declines of cork oak have developed in the stands since the end of the 1980s, with damages of variable intensity and associated with loss of vigour, defoliation and, in certain cases, mortality. The analysis of the principal causes leads to the conclusion that these interactions of different biotic and abiotic factors are becoming more complex.

In the particular case of the Maâmora forest, edaphic particularities have been accompanied by severe drought in recent years. For the Maâmora, records show that, depending on the districts, 10-44% of the trees are affected. On a national scale, the surface areas concerned are not exactly known and monitoring by aerial photography would probably be necessary.

Besides drought-related causes, also aggravated by the pumping of groundwater for irrigation and town water supply, other factors should be mentioned: attacks of defoliating insects (*Lymantria dispar*) and fungus (*Hypoxylon mediterraneum*), pruning and overgrazing in the forests.

The regeneration of cork oak

Problems

The problem of natural regeneration of cork oak stands is a very complex process which was first given attention at the beginning of this century. The most widely used regeneration system for cork oak is coppicing, but the problem of reproduction in old and declining stands is presently of high concern.

Studies on regeneration by coppicing (Marion 1955) showed the existence of a negative correlation between stump age and coppicing capacity. The number of stumps per hectare, particularly in the Atlantic and plain stands, has become highly insufficient for regeneration.

Natural regeneration of cork oak has been the objective of management practices applied to cork oak stands since 1951 but owing to the lack of appropriate techniques, it has been unsuccessful. Facing this situation, sowing and planting trials have been established during the last 5 years in the coastal, plain and Middle Atlas stands.

Existing knowledge

Regeneration trials of cork oak by sowing or planting date back to the 1920s. In spite of the lack of evaluation of these trials, lessons drawn from the first experiments illustrate the complexity and specificity of the problem.

During the past 5 years, given the extent of the degradation, sowing and planting regeneration trials were started again and a committee for the monitoring of cork oak regeneration –including managers, researchers and university teachers – was established under the Administration des Eaux et Forêts.

The results of these first 5 years are encouraging. The surface areas regenerated are close to 2000 ha/year including 1600 ha in the Maâmora forest and 400 ha in mountain forest stands (Bab-Azhar forest in Middle Atlas). However, the attack of young seedlings by the white worm remains uncontrolled and a whole research programme on its biology and possible control is underway.

Regeneration by sowing has been adopted further to the trials carried out with regard to various factors:

- selection of a regeneration plot
- soil preparation methods
- choice of acorns
- sowing
- white worm control.

These parameters were compiled into technical guidelines which were provided to managers. The technical guidelines will be updated according to research results and often pertinent observations made by field workers.

The planting techniques based on the use of 3-month-old seedlings whose hypocotyle has been reduced 1 month after germination are now being tested in the field.

Natural regeneration by sowing in mountain forest areas is not problematic once protection against cattle is ensured. However, the tending practices require further studies (spacing, density, canopy opening, etc.)

Cork oak stand management: a tool for conservation and use

The management of cork oak stands constitutes an indispensable tool for rational use through the planning of all operations to be undertaken. The site description together with dendrometric data (density, diameter, cork quality, etc.) will be a basis for the preliminary classification of stands.

At present almost 323 500 ha of cork oak stands are managed (92% of the total cork oak surface area). Non-managed forests (26 500 ha) are principally those located in the northern part of the country, for which the legal status has not been cleared yet.

Overall strategy for the improvement of forest trees species in Morocco

The regression of forest resources in general and of cork oak in particular raises the issue of genetic resources conservation and use, including the production of good-quality seeds and seedlings as an important component. The achievement of objectives implies the existence of a good strategy for the improvement of forest tree species. In this perspective, and in the framework of project UTF/MOR/011/MOR, Morocco requested a consultancy from FAO for the elaboration of this strategy. The mission was conducted by Prof. Alphonse Nanson, forest genetics expert from Belgium, in October 1995.

This strategy concerns both indigenous and introduced species. For indigenous species focus is on conservation of the existing resources, followed by improvement. In the second case, priority is given to improvement but without neglecting the conservation of the improved material.

Indigenous species

For indigenous species, the objectives are two-fold:

- conservation of existing genetic resources and their optimal use to serve the needs of national forestry
- improvement of these resources according to the means available.

It is recommended to carry out the following steps to accomplish these objectives.

Selection of populations

- analysis and synthesis of provenance trials when available
- setting up new trials with indigenous and foreign material
- comparison of various improvement products (origin, provenances, synthetic varieties, clonal varieties, etc.) from various national and eventually international regions of provenance; the comparative trials must be carried out regularly
- establishment of Regions of Provenance *sensu* OECD and EEC; these regions must take into account the ecological and genetic differences and they can not be too numerous to facilitate the use of the system
- selection within each Region of Provenance of 'identified stands' and if possible of selected 'seed stands' which will serve as seed sources for reforestation.

Individual selection

Individual selection can start according to the chronology described above only when selection is well under way and when perspectives of genetic gain are promising. The steps will be:

- selection of 'plus trees' within each Region of Provenance
- establishment of 'seed orchards', ideally one per Region of Provenance
- creation of clonal varieties for species for which preliminary studies foresee estimated gains which will compensate for the increased costs of vegetative versus generative multiplication.

Control and certification of forest reproductive materials

Improved forest reproductive material is valuable only if it reaches the forester without any error of its identity, otherwise the whole selection effort remains useless. Therefore, it is necessary, even imperative, to control the movement of forest reproductive material from harvest to delivery of seedlings to the users. All reproductive material must be accompanied by a 'provenance' certificate.

Genetic conservation

Genetic conservation can be either *in situ* or *ex situ*. For *in situ* conservation each identified or selected stand must be effectively protected to prevent the existing stand from decline due to anthropogenic factors and to secure successful natural regeneration.

Besides stands designated for *in situ* conservation, complementary *ex situ* conservation measures should be taken principally through conservation plantations, established on the basis of material collected from mature stands during good flowering years, and on a sufficient number of trees with abundant seed crop.

Besides cork oak, and in view of their economic, ecological and social importance, the following main indigenous species are subjected to breeding programmes: cedar, pines, *Thuya* and the argan tree (*Argania spinosa*). Regarding introduced species, breeding efforts focus on *Eucalyptus*, followed by pines.

Breeding programme for cork oak

Delimitation of Regions of Provenance

Conservation and improvement of production requires, among other conditions, the utilization of high-quality forest reproductive material. The first action undertaken for the implementation of the overall tree improvement strategy in Morocco was the definition and delimitation of Regions of Provenance and the identification of seed stands.

For a given species, the Region of Provenance is the territory or the group of territories subject to sufficiently uniform ecological conditions and on which stands can be found with similar phenotypic or genetic characteristics (OECD 1974). The delimitation of Regions of Provenance allows for a better movement of reproductive material and avoids problems of bad adaptation to the given ecological conditions. Any transfer of reproductive material between Regions requires knowledge that can be gained by preliminary provenance tests. Therefore, the natural area of each species must first be subdivided into homogeneous Regions of Provenance. This task is not always easy, given the high level of heterogeneity of bioclimatic conditions.

To facilitate the process, we opted for the unification of Regions of Provenance valid for all species ('partitionist concept'). The advantage of this concept lies in the facility of establishment and delimitations of the Regions of Provenance. This process was based upon studies carried out in the framework of several technical cooperation projects of GTZ (for the selection of seed stands and seed supply) and FAO (consultancy in forest trees breeding). A series of workshops was also organized at the level of the Seed and Nursery Service in Sidi Amira and of the Regional Seed Stations in Azrou, Chefchaouen and Marrakech.

To keep a sufficiently balanced subdivision of the Regions of Provenance and to facilitate their identification, an adjustment was made taking into account natural and administrative boundaries. Given the diversity of the Moroccan landscape, each Region of Provenance was subdivided into altitudinal zones (see table below). The general map of forest regions in Morocco drawn by Boudy in 1958 served as the basic terms of reference.

Altitudinal zones (m)	Abbreviation
0 - 500	0 - 5
501 - 1000	5 - 10
1001 - 1500	10 - 15
1501 - 2000	15 - 20
Above 2001	+ 20

This work allowed for the identification and delimitation of 19 sufficiently homogeneous Regions of Provenance. The Regions were named and classified for all forest species within nine large biogeographical units (see Fig. 1):

Biogeographical units	Regions of Provenance	Code
I. Rif	Rif Atlantique	I.1
	Rif Occidental	I.2
	Rif Oriental	I.3
II. East	Plaine Moulouya	II.1
	Hauts Plateaux	II.2
III. Atlantic plain	Maâmora	III.1
	Plateau Central	III.2
IV. Middle Atlas	Moyen Atlas Occidental	IV.1
	Moyen Atlas Oriental	IV.2
	Moyen Atlas Steppique	IV.3
V. Meseta	Meseta Atlantique	V.1
	Meseta Continentale	V.2
VI. High Atlas	Haut Atlas Occidental	VI.1
	Haut Atlas Central	VI.2
	Haut Atlas Oriental	VI.3
VIII. Souss	Souss Nord	VII.1
	Souss Sud	VII.2
VIII. Presahara	Le Présahara	VIII
IX. Sahara	Le Sahara	IX

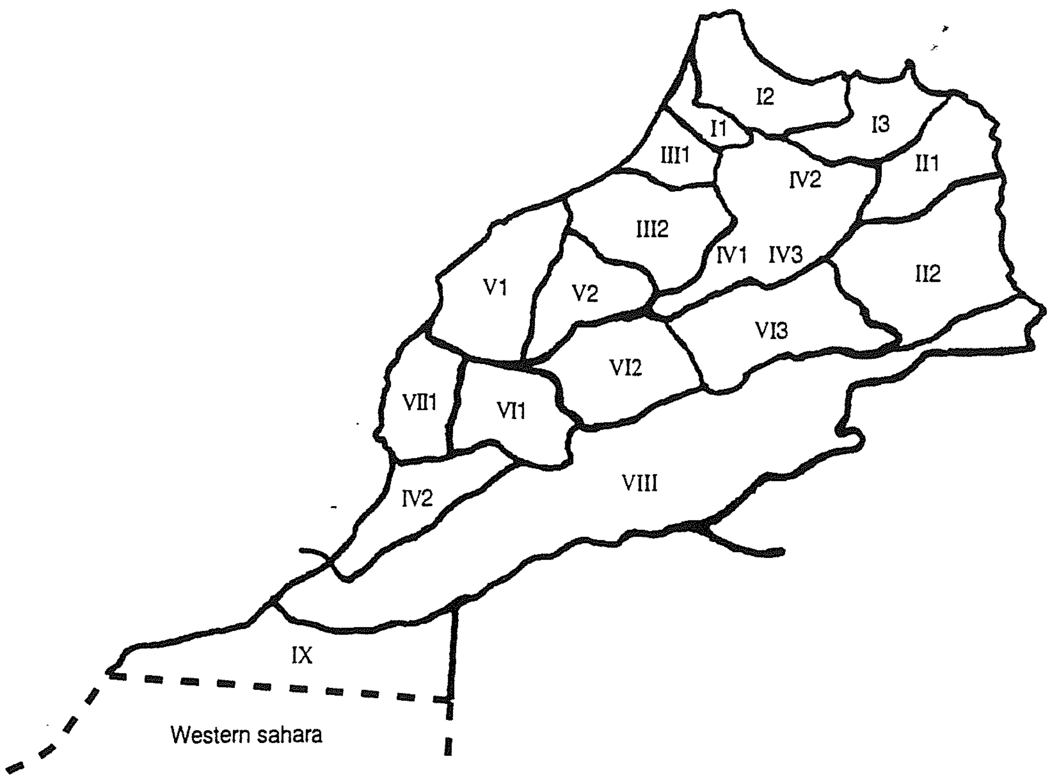

Fig. 1. Biogeographical units and Regions of Provenance within the distribution area of cork oak (*Quercus suber*) in Morocco.

Selection

As previously mentioned, successful regeneration by sowing or planting is only just starting and attacks of white worm still pose serious problems. To meet the needs of regeneration programmes, the supply of acorns should be around 200 t/year which would allow the reforestation of 1500 to 2000 ha/year. A number of stands will be selected to meet these demands and to take into consideration the task of preserving the genetic pool of cork oak stands. In the Maâmora, 19 seed stands covering 1958 ha have been anticipated.

The selection of elite (plus) trees will be based on phenotypic criteria: height, diameter, crown shape, branching, cork quality, sanitary state and fructification. The selected stands are subjected to permanent protection and to silvicultural management. For the establishment of comparative field trials on families and provenances, acorns will be collected from seed-producing trees in the selected stands. Further to the results of the family and provenance tests, seed orchards will be established.

Varieties with high qualitative and quantitative properties will be created by controlled hybridization. The activity will consist of the following steps: selection of parent trees, phenological observations, pollen collecting and conservation, induction of flowering, controlled hybridization and progeny tests.

Seed procurement and nurseries

The objectives of this foreseen activity can be summarized as follows:

- collecting and storage of acorns to overcome the problems caused by irregularity of acorn production and low fructification
- improvement of seedling production in the nurseries through the control of parameters specific to cork oak, particularly substrate and transplantation of seedlings
- vegetative multiplication techniques: trials will be carried out to study the major methods of vegetative reproduction, cutting and grafting.

Biotechnology

Given the possibilities for micropropagation of cork oak, the following issues are presently under study:

- experiments on different phases of *in vitro* propagation (primary culture, rooting, reactivation and multiplication through hyper-branching)
- removal of maturation constraints
- propagation of old elite trees
- mass production of plants.

Phytopathology

Since cork oak is affected at different development stages by attacks of several types of parasites, the programme will focus on the monitoring of the sanitary state of acorns, including inventorying of pests and parasites prone to attack the fruit at different stages and to suggest adequate measures for the control of major pathogens. Furthermore, the sanitary state of young plants will be monitored through the establishment of chemical control trials, particularly against the white worm – to determine the best season and best treatment (active product, dose, frequency of application, etc.). These studies led to the identification of three species and the larvae, already at the nymphal stage, are now being reared in the laboratory.

The sanitary state of stands, both for selected seed stands and for the rest of the cork oak stands also will be investigated. The first group will be subjected to close monitoring to avoid contamination of acorns and of seed-producing trees by pathogen agents.

In the stands, a wide programme is being carried out, focusing on:

- inventorying of xylophagous insects
- biology of *Cerambyx cerdo* and *Platypus cylindrus*
- study of the acorn fauna
- study of insect-tree interaction
- establishment of a monitoring network of cork oak stands with regard to attacks of the fungus *Hypoxylon*, *Lymantria*, *Orgya* and *Cerambyx* and the implementation of adequate control measures for each of these parasites.

Conclusion

The multiple degradation problems encountered by Moroccan forest resources require urgent and concrete measures. To respond to this urgent need, an overall strategy for the improvement of forest tree species was elaborated, for both indigenous and introduced species. In this perspective a priority programme for the improvement and conservation of cork oak genetic resources is being carried out.

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Regeneration of cork oak in Tunisia[1]

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Introduction

Natural cork oak forests in northwestern Tunisia seem to be undergoing a continuous degradation process and their regeneration is becoming very difficult. Natural regeneration is very sporadic and occurs randomly. For our cork oak stands, the only mode of regeneration posing no problem is through coppicing but it can only, at best, maintain existing situations, and can hardly reconstitute the structure of denser stands. The regeneration of these forests is a delicate issue, and all the more difficult to achieve as it was addressed quite late, when stands have already reached their limit of growth potential and even physiological resistance.

Protection and management of cork oak stands

Research undertaken

Since 1970, the Institut National de Recherches Forestières in Tunisia has studied the rejuvenation of cork oak stands. The major objective is to record the importance of sprouting and suckering for the regeneration of the cork oak stands of Bellif and Houamdia leading to a sapling stand.

An experimental design was established in two stands of coppiced cork oak to determine a method of thinning. Strong and healthy stump sprouts capable of replacing the old cork oaks were observed.

Work in progress

Present studies concern only cork oak regeneration and address mostly the rejuvenation of degraded forest. They contribute to solving the problems of natural and assisted natural regeneration of the Kroumirie oak stands.

Specific objectives are as follows:

- conservation of acorns including germination tests
- study of regeneration dynamics in time and space
- defining the best soil preparation method for the sowing of cork oak acorns under a bush layer of variable density
- comparing two artificial regeneration techniques: planting or direct sowing of cork oak.

These studies deal with regeneration of cork oak stands from seeds. To better understand this issue it was felt necessary to study the cork oak acorn behaviour in the nursery and in the wild.

Conservation of acrons

Acorn harvesting

Observations of the acorn-maturation process during three sequential years allowed the determination of the harvesting date. Harvest begins on 15 November. It is recommended to discard damaged acorns maturing earlier.

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[1] This report was presented at the meeting in Almoraima, Spain. Translated from French by Elinor Lipman, IPGRI.

It is better to harvest healthy acorns before they fall and as soon as they reach a uniform brown colour. Acorns should remain as little as possible on the ground to avoid various factors of damage and contamination.

The use of plastic sacks will be avoided since the lack of aeration favours condensation and warming. Jute sacks or (even better) openwork harvesting plastic boxes which can be used afterwards for storage are recommended.

Acorns must remain in these containers as little as possible. Exposure to sun during transport will be avoided.

Storage

To ensure full success of each sowing campaign in our cork oak stands and due to the irregularity in acorn production, it is necessary to conserve acorns in storage.

The acorn is a very special seed, since its water contents must be preserved in order for it to retain germination ability. It must be treated not as a seed but rather as a fruit. Conservation must start immediately after harvesting.

Three storage techniques were tested according to the expected length of conservation:

Open air storage for 2-3 months. Acorns are stored in jute sacks hung in the open air in a covered and well-aerated place. This conservation technique allowed a prolonged sowing period until February. After 3-4 months, however, any sowing is destined to failure. This conservation method is very easily implemented.

Storage in dry sand for a duration under 6 months. This method ensures the minimum level of aeration necessary for respiration and leads away carbon dioxide and water vapour produced by transpiration (humidification of the sand by the condensed water from transpiration must be avoided, as it would then activate the development of the embryo). Sowings carried out after the fifth and sixth months following harvest were unsuccessful, with germination close to zero. The acorn lost its germination capacity. This conservation method is very delicate and is not recommended.

Conservation in cold storage for a longer duration (1 year and more). In this method acorns are stored in small amounts of approximately 3 kg, i.e. 390 to 420 acorns, in polyethylene sacks carefully sealed and placed in a cold-storage room with a constant temperature of +3 to 4°C. However it was observed that precooling of acorns before packing (3-4 hours) reduces condensation of water on the inside layer of the sacks when they are taken into the cold room. It is better to store the polyethylene sacks in openwork, stackable transport boxes. This allows periodic control of acorns and ensures that sufficient air circulation is maintained around the sacks deposited in each box. This also facilitates handling and protects the sacks from risks of perforation.

Thermotherapy treatment for long-term storage. This method consists of soaking the acorns in water at +40°C for 2 hours, draining them, and storing them in a cold room at +4 to 5°C with 50% relative humidity.

Another possible treatment is **hydration** for 1 hour once a fortnight, followed by storage in cold room at +4 to 5°C with 50% relative humidity.

Germination

Laboratory experiments. Sowing of acorns was spread over January to June, to test the evolution of germination ability according to the method and length of storage. The results show that the percentage of germination varies for both storage methods (in dry sand and in open air). The acorns germinated only during the first 3 months. Germination varies from 36 to 96%. For conservation in cold storage, the acorns can be kept up to 12 months and the average germination is above 68%. This value is reached when there is no accident during storage. An increase of a few degrees of the cold room temperature, even for very short periods (2-3 hours) could have serious consequences even if there is no visible modification in the state of acorns.

Nursery experiments. In parallel with laboratory experiments, a comparative trial was carried out from January to June in the nursery. For each storage method, 150 acorns were sown. The aim of this trial was to reduce the length of stay of seedlings in the nursery through late sowing. The production of seedlings with a taproot of moderate length would not make transplanting too difficult. The seedlings were placed in 800-1000 cc containers above ground level.

In the nursery it was observed that germination occurs late. The development of the first stem is observed only after 35-40 days for the different storage methods. The germination follows the same ratio as that obtained for laboratory experiments.

Regeneration of cork oak forests - the case of the Bellif forest

Natural regeneration under forest cover

The main objective is to analyze the regeneration dynamics in time and space after complete protection of the site.

A trial was established in the Bellif cork oak forest to determine regeneration elements on a surface area of 0.5 ha. Each plot covers an area of 10 m^2. For this trial, several measurements were made in 1992, 1993, 1994 and 1995. A layer of seedlings covers the site. Seedlings are particularly abundant under the crowns of seed-producing trees and in small depressions. These measurements were made every year before and after summer season. The following densities were recorded:

	1992		1993		1994		1995†
	Jun	Nov	Jun	Nov	Jun	Nov	Jun
Average density/ha	150 000	117 000	102 000	97 000	92 000	85 000	77 000
No. of dead seedlings/ha	–	32 000	15 000	5 000	5 000	7 000	8 000
Mortality rate	–	21%	12%	5%	5%	7.6%	9%

† No data for November.

Artificial regeneration by sowing

The objective of the trial is to determine the most suitable soil-preparation method for sowing of cork oak acorns under a bush layer of variable density. The existing undergrowth was neither coppiced nor cleared.

The experimental design consists of three randomized blocks, with four treatments:

- localized soil preparation: planting holes (30 x 30 cm)
- surface soil preparation: simple picking
- in-depth soil preparation: utilization of an agricultural drill at 60-cm depth
- mechanical soil preparation: utilization of a triple ploughshare: sowing on the back of the furrow.

Three acorns were sown together in a horizontal position, at a depth of 3 cm. The spacing of sowing is 2 x 2 m.

Regeneration by coppicing

The major objective is to record the importance of stump sprouts and suckers in the regeneration of the cork oak stand in Bellif in order to obtain a sapling stand (Hasnaoui, pers. comm.). An experimental design was established in two stands of coppiced cork oak (Chakroun 1977, 1978) to determine a method of thinning. Strong and healthy stump sprouts capable of replacing the old cork oaks were obtained.

Generally, this work shows that several elements are in favour of this type of regeneration (Chakroun 1977, 1978; Hasnaoui Brahim 1988) and in particular:

1. Stump sprouting is very good if stems are cut in a favourable period and protected from animals.
2. Stump sprouts have a fast growth in height which represents a favourable precondition to avoid damage by animals.
3. Growth in diameter of the sprouts is faster than that of individuals reproduced generatively. An early production will be marked as soon as 16 years after coppicing instead of the 30 years needed after sowing.
4. Thinning is rather profitable and does not represent a heavy budgetary load for the management (sale of thinning products in various forms: stakes, poles, fuelwood, etc.).
5. The regeneration of the cork oak stand through stump sprouting leads to an even-aged stand in a given site.
6. Suckering may be a satisfying mode of regeneration, taking into account the physiological and environmental conditions it offers: breaking the dormancy of buds located on superficial roots; considerable supply of nutrients; sufficient light intensity, and less competition for space.

It must be noted that growth in height and diameter is fast and benefits from the considerable nutrient and water supply made possible by the powerful root system of the stump. Continuous suckering over several years can lead to a specific type of mixed coppice.

In conclusion, regeneration by coppicing requires the following steps:

1. To carry out field surveys during which the cork oak stands best suited to stump sprouting will be delimited and mapped out.
2. To cut a few trees in order to test their sprouting ability (which depends on factors inherent to the tree and to site conditions). If this test has positive results, clear-cutting should be done. However, a few plus trees (with superior phenotypic performance) should be kept for use as seed producers. Seeds could use the opportunity of the changes in environmental conditions to germinate and grow. This cutting must be executed during the period of vegetative rest.
3. To put the whole site under strict protection for an average period of 10 years so that sprouts and suckers can grow away from animal damage.

Selected bibliography

The Selected Bibliography presented here is a result of interactions among the participants and Network members in the period between the Sassari and the Almoraima meetings. The references deal primarily with issues directly linked to the conservation and use of cork oak genetic resources, but in many cases also cover broader relevant subjects such as the distribution of the species, its evolutionary history, ecology, physiology and silviculture. The original titles of publications given in French, Spanish, Portuguese, Italian or English have been maintained. The selected bibliography, being an important objective of the workplan, will be updated regularly. Requests should be addressed to the host institute, Estação Florestal Nacional in Oeiras, Portugal. The database will be available on the Internet.

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