Noble Hardwoods Network

Report of the sixth (9–11 June 2002, Alter do Chão, Portugal) and seventh meetings (22–24 April 2004, Arezzo, Italy)

M. Bozzano, M. Rusanen, P. Rotach and J. Koskela, *compilers*

European Forest Genetic Resources Programme (EUFORGEN)

IPGRI is a Future Harvest Centre supported by the Consultative Group on International Agricultural Research (CGIAR)

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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 for 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 analyse needs, exchange experiences and develop conservation objectives and methods for selected species. The Networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from participating countries carry out an agreed 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. Further information on EUFORGEN can be found from its Web site (www.euforgen.org).

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Citation:
Bozzano M., M. Rusanen, P. Rotach, J. Koskela (compilers). 2006. EUFORGEN Noble Hardwoods Network, Report of the sixth (9–11 June 2002, Alter do Chão, Portugal) and seventh meetings (22 - 24 April 2004, Arezzo - Italy). International Plant Genetic Resources Institute, Rome, Italy.

ISBN 13: 978-92-9043-682-9
ISBN 10: 92-9043-682-4

IPGRI
Via dei Tre Denari 472/a
00057 Maccarese
Rome, Italy

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Sixth EUFORGEN Noble Hardwoods Network meeting

Opening

M. Rusanen, Chair of the Network, welcomed the participants from 31 countries and thanked the National Coordinator, M.C. Varela, the local organizers and the National Forest Department for the excellent organization and for their participation.

Mr A. Cruz, the Mayor of Alter do Chão, welcomed the participants to this small agricultural town. Mr C. Morais, Director of the National Forest Services, was unable to be present but sent a letter welcoming participants and underlined the importance of forest genetic resources. Mr C. Ferreira, Director of the Coudelaria de Alter hosting the meeting also sent a note welcoming participants. He wished the participants a successful meeting and a good stay in Portugal.

M. Bozzano, on behalf of the EUFORGEN Secretariat, gave a brief overview of upcoming meetings, current activities and progress in the Programme. He also updated the participants on the progress in the recruitment of the new EUFORGEN Coordinator.

The participants introduced themselves. J. Fernandez Lopez (Spain) and J. Turok (Secretariat) were unable to attend and sent their apologies.

M. Rusanen, summarized the progress made from the last meeting, highlighting targets achieved and stressed the main current tasks of the Network: the improvement of information flow, the finalization of Technical Guidelines (TG) for long term conservation strategies and the elaboration of joint project proposals.

The agenda of the meeting was adopted.

Four rapporteurs were nominated: M. Heuertz, S. M.G. de Vries, K. Russell, and M. Bozzano.

Country reports: status and practices of gene conservation

Network members were divided in four working groups to discuss progress made in each country and a representative of each working group gave a presentation summarizing the status and progress. Updates for each country concerning conservation and use, changes in inventories, in relevant policies and legislation, research, tree improvement, public awareness and progress will be posted on the Web site: http://www.ipgri.cgiar.org/networks/euforgen/euf_home.htm. Particular progress was reported in the implementation of the EU directive 1999/105/EC concerning the trade and certification of forest reproductive material. In addition, most of the countries raised the need to improve public awareness and informed about an increasing interest in the use of autochthonous reproductive material. Changes in forest ownership in several Eastern European countries could have implications in relation to valuable genetic resources Introductory country reports from Serbia and Montenegro and Albania were presented.

M. Bozzano introduced the new information platform. Since the status of national activities on forest genetic resources in Europe is changing with increased interest, a new, user-friendly platform will be available on-line. The new EUFORGEN Web site will allow the browsing of information (particularly country reports and country updates) through a multi-criteria search. It will be easy to add to and obtain information from the Web site, which will be on-line by the end of August 2002. The importance of having standardized formats for reports and updates was stressed.

All participants will provide the Secretariat with an electronic version of their report, in the agreed format, by 30 June 2002.

Discussion on sharing of gene conservation responsibilities: The Common Action Plans

S. M.G. de Vries introduced the proposal made by the group of Network chairpersons and initiated by B. Heinze, as a new vision for future activities of EUFORGEN. This will be discussed during the forthcoming Steering Committee meeting. Recognising the achievements of the EUFORGEN Networks, a plan for the future based on providing a more direct support for implementation of gene conservation action was proposed and discussed. Networks of gene conservation units for target species within their distribution areas would be carefully designed incorporating recent research results and implemented through 'Common Action Plans', with voluntary agreements among countries, including shared costs and responsibilities. This initiative will provide the basis for recommending, coordinating and monitoring the practical gene conservation measures taken in the forests.

M. Rusanen is willing to prepare a Common Action Plan for Gene Conservation Network for *Acer platanoides*. The Secretariat will send a format for description of the conservation stands to all network members by 20 June 2002 to be completed with geographical coordinates and relevant information. These will be sent by 30 July 2002 to M. Rusanen. Meanwhile a distribution map will be prepared.

P. Rotach and many participants supported the idea that a complete European picture is needed on the species' distribution to enable holistic approaches and to develop action plans.

The network unanimously supported the concept of the Common Action Plans and will provide comments and remarks on the draft to the secretariat by 23 June 2002.

Technical Guidelines (TG)

M. Bozzano presented the results of an investigation on distribution maps using different sources of data and explained the different software packages used.

After discussions, the existing format for the TG was retained. Distribution maps of all TG species will be provided by the Secretariat by 30 August 2002 (since it was agreed already in 2001, this topic should have a priority in the Secretariat agenda). To harmonise all maps, the *Atlas Flora Europaea* (AFE) format will be used where possible. The Secretariat will contact AFE for details and agreements.

Harmonization of the 'Guidelines for genetic conservation and use' section is a priority starting with minimum approaches. The emphasis should be on the local situation with practical management advice that considers abundance and population structure. A maximum of three selected references has been suggested for further reading. All participants will send their specific comments directly to the authors by 30 August 2002. Chair and V. Chair agreed that the *Fraxinus* draft species TG would be edited with regard to style and presentation by the Secretariat and sent to the other TG' authors by 15 September 2002 as an example for adjusting their TG as appropriate. All revised TG will be sent to the Secretariat by 15 October 2002.

G. Eriksson will add the section on the objective of genetic conservation into the introduction.

The Secretariat will be responsible for producing the publication by the end of 2002. The TG can be translated and adapted by Network members as appropriate.

F. Ducci offered to coordinate the elaboration of draft TG on *Juglans regia*, *Alnus cordata, Acer monspessulanum, Acer opalus*, *Acer minor* (those *Acer spp* could be presented within the same module). F. Ducci will circulate them one month before the next NH meeting. The need for development of TG for other NH species, especially those occurring in the Balkan area, was stressed.

Research update

Seven research projects were presented and discussed. It was agreed to include a detailed report of each contribution (approximately two pages) into the published report of this meeting. Final versions should be sent to the Secretariat by 31 July 2002.

Suggested research priorities

G. Douglas presented a brief introduction to the EC Sixth Framework Programme.

The NH network expressed the wish to develop research activities to characterize the pattern and extent of genetic diversity in all target NH species in Europe. Particular emphasis should be devoted to the mechanisms responsible for adaptation, such as resistance to biotic and abiotic stresses. Functional compatibility and specificity of different populations of forest trees to their symbionts and pathogens should be investigated. The role of hybridisation, silviculture and the transfer of reproductive material are key factors affecting genetic resources. The results should provide a basis for future monitoring and prediction of the evolution of genetic resources of these forest trees in a changing environment. Further research priorities include the study of self-incompatibility in the *Rosaceae* family, investigation of the genetic resources of *Tilia* spp and research on wild relatives of fruit trees. Such research initiatives would support the future Common Action Plans. The importance of sharing research results with countries not previously involved and projects encouraging the need for joint research efforts (i.e. common trials) was highlighted.

In order to increase the awareness of Network members on forthcoming research, information on projects and relevant websites will be sent to the Secretariat by 18 June 2002. The Secretariat will then distribute this information to all Network members by 22 June 2002.

Information management and public awareness

The participants agreed to proceed with the preparation of a Photo CD, which would include characteristic images of Noble Hardwoods and their genetic resources. Main themes should include: full trees, morphological characters, landscapes, traditional uses, laboratory outputs, etc. B. De Cuyper offered to collect the images and will send instructions by 20 June 2002 to all Network members. Each country is responsible for providing at least 2 images (preferably slides) by the end of July 2002 to B. De Cuyper. The collection will be available to all members, who might use it for public awareness purposes, publications, Web pages etc. given that the author is acknowledged if it is required.

S. M.G. de Vries and T. Myking presented the status of the on-going development of the Poster on Noble Hardwood species. The revised text was adopted by the Network. It was left up to the group of four Network members (T. Myking, S. M. G. de Vries, K. Russell and J. Fennessy) to finally re-arrange the text, make a lay-out of the poster by adequately using pictures that will be sent by all the Network members to B. De Cuyper. They will also consider, in consultation with the Secretariat, whether names of species and/or names of authors should be quoted. A draft will be circulated to the Network members by the poster-group by 31 January 2003.

B. Musch proposed to present a poster (in French) on EUFORGEN at a scientific meeting with public presence in France.

To highlight EUFORGEN, it was suggested that public awareness material was made available at the DYGEN meeting to be held in Strasbourg in December 2002.

Where possible, EUFORGEN activities should be promoted through the local press. A positive example was given at the 7th *Populus nigra* meeting in Croatia in 2001.

Bibliography

M. Bozzano highlighted the EUFORGEN bibliographic database on grey literature. The Network members were encouraged to continue providing references for this database. The database has been frequently used on-line from the web page. An improved access form will be circulated to Network members by 15 August 2002. It was clarified that each member should restrict contributions to only those concerning grey literature available in her/his own country.

It was noted that contact information of the author (or contributor of the reference when author not available) should be provided for all references. The matter of duplication should be taken care of before the next meeting.

Date and venue of next meeting

The Noble Hardwood Network expressed the intention in holding the next meeting in autumn 2003, (according to the overall EUFORGEN agenda). F. Ducci offered to organise the meeting in Italy in collaboration with a subgroup of the ECP-GR Malus-Pyrus Network, which was appreciated by the participants.

The Secretariat will follow up and provide information to all countries in due course.

K. Russell suggested to have an extra day for restricted working groups on selected species in forthcoming NH meetings.

G. Eriksson pointed out that the group had earlier decided to have a presentation on the links between gene conservation, tree breeding and utilisation and this should be included in the agenda of the next meeting.

Adoption of the report

The report was adopted and distributed to the participants.

Conclusions

The local organizers were thanked for their kind efforts in preparing and organizing this meeting. It has provided the participants with an insight into the unique situation of forest gene conservation in the Mediterranean region. M. Rusanen thanked the participants for their work and declared the meeting closed.

Seventh EUFORGEN Noble Hardwoods Network Meeting

Opening

F. Ducci welcomed the participants on behalf of local organizers and provided a short presentation on the Istituto Sperimentale per la Selvicoltura (ISSA) and its work on noble hardwoods. G. Corrado (ISSA Commissioner), A. Tocci (ISSA Director), D. De Laurentis (Corpo Forestale dello Stato and EUFORGEN Italian National Coordinator) welcomed the participants on behalf of their Institutes.

M. Rusanen, Chair of the Network, welcomed the participants from 27 countries and thanked the local organizers for the arrangements. All participants then briefly introduced themselves.

M. Rusanen presented the tentative agenda of the meeting which was adopted with some amendments. L. Salek, J. Buhagiar and S. Orlovic were nominated as rapporteurs for the meeting.

EUFORGEN update

J. Koskela provided a short update on recent meetings and outputs of EUFORGEN and listed various other meetings where the Secretariat promoted the EUFORGEN Programme. He briefly highlighted the outputs of the 4th Ministerial Conference on the Protection of Forests in Europe (MCPFE) and the recent meetings.

Introductory country report by Iceland

T. Eysteinsson gave a general presentation on noble hardwoods in Iceland highlighting the role and the importance they have in the country. The present forest management goals are mainly multiple use with emphasis on timber production. Other important elements are soil conservation, land reclamation, amenity and recreation. The full country report will be published in the proceedings of this meeting.

Northern Europe (Denmark, Finland, Norway, Sweden, Iceland, Ireland and United Kingdom)

For most of the group and apart from Iceland the country reports have not changed to any notable degree since the last meeting in Portugal. However, there has been major changes in some areas, e.g. in the forest reproductive material sector. The change in European Commission (EC) regulation (1999/105/EC) on 1 January 2003, extended the regulation from the 13 ‘scheduled’ species to 47 species. There is the additional impact of increased membership of EU with the additional ten new countries extending the open market area.

Plant material of unsuitable sources will have a negative effect on forest genetic resources, and material should be clearly identified and its adaptedness should be clearly stated. This is also important for material used for landscaping and roadside planting. The group stressed the importance of informing and raising awareness among forest professionals and the general public about the impact of provenance choice. However, the group expressed a wish to avoid the popular understanding that use of local is always best.

The discussion highlighted the category ‘source identified’ as causing some confusion in a number of countries. The group discussed the sources of forest reproductive material, some favouring improved seed orchard seed while others favour a close-to-nature approach. Species diversity requirements can be beneficial for hardwood gene conservation but the group recognised the extra cost in fencing the noble hardwood plantations.

Western Europe (Belgium, Hungary, Luxembourg, The Netherlands, Germany, Austria and Switzerland)

All countries have continued surveying and inventorying noble hardwoods species with different levels of detail and different focal points.

The Member States implemented the 1999/105/EC directive in 2003, Luxembourg is still working on the update. In Hungary, the updated legislation on forest reproductive material (FRM) will be in force on joining the European Union. Switzerland revised the Forestry Act with emphasis on conserving and enhancing biodiversity. The category 'source identified' is handled differently in each country.

Research activities cover pollination and variation studies, genetic characterisation of populations and studies on 'purity' of certain species affected by continuous introgression pressure (*e.g. Malus, Pyrus*).

The practical implementations had been summarised in the tables included in the country updates.

Austria and Germany published brochures explaining the new regulations on FRM. Additionally, the same countries issued recommendations/guidelines regarding the use of local material for non-forestry purposes (*e.g.* landscape and amenity plantings).

The group felt that questions on species not listed in *Annex I* of the EC directive, as well as experience regarding noble hardwood seed orchards should be put on the agenda of the next meeting.

Central and Eastern Europe (Estonia, Poland, Czech Republic, Slovenia, Serbia and Montenegro, Croatia and Bulgaria)

The virgin forests of this sub-region have an important role in conserving noble hardwoods genetic diversity. Field inventories are based mainly on state forest service or enterprises databases, but genuine inventories per species are also done by some research institutes.

Policy and legislation follow the EC regulations and the further development of national forest programmes is in line with MCPFE resolutions. An act on FRM has been prepared and implemented in all countries (in some in 2004). Slovenia added eight species to the national list.

A majority of countries continue in their research projects dealing with noble hardwoods. No recent research activities are reported for Estonia.

Practical implementation is different in each country. Coordination is done mainly by representatives of research institutes and through personal contacts.

Public awareness concentrated on seminars, teaching and producing leaflets, also some conferences and Web sites/virtual networks.

Mediterranean region (Portugal, Spain, Italy, Malta, Turkey and France)

Current problems concern the compilation of inventories, the main difficulty being on the definition of population or stand for those noble hardwood species scattered in mixed forests. Some noble hardwoods are recorded only by genus. In addition priority assigned to a species differs between countries with Portugal inventorying only chestnut, Spain and Italy added 3 and 39 species respectively to their national lists.

The main changes in policy and legislation concern the application of the 1999/105/EC Directive by all the countries within the group. However, the species list differs considerably between members. Harmonisation of laws and methods for establishing seed stands and provenance regions was highlighted.

Research in progress concerns population genetics, breeding and selection of new basic materials and their propagation techniques for a number of noble hardwood species. *Ex situ* conservation studies are undertaken in all countries.

Conservation work on *Liquidambar orientalis* and *Corylus colurna* are under way in Turkey. Legislation for establishing special areas of conservation to protect habitats or species has been enacted in some countries. In France four *ex situ* collections of noble hardwood species (*Sorbus domestica*, *Ulmus* spp., *Prunus avium* and *Juglans* sp.) are now becoming national collections.

In general coordination in most countries is undergoing reform following application of the 1999/105/EC directive.

Documentation, information and public awareness

Photo CD

B. De Cuyper updated Network members on the progress made on the image collection database providing statistics and highlighting gaps. Morphology is currently the most represented while other topics such as management and wood utilisation are lacking. Therefore he stressed the importance of providing material to fill the gaps.

Participants were reminded that photos can be used freely for public awareness purposes provided that the name of the photographer and EUFORGEN (e.g. B. De Cuyper/EUFORGEN Noble Hardwoods Network) are acknowledged. B. De Cuyper agreed to simplify the classification and send a message with instructions to all participants via e-mail by 30 April and participants will send images to Bart by 30 June 2004. In addition B. De Cuyper will finalise the CD case cover design.

Network and EUFORGEN posters

An updated version of the Noble Hardwoods Network poster and a general EUFORGEN poster were displayed. The Network poster was originally developed for the DYGEN Conference and revised for the World Forestry Congress. These posters as well as other Network posters are available from the EUFORGEN Web site.

Noble Hardwoods poster

The poster text was originally agreed on during the sixth Noble Hardwoods Network meeting in Portugal. Relevant high-quality photos were selected by S. de Vries and T. Myking and sent to the Secretariat. The layout of two draft versions of the poster was presented and discussed during the meeting. The version showing the EUFORGEN Web-address was chosen and it was agreed to improve the layout following the suggestions provided during the meeting. The poster will be produced by the Secretariat and will also be made available through the EUFORGEN Web site.

Meetings, projects and other initiatives

CASCADE update

F. Villani presented the outputs of the CASCADE project (EVK2-CT-1999-00006), which focused on securing gene conservation and both adaptive and breeding potential of chestnut (*Castanea sativa*). The project aimed to integrate information from several research fields to devise long-term strategies for the conservation of chestnut genetic resources. The final project report has been submitted to the EC and the project partners are in the process of developing several publications based on the project. The Network participants considered the results of the project useful for the further development of the Common Action Plan-concept. More detailed information can be found at http://soi.cnr.it/~chestnut. F. Villani will provide a written update to be included to the meeting report.

EVOLTREE proposal

B. Heinze provided an update on EVOLTREE (Evolution and Management of Diversity in European Forest Trees) which was submitted as a Network of Excellence (NoE) proposal to the EU 6th Framework Programme in 2003. The proposal aimed to implement genomic approaches for monitoring, predicting and managing genetic diversity in European forest trees for sustainable resource management and environmental protection. EVOLTREE was a consortium of 32 partners from 14 different countries. The proposal was not accepted for funding because forest genomics was not considered as a high priority topic in the previous call, which had a major focus on marine ecosystems.

The EVOLTREE coordinator (A. Kremer, France) has recently sent out a message to the partners and asked them to provide feedback for the revision of the proposal. The next and final call for proposals under the 6th Framework Programme is likely to be opened in June 2004 and it will focus on terrestrial ecosystems. EVOLTREE II could be re-submitted with substantial changes.

Others

J. Svejgaard Jensen informed the Network on a recent US proposal to establish a IUFRO working party on silviculture and management of endangered species.

E. Collin presented the outcomes of the second International Elm Conferences held in Valsain (Spain) in 2003. The proceedings of the conference will be published soon.

New EC Regulations on Genetic Resources in Agriculture

J. Koskela reported on the development of new EC Regulations on Genetic Resources in Agriculture. The focus of this new Regulation will be on animal gene conservation and the budget will be € 10 million for a period of three years. The scope of the Regulation also includes crop, microbial and forest genetic resources with the focus on conservation, characterization, collection, utilization, documentation and evaluation. On-farm conservation and inventories are likely to be also eligible but research activities are specifically excluded. EUFORGEN is mentioned as the only forest-related framework in the Programme Regulation document. A first call is expected to be opened by autumn 2004, and a second call is scheduled for 2006. The Regulation is expected to provide funding for one or two forest-based projects.

E. Collin presented an elm conservation concept note to the new EC Programme on genetic resources conservation and utilisation. The possible project would be coordinated by L. Gil (Spain).

The possibility for a joint project on several species was discussed. Network members pointed out that there was too much emphasis on research in the draft and that as many countries as possible should participate in the project. Network members willing to contribute are invited to send their comments directly to E. Collin, A. Santini or L. Gil.

S.M.G. de Vries stressed on the importance of close and supportive collaboration among EUFORGEN Networks, avoiding duplicating efforts and especially not affecting negatively EUFORGEN's reputation.

J. Koskela forwarded a message from M. Lateur, Chair of the European Cooperative Programme on Genetic Resources (ECP/GR) *Malus/Pyrus* Working Group, who expressed the interest to collaborate with the Noble Hardwoods Network in developing a joint proposal for the new EC Regulation. The Noble Hardwoods Network welcomed the proposal and it was agreed that the electronic version of the proposal should be circulated to all members by the 27 April. Members should respond directly to M. Lateur copying correspondence to P. Rotach. The countries that expressed their interest in the further development of the collaboration are: Switzerland, The Netherlands, Hungary, Bulgaria, Belgium, Croatia, Slovenia, Italy, Malta, Czech Republic, Turkey, United Kingdom, France, Serbia and Montenegro, Germany, Poland and Portugal.

Progress made in the Network activities

EUFORGEN Web site, bibliography and technical guidelines

M. Bozzano presented the new structure of the EUFORGEN Web site, which is accessible directly through a new address (www.euforgen.org). The Web site is database-driven and includes a number of new features such as 'What's new'-section, species summary pages and an improved search engine. The EUFORGEN grey literature database is maintained as part of the new Web site and it currently includes nearly 2000 references. There is a need to include information on where to find given references and it was decided to indicate network members as contact persons in each country. Network members were encouraged to visit the Web site and to provide feedback to the Secretariat for further development.

M. Bozzano then provided an update of the state of the Technical Guidelines (TGs).

- The first set published in April 2003 includes *Fraxinus excelsior*, *Acer pseudoplatanus*, *Sorbus domestica*, *Prunus avium* and *Alnus glutinosa*;
- The second one, published in April 2004 includes: *Ulmus laevis*, *Malus sylvestris* and *Pyrus pyraster*, *Liquidambar orientalis*, *Castanea sativa* and *Tilia* spp. It was presented during the meeting.
- A third set is scheduled for publication later in 2004. The state of the remaining noble hardwoods TGs is as follows:
  - *Sorbus torminalis*: B. Musch has provided the final draft, ready for circulation.
  - *Alnus cordata:* F. Ducci has provided the draft, which will be circulated, after finalisation.
  - *Juglans regia:* J. Fernandez Lopez, M.E. Malvolti and K. Russell join F. Ducci in the development of the draft.
  - *Acer campestre:* L. Nagy will be supported by F. Ducci.

Deadline for sending final drafts to the Secretariat is 30 August 2004; no species modules will be accepted after this date. The Network expressed strong interest in continuing with this effort in the next phase.

M. Bozzano gave a live demonstration on the practical use of the free GIS software (DIVA-GIS) available through the EUFORGEN Web site showing some of the distribution maps developed by the Network.

It was agreed that Network members should advise potential stakeholders in their own countries on the tools and information available.

Common Action Plan (CAP)

M. Rusanen gave a presentation on CAP using *Acer platanoides* as a model species. The goal is to establish a pan-European network of primarily in situ gene conservation stands. The effort of many Networks in compiling the in situ conservation stands was acknowledged and the map showing the stands overlapping the species distribution was presented. The concept of Common Action Plan was explained and several questions were posed for discussion; an animated conversation followed the presentation. It was agreed:

- that henceforth the expression 'conservation unit' (cu) is to be used instead of conservation stand.
- that a conservation unit should ideally be dynamic.
- that a conservation unit need not necessarily be within the natural distribution area.
- that a working group (WG) (B. Heinze, T. Myking, H. Kraigher, P. Rotach, L. Nagy, M. Rusanen, M.C. Varela and J. Fernandez Lopez) will define criteria and minimum requirements for gene conservation units. A draft is to be circulated by the WG before 30 June 2004 to all Network members and any comments/ feedback is to be sent to the WG before 15 September 2004. The final version will be released before 31 October 2004.
- that M. Rusanen will continue work to further develop the Common Action Plan for *Acer platanoides* as a model and this will be presented in the next meeting.
- that J. Jensen will develop the Common Action Plan for *Tilia cordata* and this will be presented in the next meeting.

Outputs of the MCPFE process and development of EUFORGEN Phase III

After the EUFORGEN Steering Committee meeting in Sweden in June 2002, a Task Force produced a EUFORGEN strategy paper for the preparatory process of the Vienna Ministerial Conference. Based on this paper and other efforts, conservation of forest genetic resources were highlighted in the Vienna outputs.

J. Koskela presented recent outputs from the MCPFE process, i.e. the fourth Ministerial Conference (April 2003) and the Expert Level Meeting (October 2003), both held in Vienna, Austria and the *ad hoc* working group on the 'Development of pan-European understanding of the linkage between the ecosystem approach and sustainable forest management' (19 – 21 April 2004, Krakow, Poland). For more information the Web site is www.mcpfe.org.

At the Vienna Ministerial Conference, the European ministers responsible for forestry and the EC committed themselves to "take further steps to maintain, conserve, restore and enhance biological diversity of forests, including their genetic resources, in Europe and also on a global scale" (paragraph 22 of the Vienna Declaration). In Resolution 4 on Forest Biological Diversity, the ministers also committed themselves to "promote the conservation of forest genetic resources as an integral part of sustainable forest management and continue the pan-European collaboration in this area" (paragraph 16).

Following discussion on Phase III, the Network decided that it should convey the following message to the Steering Committee:

The Noble Hardwoods Network wishes to continue a species-oriented approach (without decreasing the current number of species) and contribute to thematic networks should they be established. The Network feels that there is a need to explore the opportunity of developing a working-group approach within the Network in order to focus on specific issues.

The question of sub-regional working groups was also raised, but it was agreed that the Pan-European approach is more appropriate for successful gene conservation.

Seminar on genetic conservation, tree breeding and utilization of noble hardwoods

Provenance regions for chestnut (Castanea sativa *Mill.*) *and flowering ash* (Fraxinus ornus *L.*) *in Tuscany* (*M. Vannucci*)

M. Vannucci made a presentation on the development of provenance regions in Italy with special reference to two species in Tuscany. Following the EC directive on FRM, it has become obvious that there is a need to develop a coherent system at national level to provide guidance for using the material appropriately. In Italy, the establishment of the provenance regions will be carried out independently by each region. These provenance regions should reflect species geographical distribution and variations in climatic and biogeographical factors. It is also necessary to obtain detailed forest inventory data as part of this process.

Clone selection for wild cherry (Prunus avium *L.*) *(F. Ducci and G. Signorini)*

F. Ducci and G. Signorini shared a presentation on clone selection for wild cherry using morphological traits such as stem and branch qualities as well as wood colour. Further information was provided using wood colour in clone selection and how to measure wood colour. The results of the studies indicate that clonal variation in growth and branch characteristics is considerably high (between 60 and 70 per cent) and that heritability is high for all traits. Regarding wood colour, it seems that there is no single colour that would be better than others in selecting clones. Information on colour preferences of the manufacturers should be taken into account while selecting clones. The relationship between wood colour and soil chemical composition should be further investigated.

In vitro *tissue culture in wild cherry* (Prunus avium *L.*) *(A. De Rogatis)*

A. De Rogatis presented research results on tissue culture techniques in wild cherry. The ISSA has been studying *in vitro* regeneration by somatic embryogenesis and by organogenesis from different types of somatic tissues. Isozyme studies have also been carried out among clones. Different genotypes have provided very different results in different growth media. No somaclonal variation was observed in shoots regenerated from leaf tissue. This has to be confirmed by DNA analysis.

Noble hardwoods cultivation techniques (S. Ravagni)

S. Ravagni provided an overview of cultivation techniques of noble hardwoods studied by ISSA to produce high-quality timber. She presented various alternatives for establishing single-species and multi-species tree plantations. *Quercus robur*, *Q. petraea*, *Juglans regia*, *J. nigra*, *Prunus avium*, *Sorbus* spp., *Pyrus communis* and *Fraxinus* spp. have been studied for single-species plantations. Other species such as *Alnus cordata*, *A. nigra*, *Elaeagnus* spp. *Robinia pseudoacacia* have been tested as accompanying species.

Walnut (Juglans regia *L.*) *genetic variation in Europe (M.E. Malvolti)*

M.E. Malvolti presented results from several studies on genetic diversity in walnut in Europe and Asia. The results indicate that walnut is threatened by genetic erosion and that gene conservation programmes are needed to preserve local varieties and ecotypes. These can also be used for tree improvement programmes focusing on fruit and wood production. She also informed the Network participants that the 5th International Walnut Symposium will be held in Sorrento, Italy, 9–13 November 2004 (www.walnut2004.sistemacongressi.com).

Any other business

Missing contributions (country updates and scientific papers) should be sent to the Secretariat by 30 June 2004. The importance of respecting deadlines was stressed in order not to delay the publication process. Contributions not received by this deadline will not be included in the proceedings.

Date and place of next meeting

Norway and Denmark have offered hosting the next meeting in spring/summer 2005. Both offers were acknowledged. It was agreed that the meeting will be held in Norway provided that local support will be confirmed and that the Noble Hardwoods Network will continue in its present form during Phase III of EUFORGEN. Denmark will be the alternative host.

Adoption of the summary of the meeting

The meeting summary was adopted and the Chair closed the meeting.

Introductory country reports

Noble hardwoods in Icelandic forestry

T. Eysteinsson
Iceland Forest Service, Egilsstadir, Iceland

Forests and native species

Iceland has the lowest percentage of forest cover in Europe—about 0.3% of the total land area. This translates to between 30 000 and 40 000 hectares (ha) of forested land. About two-thirds of this is native downy birch (*Betula pubescens* Ehrh.) forest and about one-third is planted forest of various species, mostly exotic conifers. In addition to this, about 100 000 ha or 1% of the total land area is classified as 'other wooded land', which is mostly downy birch scrub less than 5 m in height (Anonymous 2000).

Other native tree species in Iceland are rowan (*Sorbus aucuparia* L.), which is widespread, aspen (*Populus tremula* L.), which is very rare, and tea-leaved willow (*Salix phylicifolia* L.), which is common but only rarely attains tree height (5 m). Other native woody species never reach 5 m in height.

Forestry

Traditionally, the birch woods of Iceland were economically important as a source of building material, fuel, fodder for livestock and charcoal needed to smelt bog iron and make iron tools. Woodlands continued to be important as a source of fuel and winter fodder for sheep until the 1930s, after which wood fuel was replaced by geothermal heat and imported oil. Furthermore, birch was replaced as a source of fodder by cultivated hayfields. Thus, traditional forest utilization practices have all but disappeared.

Modern forestry is considered to have started with the planting of the 'Pine Stand' at Thingvellir in 1899, Iceland's first forest plantation. During the first decade of the 20th century, many exotic tree species were introduced including several noble hardwoods. Those that grew best and consequently became used as park and garden trees were Swedish whitebeam (*Sorbus intermedia* (Ehrh.) Pers.), wych elm (*Ulmus glabra* L.), sycamore (*Acer pseudoplatanus* L.) and, to a more limited extent, silver birch (*Betula pendula* L.) and ash (*Fraxinus excelsior* L.). Originally, seed and seedlings of these species came from Denmark or Norway but some have been propagated from Icelandic seed during the past few decades (Blöndal and Gunnarsson 1999).

After these limited early trials, emphasis in forestry shifted from planting to protecting native woodland remnants. During the first half of the 20th century the state acquired several important forests and woodlands and protected these from grazing; these now comprise our national forests. Among these, two have substantial populations of rowan.

Beginning in the 1950s, emphasis in Icelandic forestry again shifted to afforestation through planting, mostly of exotic conifers, although native birch has always comprised 10–30% of afforestation planting. *Sorbus* species—elm, sycamore, silver birch and ash—were not planted for afforestation to any extent but forestry nurseries produced them for sale to individuals who planted them in their gardens and around summer cottages. Thus, experience was gained regarding the cultivation of these species.

Today, roughly 5 million seedlings are planted annually in afforestation in Iceland, which translates to an annual increase in forest cover of 1200–1500 ha (Gunnarsson 2003). Practically none of this is in noble hardwoods, although silver birch is regularly planted and the use of native rowan in afforestation has been increasing in recent years.

Iceland Forest Service (IFS)

The Iceland Forest Service (IFS) is the state forestry authority. Established in 1907, its mandate is to protect and preserve natural forests and forest remnants, to grow new forests where appropriate and to advise on forests and forestry-related matters. To these ends the IFS manages the national forests, 40 native forests and afforestation areas, with combined conservation, recreation, research and development goals. Research is an increasingly important role of the IFS as afforestation increases. Emphasis in research is on forest genetics and adaptation, pests and diseases, afforestation conditions and establishment techniques, forest ecology, carbon sequestration and forest inventory. Forestry extension and planning has been an important part of IFS activities but this is increasingly being provided by the Regional Afforestation Projects.

For most of the second half of the 20th century, the main activities of the IFS were seedling production, with as many as six nurseries in operation, and planting trees, mostly in IFS-owned lands. Seedling production has now been privatized and planting is only a minor part if IFS activities today.

The IFS is responsible for most tree improvement activity in Iceland as well as management and conservation of forest genetic resources. There are ongoing active tree improvement projects for native birch, russian larch (*Larix sukaczewii* Dylis), sitka spruce (*Picea sitchensis* (Bong.) Carr.) and black cottonwood (*Populus trichocarpa* Torr. & Gray).

The head office of the IFS is in Egilsstadir in East Iceland and the research station—Iceland Forest Research—is located at Mógilsá just north of Reykjavík. In addition to this, there are six district forestry offices mostly located within the national forests. The IFS has a full-time staff of about 50 professionals and support staff and employs another 30–40 seasonal staff.

Regional Afforestation Projects (RAPs)

Since 1990, a total of six Regional Afforestation Projects (RAPs) have been set up, covering the whole country. The RAPs manage the government grants scheme for afforestation on farms, each in its own region of the country. They are independent of the IFS in that they each have their own board of directors, consisting mostly of local people, and an independent budget. However, the IFS has one member on each board and professional ties with the IFS are close.

The RAPs employ a total of 22 professional staff and provide grants and other assistance to over 500 landowners participating in the projects. About 70% of all tree planting in Iceland is carried out under the auspices of the RAPs.

Icelandic Forestry Association (IFA)

The Icelandic Forestry Association (IFA) is the third player in Icelandic forestry. It is an umbrella organization of over 50 local forestry societies and, with a total membership of over 7000, by far the largest environmental non-governmental organization (NGO) in Iceland. The IFA publishes *Icelandic Forestry*, Iceland's main forestry publication, and provides education and extension services. They also manage the Land Reclamation Forests programme according to a contract with the Ministry of Agriculture. This government-funded programme provides seedlings for planting on degraded and eroded land and through it, roughly 1 million seedlings have been planted per year since 1990, mostly by the local forestry societies in co-operation with municipalities.

Besides the Land Reclamation Forests programme, local forestry societies are mostly concerned with managing older forests and woodlands for outdoor recreation, some grow Christmas trees, some have small tree nurseries and one owns a large commercial nursery.

Forestry goals

In general, Icelandic afforestation is planned and cultivated forests managed with multiple-use objectives (Skulason et al. 2003). These objectives can best be described based on the four principle functions of forests: ecological (ecosystem processes, habitats, wildlife); economic (wood production, non-wood products); protective (soil and water conservation, shelter, sequestering CO_2); and social (recreation, cultural and spiritual).

In forest planning and management, greater emphasis is often placed on one or two of these functions and less emphasis on others without ignoring them entirely. Within the RAPs, the majority of afforestation plans to date emphasize timber production as a primary goal within areas where timber production is possible, the main timber species being exotic conifers. In peripheral areas, emphasis is on protective functions and in some cases ecological restoration, where the main species is native birch, or on establishing shelterbelts. A few plans have been drawn up emphasizing wildlife value, improved grazing for livestock and outdoor recreation as well.

The management goal for the majority of national forests (IFS lands) is protection of native forest and woodland ecosystems. Outdoor recreation, timber production, ecosystem restoration and research are also goals in some IFS lands, whereas erosion control and reclamation are the main aims on land managed by the Soil Conservation Service.

Rowan

It seems likely that rowan arrived in Iceland shortly after the retreat of the ice age glaciers roughly 10 000 years ago and that new genetic material has arrived sporadically ever since. The most likely vehicles for this are fieldfares (*Turdus pilaris*) and blackbirds (*T. merula*) that often get sidetracked to Iceland during their autumn migration from Scandinavia to the British Isles and southern Europe. By the same token, Icelandic redwings (*T. iliacus*) migrating to Great Britain and Ireland can easily carry seeds of rowan south.

Rowan was probably common in Icelandic woodlands at the time of human settlement 1130 years ago, as attested to by place names such as Reynines (Rowan peninsula), Reynivellir (Rowan flats) and Eskifjörður (Ash fiord—named by someone who misidentified rowan as ash, an understandable mistake). But eleven centuries of unsustainable use, principally as sheep grazing territory, have reduced woodland cover by 95% and rowan is today widespread but uncommon in Iceland. It is most often found growing in birch woods but also as single trees or shrubs in places that are inaccessible to sheep. Populations of several individuals to several hundred are found in a few places in eastern Iceland and the northwestern fiords. The number of mature individuals growing in the wild is probably less than 1000, certainly much fewer than the number planted in gardens and parks.

However, seedlings and saplings of rowan seem to be becoming increasingly common in forests and afforestation areas protected from grazing. Much of this regeneration stems from redwings feeding on berries of cultivated rowan and dropping the seeds in night roosting areas which are often in forests, either natural or planted. Rowan growing under good cultivated conditions usually flowers more profusely and produces more berries than in the wild. Thus it seems that cultivation of rowan as a park tree, which began in the 1940s, combined with forest protection and afforestation efforts are resulting in increased regeneration of rowan.

The origin of rowan cultivation in Iceland is well known. Most of the rowan grown in nurseries in south Iceland between 1930 and 1980 originated from a single tree growing on a cliff face in south-central Iceland. This was the only known rowan in all of south Iceland. Practically all of the old rowan in gardens in Reykjavik and other towns and villages in south and west Iceland are of this origin and are all phenotypically similar.

Likewise, the rowan grown in nurseries in northern Iceland originated from a single individual growing on a scree slope in north-central Iceland. This tree was thought to have magical properties and people made pilgrimages to visit it. In the late 18th century a local priest had finally had enough of this paganism and ordered the tree cut down; it re-sprouted from the stump and roots and in 1825, a farmer in the area dug up some of the root suckers and planted them in a walled garden near his house. These trees still live and they were the source of berries for propagation in nurseries beginning in 1910. The old rowans in Akureyri and other towns in north Iceland are all of this origin and they are all phenotypically similar but different from the ones in Reykjavik.

A tree nursery in east Iceland had more trees to choose from but probably only propagated berries from a few trees. Rowan of Danish origin has been available in nurseries since the 1970s. It is well adapted to the Icelandic climate and has become widespread in cultivation. Specimens are all phenotypically similar and probably originate from one cultivar. In all, rowan in cultivation in Iceland can probably be traced to fewer than 10 original parent trees, most from only two trees.

There is no programme to conserve genetic resources of rowan in Iceland and no regulations as to what genetic material should or should not be used. It is unlikely that such regulations will be instituted or that they will have any meaning even if they are. There is no reason to assume that Icelandic rowan forms a distinct population from Scandinavian or British rowan. Any measurable differences are likely to be the result of founder effects (limited genetic material arriving) rather than any meaningful evolution after arrival. Also, the very limited genetic diversity of cultivated rowan does not seem to result in any problems, such as susceptibility to disease. Thus, there seems to be no practical reason for preserving the limited genetic diversity of Icelandic rowan by keeping it separate from imported material nor is there any pressing need to increase diversity by bringing together trees from different parts of Iceland so they can interbreed. It seems that the best course of action is simply to provide opportunities for natural regeneration and not worry too much about genetics.

Rowan has not, until now, been planted much in afforestation. However, that is likely to change in the coming decades. A series of silvicultural and genetic trials is planned, with the goal of developing wood production of rowan. If successful, rowan will become both a valued and valuable component in Icelandic forests.

Exotic noble hardwoods

As stated earlier, there is a century of experience with cultivation of several noble hardwood species in Iceland and shorter experience with a few others (Table 1). Only one of the species on the EUFORGEN group's list is regularly used in afforestation in Iceland—silver birch, of which up to 50 000 seedlings are planted annually (Gunnarsson 2003). Other species are generally close to the limits of their adaptation in Iceland and therefore require sites with good shelter and high soil nutrient status, preferably on a south-facing slope. Many of them have, until now, only been grown under south-facing walls of houses. However, with a warming climate, some of these species could become more viable in Icelandic forestry, at least as amenity species and some even as components in timber production forestry. There are already plans to plant forest stands of sycamore and ash and more are likely to follow.

Table 1. Noble hardwoods in Iceland

The group's list	Experience	Potential for use
Alnus glutinosa	Limited to a few trees	Unknown, requires provenance testing
Acer pseudoplatanus	Extensive	Amenity, potential timber species
Betula pendula	Extensive	Amenity, potential timber species
Fraxinus excelsior	Several seed sources tried	Potential amenity species
Prunus avium	Limited to a few trees	Unknown, but seems promising
Sorbus aria	Recent, sold in nurseries	Good amenity species
Sorbus aucuparia	Native	Amenity, potential timber species
Tilia cordata	Limited to a few trees	Unknown, potential amenity species
Ulmus glabra	Extensive	Good amenity species
Related species		
Alnus incana	Extensive	Amenity and soil conservation
Betula pubescens	Native	Amenity, conservation, timber
Prunus padus	Extensive	Good amenity species
Sorbus intermedia	Extensive	Amenity, potential timber species

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Noble hardwoods in Serbia and Montenegro

S. Orlovic[1], N. Matovic[2] and A. Pilipovic[1]
[1]Poplar Research Institute, Novi Sad[†]
[2]Yugoslav Centre for Plant and Animal Genetic Resources, Belgrade
[†]Present address: Institute of Lowland Forestry and Environment, Agricultural Faculty Novi Sad, Antona Cehova 13 - P.O. Box 117, 21000 Novi Sad, Serbia and Montenegro

Serbia and Montenegro have 2.85 million hectares (ha) of forests, of which 50.2% are state forests. The total growing stock is about 134.39 million m^3. Forest percentage varies from about 6% in northern Serbia and Montenegro (Vojvodina Province) to about 32% in other parts of the country. Hardwoods cover 1.32 million ha. Serbia-Montenegren forests are categorized as 'high forests' and 'plantations' (49%); coppice forests (42%); and scrub and brushwood (9%). The average volume is 107 m^3 ha^{-1} and the average current volume increment is 2.6 m^3 ha^{-1} (Jovic and Medarevic 1995). Broadleaf stands cover 60.7% and conifer stands cover 4.7% with mixed broadleaf stands covering 30% and mixed stands of broadleaves and conifers covering 33% (Isajev et al. 1996).

Main species

Sycamore (*Acer pseudoplatanus*) in Serbia and Montenegro occurs as an admixed species in beech-type (*Fagetum subalpinum*) and fir-beech (*Abieto fagetum*) forests. Together with European ash (*Fraxinus excelsior*), it makes the association *Acereto–Fraxinetum serbicum* which is most commonly found in north-east Serbia. There are several varieties and forms, the most important of which are the subspecies *villosum* and var. *subtrilobum.*

Norway maple (*Acer platanoides*) is an admixed species in broadleaf forests and is frequently found in beech forests. It also makes the association *Acereto–Fraxinetum*. Norway maple thrives in areas where pedunculate oak and ash, sessile oak and hornbeam, Turkey oak and Hungarian oak, beech and fir are also found. It is not as common as sycamore.

European ash in Serbia and Montenegro grows most frequently in hilly and mountainous beech forests. In these forests, there are smaller tracts where European ash, maple, Norway maple and other species of the association *Acereto–Fraxinetum* predominate. European ash also grows in the association *Fago–Intermedio–Columetum* with beech, maple and Turkish hazel (*Corylus colurna*). There are two varieties of European ash, namely *F. excelsior* var. *pendula* and *F. excesior* var. *globosa*. Narrow-leaved ash *(Fraxinus angustifolia)* grows naturally in the associations *Genisto–quercetum roboris* and *Querco–Fraxinetum serbicum*. In Serbia and Montenegro several forms of narrow-leaved ash have been described, namely *F. obtusa, F. rostrata, F. leptocarpa* and *F. macrocarpa*.

Single specimens of wild cherry (*Prunus avium*), wild apple (*Malus sylvestris*) and wild pear (*Pyrus pyraster*) can be found in the forests of sessile oak–hornbeam, mountainous beech, maple and ash.

Single specimens of common elm (*Ulmus campestris*) can be found growing with pedunculate oak and ash together with *Q. robur, fr. angustifolia, C. betulus* and *A. campestre*. It also occurs at drier sites in the associations Turkey oak–Hungarian oak and sessile oak–hornbeam. It has several varieties and forms, namely *U. campestris, F. suberosa, U. campestris* var. *carpinifolia* and *U. campestris* var. *salicifolia*. European white elm (*Ulmus laevis* Pall.) can be found in lowland forests, plains and river valleys with willows, poplars, narrow-leaved ash, and pedunculate oak together with *S. alba, S. fragilis, P. alba, P. nigra, F. angustifolia* and *Q. robur*.

Service tree (*Sorbus domestica*) grows as an admixed species in the warmer region of the oak forest belt, e.g. Hungarian oak–Turkey oak forests. Wild service tree (*Sorbus torminalis*) is an individually admixed species in Hungarian oak, Turkey oak, sessile oak and hornbeam forests.

Common whitebeam (*Sorbus aria*) and rowan (*Sorbus aucuparia*) grow as individual trees in beech and fir forests.

The lime genus (*Tilia* spp.) in Serbia and Montenegro is represented by several species, the most important being the small-leaved lime (*Tilia cordata*), which grows in the association of sessile oak–hornbeam and in the lower region of beech; and large-leaved lime (*Tilia grandifolia*), which grows in the communities sessile oak–hornbeam, mountainous beech and lime–European ash.

Economic and social importance

The main species of noble hardwoods in Serbia and Montenegro, presented in Table 1, generally occur as individually admixed species in mixed associations. Exceptions are the species in the genera *Fraxinus* and *Acer,* which compose mixed associations. The species in these genera are at the same time economically the most significant species.

Table 1. Main noble hardwoods in Serbia and Montenegro

Species	Distribution	Economical importance
Acer pseudoplatanus	++	++
Acer platanoides	++	++
Ulmus campestris	+	-
Ulmus effusa	+	-
Sorbus domestica	+	-
Sorbus aucuparia	+	-
Sorbus torminalis	+	-
Sorbus aria	+	-
Prunus avium	+	+
Malus silvestris	+	-
Pyrus pyraster	+	-
Tilia cordata	+	+
Tilia grandifolia	+	+
Fraxinus angustifolia	+	+
Fraxinus excelsior	+	+

Conservation (*in situ, ex situ* measures) and use

In the previous reporting period, there were very few activities on *ex situ* and *in situ* conservation of noble hardwood gene resources (Tables 2 and 3). The work on the conservation of these species is far less than called for by their economic significance. The main reason for this is the complete absence of organized work on these species, along with the deficiency of financial means.

Table 2. Current status of *ex situ* conservation of noble hardwood tree genetic resources in Serbia and Montenegro

Species	Stands	Seed orchards and clone collections			Seed storage for conservation (kg)
	ha/no.	Number	ha	Number of clones/families	
Acer pseudoplatanus	-	-	-	-	-
Acer platanoides	-	-	-	-	-
Ulmus campestris	-	-	-	-	-
Ulmus effusa	-	-	-	-	-
Sorbus domestica	-	-	-	-	-
Sorbus aucuparia	-	-	-	-	-
Sorbus torminalis	-	-	-	-	-
Sorbus aria	-	-	-	-	-
Prunus avium	-	1	1	35	-
Malus silvestris	-	-	-	-	-
Pyrus pyraster	-	-	-	-	-
Tilia cordata	-	-	-	-	-
Tilia grandifolia	-	-	-	-	-
Fraxinus angustifolia	1.00/1	-	-	-	-
Fraxinus excelsior	1.00/1	-	-	-	-

Table 3. Current status of *in situ* conservation of noble hardwood tree genetic resources in Serbia and Montenegro

Species	Stands (ha/no.)	Single trees
Acer pseudoplatanus	0.22/6	8 groups
Acer platanoides	-	5 groups
Ulmus campestris	-	-
Ulmus effusa	-	-
Sorbus domestica	-	-
Sorbus aucuparia	-	-
Sorbus torminalis	-	1 group
Sorbus aria	-	-
Prunus avium	-	5 groups
Malus silvestris	-	1 group
Pyrus pyraster	-	1 group
Tilia cordata	-	1 group
Tilia argentea	1.00/1	-
Tilia grandifolia	2.60/2	2 groups
Fraxinus angustifolia	61.92/3	2 groups
Fraxinus excelsior	30.28/7	4 groups
Castanea sativa	0.38/1	-
Aesculus hippocastanum	0.38/1	-

Inventories

There is no precise inventory, either by area or by tree species, because noble hardwoods in Serbia-Montenegro occur mainly as individually admixed species.

Relevant policies and legislation

In Serbia and Montenegro, there are no legal regulations addressing gene resources issues. However, the National Centre for Plant and Animal Genetic Resources, Department of Plant Genetic Resources, oversees national genetic resources issues. In addition, the appointment of the national EUFORGEN co-ordinator is in progress.

Research

The research on noble hardwoods is rare primarily because there are relatively few researchers. In addition, there is an absence of programmes by which such research could be financed.

Tree improvement

Of all the noble hardwood species, wild cherry has been the most treated species. In 1999, a seed orchard was established that was composed of half-sib families of 35 'plus trees'.

Along with wild cherry, common elm is now being studied. The study includes the definition of variability, as well as the elaboration of the micropropagation method in the aim of establishing a clonal seed orchard from the selected genotypes as a form of *ex situ* conservation.

Last year, the selection of narrow-leaved ash genotypes was started in natural populations in the Danube and Sava river valleys; the genotypes were selected, seed was collected and is being used for the propagation of seedlings from which a seedling seed orchard will be established.

Summary and country priorities

The progress in noble hardwood gene conservation depends on financial support and co-ordination at the state level. Seed stands or plus trees have already been selected for some species of noble hardwoods; however, selection must start immediately for the remaining species. Seed orchards must be established for the species where there are plus trees. This will simultaneously cover the function of gene resource conservation and an increase in the utilization of the species' genetic potential. In addition, implementation of new methods of biochemical genetics must start immediately. These methods have been applied in agricultural species, but not in forest tree species in Serbia-Montenegro. Without more reliable data on the genetic structure, it is difficult to develop future research activities.

A Serbia/Montenegran gene conservation database does not exist; however, there are plans to develop such a database relatively soon. This development will enhance future work on gene conservation.

Until now, Serbia and Montenegro have not participated in international co-operative programmes. We can accomplish very little if we work by ourselves; we are aware that our future means co-operative work with the scientists from other countries. For this reason, we expect that the researchers from Serbia and Montenegro will be included in other international co-operative programmes in addition to EUFORGEN, such as the projects developed within the sixth research framework programme of the European Commission. Serbian and Montenegren researchers will benefit from the exchange of information and the acquisition of new knowledge.

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Research on noble hardwoods: updates presented during the sixth meeting

Genetic structure of common ash in Europe analyzed with nuclear microsatellites

***M. Heuertz*[1,2]*, J.-F. Hausman*[1] *and X. Vekemans*[2]**
[1]*Centre de Recherche Public-Gabriel Lippmann, CREBS Research Unit, Belvaux, Luxembourg*
[2]*Université Libre de Bruxelles, Laboratoire de Génétique et Ecologie Végétales, Brussels, Belgium*

Background

The investigation of the structure of diversity with nuclear genetic markers over a species' natural range has often revealed similar patterns in species that share a common life history (Hamrick and Godt 1989). For instance, many tree species have been found to present high genetic diversity within populations but low differentiation among those same populations (Hamrick et al. 1992). Some biological characteristics of tree species such as long generation time and predominant wind pollination in temperate areas (Hamrick and Godt 1989) can explain this. On a large spatial scale, the genetic diversity patterns of tree species have also been influenced by historical processes like recolonization after the last ice age (e.g. Newton et al. 1999). During the recolonization process, successive founder events led to a loss of genetic diversity because colonisers only bore a sample of the diversity of their source populations (Nei et al. 1975). On the other hand, admixture of colonisers from different glacial refuges acted against the loss of genetic diversity (Comps et al. 2001). Human impact through past and present management has also strongly imprinted diversity patterns in tree species (e.g. Köenig et al. 2001).

The common ash (*Fraxinus excelsior* L.) is a noble hardwood species that occurs in several mixed deciduous forest types found over most of Europe, with the exclusion of the most southern and most northern parts. It is a post-pioneer, heliophilous species with a complex and not yet fully characterized mating system; there is a continuum from pure male to pure female individuals with different kinds of hermaphroditic intermediates (Wardle 1961, Picard 1982, A. Lamb and D. Boshier, unpublished). Common ash is wind-pollinated and its seeds, the samaras, are wind-dispersed.

So far, the genetic diversity of common ash has been investigated with nuclear microsatellite markers on a country-wide scale in two studies (Heuertz et al. 2001, Morand et al. 2002). These studies revealed similar high within-population diversity and low differentiation among populations in Bulgaria and France; a strikingly high level of heterozygote deficiency was observed in France but not in Bulgaria.

The objective of our present work was to identify geographical patterns of within- and among-population diversity at the European scale, clarify genetic relationships between European regions and interpret the observed patterns from an evolutionary point of view.

Material and methods

We sampled an average of 30 non-adjacent common ash trees in 36 putatively autochthonous forests in the south-eastern part of Europe. DNA was extracted from buds or leaves with commercial extraction kits (DNeasy Plant mini kit from Qiagen, NucleoSpin Plant kit from Macherey Nagel) and amplified at five nuclear microsatellite loci through polymerase chain reaction (PCR). For each locus, one PCR-primer was labelled with a fluorescent dye, which allowed detection of amplified fragments on an automated DNA sequencer (ABI Prism 377 DNA sequencer). The amplified fragments were sized with the help of the sequencer software by comparison with an internal size standard.

Data analysis was performed with the programs Gen-survey (Vekemans and Lefèbvre 1997), Genepop (Raymond and Rousset 1995), Fstat (J. Goudet 2001, available from http://www.unil.ch/izea/softwares/fstat.html) and Spagedi (O. J. Hardy and X. Vekemans 2002, available from http://www.ulb.ac.be/sciences/lagev).

Results and discussion

The analysis of within-population diversity at the European scale revealed high heterogeneity in levels of allelic richness and gene diversity among populations. A geographical pattern of variation could be identified; a higher diversity existed in northern and western Europe than in the south-eastern part of the continent. Thus, there was no loss of genetic diversity through founder events that would have occurred along with the recolonization from ancient glacial refuge areas in south-eastern Europe towards recently recolonized regions located northwards. We attributed the higher diversity levels in recolonized regions to the occurrence of admixture of previously differentiated gene pools during the process of post-glacial recolonization.

We further identified a geographical gradient of variation in the inbreeding coefficient. Generally, populations in ancient glacial refuge areas were close to the Hardy-Weinberg equilibrium and a significant heterozygote deficiency was observed in recolonized areas, confirming the results obtained by Morand et al. (2002) in France. Different explanations can be put forward for this: variation across Europe in the frequency of null alleles, in the level of local mating among relatives, or in the mating system with higher levels of self-fertilization in the regions with significant heterozygote deficiency.

The analysis of genetic diversity among populations revealed higher differentiation among populations from south-eastern Europe than among populations from central and western Europe. This pattern was explained by the co-occurrence of ancient, differentiated gene pools under a stable demographic history and restricted gene flow among regions from south-eastern Europe. Conversely, in central and western Europe, extensive gene flow during post-glacial recolonization would have homogenized genetic diversity in populations from different regions. Variable levels of gene flow can indeed occur in a species in different demographic contexts; similar levels of pollen and seed dispersal into low-density stands have been shown to result in higher effective gene flow than under stable demographic conditions (Austerlitz et al. 2000).

We found that the genetic diversity in common ash is highly differentiated in regions from south-eastern Europe and suggest that these regions constitute important source populations for the conservation of genetic resources in this species.

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FRAXIGEN: Ash for the future—defining European ash populations for conservation and regeneration

J. Stewart
Oxford Forestry Institute, University of Oxford, Oxford, United Kingdom

The FRAXIGEN project started in January 2002, funded by the European Union (EU) fifth framework programme for research. Its overall aim is to increase our understanding of the genetic structure and dynamics of ash populations in Europe, as well as to investigate the forces shaping these populations, in particular the influence of reproductive processes on gene flow. The project will also provide insights into the scale at which localized adaptation occurs, using innovative networks of field trials established under woodland conditions. The research results will provide practical guidance on strategies for the collection, exploitation and conservation of ash genetic resources in Europe, with an emphasis on the selection of genetically appropriate planting material for conservation and ecological restoration plantings.

In FRAXIGEN, we are working on three native European ash species, namely: *Fraxinus excelsior, F. angustifolia* and *F. ornus.* Partners in the UK, Sweden, Spain and Greece[1] are carrying out parallel studies on the three species, with the UK and Swedish partners focusing on *F. excelsior* and the Greek and Spanish partners on the other two, more southern species. We hope to include additional partners from Slovakia and Romania[2] from late 2002, extending our research area eastwards.

There is a strong molecular component to the research. Neutral markers are being used to investigate the genetic architecture of each species and the effects of reproductive processes on this structure. At the population level, 40 trees in each of 15 populations are being sampled for each species, and nuclear microsatellites will be used to estimate both diversity within populations and differentiation between populations. In a subset of the same samples, chloroplast DNA markers will be used to characterise each population in terms of haplotype diversity, building on the results of the EU 'Measuring Molecular Differentiation of European Deciduous Forests for Conservation and Management' (CYTOFOR) project.

The nuclear microsatellite markers will also be used for more detailed studies in two populations of each species, where the mating system and patterns of gene flow will be investigated, including the relative contributions of pollen and seed to gene flow, and the level and importance of inbreeding depression. In these detailed studies, 20 mother trees and their progeny arrays (20 seeds per mother), as well as at least 150 putative sires (male and hermaphrodite), will be mapped and genotyped. The data will be used to estimate pollen:seed flow ratio as well as to carry out paternity exclusion analysis. The results will help us to design seed collection strategies to maximise genetic diversity.

The molecular studies are complemented by a strong element of field observations and experiments. In each of the intensive study populations, detailed observations of phenology, sex allocation and resource allocation (masting behaviour) will be continued over two years, as well as controlled pollinations to estimate the scope for self-fertilization. These observations will inform the interpretation of the molecular data by giving insights into the effects of reproductive biology on gene flow and the mating system.

In a separate, but closely related, component of FRAXIGEN a network of field trials will be established for each species. These will be planted in Greece for *F. angustifolia* and *F. ornus*, and in UK for *F. excelsior*. These networks are reciprocal transplant experiments (RTEs), in which seed from eight sites is planted in trials at the same eight sites, so that each trial contains both the 'home' population from that site, and the seven 'away' populations from the other sites. The sites are chosen to be at widely differing distances from one another, ranging from 5 km to over 500 km apart. The populations closest together are selected to be on contrasting sites in terms of soil or altitude. This design will give us insights into the relative contributions of localized adaptation and gene flow to population structure

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[1] UK: Oxford Forestry Institute, University of Oxford (co-ordinator) and Forestry Commission Research Agency; Greece: Forest Research Institute, Thessaloniki and Aristotelian University, Thessaloniki; Spain: Consejo Superior de Investigaciones Científicas, Valencia and University of Valencia; Sweden: University of Göteborg and Swedish Agricultural University, Umeå.

[2] Slovakia: Technical University, Zvolen; Romania: Forest Research and Management Institute (ICAS), Simeria Research Station.

and differentiation. In other words it will shed light on the question, "How local is local?" There is a strong trend towards preferring local material for planting, and this is enshrined in several European policies and directives, such as the Helsinki Guidelines adopted by the second Ministerial Conference on the Protection of Forests in Europe (MCPFE) in 1993, which state that "local provenances should be preferred where appropriate". There is still, however, very little scientific basis for defining what distance may be considered 'local'. This is a key question that FRAXIGEN seeks to address.

Overall, the project's results should provide a sound scientific basis for selection of genetically diverse and robust planting material. They will be disseminated through several 'non-technical' media including our Web site (http://www.fraxigen.net); a brochure produced in each of the partner countries' languages; and annual user group meetings in each partner country.

Report on EC Fifth Framework Project "Improving Fraxinus (ash) productivity for European needs by testing, propagation and promotion of improved genetic resources" (RAP: Realising ash's potential)

G. Douglas
Teagasc Kinsealy Research Centre, Kinsealy Biotech Unit, Dublin, Ireland

The genetic improvement of common ash (*Fraxinus excelsior*) is the main objective of the project. The work involves examining existing provenances trials, establishing a European provenance trial in six countries, improving the vegetative propagation of selected materials, studying genetic diversity and hybridization and defining effective means to communicate the research results and market situation for ash to end users. This project has 15 partners in 9 countries.

Growth data have been collected and the first analyses have shown significant variation among provenances. First results have shown significant differences between provenances for height and circumference data and genotypic inheritability. These differences varied between 0.32 ($p<0.05$) and 0.76 ($p<0.001$) at the two sites examined. The coefficient of phenotypic variation decreased with time; it ranged from 15% to 19% for data collected in 1990 to 5–6% for data collected in 2000. The analysis of data on stem form and crown form showed highly significant differences among provenances and inheritability that was characterized as medium to very low (from 0.68 [$p<0.0001$] to 0.26 [NS]). Provenance effects were also highly significant for frost damage at two sites and genotypic inheritability was good (from 0.60 [$p<0.001$] to 0.72 [$p<0.001$]). The genotypic inheritabilities and gains were computed after a multi-site analysis of all characters measured was completed. This showed that provenance effect was highly significant for height growth but not for height increment and stem girth. Height inheritabilities were 0.78 ($p<0.001$) from 1990 growth data, decreasing to 0.53 ($p<0.01$) for 2000 data. Provenance effect was at least significant for crown and stem form giving genotypic inheritabilities from 0.44 ($p<0.05$) to 0.77 ($p<0.001$).

Ash seeds have been collected, distributed and stratified by the seven partners who will establish the European provenance trial. Each partner will test a core collection of 30 provenances collected in diverse geographic regions within the natural range of European of ash. The plants are ready for field planting by several partners in spring 2004.

The first estimates of genetic diversity using microsatellites showed a high level of diversity within populations. However, more detailed analyses showed limited heterozygosity in some populations which may indicate a higher level of inbreeding than previously expected.

A fine scale genetic structure was revealed in ash populations using spatial autocorrelation tools and parentage analysis. It showed that two trees separated by less than 100 m were genetically more similar than two trees chosen at random in the same population. It means that gene flow by pollen and seeds is restricted within the stand. The neighbourhood size estimates showed that any given tree in the studied stand mates at random with 178 individuals.

Five polymorphic microsatellite loci were used in a parentage analysis of natural seedlings in four zones within a stand. It showed that the mean distance of seed dispersal was about 80 meters and followed geographic contours in two valley sites. This means that seed dispersal within the stands is significantly less than the level expected by random events. Estimates of gene flow from outside the ash stand were 58%, due to dispersal by pollen.

Plus trees were identified among the trees in provenance trials. They were conserved in the nursery by grafting. The best trees in the best provenances were used to establish shoot cultures and to develop an effective system for large-scale micropropagation. Shoot cultures were established successfully from diverse sources; dormant winter buds, shoots from grafted plants and immature embryos. Viable shoot cultures were established from 27 mature trees and from seeds collected from selected trees (16 new cultures). Shoots have been micropropagated satisfactorily on woody plant medium with cytokinin giving a range of micropropagation rates of 1.0 to 3.1 per subculture, per genotype. Scale-up of selected tree production has been underway by two commercial laboratories with 1000 plants now at the rooting stage. Rooting experiments have started giving 50–90% rooting in micro-shoots and 80–90% survival of rooted plants in the greenhouse. Somatic embryogenesis was demonstrated for the first time in *F.*

excelsior by using immature embryo as the primary explant culture on MS medium with 2.0 ml 2,4-D and 1.0 ml BA. The somatic embryos continued to develop to the maturation stage by further culturing. In addition, adventitious shoot regeneration was recorded in the axes of cultured embryos.

Studies on flower induction showed that application of drought stresses to grafted trees in the proceeding year increased tree flowering from 4 to 21% and delayed flushing date. Paclobutrazol applications reduced shoot growth and increased trunk diameter.

Cuttings from ash seedlings give 75–95% rooting. High rooting rates of 68% to 95% were recorded in cuttings from four mature trees when the cuttings were collected from micropropagated plants that had been transferred to the glasshouse. This indicates that the micropropagation step restores rooting competence to mature material. Monitoring of the levels of soluble carbohydrate in ash cuttings indicated that a low initial level of mannitol or a rapid decrease in the level of mannitol was indicative of high rooting rates.

Methods to survey the end users of ash were determined in the context of identifying the key players who will affect the adoption of any new technologies (or germplasm) in relation to ash. The existing state-of-the-art adoption models were reviewed and methods were developed to conduct the survey in relation to this project. The participants who will constitute the consultation panel of end users have been identified among the European partners in this project. They will act as an important source of information in the market for ash as well as on other aspects related to ash improvement.

Genetic resources of broadleaved forest tree species in south-eastern Europe

R. T. Volosyanchuk
Laboratory of Forest Tree Breeding, Ukrainian Research Institute of Forestry and Forest Melioration, Kharkiv, Ukraine

The first phase of the project, 'Genetic resources of broadleaved forest tree species in south-eastern Europe' financed by the Ministry of Finance of Luxembourg through the International Plant Genetic Resources Institute (IPGRI), aimed at the consolidation and development of national forest genetic resources programmes (1997–2000). It focused on three neighbouring countries with similar natural conditions and forest management traditions, namely, Bulgaria, Moldova and Romania.

A complementary approach was chosen encompassing the *in situ* conservation of genetic diversity of native forests, further development and use of technologies for *ex situ* conservation as well as genetically sustainable forest management. The project put strong emphasis on capacity building and strengthening of links between the institutions and scientists involved with their counterparts in other European countries.

In order to further disseminate the outputs of the project and to test the applicability of the results to similar situations, it was decided in consultation with partners and with the donor agency to include Ukraine in the second phase (2001–2004).

Ukraine is part of south-eastern Europe sharing similar natural conditions and forest management practices with the three countries involved so far (Bulgaria, Moldova and Romania). Ukraine is currently in a transition phase with far-reaching socio-economic implications. The Ukrainian Research Institute of Forestry and Forest Melioration in Kharkiv (URIFFM), the Institute of Mountain Forestry in Ivano-Frankivsk (URIMF), the Ukrainian State University of Forestry and Wood Technology in Lviv (USUFWT) and other institutes with experience and capacity in forest genetics and tree breeding will significantly contribute to accomplishing the wider objectives of the project. Moreover, their direct involvement will raise decision-makers' awareness about the need to sustain forestry research, which is currently at a critical stage.

Objective 1. Conservation and use of genetic resources

In situ *conservation*

Ecogeographic inventories

In 2001–2002, ecogeographic inventory activities were carried out in Ukraine. In the case of Ukraine, it was necessary to start a comprehensive inventory of the existing system of genetic reserves and other *in situ* gene conservation units throughout the country, with an emphasis on *Quercus* and *Fagus* resources. Particular emphasis was given to the inventory of rare broadleaved species in the relevant gene conservation units. All partners ensured the use of common information standards for description of the gene conservation units. The units inventoried were mapped using GPS to be associated to the maps of the relevant species' distribution.

In 2001–2002, the inventory was carried out in eight provinces: Ternopil, Chernivtsi, Zhytomyr, Rivne, Sumy, Kharkiv, Poltava and part of Crimea. To save travelling costs, *Quercus* and *Fagus* resources along with all *in situ* gene conservation units of other broadleaved tree species available in a province were inventoried. A total of 139 sample plots have been established. Brief information on the work done is presented in the Table 1. The data are ready to be added to existing EUFORGEN databases and to be made available on the Internet.

Genetic inventories

A feasibility study on genetic inventories of beech (*Fagus sylvatica*) using molecular markers (adoption and development of methods, preliminary analyses) was conducted. The first step of the study compared efficacy and applicability of two main markers, Restriction Fragment Length Polymorphism (RFLPs) and Randomly Amplified Polymorphic DNAs (RAPDs), for beech.

Table 1. ***In situ*** **gene conservation units of broadleaf species inventoried in Ukraine in 2001–02**

Species	Gene reserves	Superior ('plus') stands	Superior ('plus') trees
Quercus robur L.	71	14	382
Q. petraea Liblein.	4	–	51
Q. pubescens Wild.	2	–	–
Fagus sylvatica L.	15	–	10
F. sylvatica ssp. *taurica*	3	–	–
Fraxinus excelsior L.	3	–	2
Acer pseudoplatanus L.	1	–	1
Arbutus andrachne L.	1	–	–
Sorbus tormimalis L.	1	–	–
Alnus glutinosa L.	3	–	–
Phistacia mutica Fisch et Mey	2	–	–
Total	106	14	457

Ex situ *conservation*

Ex situ conservation is developed and applied in Ukraine, in close collaboration between URIFFM and CRP-Gabriel Lippmann (CRPGL). This Activity included joint experiments in the area of micropropagation and building of new technical capacity in Ukraine, with a focus on micropropagation of threatened oaks. An eight-week training programme for a young researcher from Ukraine at CRPGL in Luxembourg was undertaken in 2001 and included:

a) Initiation steps of *in vitro* culture;

b) Multiplication of shoots using existing culture.

Follow-up work to the training programme was carried out at URIFFM, with special emphasis on the application of the acquired skills and techniques:

c) Adaptation of oak *in vitro* culture protocols for the local conditions;

d) Choosing the most endangered plus trees of *Q. robur* in the region surrounding the location of the URIFFM;

e) Collecting the material from the trees;

f) Establishing clonal archive of the plus trees.

Objective 2. Policy issues related to the management of forest genetic resources

In order to support conservation strategies it is essential to analyze the legal and policy framework in Ukraine and propose options for further development or revision of legislation and policy systems. A translation of main legislative documents in effect in the area of FGR conservation has been done by URIFFM.

Analysis of the relevant national legislation and information on the status of FGR conservation was included in Ukraine's country report on implementation of the Convention on Biological Diversity.

Objective 3. Information management

Maps of the natural distribution range of *Fagus* spp. *and Q. pubescens* Wild. in Ukraine were developed. Forest inventory data and paper maps in scale 1:200 000 were used to prepare these maps. A data model has been developed and released in *Smallworld* GIS. A compartment was used as a cartographic unit for mapping. Since the original data are linked to a subcompartment they were generalized up to the compartment level. Special applications for linking separate parts of schemes using source and target sets of points and for geo-coding have been developed. All electronic cartographic data are stored and processed in the unified conic equidistant projection, which is the most convenient for mapping of the whole territory of Ukraine. Therefore, the original software has been developed to transform raster topographic maps from Gauss-Kruger projection to the conic equidistant one.

Data of the State Forest Inventory Service were used to develop the map. The Service updates its mapping data every 10 years. The methodology developed in the project allows updating the maps of species' natural distribution according to the new State Forest Inventory Service data, on request.

The database of the *in situ* gene conservation units inventoried is under compilation. The structure of the database has been developed. Information on the units inventoried during 2001–2002 has been recorded into the database.

In order to raise public awareness of the importance of forest genetic resources at a local level, a presentation on a seminar with forestry managers was done. Continued coverage of the activities on forest genetic resources conservation was ensured at the local, national and international level. Four articles on genetic resources of broadleaved tree species have been published at national and international level. Project activities were presented at one national and two international scientific conferences.

The RESGEN 78 EU Project

E. Collin
Cemagref, U.R. Ecosystème Forestiers, Nogent-sur-Vernisson, France

The 5-year 'Conservation of Elm Genetic Resources' project (RESGEN number CT96-78) aimed at improving the conservation, evaluation and use of the hundreds of elm clones already held in a large number of institutes across Europe. It was co-ordinated by Cemagref (France) and funded by the European Commission in the frame of a programme (EC Regulation number 1467/94) in favour of the conservation and utilization of genetic resources in agriculture.

The project started in January 1997, came to its administrative ending in December 2001, and was concluded by the presentation of its main scientific results at the second International Elm Conference held in Spain in May 2003. It included 17 partner institutes from nine western European states representing the geographic range of the European Union (EU). It was based on an existing core of *ex situ* collections in several countries, and complemented them with material originating from other EU countries where conservation actions had not yet begun. It was built upon a diverse group of scientists (pathologists, geneticists) and foresters, each of whom provided expertise and tested methodologies in the different research fields. The project enabled the satisfactory completion of following six tasks:

1. A common database was build to list and describe the 2080 clones held by project participants. This database proved particularly helpful for the selection of the priority-conservation clones to exchange between partners and conserve in different locations;
2. The molecular characterization of a large subsample of the total collection was carried out using nuclear DNA markers (RAPDs and ISSRs) and chloroplast DNA markers (PCR-RFLPs). This permitted clarification of the taxonomy of elms (and particularly the status of narrow varieties of *U. minor*), assess the extent of hybridization, and gain information on the routes followed by the elms when recolonizing Europe after the last iceage;
3. The evaluation of desirable traits was facilitated by sharing expertise and adopting common protocols for experimentation and notations; a strong variability in bud burst period and an interesting variability in tolerance to Dutch elm disease (DED) were found; knowledge on elm attractiveness for bark beetles (vectors of DED) was increased;
4. The rationalization of the European elm collection was achieved through the selection of the priority-conservation clones to conserve in a restricted 'core collection' and the identification of geographic zones where complementary sampling was urgently needed;
5. The long-term conservation of the 850 core-collection clones was ensured by their duplication for conservation in low hedges (unattractive for the vector of DED) at two different institutes, and by the cryopreservation of buds of a 450 clone subsample in liquid nitrogen;
6. The dissemination of project results is being carried out by different kinds of public: scientists, professional foresters and arborists, and the general public. Outputs are expected for amenity planting, afforestation and the reconstruction of hedges. Because it will provide methodological support for the implementation of the Noble Hardwoods Network's strategy, the project will also contribute to the identification and sustainable conservation of valuable elm genetic resources throughout Europe.

More detailed information on the project results will be available in the proceedings of the second International Elm Conference to be published in 2004, and in papers for scientific journals (publication expected for 2004).

Life history traits and genetic variability in some noble hardwoods

G. Eriksson
Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

At the Swedish Department of Forest Genetics, there are four different projects currently running with the common objective of understanding the impact of life-history traits on among- and within-population variation in juvenile growth and growth rhythm traits of noble hardwoods. One of the projects studied allozymes; in another, gene flow is being studied using microsatellites; the last project evaluates whether life history traits may be of use for sampling of gene resource populations in the absence of genetic knowledge. A complete description of all four projects follows.

The noble hardwoods covered by the projects include *Acer platanoides*, *Betula pendula*, *Castanea sativa*, *Fraxinus excelsior*, *Prunus avium*, *Prunus padus*, *Sorbus aucuparia* and *Ulmus laevis*, as well as *Fagus sylvatica* and *Quercus robur*.

Project 1

Project 1 is run in cooperation with Nordic colleagues (Sanna Black-Samuelsson, PoulErik Brander, Martin Jensen, Tor Myking, Mari Rusanen, Tore Skrøppa, Pekka Vakkari and Lars Westergaard) and includes adjacent populations of *Acer platanoides* and *Betula pendula*. Studies are conducted in field trials as well as in the growth chamber at two nutrient levels. Allozyme variation is also being studied.

Some of the growth and phenology data from the field trials supported the hypothesis that there would be a larger differentiation in the scattered and insect pollinated species, *A. platanoides,* than in the continuously distributed and wind-pollinated species, *B. pendula.* This hypothesis was also supported by the allozyme data with a larger F_{st} value in *A. platanoides* than in *B. pendula.* However, the larger variation in bud flushing in *B. pendula* than in *A. platanoides* disagreed with the hypothesis.

The family variance components were larger for most traits in *B. pendula* than in *A. platanoides* supporting the second hypothesis, that the within-population variation would be largest in the former species. However, the coefficient of additive variance was largest in *A. platanoides* both in field trials and in the growth chamber, and the expected heterozygosity and number of alleles per locus did not differ, which is in disagreement with the second hypothesis.

The final hypothesis, that phenotypic plasticity would be largest in *B. pendula*, was strongly supported by the growth chamber data.

In conclusion, the belief that *A. platanoides* with its scattered distribution and insect pollination would consist of rather isolated small populations was not supported by the findings of our study. This was particularly evident from the estimated number of migrants per generation estimated at 2.3.

Project 2

Project 2 is run in cooperation with Virgilijus Baliuckas and Tomas Lagerström. It includes most of the species listed above except *Castanea sativa* and *Ulmus laevis*. Growth and growth rhythm traits of the species were studied in nurseries. Not all nursery data have been evaluated yet but the results so far are presented below.

Each species is represented by 4–5 populations with up to 30 open-pollinated progenies from each species. Since some of the experiments were established in such a way that a conversion to seedling seed orchards would be an option, we cannot obtain any results with regards to the among-population variation for all experiments within this project. The results from this project were summarized by Virgilijus Baliuckas as follows:

1. The hypothesis on genetic structure of species based on life-history traits was confirmed to some extent by our studies;
2. Among the traits studied, bud flushing was the most heritable and stable trait across environments and years;
3. Relatively large values of coefficient of additive variation (CV_A) were found in most populations suggesting good possibilities for dynamic gene conservation;

4. Based on CV_A values, selection of populations could be done for dynamic gene conservation;
5. Within-trait correlations were strong, suggesting that assessments one year would be fairly reliable.

Project 3

Project 3 deals with among- and within-population variation in five populations of *Ulmus laevis* studied in a nursery, in cooperation with Rachel Whiteley and Sanna Black-Samuelsson. The populations originate from Sweden, the Moscow region in Russia, central Germany, and south-western France.

One unexpected observation was the early bud flushing of the French population while the late bud flushing of the German populations was expected. Growth cessation took place according to expectation, i.e. in the following sequence: Sweden/Russia – Germany – France. The German populations had the tallest trees while the Swedish and Russian populations had the shortest. Some of the populations had a considerable within-population variation. Gene flow will be studied in this material.

Project 4

Project 4 includes a growth chamber study of *Castanea sativa* run within the EU project CASCADE (EVK2-1999-00065), in cooperation with Marco Lauteri and Alfas Pliura. Because the data were reported by Alfas Pliura during the previous meeting, no further reporting will be done here.

Conclusion

In conclusion, it is of importance to jointly analyze genetic variation in adaptive traits and in neutral traits to obtain a better understanding of the observed variation in adaptive traits.

Genetic conservation, tree breeding and utilization of noble hardwoods: seminar papers and presentations from the seventh meeting

Definition of provenance regions for chestnut (*Castanea sativa* Mill.) and flowering ash (*Fraxinus ornus* L.) in Tuscany

F. Ducci and M. Vannuccini
CRA–Istituto Sperimentale per la Selvicoltura, Arezzo, Italy

Introduction

In the last decade, several European countries have delimited provenance regions (PRs) for forest species as the basic management units for forest genetic resources. This management approach has recently been set as a European standard by Council Directive 1999/105/EC which requires each country to develop a PR system for regulating the movement and use of propagation materials of *source identified* and *selected* categories. The directive defines *provenance region* as a territory subject to relatively uniform ecological conditions (including altitude, where appropriate), where populations have similar phenotypic and/or genetic characteristics.

European countries that have already established PR systems have followed one of two basic approaches: the *partitionist* and the *associative*. In the partitionist approach, the most important factor is the ecological homogeneity of the territory regarding climatological parameters, geographical criteria (such as altitude or distance from the sea), geomorphology and pedology. The factors to be considered are those that most affect the local distribution of forest species or the expression of phenotypic characters. Countries such as Germany, Norway and the United Kingdom have used this system to define their PRs. In the associative approach, which was used by France, genetic parameters are the main guide in defining PRs. In this approach, genetically similar populations are grouped in the same PR. This requires detailed information about the genetic structures of a large number of populations of each forest species.

Until recently, the Italian Register of Basic Materials based on the previous Directive (1966/404/EC) did not include either a PR system or a biogeographically comprehensive classification of the national territory. Instead, it based the management of basic materials on an accurate description of each basic material (Morandini and Magini 1975). This practice dates back to the 1970s and reflects the difficulty in delimiting ecologically homogenous districts in such an environmentally diverse territory as Italy.

In response to the application of 1999/105/EC, Ducci et al. (2003) made a first attempt to define a common national methodological frame for defining PRs, based on the proposal put forward in *Guidelines for Forest Propagation Material Use and Management* (Ducci 2003) and finished with reference to genetic structure aspects (Ducci et al. 2005). This paper attempted to classify the national territory biogeographically, according to the partitionist approach, as a first contribution to the process of identifying PRs.

That proposal is reprised and revisited in this paper, in order to define an appropriate methodology for the regional scale. The method was applied to establish PRs in the Region of Tuscany, which recently incorporated Directive 105/1999/EC into its regional forest law. The paper focuses on two species, chestnut (*Castanea sativa* Mill.) and flowering ash (*Fraxinus ornus* L.), which are widely distributed in Tuscany.

Materials and methods

Previous papers (Ducci et al. 2003, 2005) provided a general approach for the delimitation of PRs. Among the factors considered to affect PR boundaries were bioclimatic characteristics, major geolithologic systems, the ridges along major mountain chains and rivers.

In order to apply this approach to the regional scale it was necessary to have detailed environmental information and a comprehensive regional bioclimatic map at an appropriate scale. However, no such map of Tuscany exists, so the definition of PRs involved the following steps:

- analysis of the available environmental information
- evaluation of the opportunities for producing a bioclimatic map, using an empirical approach if necessary, as an operative support for the definition of PR boundaries
- definition of PR boundaries, following the procedure proposed by Ducci and Pignatti (2004)
- statistical analysis to test the validity of these PR boundaries.

Available information

A *partitionist* approach was followed to define the proposed PRs for chestnut and flowering ash. All the available ecological and climatic cartography of Tuscany was collected, as was all relevant information on the geographical distribution of species.

All the spatial information was transferred to a Geographic Information System (GIS) for subsequent processing. Analysis followed a pixel-based approach, and all information layers were presented in the resolution of 400 m, which is the size of the basic units in the Tuscany Forest Inventory.

Species distribution

The geographical distributions of chestnut and flowering ash over the region were assessed by extracting all the pixels in the Tuscany Forest Inventory database (prepared by the Region of Tuscany in 1998) in which the species were recorded (Figure 1). For each pixel, the inventory database records only the three species that cover the largest areas; so it probably underestimates the actual distribution of the species considered here (particularly flowering ash).

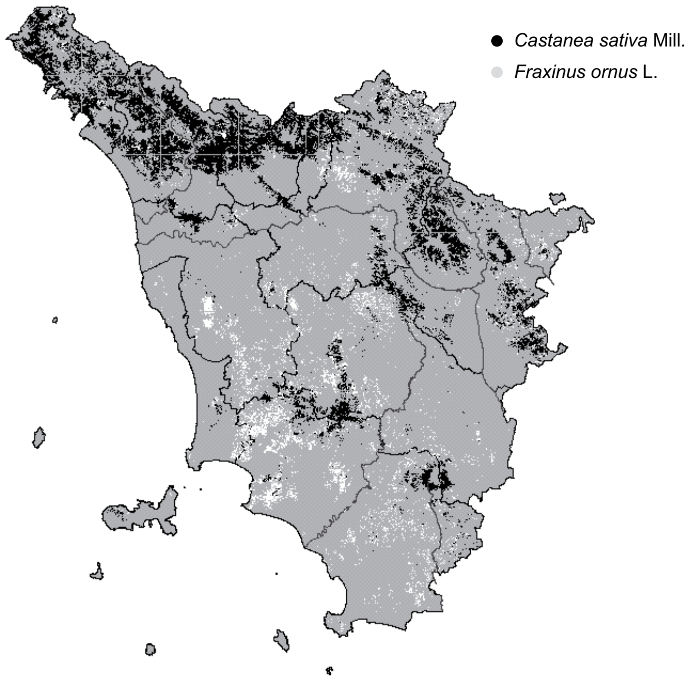

Figure 1. Geographical distribution of chestnut and flowering ash according to the Tuscany Forest Inventory.

Climatic information

Because there is no comprehensive climatic study of Tuscany, information on climate at the regional scale was derived from various climatic cartographies.

Mean annual temperature isolines were acquired from the regional climate map produced by the Italian National Research Centre (CNR) (Rapetti and Vittorini 1995). The isolines were then digitized and transferred to a GIS environment; by triangulation algorithm, a Triangulated Irregular Network (TIN) was produced and a mean annual temperature map was then obtained. The same procedure was followed to produce an annual rainfall map (Figure 2) from rainfall isolines taken from Bigi and Rustici (1984).

The regional climate map was used to produce a map of dry months; these are months in which rainfall (R, in mm) is less then twice the mean monthly temperature (T, in degrees Celsius; $R < 2T$).

Soil information

Although pedology is one of the main factors affecting biological diversity, there is little information on Tuscan soils, mainly because of the high pedologic variability, which is difficult to represent on a map. In addition, what information is available (for example, the regional lithologic map) shows fragmentation of units, making it unsuitable for work on the large scale.

Lithology, geomorphology and pedology were all obtained from examination of the ecopedologic units of the European Ecopedologic Map (European Soil Boureau 1999). At the ecopedologic unit level, the degree of fragmentation on the map is consistent with the scale of the work (i.e., each unit is significant in size, at a scale of almost 1: 250 000).

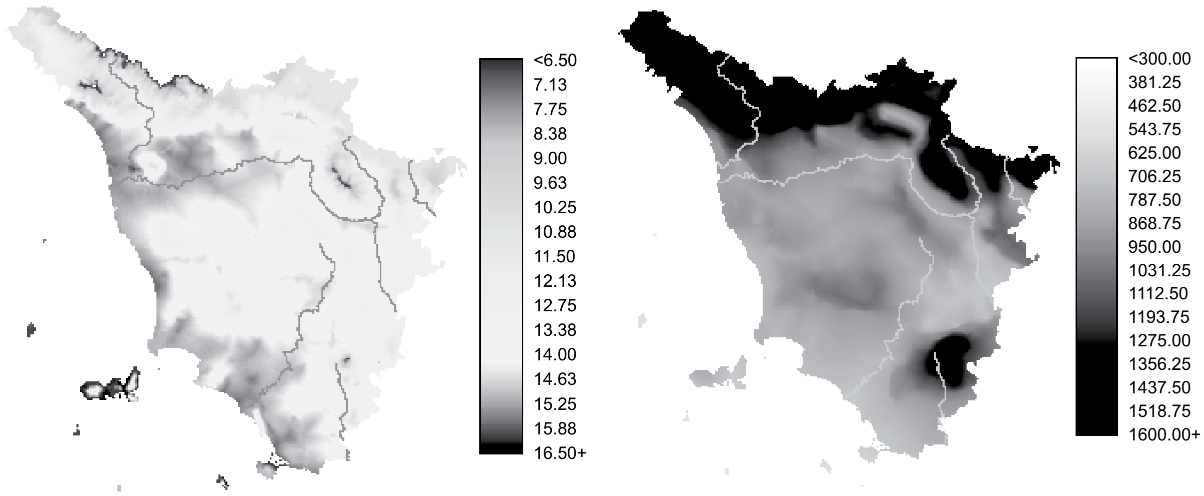

Figure 2. Mean annual temperature (°C) (left) and annual rainfall (mm) (right) in Tuscany.

Aggregation of bioclimatic information

The process of delimitating PRs involves evaluating a number of climatic factors, such as mean annual temperature, annual rainfall, summer rainfall and dry period. Bioclimatic maps (when available) can be useful tools, as they represent a vegetation-dependent synthesis of a region's main climatic characteristics.

In Tuscany, for which no regional-level bioclimatic map exists, the interpretation of each climatic parameter separately (which means evaluating a number of different factors at the same time) is not simple, especially when the aim is to obtain a clear view on a large scale.

An empirical method for aggregating climatic information into a comprehensive bioclimatic map was therefore proposed.

There are two essential bioclimatic classifications for Tuscany: the Italian bioclimatic map by Tomaselli et al. (1973), at the national scale, and the bioclimatic characteristics of the forest types described by Mondino and Bernetti (1998). These basic bibliographic sources were used to classify the regional territory into five distinct bioclimatic zones. For each zone, the mean annual temperature range, the annual rainfall range and the length of dry period were identified, as were the distinctive forest types. Maps of these last were produced from information in the Tuscany Forest Inventory database.

In order to produce a bioclimatic map with proper detail, a modelling approach based on fuzzy logic was chosen. The membership grade of each pixel to each bioclimatic zone was evaluated by applying an appropriate fuzzy set membership function, which took into account the range of values that each factor can assume inside the zone. Then, for each bioclimatic zone, the four environmental factors (standardized in the 0-255 byte range) were aggregated into a single membership grade map by the linear combination method (Eastman 1999).

The result of this analysis was a *soft classification* in bioclimatic zones, giving five membership grade maps (one for each zone). Figure 3 shows the membership grade map for the ipomesaxeric zone (Tomaselli et al. 1973). In order to make the maps easier to interpret for delimitating PRs, the five *soft* images were aggregated into a single *hard* map, by assigning each pixel to the bioclimatic zone for which it showed the highest membership grade. The resulting bioclimatic map is shown in Figure 4.

Delimitation of PRs

When defining PR boundaries, bioclimatic characteristics and soil information are very important, as are those physiographical boundaries that can act as barriers to gene fluxes, such as the Apennine and Chianti ridges and main river systems (Arno, Ombrone, Serchio and Fiora). The relative importance of each parameter depends on the species' ecological characteristics and geographical and altimetric distribution.

Soil information was obtained by evaluating the ecopedologic map at the ecopedologic unit level (which includes soil information), or at least at the sub-soil regions level (based on lithology and geomorphology). In some cases, such as Monte Amiata, ecopedologic units were one of the main discriminating parameters.

Physiographical boundaries were preferred as PR boundaries (even allowing for their lower internal homogeneity), in order to obtain the clearest boundaries possible. When a clear physiographic limit did not exist, administrative boundaries, such as between provinces and municipalities, were used. Excessive fragmentation of administrative units among PRs must be avoided in order to simplify the management and control of basic materials by provinces and mountain communities.[1]

Owing to the great environmental diversity of Tuscany, a relatively high heterogeneity in environmental parameters within each PR must be accepted.

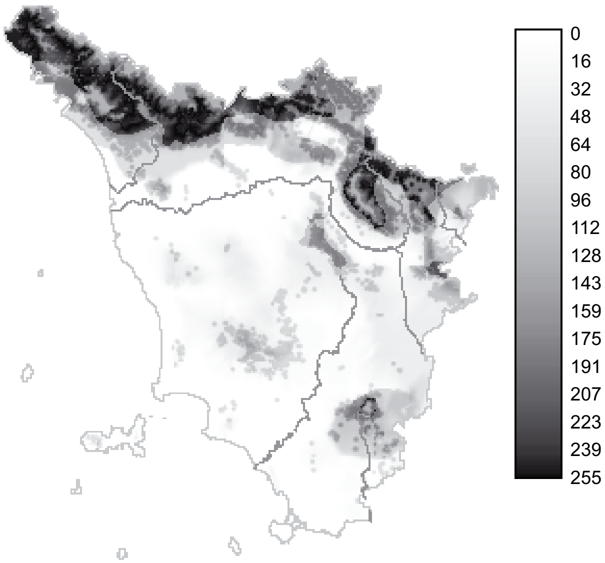

Figure 3. Sample membership grade map. The membership grade of each pixel to the temperate mesaxeric bioclimatic zone is scaled in the 8 bit range of 0-255 (255 = complete membership, 0 = no membership).

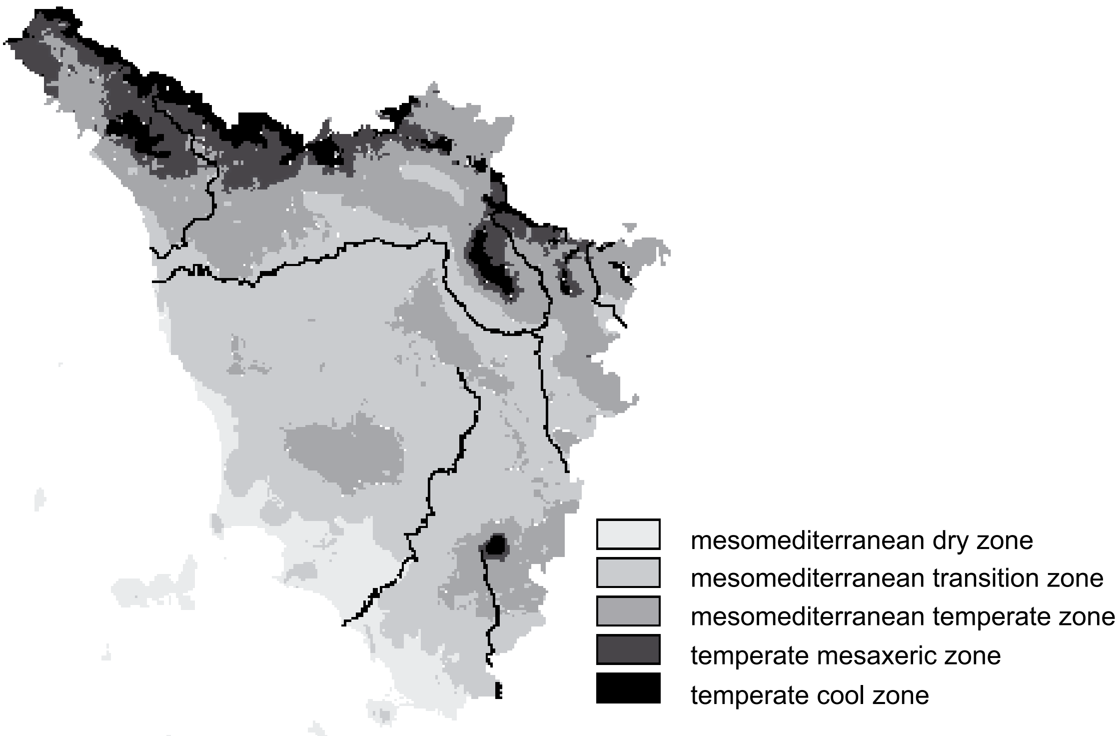

Figure 4. Bioclimatic map of Tuscany, obtained from aggregating fuzzy sets for each zone.

[1] Italian public administration is organized hierarchically in Regions, Provinces and Municipalities. Municipalities in mountain territories are associated in Mountain Communities, which have competence in agriculture and forestry.

Analysis

The PRs for chestnut and flowering ash were evaluated with pixel-by-pixel discriminant analysis.

The analysis considered four environmental parameters: mean annual temperature, annual rainfall, number of dry months, and soil suitability for chestnut and ash growth. Soil suitability was assessed by reclassifying the ecopedologic map. First, a cross-tabulation was performed between the ecopedologic units and the species distribution from the regional inventory in order to obtain the frequency of inventory units in each ecopedologic unit. Then, a score was assigned to each unit that reflects the ratio among units in terms of species frequency:

$$S = \frac{n_i}{n_{i\max}} N$$

where: S = suitability score; n_i = frequency of chestnut (or ash) inventory units in the i[th] ecopedologic unit; n_{imax} = maximum frequency of chestnut (or ash) inventory units in a single ecopedologic unit; and N = number of ecopedologic units.

Results

Figure 5 shows the subdivision of Tuscany into six PRs for chestnut, and the ecopedologic heterogeneity within each PR.

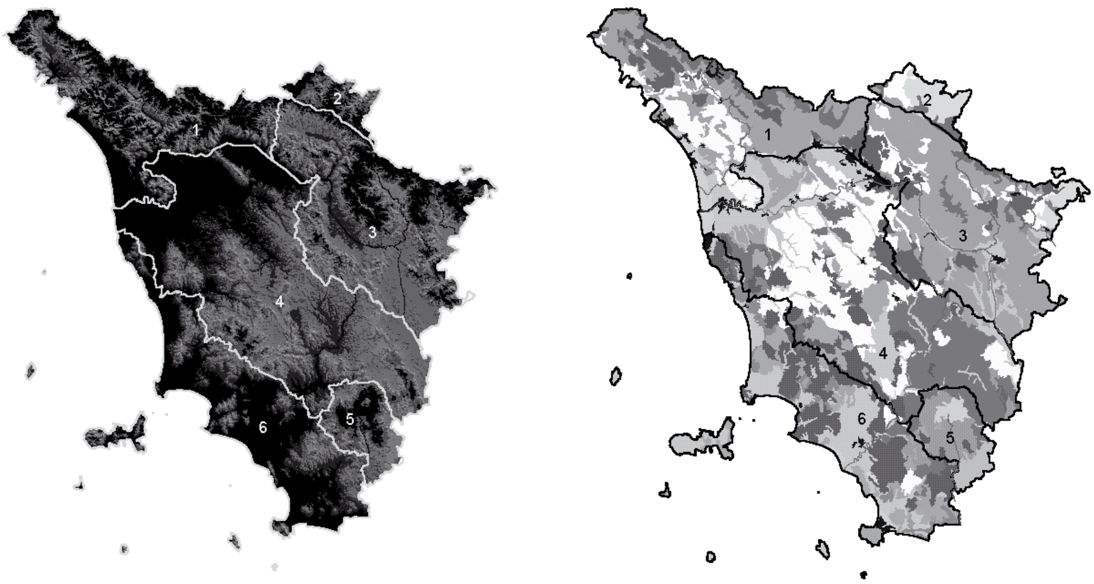

Figure 5. Provenance regions for chestnut (left) and ecopedological heterogeneity among PRs (right) in Tuscany.

Results of the discriminant analysis are summarized in Tables 1 and 2. The first discriminant function, which explains about 70% of variance, shows higher correlations with dry months and annual rainfall; these variables are also the most effective in the second function, while soil suitability is far more correlated with function 3 (which explains about 21% of variance).

Table 3 shows the percentage of cases (i.e. pixels) classified in each PR by means of discriminant functions versus current classification developed as described under 'Delimitation of PRs' (above). The environmental parameters used in the analysis were very effective in discriminating three PRs (numbers 2, 4 and 6), and gave fairly good results for another region (number 5). However, the factors considered were inadequate to describe the environmental variability between PRs 3 and 4: region 3 seems to be particularly poorly characterized by mean values of parameters such as temperature and rainfall, as significant differences with region 4 depend more on the thermopluviometric regime than on mean values. In fact, the boundary between the two regions marks the transition between a Mediterranean sub-oceanic climate and a subcontinental climate.

Table 1. Discriminant analysis for chestnut provenance regions: eigen values

Function	Eigen value	Variance (%)	Variance (% cumulated)	Canonical correlation
1	1.938	70.4	70.4	0.812
2	0.585	21.3	91.7	0.608
3	0.198	7.2	98.9	0.406
4	0.030	1.1	100.0	0.172

Table 2. Correlations between canonical discriminant functions and variables

	Function			
Variable	1	2	3	4
Dry months	-0.835*	0.515	0.142	0.133
Annual rainfall	0.807*	0.504	-0.212	0.224
Soil suitability	0.372	0.058	0.878*	0.296
Mean annual temperature	-0.472	-0.226	-0.145	0.840*

* = higher correlation between the variable and any function.

Table 3. Observed versus predicted cases (in percentages) in each group (i.e. provenance region)

Observed membership group	Predicted membership group					
	1	2	3	4	5	6
1	**48.2**	22.4	5.2	9.4	13.3	1.4
2	7.5	**88.6**	3.9	0.0	0.0	0.0
3	11.7	10.8	**34.4**	35.4	7.6	0.2
4	0.0	0.0	3.0	**76.6**	4.1	16.3
5	0.6	11.9	0.3	6.6	**57.9**	22.8
6	0.0	0.0	0.3	13.5	0.5	**85.8**

Bold = percentage of correctly classified cases in each group.

The high percentage of cases in region 1 classified by the analysis in region 2 can be explained by differences in the nature of soils, which was neither considered by the analysis nor represented on the ecopedologic map. Region 1 represents the climatic and pedologic *optimum* for chestnut and is characterized by arenaceous lithology and neutral soils, while region 2 represents the conditions of chestnut's maximum tolerance to calcareous and clayey soils (Mondino and Bernetti 1998).

For flowering ash, five PRs were recognized (Figure 6).

Discriminant analysis shows that two parameters (annual rainfall and dry months) are enough to discriminate among almost all regions, except for region 3 (Table 4).

On average, classification by means of discriminant functions is more effective for ash than for chestnut (Table 5). In all cases, the analysis fails to classify region 3 completely: only 24.5% of cases were correctly classified, while about 45% were assigned to region 4. In fact, apart from at higher altitudes (which are of no interest for ash growing), temperature and rainfall values are fairly similar in both regions. Again, soil parameters are not effective in discriminating between regions: regions 3 and 4 show high differences in lithology and pedology, as region 3 is rather homogenous and dominated by arenaceous-pelitic flysch pedogenetic substrate, while soils originating from sedimentary rocks (Holocene and Tertiary clays and sands) prevail in region 4 (which shows a higher internal lithologic complexity).

For ash, rainfall and dry period seem to be the most effective parameters, as emerges from comparison of ash distribution and climatic maps. This explains why the Monte Amiata system is not recognized as a proper PR, because ash distribution stops on the mountain's lower slopes, where the climate is still markedly Mediterranean.

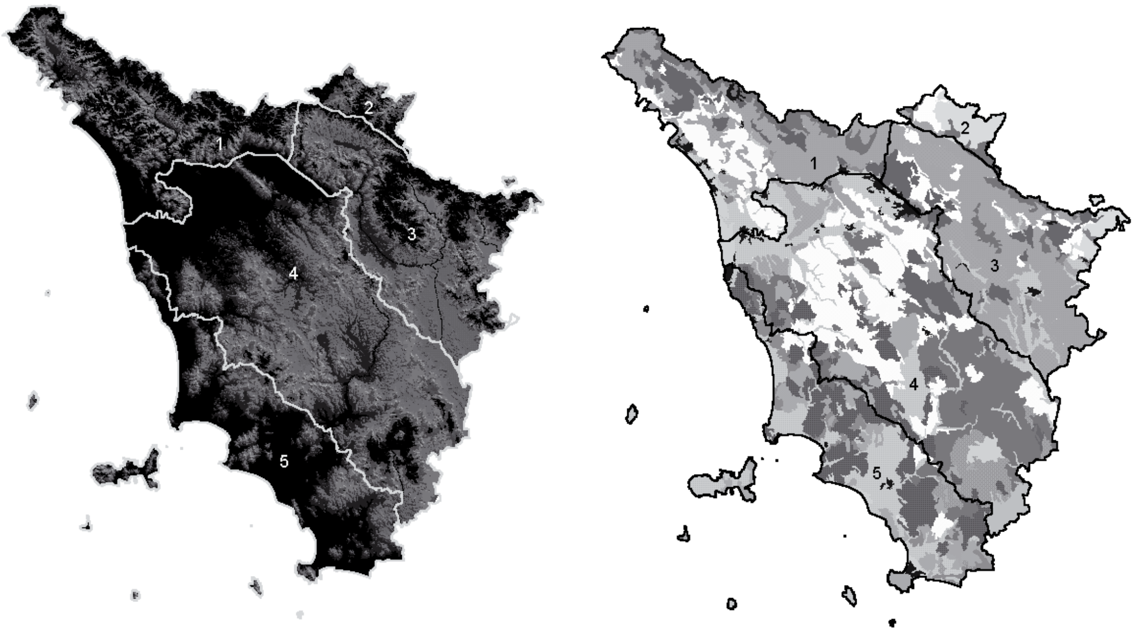

Figure 6. Provenance regions for *Fraxinus ornus* L. (left) and ecopedological heterogeneity among PRs (right) in Tuscany.

Table 4. Discriminant analysis for *Fraxinus ornus* provenance regions: eigen values

Function	Eigen values	Variance (%)	Variance (% cumulated)	Canonical correlation
1	2.098	81.0	81.0	0.823
2	0.457	17.6	98.7	0.560
3	0.033	1.3	100.0	0.178
4	0.001	0.0	100.0	0.034

Table 5. Observed versus predicted cases (in percentages) in each group (i.e. provenance region)

Observed membership group	Predicted membership group				
	1	2	3	4	5
1	**58.8**	22.3	8.1	10.6	0.2
2	21.4	**76.5**	2.0	0.0	0.0
3	16.6	13.6	**24.5**	45.3	0.0
4	3.1	1.2	8.0	**75.3**	12.5
5	0.0	0.0	0.5	21.5	**78.0**

Bold = percentage of correctly classified cases in each group.

Discussion

The proposed PRs reflect some notable aspects of the geographical distribution of chestnut and ash.

Chestnut distribution shows four well-defined sectors. The first (northwest Tuscany) is characterized by a Mediterranean oceanic or sub-oceanic climate and the arenaceous-pelitic contours of the Apennines; these represent the main lithologic character of this region, with the exception of the Alpi Apuane metamorphic system. This region represents chestnut's pedoclimatic optimum.

A second sector corresponds to the Adriatic side of the Apennines, which is characterized by a subcontinental climate and a clayey-arenaceous pedogenetic substrate. This region represents chestnut's maximum tolerance conditions to clayey and calcareous soils. In this situation, unmanaged chestnut woods tend rapidly to be replaced by hornbeam (*Ostrya carpinifolia*).

The high Arno basin represents the third sector (PR 3). The Tuscan side of the Apennines, Pratomagno and the Chianti mountains, with their common trait of arenaceous pedogenetic substrates, are the zones of main interest for chestnut. This zone also includes the Arno river terraces between Chianti and Pratomagno.

The fourth sector is characterized by a marked Mediterranean climatic regime, and includes the central sector of Tuscany. Chestnut is essentially localized in central-southern Tuscany, the Montagnola Senese and Colline Metallifere, where it is often accompanied by elements of Mediterranean flora.

The most distinctive character of the chestnut woods of Monte Amiata is its soils originating from volcanic rocks. The Amiata chestnut woods are unusual for Tuscany, and are far more similar to woods in the volcanic systems of Lazio.

Geographical distribution of flowering ash falls into four sectors, but these are never very clearly distinguished. The first sector corresponds approximately to the meso-Mediterranean dry bioclimatic zone, characterized by a dry period of about three months, where ash is associated with holm oak (*Quercus ilex*) and Mediterranean shrubs.

A second sector corresponds to the transition zone between the Mediterranean dry zone and the ipomesaxeric zone. Ash is found in several situations here, such as in mixed stands with holm-deciduous oaks or associated with hornbeam and white oak (*Quercus pubescens*). It grows on various soils, but especially on calcareous and clayey hills. A similar situation, but with more features of a continental climate, is found in Val di Chiana and the Arno and Tiber high basins, where ash is associated with Turkey oak and hornbeam on clayey and calcareous soils.

The third sector of ash distribution is clearly distinguished by both its geographical position (the eastern aspect of the Apennines) and lithology, as ash is one of the main characteristic species (with white oak and hornbeam) of the natural vegetation of the clayey-arenaceous system scysts.

All Mediterranean oceanic and sub-oceanic climate zones of northwest Tuscany (from the Lunigiana to the Bisenzio valley) can be included in one sector, which is of little interest with respect to ash. The main natural populations are differentiated essentially by soil characteristics, and two basic situations can be recognized: ash populations of the Alpi Apuane metamorphic system (associated with hornbeam on pioneer cenosis or with hornbeam and deciduous oaks on more evolved soils), and those on the arenaceous-pedogenetic substrates of the Lunigiana. This sector shows high pedologic heterogeneity, and is essentially distinguished on the basis of climate.

Conclusions

The PRs identified in this paper reflect specific sectors of species' geographical distribution, and variations in major climatic factors as well as major biogeographical phenomena at the regional scale. An ecologically accurate identification of PRs requires detailed climatic and geopedologic information. Simple temperature and rainfall mean values often give information that is of low biologic significance, as discriminant analysis illustrates: the most effective climatic parameter in the analysis was the length of dry period, and this was the only aggregated parameter considered. Analysis could therefore probably be greatly improved through the use of aggregate climatic indices, for which more climatic information (at present not available for Tuscany) is needed. The study also emphasizes the importance of detailed forest inventory data, as species' geographical distributions are the most important information when defining species-specific PRs.

As PRs will be defined at the regional level, with each region probably tending to act independently, great effort must be made to ensure that different local systems are consistent with each other. Rather than differences among bioclimatic and pedologic information, it is the lack of inventory data that could be the major difficulty in ensuring a common basis for different regions. A priority in the application of 1999/105/EC must therefore be the definition of a national framework. The overall national PR structure should be hierarchical, with regional PRs representing the basic units for managing forest propagation materials, and overall framework units ensuring compatibility among regions (i.e., by regulating the movement of propagation materials among PRs of different regions).

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Clone selection for wild cherry (*Prunus avium* L.) with special reference to some traits used

***F. Ducci*[1], *A. Germani*[1], *G. Janin*[2], *R. Proietti*[1], *G. Signorini*[1]**
[1]*CRA–Istituto Sperimentale per la Selvicoltura, Arezzo, Italy*
[2]*Formerly: INRA, Station pour la Qualité des Bois, Champenoux – Nancy, France*

Introduction

Italy has approximately eight million hectares (ha) of forests, mainly in mountainous areas (MAF 1985). The most suitable environment for noble hardwoods, mixed temperate forests, is distributed from the Alps to Calabria, along the Apennine range. Little timber is harvested from these forests, because of the need to protect the soil, because most of the forests are in protected areas and because labour costs are very high; fifty percent of national timber production is from poplars growing on about 150 000 ha, mainly concentrated in the Po Valley. While poplar is important for pulp and plywood industries, the Italian furniture market requires higher-quality noble hardwood for veneers. Noble hardwoods, mainly walnut (*Juglans regia* L.) and wild cherry (*Prunus avium* L.), are extensively used in production of high-quality furniture in Italy, but only 20% of the wood used comes from Italy. The remainder is imported at very high cost, mainly from France and Balkan countries. Import has been estimated at about 3.5 million cubic metres per year (unpublished data, Assolegno, Private Wood Industries Association, 2003).

In an effort to meet the demand for noble hardwood from national production, Italian forest administrations have been developing new plantation programmes. The CRA–Istituto Sperimentale per la Selvicoltura at Arezzo (ISSA) has been doing this since the early 1980s. About 150 000 ha have been planted with noble hardwoods since that time and 40% of this future stock consists of wild cherry and walnut. ISSA estimated the turnover for seedling supply alone exceeded € 50 million over the last fifteen years (Colletti 2001; Ducci 2003).

Problems concerning trading, quality and certification of noble hardwood reproductive materials were initially severe, due to lack of knowledge about the distribution and genetic structure of the species in Italy and to the absence of regulations (Ducci 1989). The problem of lack of regulations has since been solved, with noble hardwoods included in the list of species safeguarded by national laws (Ducci 1999, Camoriano and Ducci 2004) and by the European Directive 1999/105/EC, now implemented through national decree 286/2003.

In its natural habitat (mixed temperate forests; deciduous oak and beech mountain forests in Italy) wild cherry adopts different regeneration strategies according to the ecological succession stages. In mature forests it grows as single, scattered trees or in small groups of trees originating from seed. However, when a rapid colonisation of bare ground is required, it grows in small clumps of trees originating from numerous root suckers. Studies have shown that the genetic variation within such clumps may be very low, indicating that the trees originated from only one tree (Ducci and Santi 1997). Thus, clones in wild cherry are natural and wild cherry plantations can be established with more variability than natural populations.

This paper aims to show the opportunities offered by the establishment of wild cherry clonal plantations with special emphasis on traits used for clone evaluation. The focus of the paper is on opportunities offered by the use of clones in intensive plantations (Wright 1986; Zobel and Talbert 1984).

Materials and methods

Ten wild cherry clones were selected by CRA-ISSA and the University of Bologna using the 'independent culling' selection method (Zobel and Talbert 1986). Under this system, materials are selected for a set of initial traits and then characterized for other traits in later growth stages. For this trial, initial traits considered were for growth and architecture, with subsequent characterization for colour or aesthetic of the wood.

The clones were micropropagated *in vitro* and established between 1985 and 1986 at four pilot test sites representing a range of altitudes and soil conditions (Ducci et al. 1990) (Figure 1). Soils were ploughed to 50 cm depth and micropropagated bare root plantlets were planted on a 3 m × 3 m grid according to a randomized block design, where individual plots were initially formed by 12 trees. Each clone was represented in each site by an average of 40 to 80 plantlets, according to the number available from the laboratory.

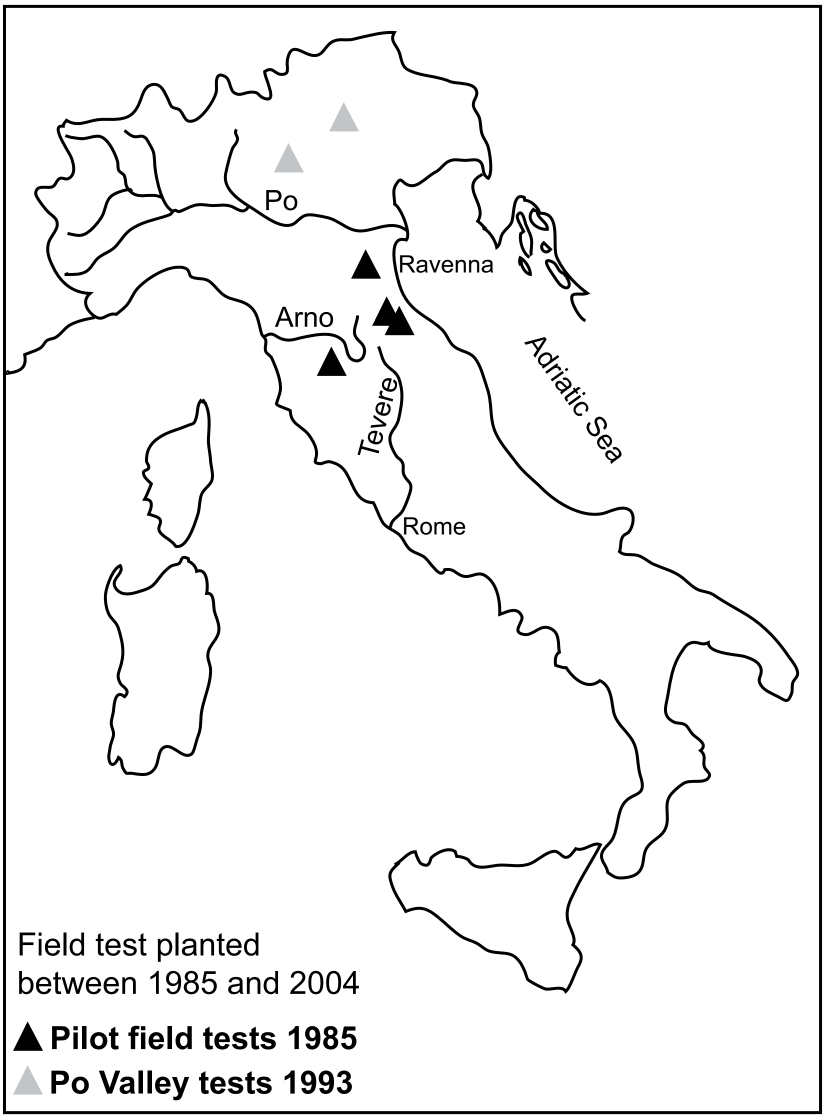

Figure 1. Location of the wild cherry experiments established in 1985/86 (black) and in 1993 (grey).

In 1993 a second trial was established in two sites in northern Italy; one near Lake Garda (Drugolo) and one near Vicenza (Montecchio Precalcino). These sites are typical of sub-Alpine conditions in the Po Valley. Drugolo is on the southern morainic hills of Lake Garda, where the climate is relatively mild and the soil very dry. Montecchio Precalcino is at the base of the Venice Dolomites, where climate is more continental, soils are alluvial and water availability is greater. This experiment tested 54 clones from eight Italian provenances in a randomized complete block design, each block consisting of one two-ramet plot per clone, according to the method proposed by Muranty (1993).

Traits measured in both trials were: height (H) and basal diameter (Diam) at planting and 2 and 4 years after planting; height and diameter at 130 cm (Dbh) at 6, 8 and 9 years after planting; straightness (1 crooked to 4 straight); branch angle averaged on the main branches in the fifth and sixth years; whorls (1 fastigiated to 7 horizontal); total number of branches at age 6; and mean number of branches/whorl. Four additional traits were studied in the second trial: H ($H_{92\text{-}93}$) and Diam ($Db_{92\text{-}93}$) increments averaged over two years after plantation (as a measure of transplantation stress); and mean H and Dbh increments, averaged between the third and the ninth year of growth.

A final group of traits was examined at the site near Ravenna, where growth was fast. The colour of the wood was assessed using a DATACOLOR® Portable colorimeter and the CIELab colorimetric space (Ducci et al. 1988). This space is three-dimensional and each specimen can be quantitatively described by co-ordinates such as *L** (luminance, white to black), *a** (chromatic component, green to red) and *b** (chromatic component, blue to yellow). Other parameters such as C* (saturation, $C^* = (a^{*2}+b^{*2})^{1/2}$) and h (colour angle, $h = arctg(b^*/a^*)$) were calculated to express the aesthetic value of veneers.

Data were analysed for both the experiments following methods proposed by Ducci et al. (1990), Santi et al. (1998) and Muranty et al. (1998) in order to carry out the analysis of variance, and to determine the variance components for each trait and the clonal heritability or broad sense heritability. Differences among blocks were significant at some sites, probably because of differences in soil fertility caused by previous land use, and hence intra-site factor analyses of variance were performed to evaluate significance of clone × block interactions (Table 1).

Table 1. Analysis of variance models used for the % component analysis

Within sites

Source of variation	d.f.	Variance components
Among blocks	b - 1	$\sigma^2_E + n'\sigma_{(bc)} + n'c\sigma^2_b$
Among clones	c - 1	$\sigma^2_E + n'\sigma_{(bc)} + n'b\sigma^2_c$
Blocks x clones	(b-1)*(c-1)	$\sigma^2_E + n'\sigma_{(bc)}$
Error	N-bc	σ^2_E

Where: b = number of blocks/clone; c = number of clones/site; n' = mean correct number of trees/plot.

Among sites

Source of variation	d.f.	Variance components
Among sites	s - 1	$\sigma^2_E + n'\sigma_{(bc)} + pc\sigma^2_s$
Among clones	c - 1	$\sigma^2_E + n'\sigma_{(bc)} + ps\sigma^2_c$
Sites x clones	(s-1)*(c-1)	$\sigma^2_E + n'\sigma_{(bc)}$
Error	N-bc	σ^2_E

Where: s = number of sites; c = number of clones; p = mean correct number of plots/site.

The set of traits assessed was considered meaningful for silvicultural (i.e. planting, spacing and pruning) and industrial purposes (i.e. knots and colour), but evaluating clones for each trait separately was practically impossible. It was therefore necessary to combine information on all traits of interest into a single index. A Selection Index (Zobel and Talbert 1986) was computed and tested for the first time in the trials at Drugolo and Montecchio Precalcino. Because of the difficulty of establishing economic weights for each trait, the index was synthesized from clone performances within each site as follows:

SI = *Perf.* Htot + *Perf.* Stem form * (1.30 *dom*) + [(*Perf.* Branch angle + Branches per whorl + $H_{92\text{–}93}$)/3];

where *Perf.* is the performance of the clone in the experiment, *Htot* is a production and adaptation indicator; *Stem form* is important for final products; and *dom* is dominance. As cherry clones are generally characterized by good levels of dominance, this trait was included to improve the *Stem form* effect by 30%. The influence of traits enclosed in square brackets was weighted at about 30%. Branch angle is important for pruning, branches per whorl is a measure of wood quality and $H_{92\text{-}93}$ is an indicator of ability to recover from planting stress. Clones showing an SI greater than 10% of the test site mean were selected.

Main results

Variance components (%)

Differences between clones accounted for a large part of the observed variance in the traits measured. At the best, most homogeneous sites of Forestello and Ravenna the clone effect was particularly evident (Table 2).

Table 2. Percentage of observed variance in height, diameter at 130 cm and branch angle attributed to block and clone effects in 3- and 19-year-old wild cherry trees at Forestello and Ravenna sites, Italy

	Block effect (%)		Clone effect (%)	
Trait	3 years old	19 years old	3 years old	19 years old
Height	0.27–4.56	1.8–7.4	70.0–72.2	67.3–69.4
Dbh	1.14–2.3	2.5–12.6	63.2–65.42	58.5–61.2
Branch angle	0.5–2.0	1.2–2.1	51.2–65.4	49.2–63.1

The block effect was very low, indicating that the sites were homogeneous. Variations among 19-year-old trees were related to differences among plantlets and microenvironmental variation within individual plots.

Differences were also observed in height and Dbh when these traits were measured in trees one year after planting. The error effect was generally higher in this year; the highest values were observed at the worst mountain sites, 32.8 and 35.4 in Forestello and Marani, respectively, and 49.4 to 53.6 in the other sites. This may have been the result of micro-site variations and differences in the size of plantlets issued from the laboratory and in nursery adaptation of the plantlets. Post-plantation stress was probably greater during the first summer after establishment, especially at the mountain sites.

Clonal (broad sense) heritability of the main traits

Theoretically, when vegetative propagation techniques (grafting, cuttings, micropropagation etc.) are used, the complete genetic variance (additive and non-additive) is transferred from the ortet to ramets. The only source of variation would be determined by the environmental component (σ^2_E).

Heritability is calculated as:

$$H^2 = \sigma^2 G / \sigma^2 F = (\sigma^2_A + \sigma^2_{NA}) / (\sigma^2_A + \sigma^2_{NA} + \sigma^2_E)$$

Where σ^2G is the genetic variance and σ^2F is the phenotypic variance; σ^2_A is the additive component of variance; σ^2_{NA} is the non-additive component of variance and σ^2_E is the environmental component. Values of H^2 vary between 0 and 1.

It is the parameter generally used to estimate how much of the variation within a population is the result of genetic differences among individuals. It expresses the extent to which parents transfer their traits to their offspring and is an important tool for estimating gains that may be obtained from genetic improvement and breeding programmes (Zobel and Talbert 1986).

The breeder–silviculturist has to improve also σ^2_E in order to maximize $H^2 = 1$. In general this is achieved when site fertility is high and homogeneous. These site characteristics minimize variation due to environmental factors and allows clones to express their genetic traits fully, as was observed in the trials presented here (Table 3).

Similar H^2 values were obtained in the wider trial at Drugolo and Montecchio Precalcino.

The broad-sense heritability values found in these trials are similar to those found by Santi et al. (1998) for similar traits: 0.45 for H increment 94-01; 0.48 for Dbh increments; 0.34 for branch number; 0.50 for branch angle; 0.54 for branch size and stem straightness; and 0.60 for flower bud breaking.

Table 3. Variation in broad-sense heritability (H^2) in 3- and 19-year-old wild cherry trees at four sites in central Italy

	Best locations		Poorest locations	
Trait H^2	**Forestello**	**Marani**	**Ortali-Ruscello**	**Mangano**
H tot	0.69–0.71	0.73–0.77	0.41–0.44	0.47–0.49
Dbh	0.62–0.63	0.53–0.54	0.30–0.32	0.31–0.34
D base	0.65	0.50	0.33	0.25–0.28
Branch angle	0.51	0.67	0.52	0.44

Relationships between growth performance and the other traits

Correlation analysis showed that growth-related traits such as height and diameter increments are significantly related (Table 4). Correlation between increments immediately after planting and those in later years are low.

Table 4.Relationships between main traits studied in wild cherry clones in Italy

Drugolo

	Incr. H 92–94	Incr. H 94–01	Incr. dbase 92–94	Incr. dbase 98–94	Incr. Dbh 98–01	N. Branch/ whorl	Total Branch at age VI	Stem form	Branch angle
Incr. H 92–94	1	0.192(**)	**0.705**(**)	**0.493**(**)	0.131(**)	0.186(**)	0.370(**)	0.147(**)	0.151(**)
Incr. H 94–01		1	0.316(**)	0.654(**)	**0.699(**)**	0.015 (ns)	0.285(**)	0.204(**)	0.010 (ns)
Incr. H 94–01			1	0.583(**)	0.213(**)	0.190(**)	0.443(**)	0.090(*)	0.131(**)
Incr. dbase 98–94				1	**0.598**(**)	0.200(**)	0.564(**)	0.156(**)	0.104(*)
Incr. Dbh 98–01					1	0.096(*)	**0.342**(**)	0.080 (ns)	0.034 (ns)
N. Branch/ whorl						1	**0.323**(**)	-0.063 (ns)	0.310(**)
Total Branch at age VI							1	0.061 (ns)	0.300(**)
Stem form								1	-0.016 (ns)
Branch angle									1

* = $P \leq 0.05$; ** = $P \leq 0.01$; ns = not significant.

Montecchio Precalcino

	Incr. H 94–01	Incr. H 92–94	Branch angle	Incr. Dbh 94–01	Incr. Dbh 92–94	Branch size	Dominance	Stem Form
Incr. H 94–01	1	-0.231(**)	0.071 (ns)	0.495(**)	-0.211(**)	0.020 (ns)	0.191(**)	0.122(**)
Incr. H 92–94		1	0.209(**)	0.209(**)	0.736(**)	-0.097(*)	0.060 (ns)	0.055 (ns)
Branch angle			1	0.129(**)	0.138(**)	0.046 (ns)	0.089(*)	-0.065 (ns)
Incr. Dbh 94–01				1	0.202(**)	-0.173(**)	0.002 (ns)	-0.011 (ns)
Incr. Dbh 92–94					1	-0.170(**)	0.010 (ns)	-0.006 (ns)
Branch Size						1	**0.307**(**)	**0.301**(**)
Dominance							1	0.395(**)
Stem Form								1

* = $P \leq 0.05$; ** = $P \leq 0.01$; ns = not significant.

Branch traits

Branch angle

This trait was largely unaffected by block and site effects. No provenances were characterized by horizontal branches or fastigiated ones.

Branch size

There was a significant negative correlation between relative branch size and stem diameter at the same height. Most of the clones tested had thin branches. Clones with small branch diameters were not necessarily the best in terms of growth.

Mean number of branches per whorl

The mean number of branches per whorl is another important trait for pruning and wood quality, as workability of the timber is directly related to knot occurrence. This trait was independent of clone × site interaction. The number of branches varied between about 2.0 and 4.5 per whorl and was significantly correlated with branch angle; on clones with a large number of branches were more likely to have branches that grew horizontally. No geographic trends were detected, and no correlation was observed with growth traits.

Stem dominance and form

Stem dominance is less important as a trait for cherry selection than stem form because dominance is generally common in wild cherry. Stem dominance was negatively correlated with growth in several clones: slow-growing clones commonly have better stem form (straightness). Growth of some clones varied significantly between sites, and the stem form of these also varied between sites. Other clones showed good stem characteristics and good growth at all sites.

Selection Index

The Selection Index showed good efficiency for selecting clones (Figure 2). The index allowed clones to be divided into those that interact with sites and those that do not, and permitted selection for characteristics to suit local site conditions.

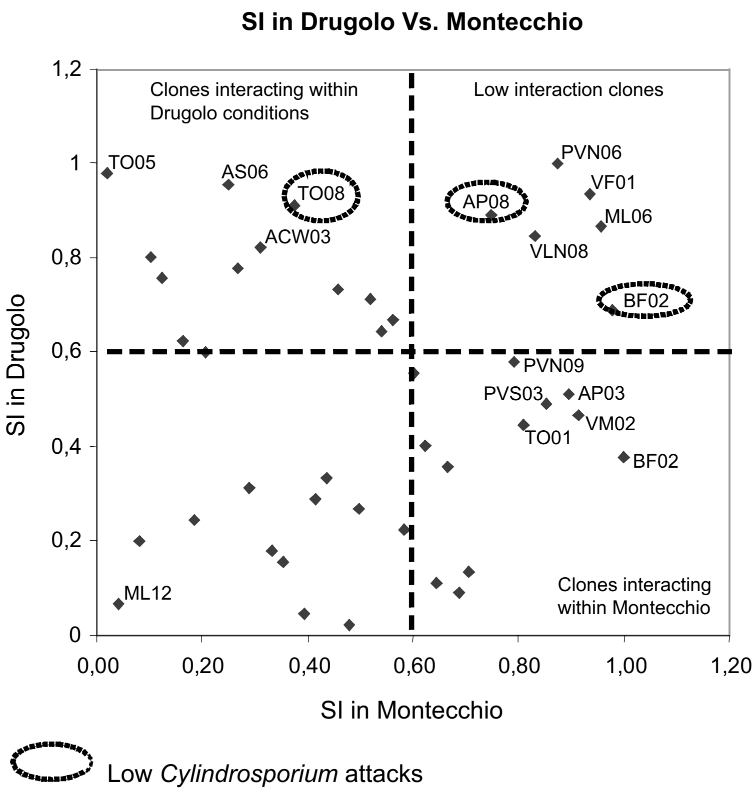

Figure 2. Selection indexes for wild cherry clones grown at Montecchio Precalcino and Drugolo, Italy. Clones showing an SI more than 10% above the plantation average were selected. The lower left-hand quadrant shows low interaction clones to be discarded because of their bad quality

Wood colour

Clone had a greater effect on the colour of sapwood than on the colour of heartwood (Table 5).

Table 5. Percentage of variation in heartwood and sapwood colour accounted for by L*, a*, b*, White Index (C) and colour angle (h) (see text for explanation) in wild cherry clones in Italy. Comparison between clone and ramet (error) components is for sapwood only, as differences between clones were not significant for heartwood

	L*	a*	b*	White Ind.	C	h
Heartwood	17.0	18.2	11.4	18.8	10.9	30.8
Sapwood	67.8	53.6	43.3	57.6	52.5	27.9
Sapw.ramets	4.6	4.2	3.3	3.8	3.8	2.9

The colour of heartwood is influenced by the accumulation of salts, organic matter and other chemicals, hence environment has a stronger effect than does the clone. The variance component related to ramets (individual and microenvironmental error) was very low. Broad-sense heritability values varied between 0.57 and 0.7; this reflects the great homogeneity within clones.

Discussion and conclusions

Plantations of wild cherry clones are still not widely used for production of wood and more experience in their use is needed in Europe. Both the experiments reported here supplied useful information about the use of clones for this purpose:

- the clonal effect on growth parameters such as total height and diameter can be very high, but may be larger on stem form, angle and size of branches;
- the genotype × environment interaction is generally low for both the worst and the best materials;
- clones can be divided into those that interact with the environment and those that do not;
- correlations among stem form, branch traits and woody biomass traits (H and Dbh) are frequently low. This is probably due to variation among ramets (essentially their vigour) and micro-site variation;
- broad sense, or clonal, heritability can be very high for many traits useful for selection;
- clonal heritability is higher when environment and soil conditions are homogeneous and clones can express their real potential;
- methods of clone selection using multiple traits, such as a selection index, can solve a lot of practical problems when a set of several parameters must be evaluated.

Earlier experience with large-scale cultivation of poplars in monospecific stands showed a lot of problems, and the use of wild cherry clones should also be restricted to limited areas within the optimum range for the species. Moreover, the use of a very restricted range of clones within plantations should be avoided to reduce monoculture risks.

In general, it is suggested that clones characterized by low genotype × site interaction be used. However, including clones that are specifically adapted to the local conditions (e.g. for growth, architecture, colour, etc.) would also improve within-plantation variation.

Where water may be in short supply during summer plantations should be established using seedlings rather than plantlets from micropropagation, as seedlings will develop a better tap-root system. The pseudo-fasciculate root system obtained via micropropagation or cutting is better suited to alluvial or relatively deep sandy soils where water supply is constant.

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Experiences with wild cherry (*Prunus avium* L.) tissue culture

A. De Rogatis, S. Guerri and R. Proietti
CRA–Istituto Sperimentale per la Selvicoltura, Arezzo, Italy

Introduction

In Europe, wild cherry (*Prunus avium* L.) is one of the most important hardwood trees for producing timber for furniture. Its natural habitat extends across Europe, from Scandinavia to southern Russia and from the mountains of North Africa to West Asia (Ghani and Cahalan 1991).

Most wild cherry plantations in Europe are made up of diverse, genetically unimproved plants, so yields are unpredictable and less than optimal (Hammatt and Grant 1998). In response to this, wild cherry genetic improvement programmes have been started in Italy (Ducci et al. 1987; Ducci et al. 1988; Ducci 1989; Ducci and Santi 1996; Ducci and Veracini 1990) and other European countries, including France (Santi 1988; Santi and Lemoine 1990), the United Kingdom (Nicoll 1993), Belgium, Germany, the Netherlands, Denmark, Sweden and Norway (Grant et al. 1998).

Regeneration could complement conventional breeding to speed up the processes of traditional plant improvement. Genetic transformation with *Agrobacterium* could be used to develop lines resistant to pests and diseases such as bacterial canker, *Pseudomonas syringae* pvs. *morsprunorum* and *Cylindrosporium padi* (da Câmara Machado et al. 1995; Grant et al. 1998).

The micropropagation of wild cherry is now widely applied to both young (Barzanti et al. 2004; Chaix 1981; Cornu and Chaix 1981; Cornu et al. 1981; Druart et al. 1981; Grant and Hammatt 1999; Riffaud 1980) and mature plants (Biondi et al. 1990; Feucht and Dausend 1976; Hammatt and Grant 1993; Hammatt 1994a and 1994b; Hammatt and Grant 1997; Harrington et al. 1994; Ranjit et al. 1988; Riffaud and Cornu 1981; Snir 1982). Several authors have reported somatic embryogenesis of *Prunus* spp. (Durzan et al. 1990; James et al. 1984) and of *P. avium* (De March et al. 1993; De Rogatis and Rossi 1992; Garin et. al. 1997; Pedrotti et al. 1992). In addition, adventitious shoot regeneration from cotyledons (De Rogatis and Fabbri 1994–95 and 1997; Pedrotti et al. 1992; Yang and Schmidt 1992), from leaves (Escalettes and Dosba 1993; Hammatt and Grant 1998), from roots of micropropagated plants (Pedrotti and Cornu 1991) and from protoplasts (Ochatt 1991) has been observed.

In forestry, it is particularly important to use sufficient numbers of genotypes when conserving genetic variability. The Italian Genetic Improvement Programme uses several clones that are selected on the basis of growth and architecture characteristics. The Programme also includes *in vitro* regeneration by organogenesis and somatic embryogenesis from different types of somatic tissues to obtain protocols for use in future breeding. For example, *in vitro* selection for disease resistance (*Phytophthora* spp.) is applied to regenerated cherry clones.

In vitro regeneration can create problems for cultured tissues. When cells are removed from their normal conditions and placed in a tissue culture environment, the genome may restructure in response to the new conditions of stress (Ahuja 1998). Variations that originate in somatic tissue grown *in vitro* may derive from altered genotype, altered phenotype or both. The origin of new variants may be traced back to a gene mutation or an epigenetic change. Epigenetic changes are caused by altered gene expression without changes to the gene structure. They are not transmitted to the progeny and occur in response to specific events (inducers) (Ahuja 1998). In woody plants, such changes can be transmitted stably across generations of somatic cells. Variation may also be caused by changes in gene structure, by gene mutation, by changes in the number or structure of chromosomes or by changes in the DNA (Ahuja 1998; Karp and Bright 1985). The two main types of change can be differentiated through progeny tests.

Somaclonal variations are undesirable when the plants grown *in vitro* are meant to be identical to the genotypes selected for the silviculture cloning programme, or when they are for germplasm conservation. Some somaclonal variations may, however, be useful in breeding and improvement programmes.

There are several ways to detect somaclonal variations: phenotypic and morphologic measurements, progeny tests, electrophoretic analysis of isozyme systems, DNA analysis, and studies of the differences in chromosome numbers or structure.

The authors of this study focused on shoot regeneration from cotyledon and leaf tissue, the factors affecting regeneration of different genotypes, and the interactions among these factors. Somaclonal variation was investigated by electrophoretic analysis of isozymes only as initial screening.

Adventitious shoot regeneration

Regeneration from cotyledon tissue

Cotyledon tissue was extracted from seed collected at different stages of development from nine promising wild cherry clones in the Tuscan Apennines and the Po valley (Italy).

The following factors were investigated; four basal media (MS [Murashige and Skoog 1962], MB [Druart 1980], DCR [Gupta and Durzan 1985] and DKW [Driver and Kuniyuki 1984]); concentrations (0.05 to 8.00 mg/l) and combinations of six growth regulators (indole-3-acetic acid [IAA], naphthalene acetic acid [NAA], 2,4-dichloro-phenoxy-acetic acid [2,4-D], zeatin: 6-4-hydroxy-3-methyl but-2-enyl amino purine [ZEA], kinetin: 6-furfuril amino purine [KIN] and 6-benzyl amino purine [BAP]); environmental conditions (various periods of darkness, temperature and light intensity); types of container for growing explants; and sizes of explants.

For a large number of cherry clones, seeds reached full maturity 68 days after anthesis. Only immature cotyledons produced significant shoot regeneration (35 or 50 days after anthesis), but there was no significant correlation between seed size and regeneration frequency or mean number of regenerated shoots (Figure 1). In the first period, the explants were grown in darkness because light inhibited shoot development. When the explants had produced shoots they were kept at a 16 h photoperiod and temperature of 21 ± 1 °C.

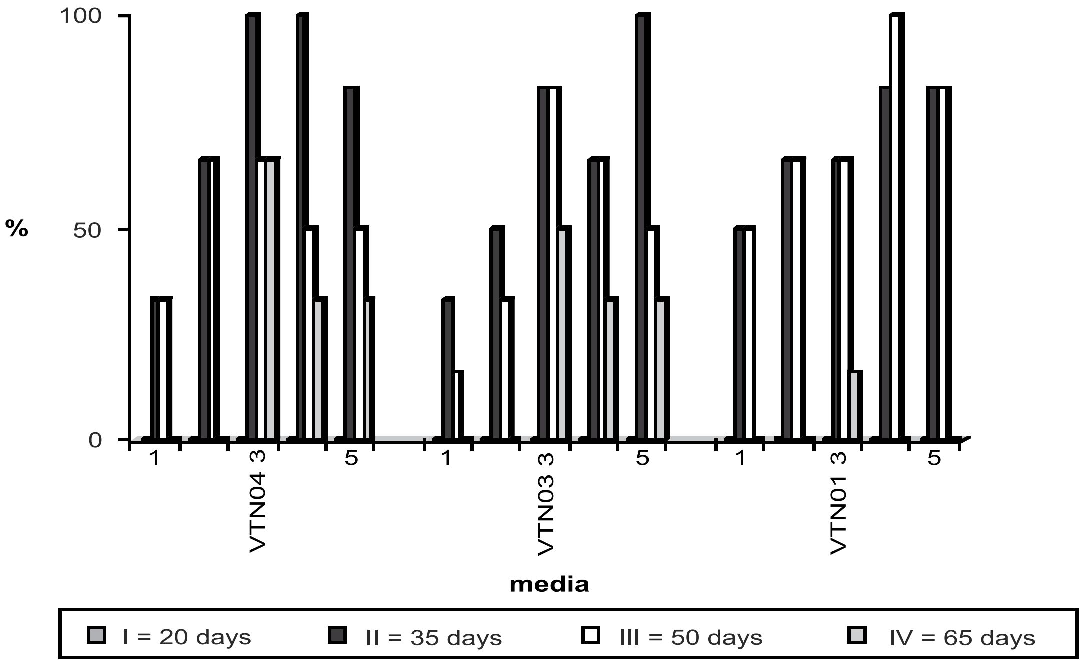

Figure 1. Regeneration frequencies during the cotyledon maturation.

The genotypes behaved differently under different growth regulators and at different light intensities (50 or 70 µmol m^{-2} s^{-1}). This was probably because of interactions between these two factors or different concentrations of endogenous hormones.

The results obtained depended on the genotype. The clones produced high percentages of regeneration (Table 1; Figure 2) and good mean numbers of regenerated shoots (Table 2) on basal media MS and DCR. However, there were no statistically significant differences among the effects of the different basal media.

Table 1. The most important regeneration frequencies of different clones (21 °C at 70 µmol m^{-2} s^{-1})

Clone	Medium	% MS	% MB	% DCR
VTN04	A	100		
	B	100		
VTS01	A	100		
	B	100		
VTN03	A	100		
	B	100		
ACW10	C			100
ACW03	C			100
VTS01	D		100	

% = regeneration frequency
A = BAP 22.19 µmol + IAA 2.87 µmol
B = BAP 22.19 µmol + 2,4-D 2.26 µmol
C = BAP 35.52 µmol + IAA 11.50 µmol
D = BAP 17.76 µmol + IAA 11.50 µmol

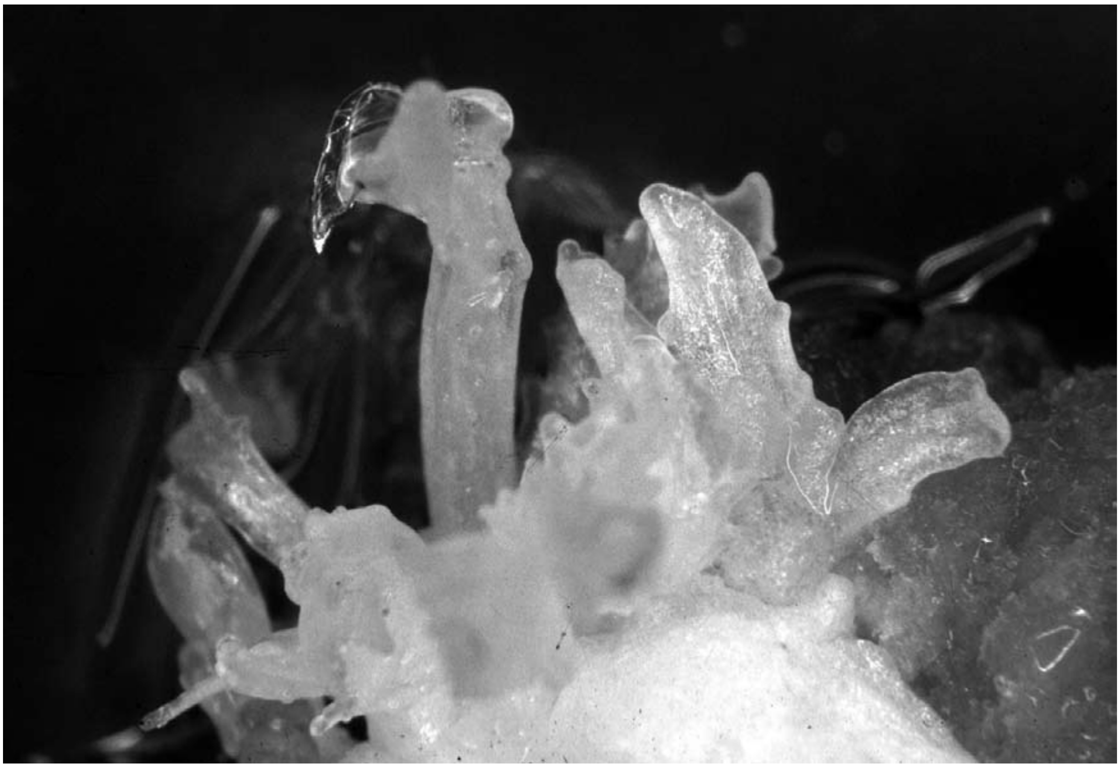

Figure 2. Shoot regeneration from cotyledon tissue.

Significant differences were found among the regeneration frequencies of different genotypes (P<0.005) and different growth regulator concentrations (P<0.001). Significant interactions were found between genotype and growth regulator (P<0.001) and between genotype and medium (P<0.001), but there was no significant interaction between basal medium and growth regulator concentration (Table 3).

The mean number of regenerated shoots per explant differed very significantly depending on the growth regulator concentration used (P<0.00001) (Table 4). There were also significant interactions between basal medium and genotype and between basal medium and growth regulator concentration (P<0.00001) and a significant interaction between genotype and growth regulator concentration (P=0.01).

Table 2. Regeneration percentage of different clones (21 °C at 50 µmol $m^{-2} s^{-1}$)

Clone	Medium	MS % (N)	DCR % (N)
ACW10	2	36 (5.44)	80 (6.70)
	3	76 (4.95)	80 (6.65)
	4	76 (6.53)	72 (5.22)
	6	68 (2.82)	40 (3.10)
VTN04	3	56 (4.93)	72 (8.28)
	5	52 (3.31)	52 (3.08)
VTS01	1	64 (8.69)	36 (8.11)
	2	52 (7.69)	16 (6.75)

% = regeneration frequency
N = mean number of regenerated shoots
1 = BAP 22.19 µmol + 2,4-D 2.26 µmol
2 = BAP 22.19 µmol + IAA 5.75 µmol
3 = BAP 17.76 µmol + IAA 22.84 µmol
4 = BAP 8.88 µmol + IAA 22.84 µmol
5 = BAP 22.19 µmol + NAA 5.37 µmol
6 = BAP 4.44 µmol + NAA 10.74 µmol

Table 3. Statistical analysis of regeneration frequency

Factor	d.f.	d.r.
Genotype	2	10.00**
Medium	1	0.12
Hormone concentration	5	11.62***
Genotype × medium	2	12.67***
Medium × hormone concentration	5	1.17
Genotype × hormone concentration	10	6.31***

** = significant at P<0.005; *** = significant at P<0.001

Table 4. Analysis of variance of number of regenerated shoots

Factors	d.f.	F	P
Genotype	2	1.370	0.2547
Medium	1	0.005	0.9458
Hormone concentration	5	13.999***	0.0000
Genotype × medium	2	13.505***	0.0000
Medium × hormone concentration	5	7.128***	0.0000
Genotype × hormone concentration	10	2.998*	0.0108

* = significant at P<0.05; *** = significant at P<0.001

Regeneration from leaf tissue

Research was carried out on the leaf tissue of seven micropropagated clones in the multiplication phase. The leaf tissue was collected from the same areas of Italy as the seeds were. The following factors were investigated: basal media (MS, DCR and WPM) (Lloyd and McCown 1981); concentration of growth regulators (0.02 to 5 mg/l) (IAA, NAA, 2,4-D, ZEA, BAP and TDZ [Thidiazuron: 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea]); temperature; light intensity; container used before induction; period in the last medium before induction; and size of leaf.

The results of this research are still being analyzed, but it can already be seen that the Coulter of Greiner was the most suitable container for preparing shoots for the induction of regeneration. TDZ was the only growth regulator to produce significant regeneration (Figure 3), while the other hormones produced adventitious shoots only occasionally. The genotypes differed markedly in their ability to regenerate, and different genotypes produced very different results in the different media. Light intensity, temperature and basal medium also affected the ability to regenerate (Table 5; Table 6). Before induction, shoots can stay in the last fresh medium for no longer than one month. The best leaf size for induction was 0.5–1 cm. Explants produced significant regeneration when leaves, without petioles, where incubated in 16 hours of light at an intensity of 50 µmol m^{-2} s^{-1} and a temperature of 25 ± 1 °C.

The MS medium gave consistently poor results while the DCR medium gave good results with all clones, the best clones were CA with 80% regeneration and VM02 with 75%. The clone PC gave the highest percentage of regeneration (85%) on WPM. The highest numbers of shoots per explant and the highest multiplication rates of regenerated shoots were obtained on VTS02.

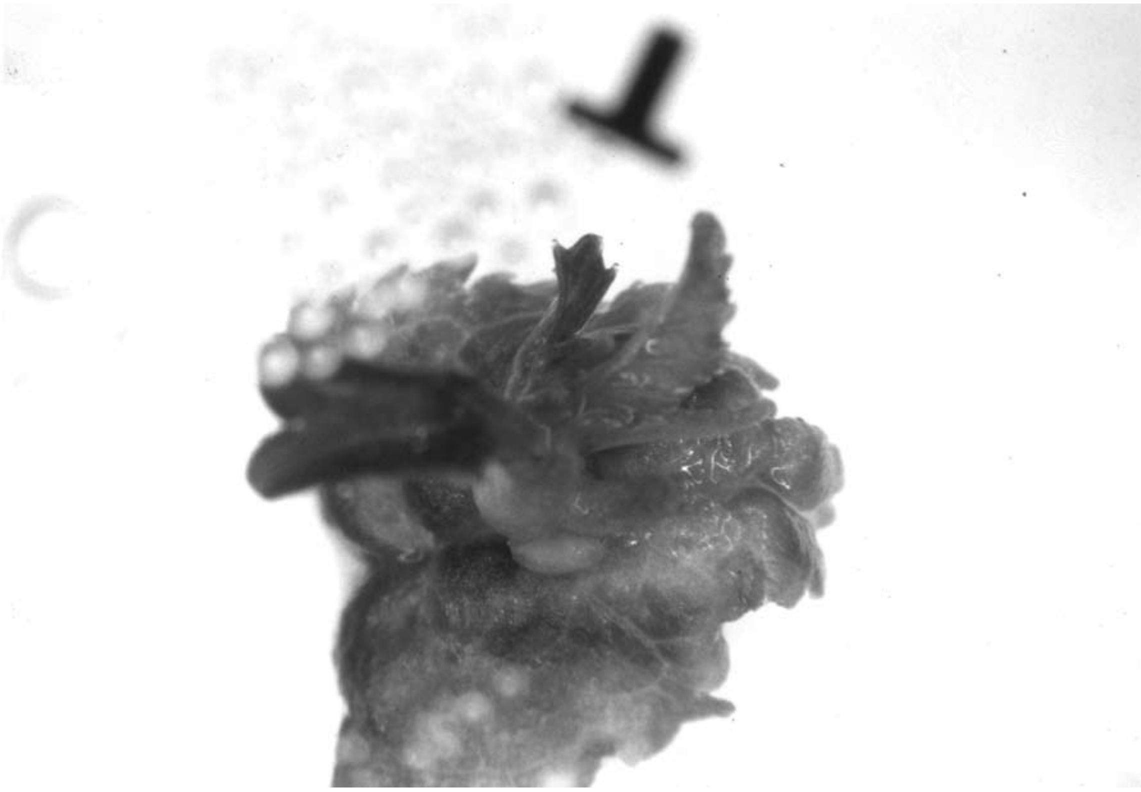

Figure 3. Shoot regeneration from leaf tissue.

Table 5. Regeneration percentage of different clones (25 °C at 50 µmol m^{-2} s^{-1})

Clone	Medium	% WPM	% DCR
PC	A	70	25
	B	85	20
	E	70	50
CA	A	20	40
	B	75	80
	E	55	75
VM02	A	15	55
	B	55	75
	E	60	60
ACW03	A	10	5
	B	45	15
	E	30	60

% = regeneration frequency
A = TDZ 0.45 µmol
B = TDZ 2.27 µmol
E = TDZ 4.54 µmol

Table 6. Regeneration percentage of different clones, mean number of shoots per explant and multiplication rate (25 °C at 70 µmol m^{-2} s^{-1})

Clone	%/N	N	N	N
	DCR B			
PC	40/1.6 (16)	1.75 (28)	4.5 (126)	3.65 (460)
CA	40/2.2 (22)	1.95 (43)	3.79 (163)	3.74 (610)
VTS 02	24/3.33 (20)	1.45 (29)	10.03 (291)	4.11 (1196)
VM 02	28/2.5 (18)	1.55 (28)	1.89 (53)	3.33 (177)
ACW 03	32/2.4 (19)	1.68 (32)	1.59 (51)	2.33 (119)
FL 02	4/4.0 (4)	1 (4)	0.5 (2)	7 (14)
	DCR E			
PC	32/2 (16)	1.68 (27)	4.26 (115)	3.23 (372)
CA	72/2.6 (47)	1.85 (87)	3.4 (296)	2.72 (804)
VTS 02	56/2.2 (31)	2.9 (90)	7.83 (705)	3.46 (2438)
VM 02	64/2.6 (42)	1.9 (80)	5.31 (425)	3.19 (1356)
ACW 03	48/2.6 (32)	1.91 (61)	3.88 (237)	3.3 (783)
FL 02	24/1.2 (7)	1.57 (11)	2 (22)	2.82 (62)

% = regeneration frequency
N = mean number of shoots per explant
B = TDZ 2.27µmol
E = TDZ 4.54 µmol
Figures in brackets refer to the number of multiplied shoots.

Monitoring somaclonal variations

Isozyme electrophoresis was used in a first screening of somaclonal variation in shoots and acclimatized plants regenerated by organogenesis from leaf tissue. The seven most interesting wild cherry clones, ACW03, PC, CA, FL07, VM02, FL02 and VTS02, were tested. The plants showed no morphological changes. Allozyme analysis was performed on leaf samples of the regenerated shoots two months after their development i.e. at formation. All the regenerated shoots of every clone (at least 35) were examined by horizontal starch gel electrophoresis. The electrophoretic and staining procedures were performed according to the guidelines of Santi (1988) and Santi et al. (1990). Samples were extracted after centrifugation of the homogenated tissue for 10 minutes at 10 000 rpm. Nine enzyme systems coded for 18 loci were analyzed: isocitric-dehydrogenase (IDH, EC 1.1.1.42), malic-dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate-dehydrogenase (6-PGD, EC 1.1.1.44), phosphoglucose-isomerase (PGI, EC 5.3.1.9), shikimate-dehydrogenase (SKDH, EC 1.1.1.25) glutamate-oxaloacetate-transaminase (GOT, EC 2.6.1.1), glutamate-dehydrogenase (GDH, EC 1.4.1.3), phosphoglucomutase (PGM, EC 2.7.5.1) and aconitase (ACO, EC 4.2.1.3).

In the zymograms, the enzyme systems showed the following different types (Figure 4):

- MDH: Type 1 on ACW03-CA – FL07 – VM02 – VTS02, Type 2 on FL02 – PC, 6-PGD: Type 1 on ACW03-CA – FL07 – VM02 – PC, Type 2 on FL02 – VTS02;
- GOT: Type 2 on FL02 – VM02, Type 3 on CA – PC, Type 4 on VTS02 – FL07;
- IDH: Type 2 on FL02 – FL07 – VM02 , Type 3 on VTS02 – PC – CA – ACW03;
- ACo: Type 2 on FL07 – VM02, Type 3 on VTS02 – PC.

There were differences in isozyme systems among clones, but not among shoots that had been regenerated from the same leaf or from different leaves of the same clone (data not shown). There were no differences among shoots grown on different media, nor between the *in vitro* shoots and the same plants in the greenhouse. Nevertheless, as the results show, isozyme analysis cannot be used to test the whole plant genome, but only a small part of it. DNA analysis is probably the only way of detecting all variations.

Discussion and conclusions

The clones with the highest regeneration frequency were those from cotyledon tissue ACW10 and VTN04, and leaf tissue PC, CA. For high multiplication rates of regenerated shoots, clones VM02 and

VTS02 were the best. It is interesting that the genotypes that produced high regeneration frequencies were also the most productive clones for the purposes of improvement.

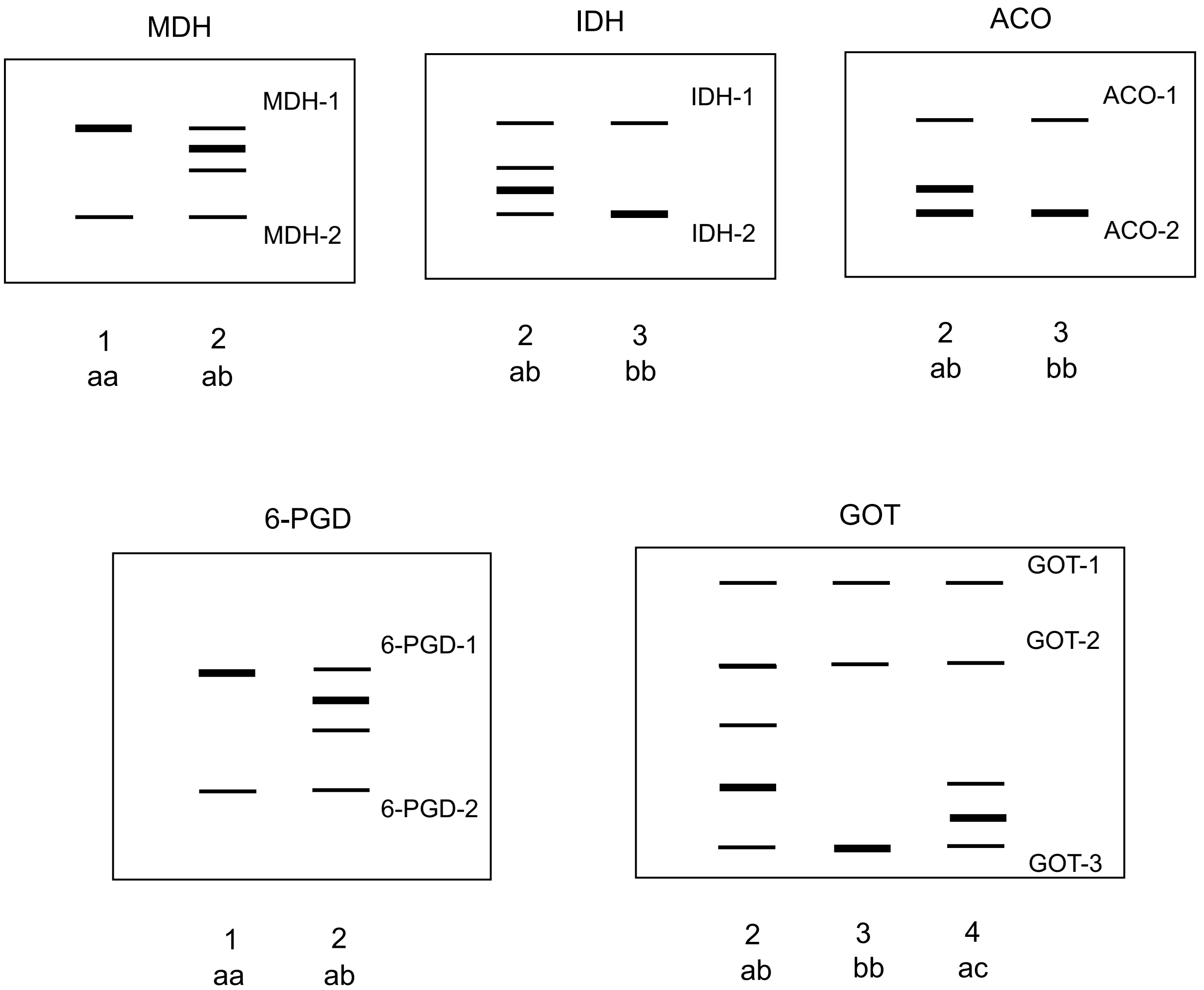

Figure 4. Zymograms of enzyme systems studied.

The shoots regenerated directly from the leaf surface and from the cotyledon surface, but the elimination of embryos was determinant (De March et al. 1993; De Rogatis and Fabbri 1997; Pedrotti and Cornu 1991; Pedrotti et al. 1992).

Genotype was observed to have considerable influence on regeneration ability. This seems to be regardless of the physiological conditions of the material at harvest, because all the material was taken from only one collection and at the same time. James et al. (1984), De Rogatis and Fabbri (1994–95; 1997) and Grant et al. (1998) observed variability in the organogenic capacity of genotypes, while De March et al. (1993) observed variability in embryogenic ability. The greatest differences were obtained among different genotypes and different concentrations of growth regulator. These differences may be connected with the interactions between genotype and growth regulator concentration, probably owing to the presence of different endogenous growth regulators or to genetic effects. Escalettes and Dosba (1993) pointed out considerable variability of results from different experiments carried out on leaf explants.

The composition of the basal medium did not significantly affect the results, although the highest regeneration frequency was found on DCR. Grant et al. (1998) obtained results with WPM only. Regenerated shoots that were induced on DCR had better subsequent development, while those induced on MS became vitrified.

The nature of the growth regulator is decisive. In the authors' experiments, the best results were obtained with BAP for cotyledon tissue and TDZ for leaf tissue. Pedrotti et al. (1992) considered the latter cytokinin necessary to induce buds from the roots of wild cherry, and James et al. (1984) from Colt cherry rootstock. Yang and Schmidt (1992) obtained results with BAP and TDZ on sweet cherry. Regarding the hormone combination, IAA with cytokinins produced the best results.

In order to induce regeneration, embryogenesis or organogenesis, it is necessary to have a specific balance of growth regulator in the fruit species, as da Câmara Machado et al. (1995) show for *P. subhirtella autumno rosa.* James et al. (1984) observed a clear genetic control of the embryogenic or organogenic ability in Colt cherry rootstock.

On cotyledon tissue, the explants with the highest mean number of shoots did not always have the highest percentage regeneration. The determinant factor seemed again to be the genotype, which had a significant effect on the number of shoots. The genotype's interactions with the different basal media and growth regulators produced significant differences.

The goal of these studies was to obtain regenerated plants that were identical to the starting material. The results suggest that there were no somaclonal variations in the shoots regenerated from leaves, but this can be confirmed only after DNA analysis.

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Noble hardwood cultivation techniques

S. Ravagni and E. Buresti Lattes
CRA–Istituto Sperimentale per la Selvicoltura, Arezzo, Italy

Introduction

The aim of this paper is to show the most important cultivation techniques used in Italy for the farming of noble hardwoods. Tree farming consists of a set of cultivation practices aimed exclusively at producing quality timber. Many tree species can produce high-quality timber that obtains high prices on the European market. Among these species are wild cherry (*Prunus avium* L), pear (*Pyrus communis* L.), *Sorbus* spp., chestnut (*Castanea sativa* Mill.), poplars (*Populus* spp.) and walnut (*Juglans regia* L.). In Italy, walnut is the most requested of these (ARSIA 2003; 2004).

We started our study of tree farming from our knowledge of silviculture, fruit growing and Italian poplar cultivation, all of which share some common features with tree farming.

The three production steps in tree farming

Tree farming follows three production steps: rooting, qualification and dimensioning (Figure 1). Each step has its own aims and specific cultivation needs.

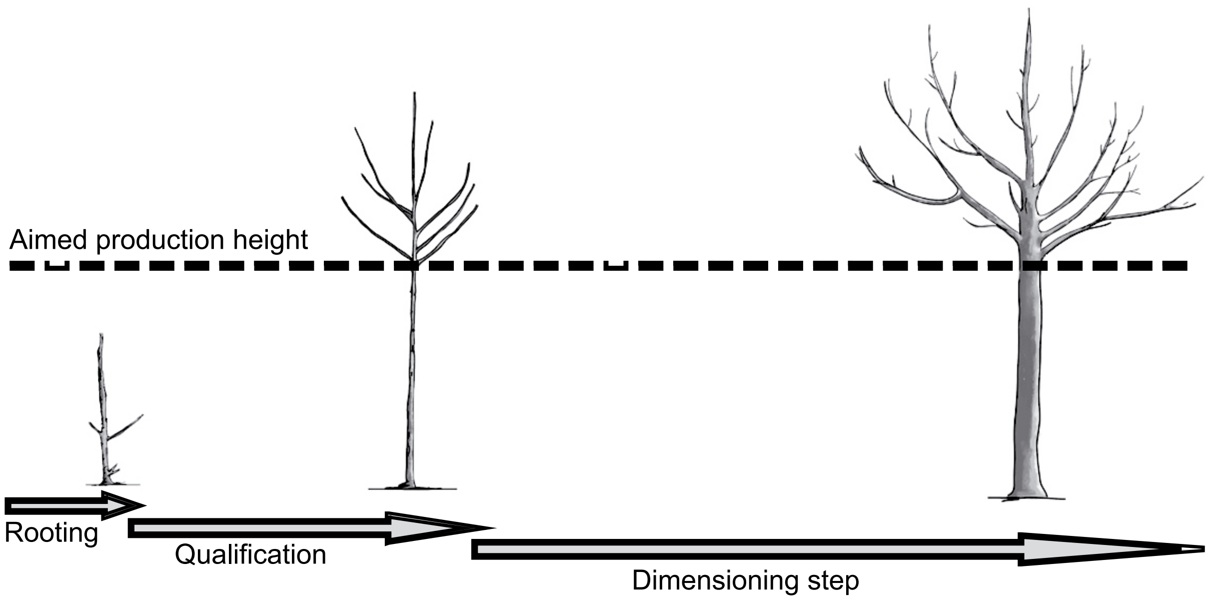

Figure 1. The three production steps in tree farming: rooting, qualification and dimensioning.

Rooting

During the rooting step, plants overcome the stress of transplanting (ARSIA 2004), so that they can produce strong and wide root systems.

This step can vary in length, from one to three years, according to plant characteristics, climatic and environmental conditions and cultivation practices. Usually, the appearance of a shoot of 50 cm or more indicates that the plant has overcome the stress of rooting.

In order for this step to be successful, it is necessary to have:

- well-prepared soil with deep and shallow furrows
- well-developed plants that have strong stems and well-developed root systems, and that have been planted correctly and at the right time

- careful cultivation after plantation so that plants do not have to compete with spontaneous vegetation during their first year.

During the rooting step, the plant should not be pruned. This is to avoid increased stress with reduced photosynthetic surface.

Qualification

The qualification step is devoted to obtaining a stem that is at least 250–300 cm tall. The stem should not have branches until it has reached a diameter of 8–10 cm (ARSIA 2004).

In order for this step to be successful, it is necessary to:

- choose the right pruning technique for the species
- choose the right pruning technique for the strength and architectural structure of the individual plant, and for the knowledge and experience of the pruner
- continue cultivation
- check shelters and stakes regularly to prevent them from damaging the plant or causing its stem to bend or narrow.

Tree farming makes use of three pruning techniques: pivot pruning, progressive pruning, and reiterative pruning. These techniques have been coded for walnut, but not for the other species. We are therefore testing pruning techniques for *Prunus avium, Quercus* spp., *Sorbus* spp. and *Pyrus communis* at the Experimental Silviculture Institute in Italy.

We have already tested reiterative pruning for walnut. This technique causes the walnut plant to raise its crown and build up an architectural pattern that is characterized by:

- an apical shoot where the crown will be in future years
- at the beginning of pruning, a crown on the two-year-old stem of vigorous plants and on the two or three-year-old stem of plants of medium/low vigour
- a stem without branches (ARSIA 2004) (Figure 2a).

The same pattern should be followed in the following years until the end of the qualification step (Figure 2b and c).

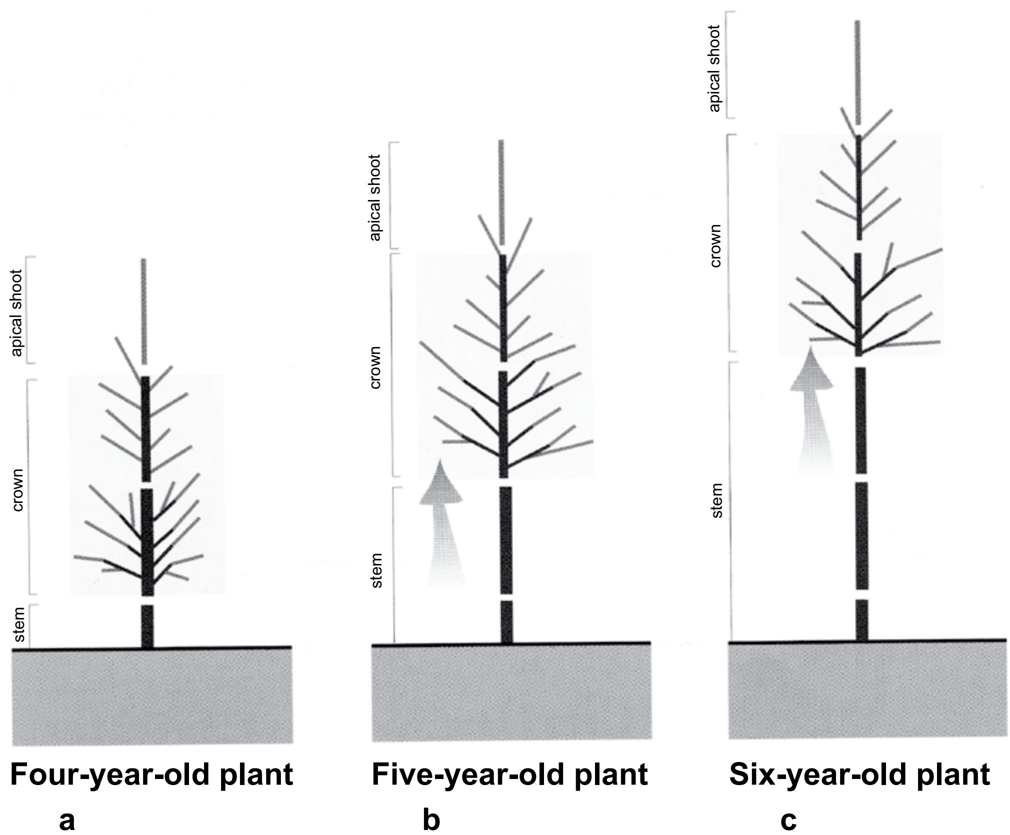

Figure 2. Reiterative pruning.

To obtain this structure it is necessary to prune:

- the top branches
- branches that are too thick or too upright
- the one-year-old branches of vigorous plants
- the 1.5–2-year-old branches of plants of medium vigour
- the 2.5–3-year-old branches of plants of low vigour.

Dimensioning

The dimensioning step aims to obtain trees with stem diameters of 30–40 cm and a sustained and regular growth pattern. Regular growth of the stem diameter is obtained by allowing the plant to extend its roots and crown into progressively wider spaces (ARSIA 2004).

In this step it is very important to monitor the plants' dimensions so as to be aware of any reductions in growth. In order to obtain progressively wider spaces and avoid competition among plants, various thinning techniques can be used.

In *geometric thinning*, 50% of the trees along fixed alignments (for example, on alternate diagonals) are cleared. This technique is possible on homogeneous plantations where there are few differences among plants. It has the advantage of being technically very simple and can be carried out by a worker with a chain saw. However, it does not allow selective criteria to be applied to the plants (Figure 3).

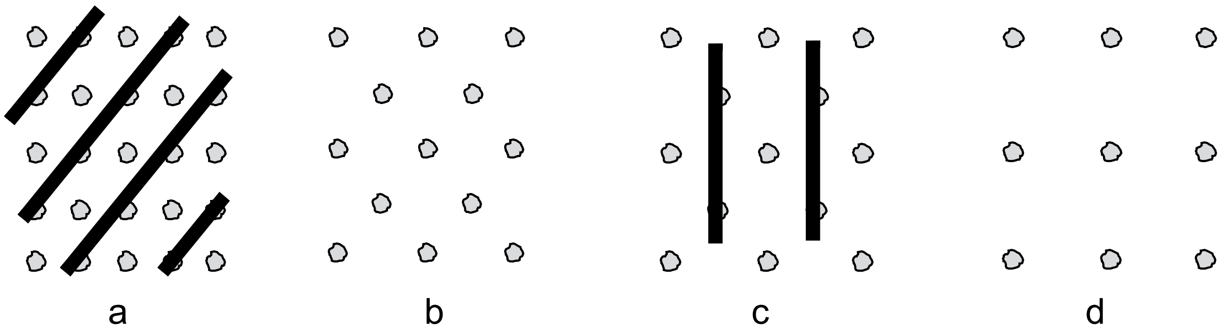

Figure 3. The evolution of a plantation: after the first geometric thinning along alternate diagonals (Figure 3a), a second geometric thinning is carried out along alternate columns (Figure 3c).

In *selective thinning*, the better plants ('candidates') are retained and surrounding plants that could cause problems for these are felled (Figure 4). It is not always easy to select candidates, but this method makes it possible to maintain the better plants in the stand. Usually, from 70 to 120 plants ha^{-1} are chosen, depending on the species and type of pruning. The selection of candidates is based on the plants' stem quality, architectural structure and strength.

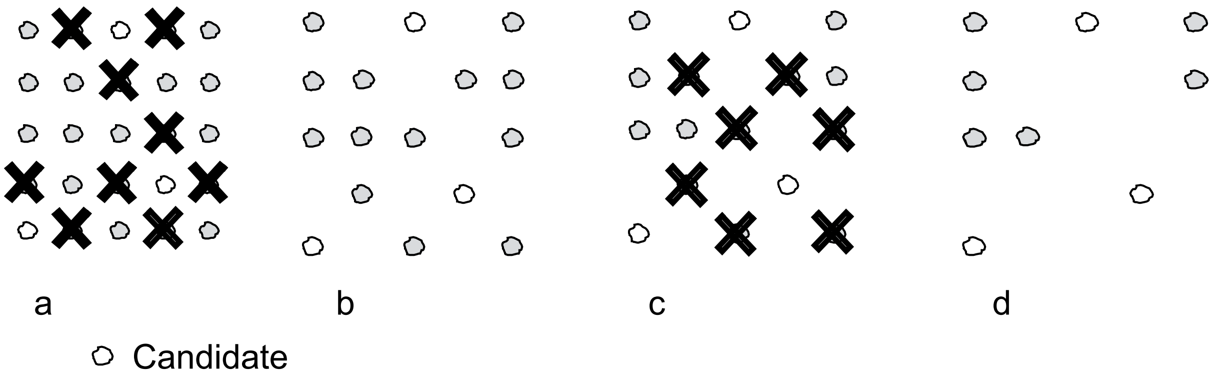

Figure 4. The evolution of a plantation after two selective thinnings (Figures 4a and 4c).

Mixed thinning is like geometric thinning with 'jumps'. Again, about 50% of the trees on fixed alignments are cleared, but the better plants on these alignments are left. These are the 'jumped' trees, and two plants around each of these are felled to leave enough space for the better trees. This makes it possible to select the better plants in a way that simple geometric thinning does not allow (Figure 5).

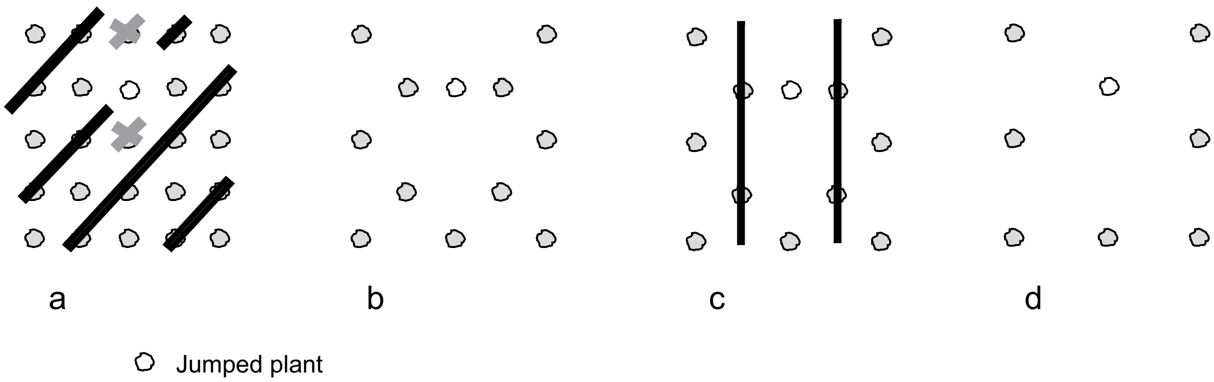

Figure 5. Geometric thinning with 'jumps' (Figure 5a); the second thinning is a geometric thinning (Figure 5c).

The planning of noble hardwood plantations

The planning of noble hardwood plantations has evolved. Twenty years ago, pure stands were planted. Initially these were of *Quercus robur*, with 3x3 m spacing between plants and 1100 plants ha^{-1}. This spacing and density made it possible to use tractors to till the soil and carry out plant selection and thinning. The latter was particularly important at the time because little was yet known about how plantations develop and which are the best cultivation techniques (for example, pruning) for obtaining good plants. High-density plantations provided a sort of insurance against selection and other mistakes in plantation management.

In this plantation model it was not possible to identify which were the main plants at the beginning of the cultivation cycle, because this does not become clear until later on. In pure stands of high-density plantations the plants that will survive all the thinnings and reach the end of the cultivation cycle cannot be identified. They cannot therefore be referred to as 'main plants' but as 'potential main plants'.

Later, we started to create pure stands with accessory plants. The purpose of these accessory plants is to help the farmer to work and the main plants to develop; accessory plants are not subject to cultivation practices such as pruning, for example.

The accessory plants that we usually used were *Alnus cordata* Desf., *A. glutinosa* Gertner., *Elaeagnus* spp., *Sambucus nigra* L., *Corylus avellana* L., *Robinia pseudoacacia* L., *Carpinus* spp., *Quercus cerris* L., *Ostrya carpinifolia* Scop., *Ulmus* spp. and *Salix* spp.

The next step was to plant mixed stands of potential main tree species, with or without accessory plants. In this case, the main plants can be identified when the plants are in their final spacing, after one or more thinning.

Mixed stands have many benefits:

- increased soil fertility, especially in mixtures with N-fixing plants such as *Alnus* spp.
- better stem quality, because the short distance between plants at the beginning of the cycle makes plants produce better forms of stem, smaller branches and stronger growth in height
- easier cultivation, because smaller branches make pruning easier, while greater shade on the soil reduces the competition from weeds, and consequently the need for tilling
- higher resistance to diseases, especially for specific pathogens
- less competition among individual trees when species with different root system morphologies, crown shapes and nutritional needs are used
- diversification of production in mixtures where the main plants are of more than one species.

However, farmers have to cultivate all the plants in this type of plantation because they do not know which will be the main plants until late in the cultivation cycle. This makes plantation management costly.

Currently, plantations are planned with main plants at low densities; from the outset of the plantation, the main plants are at their final distances. This makes it possible to concentrate cultivation on those plants that will reach the end of the cycle.

In this plantation model it is not possible to select the main plants. To solve this problem, two main trees are planted about 0.5 or 1 m away from each other, and the better is selected as the main plant when they are between 4 and 6 years old, depending on the species.

The authors experimented with this plantation model for different species, obtaining the best results with *Quercus* spp., *Fraxinus oxycarpa, Prunus avium, Tilia cordata* and *Juglans regia*. When planted in pairs, these tree species remain straight, despite the short distance between them, and grow in diameter and height at least as well as single plants do (Buresti et al. 2001b; 2002; 2003). We are currently testing the use of main plants of different species that reach commercial maturity at different times (Buresti et al. 2001a; Ravagni and Buresti 2003). This produces profits at different times, which is an advantage for the farmer. Among the mixes being experimented are plantations of poplar clones and walnut. Poplar clones reach commercial maturity in 8–10 years, while walnut needs 25–30 years, depending on the environmental conditions. In these situations, it is very important to plan the plantation properly and choose the right distances between plants, because there must be space to fell poplar without damaging walnut, and the two species must not be forced into competition.

Conclusion

This article has provided a very general description of different aspects of the cultivation of noble hardwoods to show all the sectors involved in arboriculture. It does not claim to provide complete knowledge of arboriculture, but identifies cultivation techniques that should be investigated further.

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Genetic variation of walnut (*Juglans regia* L.) in Europe

M.E. Malvolti, P. Pollegioni and S. Bartoli
CNR Institute of Agro-Environmental and Forest Biology, Porano, Italy

Introduction

Walnut (*Juglans regia* L.) is a multipurpose species utilized for fruit, timber, colouring and medical compounds. Although it has probably been present in Europe since 5000 BC (Huntley and Birks 1983), its present distribution is mainly the result of human activity (Figure 1) and walnut is one of the more manipulated agroforestry species. The selection and diffusion of the most valuable genotypes to improve and increase the quality and quantity of products can lead to reduced genetic variability, the loss of useful genes and genetic erosion. "The evolutionary potential and resilience of species depend on the amount and structure of genetic variation at intraspecific level" (Millar and Libby 1991), so conservation of genetic resources should be considered for even widespread non-endangered species, particularly if their intraspecific genetic variability is low. This is the case of the multipurpose tree species such as walnut (Fornari et al. 1999).

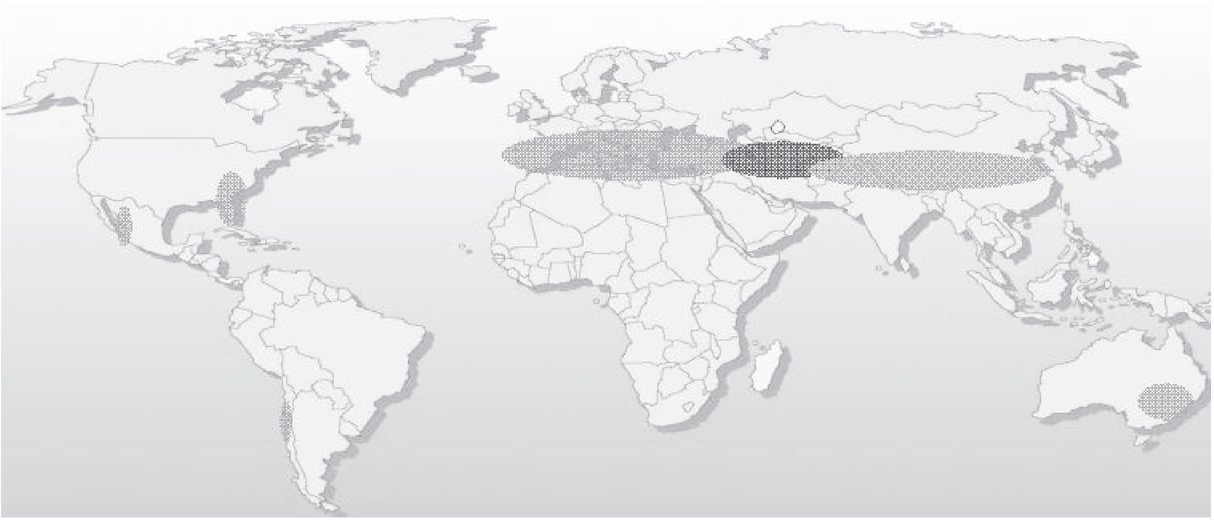

Figure 1. Worldwide distribution of walnut (*Juglans regia* L.) (◯ present distribution; ⬤ origin).

Since 1987, CNR–IBAF (formerly the Agroforestry Institute) has been carrying out studies on walnut genetic resources (natural and naturalized material, as well as selected germplasm), utilizing different kinds of genetic markers (isozymes, random amplified polymorphic DNA [RAPD], inter-simple sequence repeat [ISSR] and simple sequence repeat [SSR]). This research has been developed in the framework of national and international projects and bilateral cooperation activities.[1]

Research has been carried out in the following areas:

- analysis of walnut genetic resources in the Eurasian continent
- analysis of interspecific hybrids *J. nigra* × *J. regia* for wood production

[1] Such as: AIR3 CT92 0142; FAIR III PL96 1887 'W- BRAINS', MIRAAF 'Arboricoltura da legno' sottoprogetto 'Latifoglie Pregiate', MIPA 'Analisi di caratteri complessi in piante forestali mediante tecniche molecolari', CNR (Ag. 2000), MiPAF 'Riselvitalia' Sottoprogetto 1.1 'Biodiversità e Produzione del materiale forestale di propagazione'; MiUR 'Biofrum', MAE (L. 401/1990) (2003 and 2004). Agroforestry: agro-food, environment and bilateral cooperation agreements with the Hungarian Academy of Forestry (MTA), the Chinese Academy of Agricultural Sciences (CAAS) and the Chinese Academy of Forestry (CAF).

- evaluation of genetic diversity among and within fruit varieties
- study of mating systems
- construction of an integrated genetic map for quantitative trait loci (QTL) analysis.

Research activities

Analysis of walnut genetic resources in the Eurasian continent

Bud or young leaf material was collected from natural and naturalized walnut accessions in Italy, France, Spain, Greece, Hungary and three zones in China. Prof. Germain (of the National Institute for Agricultural Research [INRA], Bordeaux, France) provided samples from the Caucasus (Georgia) that were conserved in the French germplasm repository, and Dr Pei Dong (CAF, Beijing) provided samples from Tibet. The Spanish material was sampled and tested by Dr Neus Aletà of the Institute Mas Bovè, Reus, Spain. The germplasm was analysed using isozymes and RAPD markers. The indices of genetic variability reported in Table 1 show that the genetic erosion of walnut has been higher in European populations than in the Caucasus and China.

Table 1. Indices of genetic variability in walnut accessions

Subcontinental groups	Na	Ne	Sk	Ho	He	F
Europe	2.24	1.66	0.47	0.380	0.378 (0.021)	0.066 (0.010)
Asia (China and Caucasus)	2.38	1.78	0.43	0.433	0.435 (0.013)	0.012 (0.053)
All Populations	2.27	1.68	0.46	0.391	0.402 (0.019)	0.036 (0.012)

Na: mean actual allele multiplicity
Ne: mean effective allele multiplicity(harmonic averages)
Sk: skewness of allele distribution
Ho: observed heterozygosity
He: expected heterozygosity
F: fixation index

As the Chinese population is a potential source of useful genes, the researchers deepened their analysis by using more variable and informative RAPD markers. The results obtained confirm that the Chinese accessions are characterized by a higher degree of genetic variability than the European ones. This suggests that appropriate programmes of gene conservation should be established for these populations.

Analysis of interspecific hybrids J. nigra × J. regia *for wood production*

This study had two phases: the first aimed to differentiate the French germplasm (parental genotypes and half sib hybrids); and the second aimed to genotype Italian *J. nigra* and *J. regia* cross-fertile plants and their progenies.

The French material consisted of: (i) a *J. nigra* mother, (ii) four selected putative *J. regia* fathers, and (iii) 60 hybrid plants. Germplasm was analysed by RAPD markers. In order to identify common and individual bands of the studied genotypes, a band map was constructed (data not shown). More than 67% of hybrids showed an intermediate profile between *J. nigra* and *J. regia* parent species. In order to visualize the relationships between the parents and hybrids, principal component analysis was performed. The results showed a clear division between *J. nigra* and *J. regia.* Hybrid plants occupied various positions: a considerable group showing an F_1 nature were between the parental species. Other individuals were genetically nearer to the *J. nigra* mother or the group of *J. regia* fathers, and could be considered back-crosses or F_2.

Italian germplasm was analysed by RAPD, ISSR and SSR markers that produced congruent results. One hybrid plant, N21, was isolated from the others. SSR markers and chromosome analysis demonstrated that this individual was a triploid plant with two genomes from *J. nigra* and one from *J. regia*. Utilization of this plant should be tested, as it is very promising for its unusually fast growth. The results of this kind of study can be applied practically in the characterization of material for legal registration, utilization by breeders and the marketing of selected plants.

Evaluation of genetic diversity among and within fruit varieties in Italy

During the last 30 years, walnut cultivation has declined in Italy, mainly because of the abandonment of agricultural activities, the mechanization of agriculture, the high cost of labour and the cutting of existing trees for timber (Forte 1993). In addition, local varieties have been progressively neglected because of their irregular fruit sizes and limited market demand. These factors have caused the erosion of Italian walnut genetic resources. The germplasm still present in agricultural areas therefore represents a valuable genetic resource that should be developed.

Classification of Italian walnut varieties is sometimes unclear because consistent and well-defined characters (varieties, population) are lacking. This research aimed at using molecular markers to differentiate four 'model' Italian varieties, 'Sorrento' and 'Malizia' from southern Italy and 'Bleggiana' and 'Feltrina' from northern Italy. The research was developed to select a set of molecular and biochemical markers that would be useful in the identification and early selection of 'new' or neglected walnut ecotypes.

Samples were taken from several varieties from Campania ('Montella', 'Cervinara', 'Fisciano', 'Trescine', 'Mesanole', 'Marna' and 'Conceria'), and analysed using molecular markers (ISSR) and morphological and biochemical traits (fruit morphology, tocopherol content, quantitative and qualitative composition of oil). The same analysis was also carried out on the four Italian model varieties. One hundred and thirty-four ISSR markers grouped varieties from northern and southern Italy; each variety was well differentiated within each cluster. Among the other accessions, 'Montella' (from the southern Apennines at 800 m above sea level) occupied an intermediate position between the two model variety groups, partially overlapping with 'Bleggiana'. Biochemical analysis detected a reasonable level of linoleic acid in the cotyledons of 'Montella' samples. Linoleic acid plays a major role in the mechanism that regulates the fluidity of membranes, which is essential for photosynthetic activity and for specific signals in seed development. The fluidity of the membranes was kept constant by decreasing the temperature, which provoked increased quantities of unsaturated fatty acids. The 'Montella' accession originates from a mountain valley, so these results may indicate that the trees are naturally adapted to cool climates. Genetic and biochemical results, as well as the morphological characteristic of the fruits, support the hypothesis of a 'Montella ecotype', which should be tested further.

Study of mating systems

Walnut belongs to a monoic wind-pollinated tree genus characterized by a reproductive strategy based on nearly complete outcrossing. This is achieved through heterodichogamy to prevent self-pollination (Luza and Polito 1988) and through reduced fertility of female flowers when there is an excess of pollen grains on pistils (Beineke and Masters 1976; Szentivanyi 1990). This is may be a built-in mechanism to hinder inbreeding, as can be seen in the limited dispersal distance of walnut seeds.

The mating system affects the genetic structure and dynamics of plant populations. Good estimates of mating system parameters are essential for breeding programmes and for the selection of varieties/cultivars in orchards.

Research was carried out to evaluate how the mating system of walnut behaves in temperate zones. As a case study, we selected an isolated population of 118 trees in central Italy (Baschi, Terni). All the plants were analysed using molecular markers in order to identify the alleles circulating in the pollen cloud.

In order to test the effect of different weather conditions on the mating system, a subsample of 50 trees was randomly selected; 30 seeds were collected from around the crowns of each tree for three consecutive years. The seeds were planted in the nursery; germination rate was more than 80% every year. Molecular analyses of the progeny plantlets were repeated every year.

Statistical methods were used to assess the genetic variability of the whole population—the mother trees and the 'triennial progenies'. The fixation index of mother trees was negative, demonstrating an excess of heterozygotes, which is typical of forest trees. Because the fixation index of the progenies was also negative (Table 2) it could be supposed that the reproductive biology of this population did not give rise to inbreeding and that natural selection pressure favours heterozygotes during post-embryonic development.

Table 2. Mating system parameters for walnut trees in Italy

Genetic variability parameters of mother trees		Genetic variability parameters of the progenies								
		1° Year			2° Year			3° Year		
mean He	mean F	Ho	He	F	Ho	He	F	Ho	He	F
0.340	–0.184	0.412	0.464	–0.113	0.374	0.378	–0.032	0.364	0.373	–0.032

He (expected heterozytosity)
Ho (observed heterozygosity)
F (Fixation index)

Ritland's multilocus model (Ritland and Jain 1981) was used to test the crossing rate (tm) of the population (Table 3). The high tm value observed for three years indicated complete outcrossing, and the constant reproductive behaviour showed no significant climatic variation over this period. This result demonstrates that at temperate latitudes, during the exchange of alleles among trees, the blooming habit promotes outcrossing, thereby avoiding inbreeding. This conclusion could be useful in practical applications; a plantation for seed production, made up of selected walnut trees and located in a temperate area, could produce progeny that are derived totally from outcrosses of the gene pool.

Table 3. Estimation of outcrossing rates in walnut trees in Italy (multilocus model of Ritland and Jain [1981])

	1° Year	2° Year	3° Year
Number of individuals	368	849	836
tm (multilocus autcrossing rate)	1.033 (0.036)	1.037 (0.027)	1.018 (0.029)
ts (minimum average variance)	1.103 (0.041)	1.065 (0.042)	1.061 (0.042)

Preliminary construction of an integrated genetic map

In the last decade, molecular markers have made it easier to construct genetic maps for many plant species, including forest trees. Such maps allow a new and more effective approach to genetic analysis and the breeding of quantitative traits through their division into Mendelian components (quantitative trait loci, QTL). In outbreeding forest trees, which are highly heterozygous, dominant markers such as RAPD are widely used for constructing genetic maps through analysis of the plants' recombination in the progenies of parents in pseudo-testcross configurations (Grattapaglia and Sederoff 1994).

In order to identify QTLs for wood quality in walnut the authors selected and characterized molecular markers for evaluating genetic polymorphism among walnut trees, analysed the inheritance of these markers in the intraspecific cross progeny, constructed an integrated genetic map, and used the linkage map to identify the genes that control wood quality in walnut. Because of the lack of controlled interspecific progenies, we utilized an intraspecific controlled cross J. regia × J. regia (82 plants) and the reciprocal cross (40 individuals). The material was analysed by ISSR, RAPD and isozyme markers. More than 500 random decamer RAPD primers, 100 ISSR and 12 enzymatic systems were screened. We then selected the markers with parents in pseudo-testcross configuration, and the linkage groups among these markers were identified. In order to pool co-dominant and dominant markers, isozyme data were coded as presence/absence patterns (heterozygous and homozygous individuals as 1 and 0, respectively). From the whole data set, markers were selected with parents in '1–0' configuration and segregating progeny. Three classes were then created: 01 (00 x 10) and 10 (10 x 00) in pseudo-testcross configuration, and 11 (10 x 10/01 x 01) in intercross configuration (Table 4). For each class, the Mendelian segregation (ratio 1:1 in pseudo-testcross; ratio 3:1 in pseudo-intercross for dominant markers; and ratio 1:2:1 for co-dominant markers) was tested by χ^2 test. Two statistical programs were used to elaborate the data: MAPMAKER version 3.3 (Lander et al. 1993) to obtain female and male maps, and JOINMAP version 3.1 (Stam et al. 1993) to identify the common linkage groups. Fjellstrom and Parfitt (1994) stated that "The size estimates…suggest that Walnut genetic map spans approximately 1660 cM, with each chromosome averaging 104 cM in length" and that "the Walnut map would need 138 markers to cover 95% of the genome within 20 cM of each marker".

Table 4. Selected markers in different configurations

	Configuration			
	Pseudo-test cross		Pseudo-inter cross	Total
Classes of the parental genotypes	01 (00 x 10)	10 (10 x 00)	11 (10 x 10)	
RAPD	63	72	84	219
Isozymes	2	2	0	4
ISSR	39	34	40	113
Total	104	108	124	336

Based on this evaluation, the authors calculated that their preliminary map corresponded to the following sizes (Chakravarti 1999): female map average size: 1373.45 cM, corresponding to 82.7% of the genomic size; male map average size: 712.1 cM, corresponding to 42.9% of the genomic size. Thus, the six common groups detected in this study cover 32.2% of the estimated genomic size.

Discussion and conclusions

The presence in a few loci of different allele pools in Asia and Europe and the lower differentiation level detected among European populations suggest that the species has been present in Europe for a long period and has survived in remote areas in Italy and the Balkans. The hypothesis of species extinction in Europe and subsequent recolonization from Asia depends on accepting that the 10 000 years since the last glacial period were sufficient to give rise to the differentiation found among European and Asiatic populations (Fornari et al. 1999). This is difficult to believe of such a long-lived tree species. The authors' results show that walnut could maintain relative differentiation at the intrapopulation level, which can be explained by its reproductive strategy based on outcross pollination. Nevertheless, the proportion of polymorphic loci ($P = 0.47$), the mean number of alleles per polymorphic locus ($Na = 2.27$) and the total differentiation index ($Ht = 0.214$) are lower than the mean values reported for other widespread plant species (Hamrick et al. 1991; Millar and Libby 1991). Moreover, the polymorphic and monomorphic loci are the same in all populations. These factors explain the reduced levels of genetic diversity at the intraspecific level in walnut, compared with other widespread species, and suggest that walnut should be considered an endangered species.

Walnut is subject to strong genetic erosion in Europe, whereas in western China variability among accessions seems still to exist. Considering this, appropriate gene conservation programmes are necessary, and could include the preservation of local varieties/ecotypes. Germplasm could also be used for genetic improvement programmes to obtain the best genotypes for fruit, wood and double aptitude plantations, while respecting environmental safety.

Integrated multidisciplinary research appears to be the most useful approach to the sustainable utilization of walnut. Such research should be developed through international cooperation among countries and should involve scientists, politicians, farmers and local organizations. This approach seems to be the more appropriate for establishing effective directives and making the public aware of the conservation strategies and sustainable exploitation of the species. Modern agriculture is part of a complex system constituted by economic activities and a thick net of relationships among various activities and sectors. These elements should all be merged in order to create security and well-being at the global level.

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Conservation and management of European chestnut (*Castanea sativa* L.) genetic resources: outputs of the CASCADE project

***F. Villani*[1] *and G. Eriksson*[2]**
[1]*CNR–Istituto di Biologia Agroambientale e Forestale, Porano, Italy*
[2]*Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden*

Focus on gene conservation

European chestnut (*Castanea sativa*) is grown extensively for its fruit and timber. It is important not only for rural diversification, local economies and cultures, but also for its contribution to the landscape and environment. Across Europe, its future is threatened by human activities, the ravages of fungal attacks such as *Phytophtora* spp., which causes ink disease, and likely global warming. For these reasons it has been proposed as a model species for the development of long-term strategies aimed at combining the conservation of evolutionary and adaptive potential, the exploitation of breeding stocks and the preservation of Mediterranean landscapes.

CASCADE (EVK2-CT-1999-00006) was a European Union (EU) project funded within the Fifth Framework Programme (Key Action: Global Change, Climate and Biodiversity) and running for four years, from 2000 to 2004. CASCADE was a multidisciplinary project implemented by scientists from 11 European research institutions and five European countries (for details see Table 1 and the CASCADE Web site, http://soi.cnr.it/~chestnut). It involved such fields of research as ecology, ecophysiology, pathology, molecular genetics, population genetics, quantitative genetics, gene conservation, tree breeding, environmental economics and forest management.

The general objective of the CASCADE project was to develop a strategy for long-term gene conservation and management of European chestnut. Within this context, it set out to:

- identify the environmental conditions that limit the growth of sweet chestnut
- estimate the genetic variability of orchard, coppice and naturalized populations and the exchange of genetic material among these three types of population
- delineate zones for breeding sweet chestnut
- identify the socio-economic impact of commodities from chestnut forests and orchards.

For genetic studies, 82 populations were selected in France, Greece, Italy, Spain and the United Kingdom, representing different levels of domestication, i.e., major management regimes (naturalized stands, coppices, fruit orchards), and covering a broad range of xerothermic indices. Such indices were based on the assumption that drought and temperature during the growing period are of importance for the growth of chestnut trees. The mean temperature (TM) for the period June–September was included in each index, together with total precipitation (P) during the period. The xerothermic index, Xi, is:

$Xi = 2TM - P$

If $P > 2TM$, the xerothermic index is zero.

Scientific achievements

Variation within and among populations

A detailed description was obtained of the ambient conditions and management characteristics at the 82 localities, at both site and tree levels. This showed that chestnut is adapted to a broad range of climatic conditions but requires more specific edaphic conditions. The three main domestication levels—orchard, coppice and natural/naturalized—could be distinguished, although transition zones occurred within the naturalized group.

The European stands studied retain a fairly high level of genetic variation. Some general trends have been detected at the European level; the effective number of alleles is fairly high in southern

Table 1. Institutions and scientists participating in the CASCADE project

Institution/organization	Street name and number	Post code	Town/city	Family name	First name	Telephone number	Fax number	E-mail
CNR– Istituto per la Biologia Agroambientale e Forestale	Viale Marconi, 2	I-05010	Porano (TR)	Villani	Fiorella	+39 0763 374674	+39 0763 374330	f. ias.tr.cnr.it
Università degli Studi della Tuscia, Dipartimento di Protezione delle Piante	Via San Camillo de Lellis	I-01100	Viterbo	Vannini	Andrea	+39 0761 357449	+39 0761 357473	unitus.it
Swedish University of Agricultural Sciences, Department of Forest Genetics	Box 7027, Baeckloesavaegen 2A	S-75007	Uppsala	Eriksson	Gösta	+46 18 671240	+46 18 673279 or +46 18 672718	gosta. sgen.slu.se
Horticultural Research International, Department of Plant Breeding and Biotechnology	West Malling ME19 6BJ		East Malling, Kent	Russell	Karen	+44 1732 843833	+44 1732 521557 or +44 1732 849067	karen. hri.ac.uk
Università degli Studi di Torino, Dipartimento di Colture Arboree	Via Leonardo da Vinci, 44	I-10095	Grugliasco (TO)	Botta	Roberto	+39 011 6708800	+39 11 6708658	agraria.unito.it
University of Newcastle upon Tyne, Department of Agricultural Economics and Food Marketing – Agriculture Building	Kings Road, NE1 7RU		Newcastle upon Tyne	Garrod	Guy	+44 1912 227552	+44 1912 226720 or +44 1912 225219	guy. newcastle.ac.uk
CNRS Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UPR.9056	1919 Route de Mende	F-34293	Montpellier Cedex 5	Romane	Francois	+33 4 67613277	+33 4 67412138	cefe.cnrs-mop.fr
INRA Bordeaux	BP81 71, Avenue Edouard Bourleaux	F-33883	Villenave D'Ornon	Robin	Cécile	+33 557 122613	+33 557 122621	bordeaux.inra.fr
Centro de Investigaciones Forestales y Ambientales de Lourizàn, Xunta de Galicia	Carretera de Marìn, Km. 4, Apt. 127	E-36080	Pontevedra	Fernandez Lopez	Josefa	+34 986 856400	+34 986 856420	nia.es
Aristotele University of Thessaloniki – Department of Forestry and Natural Environment, Laboratory of Forest Genetics and Tree Breeding	PO Box 238	GR-54006	Thessaloniki	Aravanoupoulos	Phil	+302 310 992778	+302 310 992777 or +30 310 452081	for.auth.gr
NAGREF National Agricultural Research Foundation – Forest Research Institute		GR-57006	Vassilika Thessaloniki	Diamandis	Stephanos	+302 310 4611713	+302 310 461341	ri.org

European regions, and decreases towards the north and west. Analogously, allele frequencies are more heterogeneous at the local level in southern European regions, particularly Spain, southern Italy, southern Greece and western Turkey. On the other hand, stand genetic diversity seems to increase towards the north. Despite the great impact that human activities and the transfer of genetic material have had on chestnut genetic stocks, the same scenario seems to hold for chestnut as for other forest tree species in Europe. Three main gene pools were detected in the range considered: (a) the north-eastern Turkish pool, located in the region thought to be the likely centre of diffusion of the species; (b) a Greek gene pool, located in a region that had not previously been investigated; and (c) the Mediterranean Turkish gene pool, from where the European pools seems to derive. Populations from these regions should therefore be considered both as targets for the conservation of genetic resources and as sources of useful variation for breeding.

The dynamics of genetic diversity within stands were investigated through paternity analysis (to estimate the mating pattern within populations) and spatial analysis of genetic diversity patterns (to estimate intra-stand isolation by distance). This showed a level of incoming migrants per generation large enough to counterbalance the loss of diversity through inbreeding and drift. Mating probability dropped to less than 0.01% for distances of more than 2 km, increasing to about 10% for distances less than 300 m. This indicates that when there is some clustering of similar genotypes, a certain amount of inbreeding is expected. Indeed, a weak but significant spatial structure has been detected for naturalized stands and managed coppices, the correlation of genotypes being positive up to 35–45 m.

Genetic variability among and within populations representing a broad span of xerothermic indices was demonstrated for juvenile growth, phenology and carbon isotope discrimination. A strong and positive relationship between the phenotypic plasticity of carbon isotope discrimination and the xerothermic index was observed. Six field experiments were established, and provide good starting points for continued studies of growth, phenology and stem form. The development of a saturated map and the identification of quantitative trait loci (QTLs) were great achievements.

Significant variability in susceptibility to *Phytophthora cambivora* was observed between populations. This variability was lower between different domestication levels than within populations, suggesting that most variability was within populations rather than between them.

A European map of *P. cambivora* diffusion in chestnut areas and the distribution of associated *Phytophthora* species was provided. The effects of site (i.e., the complex of climatic factors) on *Phytophthora* distribution and potential diffusion were also shown. In particular, the risk of spread of the most harmful species, *P. cinnamomi,* was forecast on the basis of site factors such as minimum and maximum temperatures and soil pH.

Enquiries among more than 1000 households in the chestnut areas revealed that restoration of high forests was a top-ranked preference. Willingness to pay was also greatest for the restoration of high forests.

A network of gene resource populations

In forest tree gene conservation it is very important that the method used allows the continued adaptation of the species. Gene conservation must therefore be dynamic so that species can benefit from natural and artificial selection, thereby adapting to environmental conditions. The Multiple Population Breeding System (MPBS) allows plants to adapt to environmental change and permits breeders to respond swiftly to possible changes in the value of different characters. In MPBS, the gene resource population is split into approximately 20 subpopulations, which are usually selected in such a way as to cover the distribution range of the species. Each subpopulation should contain at least 50 trees that actively participate in the mating within the subpopulation. This should ensure that enough genetic variability is maintained in each of the subpopulations. MPBS thus fulfils the criteria for dynamic gene conservation by including the maximum existing variation among populations, while keeping enough variability within each subpopulation to allow for natural or artificial selection.

In this project, Gabriele Bucci of the Istituto di Genetica Vegetale, of the National Research Centre (CNR), Italy, developed the concepts of additive trait conservation value (ATCV), pathogen tolerance conservation value (PTCV) and marker trait conservation value (MTCV).

The ATCV was based on the *evolutionary potential*, which is the ability of a population to respond genetically to changed environmental conditions, or *population divergence*, which is based on how much a specific population differs from the other populations studied. Three adaptive traits were included in these estimates: carbon isotope discrimination, juvenile height and juvenile dry matter production. The Greek population from Paiko was the only one to show low evolutionary potential. The Spanish population from Coruna and the Greek population from Hortiatis showed the greatest population divergence, the former because of good growth and the latter owing to poor growth.

The PTCV was based on inoculation of the material with one strain of *Phytophtora cambivora;* it was calculated separately for the three domestication levels. The PTCV was calculated in such a way that a high PTCV value indicates good tolerance of *P. cambivora* and high evolutionary potential for the improvement of tolerance. The two coppice populations from Greece showed high PTCVs, as did the naturalized Greek population from Hortiatis. The French populations and the Spanish orchard populations had low PTCVs.

Three estimates were used to derive the MBCV: richness of genes, population divergence and effective population size. For the markers, it turned out that gene richness contributed most to the MBCV. In general, fruit orchard populations showed the lowest gene richness, as expected for grafted material. The southern Greek populations, including the one from Lesvos island, had a different genetic constitution from most of the other populations. This means that they have a special value in the network of gene resource populations. The gene richness of all the UK populations was notably high; these are all coppice forests.

The different conservation values cannot be fully analysed here because too few populations were studied for ATCVs and PTCVs. However, the calculations used in the CASCADE project can be applied in future detailed studies of sweet chestnut or any other tree species.

Based on the information available in early 2004, the authors suggest that there should be separate MPBS gene conservation programmes for wood production and nut production. The suggested network of gene conservation subpopulations from the area studied is illustrated in Figure 1.

Figure 1. Suggested network for gene conservation of chestnut populations for timber production in countries involved in the CASCADE project.

Annexes

Sixth EUFORGEN Noble Hardwood Network Meeting

Alter do Chão, Portugal (9 – 11 June 2002)

Saturday 8 June

Arrival of participants

Sunday 9 June

0900 Opening of the meeting
Welcome (Host country and Chair of the Network)
Introduction
Adoption of the agenda and nomination of rapporteurs
1030 Break
1100 Status and progress of gene conservation in each country
Discussion on the progress made – in working groups
1300 Lunch
1400 Introductory country reports
1630 Break
1700 A concept for the practical implementation of genetic conservation strategies and guidelines on Noble Hardwoods in Europe
Brief introduction of the concept
Link with the gene conservation strategies produced by the Network for different Noble Hardwoods species/ genera
Discussion (including conservation objectives, sampling strategies, setting of priorities, conservation incentives)
2000 Dinner

Monday 10 June

0900 Research updates
Overview of ongoing research projects and their relevant results
Discussion on research needs and priorities (including provenance research)
1030 Break
New joint project proposals
Sixth Framework Programme of the EU for research (development of a "network of excellence" on population and conservation genetics of European forest trees, possible linkages with the Noble Hardwoods Network)
1300 Lunch
Documentation, information and public awareness
EUFORGEN Database/ Information platform
Communication issues
Bibliography update
1630 Coffee break
Technical guidelines (Presentation of the first set of species modules, discussion of the outcomes, dissemination, adaptation, formulation of needs and further steps)
2000 Dinner

Tuesday 11 June

	Field trip (half day)
1530	Conclusions
	Adoption of the report
	Date and place of next meeting
2000	Farewell dinner

Wednesday 12 June

Departure of participants

Seventh EUFORGEN Noble Hardwoods Network meeting

Arezzo, Italy, 22-24 April 2004

Theme: Genetic conservation, tree breeding and utilization of noble hardwoods

21 April 2004

	Arrival of Participants

22 April 2004

0900	Opening of the meeting
	Welcome address (D. De Laurentis, Corpo Forestale, Italian EUFORGEN National Coordinator)
	Welcome by local organisers (Director/F. Ducci)
	Welcome by Chair (M. Rusanen)
	Adoption of the agenda and nomination of rapporteurs
0930	EUFORGEN update (J. Koskela)
0945	Introductory country report: Iceland (T. Eysteinsson)
1000	Country updates: discussion on the progress made in sub-groups
1030	Coffee/tea break
1100	Country updates: discussion continued and sub-groups presenting highlights
1300	Lunch
1400	Documentation, information and public awareness
	Photo-CD (B. De Cuyper)
	Network poster (M. Rusanen)
	Poster on Noble Hardwoods species (T. Myking)
	Other initiatives (if any)
1430	Meetings, projects and other initiatives
	Outputs of the CASCADE project (F. Villani)
	EVOLTREE proposal (Berthold Heinze)
15:00	Outcomes of 2nd International Elm Conferences (E. Collin)
1530	Coffee/tea break
1600	New EC Regulation on genetic resources (J. Koskela)
	Discussion

23 April 2004

0830	Technical Guidelines (M. Bozzano)
	Presentation of the published modules
0900	Common Action Plans for NH species (M. Rusanen)
	Acer platanoides as a pilot species
0930	EUFORGEN Phase III (J. Koskela)
	Vienna MCPFE outputs and development of a proposal for Phase III (2005-2009)
1000	Discussion on Common Action Plans, future activities, Phase III and the EC Regulations
1030	Coffee/tea break

1100 Further discussion on Common Action Plans, future activities, Phase III and the EC Regulations

1300 Lunch

1400 Seminar: Genetic conservation, tree breeding and utilization of Noble Hardwoods

Seed zones/provenience regions (F. Ducci, M. Vannuccini)

Clone selection for wild cherry and walnut (F. Ducci, R. Proietti, G. Signorini)

1530 Tea/coffee break

1600 Noble hardwoods and in vitro tissues culture (A. De Rogatis)

Noble hardwoods cultivation techniques (E. Buresti)

Walnut genetic variation in Europe (M.E. Malvolti)

Seminar wrap-up and recommendations

2000 Dinner

24 April 2004

Field trip (half day)

1500 Wrap-up session

Any other business

Date and place of next meeting

Adoption of the summary of the meeting

2000 Social dinner

25 April 2004

Departure of participants

Combined list of participants

Mr Ylli Hoxha (Sixth meeting only)
Instituti I Kerkimeve Pyjore e Kullotave
Forest and Pasture Research Institute
Rruga "Halit Bega", nr 23 - Kutia Postare 8367
Tirana
Albania
Tel: (355-4) 371237
Fax: (355-4) 371242
Email albaniaonline.net

Mr Ferdinand Müller (Sixth meeting only)*
Institute of Silvicolture
Federal Forestry Research Center
Hauptstrasse 7
1140 Wien
Austria
Tel: (43-1) 87838 2206
Fax: (43-1) 87838 2250
Email: Ferdinand. bva.bmlf.gv.at

Mr Berthold Heinze (Seventh meeting only)
Department of Genetics
Federal Research Centre for Forest, 5
Hauptstrasse 7
1140 Wien
Austria
Tel: (43-1) 878382219
Fax: (43-1) 878382250
Email: Berthold. bfw.gv.at

Mr Bart De Cuyper
Institute for Forestry and Game Management
Gaverstraat 4
9500 Geraardsbergen
Belgium
Tel: (32-54) 436177
Fax: (32-54)436160
Email: bart. lin.vlaanderen.be

Mr Alexander H. Alexandrov
Forest Research Institute
Kliment Ohridski Blvd. 132
1756 Sofia
Bulgaria
Tel: (359-2) 962052
Fax: (359-2) 9620447
Email : bulnet.bg

Mr Davorin Kajba
Dept. of Forest Genetics & Dendrology
Faculty of Forestry, University of Zagreb
Svetosimunska 25
10000 Zagreb
Croatia
Tel: (385-1) 235 2427
Fax: (385-1) 235 2505
Email : davorin. zg.htnet.hr

Mr Lubomir Salek
Forest Management Institute
Branch Office Kromeriz
nam. Miru 498
76701 Kromeriz
Czech Republic
Tel: (420-495) 860 111
Fax: (420-495) 262 391
Email : esycr.cz AND hotmail.com

Jan Svejgaard Jensen
Royal Veterinary and Agricultural University
Forest and Landscape
Hørsholm Kongevej 11
2970 Hørsholm
Denmark
Tel: (45) 35281503
Fax: (45) 35281517
Email: kvl.dk

Mr Ülo Tamm (Sixth meeting only)*
Forest Research Institute
Estonian Agricultural University
Kreutzwaldi 5
51014 Tartu
Estonia
Tel: (372-7) 313168
Fax: (372-7) 313153
Email: eau.ee

Mr Raul Pihu (Seventh meeting only)
Forest Research Institute
Estonian Agricultural University
Kreutzwaldi 5
51014 Tartu
Estonia
Tel: (372) 7313145
Fax: (372) 7313156
Email: eau.ee

* contact details at the time of the meeting

Ms Mari Rusanen
Forest Geneticist
Finnish Forest Research Institute
Vantaa Research Centre
P.O. Box 18
01301 Vantaa
Finland
Tel: (358-10) 211 2518
Fax: (358-10) 211 2205
Email: mari. metla.fi

Mr Eric Collin
CEMAGREF
U.R. Ecosystème Forestiers
Domaine des Barres
45290 Nogent-sur-Vernisson
France
Tel: (33)238950968
Fax: (33)238950346
Email: eric. cemagref.fr

Ms Brigitte Musch (Sixth meeting only)
Conservatoire Génétique des Arbres
Forestiers, ONF - Campus INRA
45160 Ardon
France
Tel: (33)238417816
Fax: (33)238414800
Email: brigitte. orleans.inra.fr

Mr Armin König
Institute for Forest Genetics and Forest Tree
Breeding, BFH
Sieker Landstrasse 2
22926 Grosshansdorf
Germany
Tel: (49-4102) 696107
Fax: (49-4102) 696200
Email: holz.uni-hamburg.de

Mr László Nagy
Forestry Research Institute, Experimental Station
Várkerület 30/a
9601 Sárvár
Hungary
Tel: (36-95)320070
Fax: (36-95)320252
Email: ertisarvar.hu

Mr Thröstur Eysteinsson (Seventh meeting only)
Iceland Forest Service
Midvangi 2-4
Box 98
700 Egilsstadir
Iceland
Tel: (354-471) 2100
Fax: (354-471) 2172
Email : skogur.is

Mr John Fennessy
Research Programme Manager
COFORD (National Council for Forest Research
and Development)
Arena House
Arena Road
Sandyford, Dublin 18
Ireland
Tel: (353-1) 2130725
Fax: (353-1) 2130611
Email: john. coford.ie

Mr Fulvio Ducci
CRA - Consiglio per la Ricerca e Sperimentazione
in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490
Email: ducci. icercaforestale.it and fulvio.
entecra.it

Mr Alfas Pliûra (Sixth meeting only)*
Dept. of Forest Genetics and Reforestation
Lithuanian Forest Research Institute
Liepu-1
53101 Girionys, Kaunas reg.
Lithuania
Tel: (370-37)547289
Fax: (370-37)547446
Email: mi.lt

Ms Myriam Heuertz (Sixth meeting only)
Centre de Recherche Public - Gabriel Lippmann
CREBS Research Unit
41, rue du Brill
4422 BELVAUX
Luxembourg
Tel: +352 47 02 61 458/459
Fax: 352-47-0261389
Email: ippmann.lu and
gmx.net

* contact details at the time of the meeting

Mr Sascha Wernicke (Seventh meeting only)
Administration des Eaux et Fôrets
16 Eugene Ruppert
2454 Luxembourg
Luxembourg
Tel: (352) 4022011
Fax: (352) 402201250
Email: sascha. ef.etat.lu

Mr Joseph Buhagiar
Argotti Herbarium (UOM section)
University of Malta
Triq Vincenzo Bugeja
VLT16 Floriana
Malta
Tel: (356-21) 243984
Fax: (356-21) 21248984
Email: Joseph. um.edu.mt and um.edu.mt

Mr Sven M.G. de Vries
Centre for Genetic Resources the Netherlands, Wageningen University and Research
c/o ALTERRA
P.O. Box 47
6700 AA Wageningen
The Netherlands
Tel: (31-317) 477841
Fax: (31-317) 424988
Email: sven. wur.nl

Mr Tor Myking
Norwegian Forest Research Institute
Fanaflaten 4
5244 Fana
Norway
Tel: (47) 5511 6229
Fax: (47) 5511 6245
Email : tor. skogforsk.no

Mr Leszek Bednorz
Department of Botany
August Cieszkowski Agricultural University
71c Wojska Polskiego Street
60-625 Poznan
Poland
Tel: (48-61) 8487699
Fax: (48-61) 8487145
Email : au.poznan.pl

Ms Maria Carolina Varela
Estação Florestal Nacional
INIA
Quinta do Marquês
2780-159 Oeiras
Portugal
Tel: (351) 21 4463700/4463783
Fax: (351) 21 4463702
Email : carolina. efn.com.pt and yahoo.com

Mr Lucian Dinca (Sixth meeting only)
ICAS Bucharest
Research Station Brasov
59 Fagetului Street
500040 Brasov
Romania
Tel: (40-268) 419936, (40-723) 327548
Fax: (40-268) 415338
Email: rdsbv.ro

Mr Saša Orlovic
Institute of Lowland Forestry and Environment
Agricultural Faculty Novi Sad
Antona Cehova 13 - P.O. Box 117
21000 Novi Sad
Serbia and Montenegro
Tel: (381-21) 540383
Fax: (381-21) 540385
Email : uns.ns.ac.yu

Mr Roman Longauer (Sixth meeting only)
Forest Research Institute
LVU
T.G. Masaryka 22
960 92 Zvolen
Slovakia
Tel: (421-45) 5314327
Fax: (421-45) 5321883
Email: roman. fris.sk

Ms Hojka Kraigher
Dept. of Forest Biology & Ecology
Slovenian Forestry Institute
Večna pot 2
1000 Ljubljana
Slovenia
Tel: (386-1) 2007820
Fax: (386-1) 2573589
Email: hojka. gozdis.si

Ms Josefa Fernández-López (Seventh meeting only)
Centro de Investigaciones Forestales e
Ambientais de Lourizan
Carretera de Marín km 4 - Apartado 127
36080 Pontevedra
Spain
Tel: (34-986) 805002
Fax: (34-986) 856420
Email: ffina. siam-cma.org

Mr Gösta Eriksson (Sixth meeting only)
Department of Plant Biology and Forest Genetics
Swedish University of Agric. Sciences
Box 7080
750 07 Uppsala
Sweden
Tel: (46-18)671240
Fax: (46-18)672718
Email: Gosta. vbsg.slu.se

Lennart Ackzell (Seventh meeting only)
Senior Advisor
The National Board for Forestry
DG-Secretariat
Vallgatan 8
551 83 Jönköping
Sweden
Tel: (46-36) 155706
Fax: (46-36) 166170
lennart. svo.se

Mr Peter Rotach
Chair of Silviculture
Swiss Federal Institute of Technology
Department of Environmental Sciences
ETH-Zentrum /Rämistrasse 101
8092 Zürich
Switzerland
Tel: (41-1)6323210
Fax: (41-1)6321033
Email: peter. env.ethz.ch

Mr Murat Alan
Forest Tree Seeds and Tree Breeding
Research Directorate
P.O. Box 11
06560 Gazi, Ankara
Turkey
Tel: (90-312) 2126519
Fax: (90-312) 2123960
Email : yahoo.com

Mr Roman T. Volosyanchuk (Sixth meeting only)
Laboratory of Forest Tree Breeding
Ukrainian Research Institute of Forestry and
Forest Melioration
Pushkinska, 86
61024 Kharkiv
Ukraine
Tel: (380-57) 7041002 direct line (380-57) 7078077
Fax: (380-57) 7041009
Email: hotmail.com AND
uriffm.org.ua

Ms Karen Russell
East Malling Research
New Road, East Malling
Kent ME19 6BJ
United Kingdom
Tel: (44) (0) 1732 8843833
Fax: (44) (0) 1732 521557
Email: karen. emr.ac.uk

Observers

Mr Gerry Douglas (Sixth meeting only)
Teagasc Kinsealy Research Centre
Kinsealy Biotech Unit
Malahide Road
17 Dublin
Ireland
Tel: (353-1) 8460644
Fax: (353-1) 8460524
Email : kinsealy.teagasc.ie

Mr Enrico Buresti Lattes (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490

Mr G. Corrado (Seventh meeting only)
Commissioner ISSA
Istituto Sperimentale per la Selvicoltura
Forest Research Institute
Viale S. Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39 0575 353490
Email: ats.it

Ms Serena Guerri (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490

Ms Christel Palmberg-Lerche (Seventh meeting only)
Via del Salvatore 12
00060 Mazzano (RM)
Italy
Tel: (39) 06 9049324 or 06 59603753
Fax: -
Email: christel_ hotmail.com AND christel. iscali.it

Ms Roberta Proietti (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490

Mr Alberto Santini (Seventh meeting only)
Istituto per le Protezione delle Piante - CNR
Via Madonna del Piano snc
50019 Sesto fiorentino (FI)
Italy
Tel: (39) 0555225586
Fax: : (39) 0555225666
Email: a. pp.cnr.it

Mr Pierre Sigaud (Seventh meeting only)
Forest Resources Development Service (FORM)
Forest Resources Division, Forestry Department, FAO
Viale delle Terme di Caracalla
00100 Roma
Italy
Tel: (39) 06 57055135
Fax: (39 06 57055137
Email: pierre. fao.org

Mr Augusto Tocci
Director ISSA
Istituto Sperimentale per la Selvicoltura
Forest Research Institue
Viale S. Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490
Email: ats.it

Mr Mario Vannuccini (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021
Fax: (39) 0575 353490

Ms Maria Emilia Malvolti (Seventh meeting only)
CNR-IBAF
Institute of Agro-Environmental and Forestry Biology
Viale Marconi, 2
05010 Porano (TR)
Italy
Tel: (39) 0763 374 911
Fax: (39) 0763 374 980

Ms Serena Ravagni (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021

Ms Anna De Rogatis (Seventh meeting only)
CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura
Istituto Sperimentale per la Selvicoltura
Viale Santa Margherita 80
52100 Arezzo
Italy
Tel: (39) 0575 353021

Ms Fiorella Villani (Seventh meeting only)
Istituto di Biologia Agroambientale e Forestale
C.N.R.
Viale Marconi, 2
05010 Porano (TR)
Italy
Tel: (39) 0763 374 911-916
Fax: (39) 0763 374 980
Email: f. cnr.it

Mr Gheorghe Postolache (Sixth meeting only)
Botanical Garden (Institute)
Academy of Sciences of Moldova
Str. Gh Asachi, 62/4, ap.6
2028 Chisinau
Moldova
Tel: (373-22) 720269
Fax: (373-22) 281828
Email: mail.ru AND ist.ru

Ms Janet Stewart (Sixth meeting only)
Oxford Forestry Institute
University of Oxford
South Parks Road
OX1 3RB Oxford
United Kingdom
Tel: (44) 1865 275126
Fax: (44) 1865 275074
Email: janet. plants.ox.ac.uk

EUFORGEN Secretariat/IPGRI

Mr Jarkko Koskela (Seventh meeting only)
EUFORGEN Coordinator
Via dei Tre Denari, 472/a
00057 Maccarese (Fiumicino)
Italy
Tel: (39) 06 6118223
Fax: (39) 06 61979661
Email: j. cgiar.org

Mr Michele Bozzano
Programme Specialist
Via dei Tre Denari, 472/a
00057 Maccarese (Fiumicino)
Italy
Tel: (39) 06 6118221
Fax: (39) 06 61979661
Email: m. cgiar.org

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Cover photo: M. Idzojtic/Norway maple (*Acer platanoides*)/EUFORGEN Noble Hardwoods Network

ISBN-13: 978-92-9043-682-9
ISBN-10: 92-9043-682-4