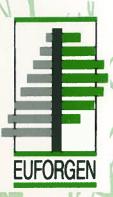


Report of the fifth meeting — 5-8 May 1999 — Kyiv, Ukraine

J. Turok, F. Lefèvre, S. de Vries, B. Heinze, R. Volosyanchuk *and* E. Lipman, *compilers*





IPGRI is an institute of the Consultative Group on International Agricultural Research (CGIAR)

Populus nigra Network

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ΕN (EUFORG Programme ഗ esource ſ enetic വ Forest European

The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of genetic diversity for the well-being of present and future generations. IPGRI's headquarters is based in Rome, Italy, with offices in another 15 countries worldwide. It operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme, and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).

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The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe. It was established to implement Resolution 2 of the Strasbourg Ministerial Conference on the Protection of Forests in Europe. EUFORGEN is financed by participating countries and is coordinated by IPGRI, in collaboration with the Forestry Department of FAO. It facilitates the dissemination of information and various collaborative initiatives. The Programme operates through networks in which forest geneticists and other forestry specialists work together to analyze needs, exchange experiences and develop conservation objectives and methods for selected species. The networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme. EUFORGEN is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries.

The geographical designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of IPGRI or the CGIAR concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. Similarly, the views expressed are those of the authors and do not necessarily reflect the views of these participating organizations.

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Contents

Summary of the meeting	1
Reports on the progress of activities on Populus nigra in countries	6
Austria, Berthold Heinze	6
Belgium, Jos Van Slycken and An Vanden Broeck	7
Croatia, Davorin Kajba	8
France, François Lefèvre	9
Germany, Rolf Schulzke	11
Hungary, Ernö Gabnai, István Bach and Sándor Bordács	12
Italy, Luisa Cagelli, Stefano Bisoffi and Lorenzo Vietto	12
The Netherlands, Sven M.G. de Vries	14
Poland, Jaroslaw Figaj	15
Portugal, Maria Carolina Varela	15
Slovakia, Ladislav Varga	16
Slovenia, Gregor Božič, Igor Smolej, Robert Brus and Hojka Kraigher	20
Spain, Nuria Alba and Carmen Maestro	24
Ukraine, Igor Patlaj, Valentyna Rudenko and Roman Volosyanchuk	25
Introductory country information on Populus alba	26
Austria, Berthold Heinze	26
· Belgium, Jos Van Slycken and An Vanden Broeck	27
France, François Lefevre	27
Hungary, Ernö Gabnai, István Bach and Sándor Bordács	27
Italy, Luisa Cagelli, Stefano Bisoffi and Lorenzo Vietto	28
Russian Federation, Ivan Popivshchy	28
Spain, Nuria Alba and Carmen Maestro	32
In situ conservation of Populus nigra in the riparian ecosystem	35
Introduction	35
Indicators for monitoring the evolution of <i>Populus nigra</i> genetic diversity <i>in situ</i> <i>François Lefevre and D. Kajba</i>	36
In situ restoration genetics of riparian populations of P. nigra	
Berthold Heinze and François Lefèvre	43
Case study: Inventory and characterization of <i>Populus nigra</i> resources within nature reserves in France	
Bernard Pont, Stéphane Pissavin and François Lefevre	51
Overview of <i>in situ</i> gene conservation activities on <i>Populus nigra</i> (status May 1999)	62
Succession in riparian forests of the Croatian Danube region Joso Vukelić, Davorin Kajba and Dario Baričevič	64
<i>Ex situ</i> conservation: update on the EUFORGEN core collection and the	
database of clones	
Luisa Cagelli, Stefano Bisoffi and Lorenzo Vietto	70

Discriminant analysis of leaf morphological characters of the European black poplar (<i>Populus nigra</i> L.) in natural populations in Croatia	
Davorin Kajba, Marilena Idojtić and Saša Bogdan	73
Bibliography – Addendum 1998–1999	77
Appendix I. Black poplar (<i>Populus nigra</i>) and the WWF European Freshwater Programme	85
Programme	86
List of participants	87

Summary of the meeting¹

Introduction

The meeting was attended by 16 participants (see List of participants at the end of this volume). R. Volosyanchuk welcomed the participants and wished them a successful and pleasant meeting. The agenda was introduced by F. Lefèvre, Chair of the Network. J. Turok welcomed the participants on behalf of IPGRI and presented the main outcomes of the second EUFORGEN Steering Committee meeting (held in Vienna, Austria, in November 1998) and other international developments relevant to the Network.

Reports on the progress of activities in countries

Introductory country reports from Portugal and Slovenia were presented. All other countries briefly reported on the progress made in the conservation of *Populus nigra* at the national level during the past 1.5 years. The reports also covered *P. alba*, where relevant.

During this period significant progress has been made in field inventories and collecting of material for *ex situ* conservation. Intensive research was carried out in a number of countries, particularly those involved in EUROPOP. Various meetings, presentations, leaflets and articles in journals contributed to raising public awareness in at least seven countries.

Synthesis of in situ conservation measures and activities

The overview table compiled in October 1997 was presented, with additional information received from Ukraine and Yugoslavia. It was agreed that the overview should be revised and/or completed by the participating countries, preferably during the meeting. Substantial progress had been achieved in countries regarding *in situ* conservation activities during the period since the last two meetings.

In situ conservation strategies

Three presentations were made:

- 1. Effects of ecosystem management on dynamic processes in *P. nigra* populations (I. Popivshchy and S. de Vries).
- 2. Restoration of riparian ecosystem (B. Heinze).
- 3. Indicators for monitoring the evolution of diversity in riparian ecosystem (F. Lefèvre and D. Kajba).

These three elements of an *in situ* conservation strategy of the Network were discussed and three overview tables produced during the meeting.

List of descriptors for inventories of stands

F. Lefèvre reported on the results of an inventory of *P. nigra* resources in existing nature reserves in France. This was the first inventory which used the list of stand descriptors previously proposed by the Network. Although the study revealed that *P. nigra* was only represented in a small number of reserves, the list was found useful for field surveys by reserve managers not specialized in forestry. However, multiple response should be allowed for several items, and quantification of the item describing regeneration should be introduced in the future.

Several modifications in the list of descriptors were agreed upon and supplementary information on climate types was presented. In particular, I. Popivshchy and

¹ This summary was adopted and distributed at the meeting.

R. Volosyanchuk provided additional feedback on the climatic classification used in their countries.

Joint research and research needs

During the last meeting it was agreed that progress made by the EUROPOP project be briefly reported at each Network meeting. An introduction to EUROPOP is given in the report of the fourth Network meeting. S. de Vries presented a summary of the progress made in EUROPOP. During the second meeting (held in Wageningen in January 1999), it was concluded that the project was on schedule:

- Results of the isoenzyme analysis of the EUFORGEN reference collection were discussed and the results achieved by the participating labs were very similar. AnFLP analysis could not be standardized. For this reason each participating laboratory will analyze one primer set for all samples.
- Cuttings from the selected trees in populations, genebanks and the reference collection were collected during the winter of 1998/99. These cuttings were multiplied during 1999 and the field trial was established with these cuttings in the spring of 2000. The trial contains 2×60 clones from populations, 100 clones from the genebank and 15 clones from the EUFORGEN reference collection (in total 235 clones).

CpDNA polymorphism in the EUFORGEN core collection was analyzed. It turned out that polymorphism in *P. nigra* is low. One of the universal primers has been adapted to detect more polymorphism. Six primer sets/restriction enzyme combinations were selected. The protocol available for oak has been adapted for black poplar.

Microsatellite-containing fragments were isolated and approximately 20 different fragments are available for sequencing. Four primers were selected from the homepage of Poplar Molecular Genetics Cooperative and these primers described for hybrid poplars were tested for *P. nigra*. As the results were satisfying, these primers will be used to start standardization for microsatellites.

All partners selected two populations which meet the selection criteria. Trees were selected and mapped. All partners took cuttings and samples from their respective genebanks.

An update of the EUROPOP homepage has been loaded at <http: //www.ibn.dlo.nl/europop>; and a discussion site on the Internet has been opened at <http://www.ibnresearch.nl/europop>.

It was agreed that after publication the characterization data of the core collection will be distributed to all those who contributed to the collection.

A proposal for a black poplar conservation project along the River Danube was introduced. It could be submitted to the Fifth Framework Programme involving central and east European countries.

While a number of urgent research topics which were identified previously by the Network (see Report of the Second Meeting) are currently covered within the framework of EUROPOP, the needs for further research were discussed and listed as follows:

- Study of local adaptation in *P. nigra* through a provenance experiment as opposed to the traditional research at the individual (clonal) level. This may be achieved through a set of common controls, i.e. the reference clones or the core collection.
- Recurrent selection programmes. Long-term breeding programmes should benefit from the genetic material collected and characterized.

- Forest ecology of riparian ecosystem and the impact of management practices on *P. nigra* genetic resources. It was decided to contact several specialists in this field. J. Turok will contact several persons as indicated by Network members and will inquire about their interest in presenting the ecology aspects to the Network at its next meeting.
- Factors influencing amount and risk of introgression and its impact.
- Minimum viable population size.

The last two items are directly related to forest practices and restoration of the riparian ecosystem.

Coordinating activities on Populus alba genetic resources in Europe

In view of the increasing involvement of the Network in the area of *in situ* conservation in the riparian ecosystems, participants of the previous meeting agreed to broaden the scope of the Network to include *P. alba*. This was endorsed by the Steering Committee Meeting in Vienna in November 1998. The main arguments for including *P. alba* into the Network are:

- ecology of the riparian ecosystem *P. nigra* and *P. alba* represent different stages in the development of this ecosystem;
- similar biological features of both species including interspecific hybridization.

While *P. nigra* remains the main priority species for the Network in the future, it was agreed that the Network will directly address the questions of *in situ* and *ex situ* conservation of *P. alba* and will only indirectly consider *P.* × *canescens* within the agenda for *in situ* management of the riparian ecosystems which include *P. nigra* and *P. alba*.

Every country briefly described the situation of *P. alba* genetic resources and expressed its needs for coordinated action at the level of the Network. The importance of *P. alba* varies significantly among European countries. While Belgium and The Netherlands are not concerned with the genetic resources of the species, *P. alba* is important for forestry practice in countries along the Danube (Austria, Hungary, Slovakia) and in Spain. The genetic resources of *P. alba* are not threatened but its use in breeding programmes and for reforestation require the attention of the Network. It was agreed that the following steps be taken before the next Network meeting:

- 1. A database of *P. alba* clones will be compiled following the same structure as that for *P. nigra*. Countries with existing collections will provide data to N. Alba, who offered to take responsibility for establishing and maintaining this database. The structure of the database with first data included will be reviewed at the next Network meeting and will then be uploaded on the Internet, along with the *P. nigra* database (see below).
- 2. Technical recommendations for the propagation and conservation of *P. alba* will be developed on the basis of a literature survey of the techniques used. Morphological descriptors for nursery evaluation and taxonomic characterization will be developed, aiming at the discrimination of *P. alba* subspecies as well as *P. × canescens* hybrids. R. Schulzke offered to develop both draft documents. They will be circulated to all Network members one month before the next meeting.

Following the adoption of these two outputs by the Network, exchange of material and establishment of a core collection will be discussed at the next meeting. E. Gabnai and L. Varga will prepare guidelines for safe movement of material to be discussed at the next Network meeting (to be circulated one month before the next meeting).

Presentation of Ukraine's national programme on forest genetic resources

R. Volosyanchuk presented the national programme on forest genetic resources which has been implemented since the 1960s. It consists of four main elements: gene reserves, *ex situ* conservation measures, tree breeding, and introduction of new species and provenances. A number of constraints are being faced in the present socioeconomic situation. The main opportunities for collaboration with partners in Europe include: further exchange of information, databases, common conservation strategies, and transfer of modern molecular genetic technologies.

A short field trip was organized to natural riparian black poplar stands on the River Dnipro. The central botanical gardens in Kyiv were also visited which host a collection of specimens from the family Salicaceae.

EUFORGEN core collection

J. Turok on behalf of S. Bisoffi presented an update on the core collection including a list of clones sent from Casale Monferrato to The Netherlands, Belgium and France during the past 1.5 years. It was agreed that the most relevant information was related to the institutions to which requests for material need to be sent.

A total of 17 countries have so far each sent two clones to the collection. One country sent only a single clone. It was requested that all Network members either send their two clones or complete their number to two. J. Turok will invite persons in countries not participating in the Network to join the core collection. Passport data according to the Network's list must accompany all clones.

It was furthermore agreed that all Network members hosting the core collection or part of it send their updated information to S. Bisoffi. This information will be published in the Report of the meeting. F. Lefèvre proposed Network members who take part in the EUROPOP project to plant cuttings of the core collection together with the cuttings they need to plant in relation to Task 1 of EUROPOP, in order to be able to compare morphological and phenological characters of all clones. It was also suggested to Network members that they plant some cuttings of the core collection on a location where the trees can grow old (e.g. arboreta, botanical gardens, forestry school yards), in order to have mature trees from a large portion of the distribution range.

European database of clones

J. Turok presented the European database of clones which was updated and sent by S. Bisoffi. The database contained 2257 entries as of May 1999. Passport data for the clones in the core collection have been included, but some relevant information is still missing for individual clones. The database has been available on the Internet since 1997 as a downloadable Excel file. J. Turok offered the possibility to change this into a searchable Access database to be accessible from the Internet before 31 May 1999.

It was agreed that the information be regularly updated. Latest updates were requested to be sent to S. Bisoffi by 15 May 1999 (as Excel files according to the previous instructions). From then on, all updates will be sent annually to S. Bisoffi by 15 June. Further instructions on how to send the updates will be provided by S. Bisoffi and J. Turok. It was decided that a complete version of the respective national database be sent every time an update is given (instead of sending individual changes).

Public awareness

Several countries sent slides for the collection, among which 134 were chosen to best represent the items identified previously and the countries that contributed. This set of slides was scanned and electronically stored on a Photo-CD which was briefly demonstrated during the session. Missing items will be treated in the same way. These are the following:

- use of young shoots for animal fodder;
- pollen;
- vines growing on the support of poplars;
- species-mixed riparian ecosystems and forest associations (particularly in dry Mediterranean oak forests);
- biodiversity in black poplar stands (e.g. butterflies that only feed on *P. nigra*);
- particular landscapes (e.g. North Africa);
- drawings from the Identification Sheet;
- restoration planting techniques; and
- poplars in the art.

Individual participants offered to supply slides on these items. New slides from Croatia and Hungary and photographs from Slovakia were shown.

The whole 'basic' set of slides on CD will be sent to all Network members by S. de Vries for use in public awareness activities. There is no copyright on the set of slides; the EUFORGEN *Populus nigra* Network should be cited as source. J. Turok will undertake a conversion of pictures and text into an interactive CD-ROM format with public awareness professionals. A draft proposal will be presented at the next Network meeting.

The WWF Danube–Carpathian Programme has addressed the Network with an outline for a European Freshwater Public Awareness Campaign (see Appendix I). Black poplar was identified as a 'flagship' in the campaign for freshwater habitats, but the conservation of the species itself should also be promoted. B. Heinze, F. Lefèvre, S. de Vries and J. Turok will keep up this contact and will investigate the possibilities for collaboration.

Bibliography

A preliminary addendum to the list of references already published in previous reports was distributed by F. Lefèvre. Other references were provided by R. Schulzke, B. Heinze, G. Božič and other participants.

The whole list of references will be compiled into a single file and provided to J. Turok for inclusion on the EUFORGEN Web site.

The list of references will be progressively turned into a searchable database, following the structure established for the Norway Spruce Bibliography, and made available on the Internet.

Overview of a study on leaf morphology

D. Kajba presented an overview on leaf morphology in *P. nigra*.

Conclusions

Election of Chair and Vice-Chair: F. Lefèvre who acted as Chair of the Network since its establishment in October 1994 was thanked by Network members for his leadership in the development of the Network. A number of practical outputs were provided and considerable impact achieved in countries by the *Populus nigra* Network during this period.

The participants elected S. de Vries as Chair and D. Kajba as Vice-Chair of the Network for the period until the Seventh Network meeting.

Next Network meeting: it was proposed that the next meeting be held in Avignon, France, in conjunction with the planned EUROPOP project meeting. The Sixth Network meeting will be held from 3 to 6 February 2000. The last day of the meeting (6 February) will consist of a joint session with the EUROPOP participants and a field trip.

The report of the meeting was adopted.

Reports on the progress of activities on *Populus nigra* in countries

Austria

Berthold Heinze Institute of Forest Genetics, Federal Forest Research Centre, Vienna, Austria

Research

The two-year project on genetic research at our Federal Forest Research Centre (FBVA) came to an end in early 1998. Two reports have been published (Heinze 1998a,b). The Austrian Research Centre at Seibersdorf is a partner in EUROPOP and its activities are well underway. The National Park of the Danube Floodplains is starting up a research programme in which *P. nigra* will receive some emphasis.

An output of the FBVA project is the Austrian core clone collection, consisting of about 180 clones, in one of our nurseries. It was established in early 1998 and most of the clones are already producing cuttings.

Public awareness

Several articles in Austrian journals with a readership consisting mainly of conservationists yielded much higher response than previous ones in forestry journals. In this way, *P. nigra* is becoming more integrated in public relations activities of conservation groups. The Austrian branch of the World Wide Fund for Nature (WWF) is currently campaigning for rare forest trees, among which the black poplar is included as a 'flagship' species (see Appendix I).

Implementation of conservation

Management plans for the Danube Floodplains National Park east of Vienna have been designed. Black poplar conservation features high in the agenda. Hybrid poplars will be removed, female clones first. Thinning of such stands is not recommended, as the remaining hybrids might flower more intensively after thinning. Hybrid stands may also be left for breakdown, for example, because of heavy infestation with mistletoe. One problem is that hybrid poplar crowns are preferred nesting sites for several bird species; therefore, at some sites, the hybrids have to be preserved until suitable black poplar crowns have developed. All this will be accompanied by planting of native tree species, including of course *P. nigra*. Under 'general rules', the management plan suggests using soil wounding to encourage the natural regeneration of silver and black poplars. The initial planning has set a timescale of 5 and 30 years, respectively, for management measures to be taken in core zones and surroundings. This may prove too short to establish viable black poplar stands where they should be, and may need revision. Nevertheless, planting autochthonous species from local sources of plant material is encouraged.

Closer to the centre of Vienna, the new hydroelectric power station has commenced operation. Landscaping the new riverside, ample space was left for natural regeneration on gravel, with trees planted only at very wide spacing – conditions readily accepted by spontaneous poplar and willow seedlings now growing in dense strips. It will be interesting to observe these further; although there are small water bypasses around the dam, it remains to be seen whether this effectively mimics natural flooding, and if those responsible will really leave such uncontrolled vegetation development untouched.

Several local groups have taken on the conservation of black poplar trees at certain river margin sites (for example, initiatives in Salzburg and Upper Austria). Although some of these sites are rather small and contain only few trees, they still contribute to genepool conservation locally. At the Salzburg site (River Salzach), river restoration measures are discussed, including measures to allow more frequent flooding.

The Austrian clone collection has already been used to provide cuttings for people interested in planting a few poplars on their land. It is hoped that once the collection is fully productive, more 'customers' will turn up.

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Belgium

Jos Van Slycken and An Vanden Broeck

Institute for Forestry and Game Management, Geraardsbergen, Belgium

Research activities

Most research activities were undertaken in the context of EUROPOP, in which the Research Institute for Forestry and Game Management participates.

Isoenzyme analysis of the ex situ collection

All individuals of the *ex situ* collection were analyzed for eight different enzyme systems. Four of them (6PGD, LAP, PGI and PGM) were useful to determine the purity of the species. A total of 180 individuals were analyzed and 21 of them with a deltoides allele could be discarded. The enzyme systems MDH, IDH, 6PGD and SKDH are useful for the study of the genetic diversity within the species. Data still have to be completed and analyzed.

Isoenzyme analysis of the Hungarian ex situ collection

In the context of a cooperation programme between Flanders and Hungary, 40 individuals of the Hungarian core collection were analyzed with ten enzyme systems.

DNA analysis of the ex situ collection

Leaf samples were taken and DNA analysis (AFLP, microsatellites, cp-DNA) is taking place at the Laboratory of Genetics of the University of Ghent.

Flowering phenology

In order to assess the risk of introgression of hybrid poplars and *P. nigra* 'Italica', flowering was monitored in a genebank during spring 1999. Conclusions so far are that *P. nigra* 'Italica' flowers before the other black poplars, so no danger for introgression occurs. Also the *P.* × *euramericana* clones (22 clones) generally flower before the native *P. nigra* (51 clones). However as they showed a considerable amount of variation, introgression is not to be excluded.

Study of the aromatic compounds of bud exudate of P. nigra

The phenolic compounds of buds of *P. nigra* and *P. × euramericana* were studied at the Laboratory of Biotechnology of the Institut Supérieur Industriel at Huy, using gas chromatography/mass spectrometry (GC–MS). This study revealed that GC–MS is a reliable method for identification of species and clones and could be used for genetic diversity studies.

Practical conservation activities and field inventories

In situ conservation

No special activities were undertaken for *in situ* conservation except that owners, mostly farmers, were informed about the rarity of black poplar in Belgium and about the current conservation programme.

Ex situ conservation activities

New prospection and updating of the national database – During the last year further prospection of relicts of the species was undertaken. The activities resulted in 19 new accessions on 11 different locations in 1998. Thirteen individuals were found in the western part of Belgium in the Ijzer valley (five locations). Six other individuals were found in the valley of the River Dender (six locations). This recent prospection brings the *ex situ* collection for Belgium to a total of 159 trees on about 50 different locations. We expect that this collection contains a certain number of genetically identical individuals due to vegetative multiplication by farmers, which was and still is a common method of propagating poplars. Efforts were made to complete the database, especially regarding the sex of the trees.

Exchange of genetic material – Cuttings of *P. nigra* clones were received from The Netherlands, originating from common river systems (Maas and Schelde).

Public awareness

A workshop on the conservation and use of native forest genetic resources was held in February 1998 in Flanders in cooperation with the Forest Administration. Topics were international context, legislation, importance for forestry and nature conservation, results of inventories, and a strategy for the future.

The Institute for Forestry and Game Management started a newsletter, focusing on the broad public. Conservation of forest genetic resources, especially the activities developed for the conservation of genetic resources of *P. nigra* have been stressed.

Special efforts have been made to involve private as well as public land managers in the forest genetic resources conservation programme.

Croatia

Davorin Kajba

Faculty of Forestry, University of Zagreb, Zagreb, Croatia

Ex situ conservation of the European black poplar genetic resources started with the selection and autovegetative propagation of old trees. Selection has been carried out over the past five years in the regions along the Sava, Drava and Mura rivers. The selection planned this winter in the region of eastern Croatia along the Drava and Danube river basins was impossible because of the high water level after flooding. Valuable riparian forests with the most beautiful and oldest trees of European black poplar exist in Croatia. In the framework of the programme, selection and vegetative propagation of trees from Bosnia and Herzegovina

were also carried out. In total 54 trees from Bosnia and Herzegovina were selected and propagated with a somewhat poorer rooting success; they are still in reproduction and will be included in clonal archives later.

The poplar and arborescent willow clonal archive was established in 1995 by the River Mura (Podturen) in the northern part of Croatia, on 3 ha. Part of this is a black poplar clonal archive with 83 clones of European black poplar. The surface of this clonal archive is limited; therefore in 1998 we established another European black poplar clonal archive in the region of Baranja in Darda (eastern Croatia). This clonal archive was completed during the spring of 1999 with new clones and it now contains 63 clones of black poplar. The nursery reproduction currently contains 37 clones of black poplar from Croatia and Bosnia and Herzegovina.

Under the Nature Conservation Act of the Republic of Croatia the floodplain forest ecosystems are protected and of particular importance for *in situ* conservation of black poplar, such as the Natural Park of Kopački rit. This Natural Park covering 17 770 ha is located in Baranja, in an area bounded by the Rivers Danube and Drava. The occasionally flooded terrain favours the development of diverse vegetation, like stands of black and white poplar with other willows. Due to its outstanding importance and well-preserved wetland biotops, the park was included in the list of Wetlands of International Importance (Ramsar Convention) in 1993. The most valuable riparian forests are in the Danube region (in the outlet of the River Drava in the Danube and Kopački rit, and along the Danube riverbanks from Erdut to Ilok).

According to the Nature Protection Act of the Republic of Croatia, parts of nature could be protected as special reserves of forest vegetation. In the Vukovar Danube area 115 ha are covered with very valuable stands of poplars and willows.

In the lowland forest in the central stream of the River Drava (Slatina), the natural mixed stands of European black poplar, white poplar and white willow are protected and excluded from regular forestry management on a total of 620 ha. A petition has been addressed for the protection of 250 ha of riparian softwood stands such as a special reserve of forest vegetation by the River Drava in the eastern part of Croatia (Osijek).

Croatia has well-preserved floodplain forest ecosystems, owing to a sustainable management method and continuous legal protection, which present significant biological and ecological values of national and international importance.

France

François Lefèvre Unité de Recherches Forestières Mediterranéennes, INRA, Avignon, France

Research

Most of the research activities in France were related to the FAIR-EUROPOP project. Protocols for isoenzyme analysis have been standardized, and 100 clones from the national collection were characterized. Populations to be studied were selected along the River Drome.

In the frame of the poplar breeding programme, 18 intraspecific progenies were obtained by controlled crossing, and a progeny field trial was established in the nursery. Concerning pathological aspects, a regular survey of the *Melampsora* rust populations in the riparian sites is conducted by INRA Nancy. Related to the riparian ecosystem, new research activities concern the effect of hydraulic perturbations on black poplar mycorrhizae (CNRS Toulouse).

Practical conservation

Ex situ activities

Collecting was carried out in the regions that were not sufficiently represented in the collection (North-East, West, South-West) and 124 new clones are currently being multiplied (not included in the current database until they are successfully introduced into the stoolbed). The key point was to obtain information about scattered individual trees, and much effort was put into public awareness.

Moreover, 29 clones from the EUFORGEN core collection were received from the Poplar Research Institute (ISP, Casale Monferrato, Italy) and from the Institute for Forestry and Nature Research (IBN-DLO, Wageningen, The Netherlands).

In situ activities

In order to start operational *in situ* conservation, it was first noticed that no inventory of *P. nigra* stands was available, and that riparian forests in general are rarely managed by National Forest Services. Therefore the *in situ* methods already operational in our country for beech and fir, for example, were not applicable. We also noticed that Nature Reserves could include some interesting *P. nigra* resources. It was then decided to make an exhaustive inventory of the *P. nigra* resources included in the Nature Reserves all over the territory. The list of stand descriptors prepared by the Network was distributed to 250 Nature Reserves (see Pont *et al.*, this volume). From this inventory, and on a voluntary basis, a first set of protected areas will be selected to start the *in situ* network. Reserve managers will actively contribute to the definition of management rules, and close relationship is expected with the EUFORGEN Network and the EUROPOP research project.

Field inventories

This survey was the first inventory of *P. nigra* stands at the national scale. We can also consider that the numerous contacts used for collecting new clones provide a certain kind of inventory of *P. nigra* individual trees. Both confirmed the rarity of the species in the western and northern parts of the territory.

Public awareness

In order to enhance contacts and to inform people on both *ex situ* and *in situ* activities, an illustrated leaflet presenting the species and the objectives of conservation was produced. The identification sheet and the list of descriptors issued by the EUFORGEN Network were also effectively distributed. A conference was held in Nantes, in a region traditionally devoted to intensive poplar cultivation and where *P. nigra* has almost disappeared, for a very broad audience ranging from representatives of the Forest Administration and poplar growers to botanists, ecologists and just 'curious people', most of them hearing for the first time about the species.

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Germany

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Activities have been carried out by the state organizations involved in the conservation of forest genetic resources and by the Federal Ministry of Food, Agriculture and Forestry and its subsidiary institutions. The objectives of the *Populus nigra* Network received strong support as there is common awareness about the situation of this endangered species and its ecological importance, as a characteristic species for a very sensitive and threatened forest ecosystem. The current situation is reported below.

Research activities

Two German institutions participate in the EUROPOP project. The project started in 1998. Initial experience shows that there is good cooperation within the project and between EUFORGEN and EUROPOP.

In situ conservation

The field inventories carried out so far brought the number of trees that could be classified as pure *P. nigra* up to 3165.

A first identification had been done in the field by morphological characteristics and was later confirmed using isoenzyme and DNA methods.

Many of those trees were very old and showed poor vitality. Although the riparian forests are under special protection by environmental and nature conservation regulations, it is clear that additional measures are needed to preserve the genetic resources of *P. nigra*. In particular it is necessary to develop a programme for the re-establishment of black poplar stands with autochthonous material from nurseries.

Ex situ conservation

As a consequence of this situation, efforts have been undertaken to collect material from adult trees and to propagate it for preservation and regeneration purposes. The methods used include grafting, cuttings and *in vitro* culture according to the physiological status of the material available, which is often very poor, so that propagation is rather difficult. The following are now established:

- one seed orchard consisting of 40 clones; and
- clonal archives in five locations with a total number of 330 clones.

Public awareness

A symposium on problems and possibilities of black poplar preservation was held in May 1998 in Hann. Münden, bringing together about 100 experts from forestry, nature conservation, water management and engineering, forest genetics and other related

institutions. Proceedings of this symposium have been published and are available (Weisgerber and Janssen 1998; articles published in these Proceedings are listed in the Bibliography at the end of this volume).

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Hungary

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Latest results of *Populus nigra* gene conservation programme

The survey of registered *P. nigra* occurrences by the national inventory of forests (4390 ha) has been proceeding for the past two years. Black poplars are found mainly in the forested areas as an admixed tree, so the investigation of species identity and purity will take a long time. An accurate morphological description is needed in this respect. In mixed forests *in situ* conservation of black poplar is possible where the extent of the population is greater than 0.5 ha, and if the owner is interested in its conservation.

In the past two years, 162 *P. nigra* plus trees were designated. These trees were given protection status with the agreement of the owner; it is, however, also important to produce propagation material from these trees, because in some cases the plus trees were destroyed by events such as flood, storm or fire.

A total of 135 clones were taken for *ex situ* conservation. This conservation method is realized in two ways: stool-bed and tree-shaped collection.

Reforestation with *P. nigra* was started in the South Danube region's strictly protected area on 1.5 ha.

The National Institute for Agricultural Quality Control has submitted an application to the National Environment Protection Fund for a gene conservation programme for native trees, which includes *P. nigra*.

Research activity will take place at the DNA laboratory of the National Institute for Agricultural Quality Control. This laboratory also carries out control tests of *P. nigra* seedlings (sampling method). These tests are in conformity with the basis of the new law on reproductive materials.

The plan of the first *P. nigra* seed orchard is ready. It is 3 ha in size, and will be established in two or three steps. The first set of trees (1 ha) was planted in the spring of 1999.

Italy

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In 1998 the activity of ISP included the maintenance of the *P. nigra* collections, as well as the updating of the *P. nigra* database on behalf of the Network (see this volume).

Research activities

About 70 clones of *P. nigra* selected in a breeding programme of *P.* × *euramericana* within the *P. nigra* females progenies were tested in nurseries established in two localities (northern and central Italy) in 1998. The tolerance behaviour towards *Melampsora larici-populina* and *Melampsora allii-populina* was rated in September 1998; most of the individuals showed a good level of tolerance to both rust species.

To evaluate the behaviour towards *Melampsora* spp., three families of *P. nigra* (about 300 genotypes for each family) were obtained by breeding parents highly resistant and highly susceptible to rusts. These were planted in nurseries in 1999. Other progenies were obtained by breeding parents characterized by different behaviours to *Marssonina* spp. and *Phloeomyzus passerinii*. These were propagated in stool-beds and will be evaluated during the year 2000.

Ex situ collections: genebanks as stool-beds

ISP collection

In 1999 most of the clones included in the ISP collection (525 genotypes of Italian origin) were propagated in a stool-bed at the Mezzi farm in Casale Monferrato; about 30 cuttings of each clone were planted. Because of the poor conditions of the material growing in the clonal bank and in the stool-bed established in 1997, it was not possible to propagate all the 600 clones. The clones which were not propagated will be collected from adult trees in the year 2000.

Other collections

- A collection of *P. nigra* genotypes is maintained at the University of Tuscia (Viterbo). Seeds were collected from 13 different stands in northern Italy in 1993. To date over 500 genotypes from 51 families are in the collection; morphological traits, phenology and resistance to *Melampsora* spp. have been considered in order to evaluate variability.
- ISP hosts the EUFORGEN core collection.

Environmental restoration

In order to produce plants for environmental restoration, a nursery with 25 *P. nigra* genotypes of Italian origin was established in Casale Monferrato in 1998 and 1999. These genotypes were chosen among the clones selected from nursery test in 1994.

Public awareness activities

An article about the *P. nigra* conservation programme was published in September 1998 in an Italian journal dealing with forestry issues (Cagelli *et al.* 1998).

A meeting on forest genetic resources in Italy, organized by the Italian Society of Forestry and Ecology, was held in Rome in December 1998. The conservation of the Italian forest genetic resources and methods for the analysis of the variability were discussed. On that occasion the situation of *P. nigra* in Italy and the EUFORGEN Network activities were illustrated.

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The Netherlands

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Research

Several research projects were initiated at national level during the past two years, since the last meeting of the Network.

At international level, EUROPOP was initiated in the beginning of 1998. This project is coordinated by The Netherlands; two different Dutch research institutions take part in the research. Funding is provided at 50% by the European Union and at 50% by the Dutch Government.

Field inventories

While a relatively great number of individual trees could be identified as *P. nigra*, it seemed impossible to find the two stands that should have been included in the EUROPOP research project. However, one stand near Gendt turned out to be sufficiently large for the needs of the project. The second stand had to be selected just across the border in Germany, along the River Rhine.

The majority of the selected individual trees could be found in a Nature Reserve called 'The Biesbosch', an area where two river systems join. This is an interesting area with regard to identification of genetic material between different river systems.

Implementation

The genebank of *P. nigra* that already contained many mature individuals had to be moved to a different location in the Polder area. The impact on the stool-bed level was not too high, although some of the entries were lost due to difficult growing conditions in the beginning. However, the impact on adult trees was more significant; it will take at least ten years before the trees are old enough to flower and are mature enough for evaluating shape, growth etc.

Concerning river management, we currently face in The Netherlands a completely different approach compared with the past. Management today gives higher priorities to nature development. In general this could offer better possibilities for floodplain forests. However, trees still seem to be in contradiction to what river managers have in mind when they think of quick release of huge water supplies in the late winters and early springs. Research will be needed to show whether this is a valid opinion or not. The opportunities for *in situ* gene conservation of *P. nigra* in the future are, however, improving.

Public awareness

At the presentation of the Governmental research programme 'Forest Related Research in The Netherlands' in Wageningen in 1998, a special session was dedicated to the work carried out in relation with EUFORGEN in general. Information was given on the three Networks in which The Netherlands participate: *Populus nigra*, Noble Hardwoods and Social Broadleaves Networks.

In May 1998 the author gave a presentation at the symposium in Hann. Munden, Germany, specially dedicated to *P. nigra* under the title: 'Activities of the EUFORGEN *Populus nigra* Network and international cooperation in research'.

In August 1998 a presentation was given at the IUFRO Division II meeting in Beijing, China, under the title: 'European Network on the conservation of genetic resources of *Populus nigra* in the framework of EUFORGEN'.

Poland

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During 1998, a field inventory of plants in the alluvium zone of the River Odra was completed by the Academy of Agriculture in Poznan (Dr Danielewicz). *Populus nigra* is a common tree along the river. Particular individuals, for example very old trees or trees with high dimensions are protected. *Populus nigra* is also protected as part of the numerous reserves, bird refuges etc. along the river. The full report of the inventory will be published in late 1999.

After the summer flooding in the south-western part of Poland in 1997, engineering works on the river regulation have been intensified. It will be necessary to ensure that riparian plant communities are adequately protected. The Institute of Dendrology has established a programme to address this issue.

Portugal

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General aspects and distribution

Populus nigra is a minor species in Portugal. Together with *Fraxinus angustifolia, Alnus glutinosa* and *Salix* sp. it occupies a riparian ecosystem, both along major rivers and temporary streams.

In Mediterranean landscapes dominated by species adapted to two to five summer months of severe drought, the water streams, even temporary, give origin to refuges colonized by *P. nigra* and other species. In such microenvironments a rich biodiversity develops, contrasting with the surrounding typical dry areas with oaks and pine stands in Mediterranean climates and contributing to a priceless diversification of landscape.

Economic interest

The economic interest in poplars is nowadays limited in Portugal. The species used to be cultivated for matches and toothpick industries. The matches industry moved to overseas countries while the toothpick industry requires low amounts of raw material. However, the species is still procured for various uses, namely:

- the veneer industry;
- the furniture industry;

- wooden roofs also make use of poplar wood, due to its reputation of resistance to insect attack;
- cattle feed (green feed during summer);
- vine supports in the north-west of the country ('vinho verde' vineyards);
- wind protective belts to protect agriculture; and
- landscaping in towns, parks and other urban-related uses.

Ecological value

The ecological value of *Populus* sp. holds for biodiversity. Birds nest in the dense canopies where availability of forage is high, due to insects and other species that are attracted by the shelter and mild temperature provided by the ecosystem resulting from the stream bed and the trees. During the suffocating days of the long Mediterranean summers, *Populus* sp. forest is a priceless refuge for wildlife, cattle and also human activities.

In Mediterranean countries where rainfall can assume a violent, sudden and concentrated profile, protection of riverbeds by trees against soil erosion, agricultural activities, pasture and human occupation of riparian zones is indispensable. *Populus* sp. assumes this role together with other species, but in some parts of the country it may be the most important species.

Threats

Agriculture, urbanism and afforestation with economically more attractive species such as *Eucalyptus* are the major threats to the species. Yet in some cases, release of agriculture due to rural abandon may also contribute to natural recovering of the species.

During the 1930s to 1950s, the state services for hydrology distributed hybrids widely and free of charge. The existence of native pure species is therefore unknown. Records and information on these procedures require time-consuming search in archives, as well as field inventories.

Perspectives

Portugal is interested in performing inventories of the genus *Populus* and in participating in the European core collection. Inventories are dependent on specific funding. Portugal is interested, at least in the short term, in providing material for the characterization through genetic markers of the various *Populus* species spreading in the country, gene flow and mating system in cooperation with other countries' laboratories or ongoing projects.

Slovakia

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Autochthonous plant communities on alluvial sediments on downstreams of rivers in Slovakia were dominated by oak, ash, elms, alder and native poplars. Besides direct human interventions, regulation of rivers, construction of dams and channels as well as construction of power stations influenced markedly the level of underground water tables. Their effects promoted considerable changes in the tree species composition. Extensive conversions were carried out later. Inferior stands of coppice origin have gradually been converted into a high forest. The black poplar (*Populus nigra* L.) is an important stabilizing and landscape-forming element in the lowlands and hilly regions. In comparison with the most productive Euroamerican poplars, it is less demanding in moisture and soil nutrients. It has a richer, more branched root system with predominating thin roots. Thus, in contrast to Euroamerican and Interamerican hybrids, it is fully adapted to our conditions. Individuals with valuable growth or other characteristics are considered a genetically irreplaceable breeding material. The black poplar does not occur in continuous stands but usually in groups and as an individual admixture within the forest types *Querceto-Fraxinetum*, *Ulmeto-Fraxinetum carpineum* and *Fraxineto-Alnetum*. However, due to anthropic interference, the occurrence of black poplar has been significantly reduced. At present, it grows on an area of about 350 hectares only.

The conservation programme of *Populus nigra* L. has been implemented in Slovakia with some interruptions since 1965. It is aimed mainly at:

- establishment of fragments of gene bases;
- advance growths *in situ* and reproduction plantations *ex situ* (plus trees, clonal archives, stool-beds field experiments); and
- intraspecific crossing.

The fragments of gene bases play an important role in the conservation of forest tree species in the lowlands. They can be defined as a cluster of stands of autochthonous forest tree species representative of their regional population, which have been preserved in intensively managed agricultural landscape. In spite of their semi-natural condition, they do not guarantee full extent of regeneration *in situ* due to their limited sizes, ranging from 2 to 50 hectares. In the mid-1990s, 19 fragments of gene bases were delineated in southern Slovakia with an area of 211 hectares (Table 1). Within these, 112 plus trees were selected. Another 76 plus trees were selected outside the fragments. By 1 January 1999 there were 188 plus trees recorded.

Based on the typical phenotypic traits, the plus trees have been classified into two groups. The first group includes phenotypically superior types and the second is the group of productive clones. The phenotypically superior types are characteristic with a straight cylindrical stem, narrow fan-shaped or pyramidal crown, and relatively thin branches at an acute angle (within 45°). The rhytidome is relatively shallowly grooved. Natural pruning of individuals in the canopy is relatively good. The productive types have a straight, sometimes slightly twisting full stem, forming at about a half of the tree height, larger, fan-like or oblong crown.

Generative *in situ* reproduction was started in four phenotypically superior stands of black poplar. Prior to natural seeding, grass detritus was removed in parts of stands with stocking level about 0.6 as well as on western edges of the stands. The size of reproduction plots was 1×1 m. Plots were arranged in a chessboard with 8 (12) plots at each parent tree. Weeds were regularly controlled during the first two years and a fungicide applied when necessary. There were also reference plots without treatment. The results were compared with sowings in a nursery. The results presented in Table 1 describe the situation at the end of the fourth growing season.

	Plots under pa	arent trees	Nurse	ry
	Mechanical and chemical		Mechanical and chemical	
Assessed parameters	treatment	Untreated	treatment	Untreated
Average number of individuals	9	0	19	0
Mean height	112	0	201	0
Health condition	3	0	2	0

Table 1. Generative in situ reproduction: results at the end	of the fourth growing season
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The analysis of quantitative and qualitative parameters indicates that appropriate silvicultural treatment makes natural regeneration of black poplar possible. It must be mentioned that the high costs of related silvicultural operations make their wider application almost impossible. The natural regeneration obtained comprises progenies of unclear genetic origin due to multiple crossings, putatively with hybrid poplars. They are affected by fungal diseases and leaf pests, and usually form curved-up to very twisting stem. Regarding growth they fall behind the standard poplar clone 'Robusta'.

In recent years, greater attention has been paid to *ex situ* conservation: regular complementation of central poplar stool-bed, clonal archives, establishment and complex assessment in field experiments. The main purpose of field experiments with autochthonous black poplar is the selection of three (five) resistant clones with at least the same growth parameters as standard poplar 'Robusta'. At present we have one central poplar stool-bed and nursery, four clonal archives with an area of 7.5 ha and 12 field tests. On these plots, 330 clones have been tested. The clones are either ortets of plus trees or originate in intraspecific hybridization.

The selection of European black poplar has been divided into two stages. The first stage is carried out in the nursery of the Forest Research Station Gabčíkovo. The period of testing lasts for two years. Each clone is represented by the number of plants which allows for statistical comparison. The survival is recorded in mid-May, and biometric measurements are carried out at the end of the second growing season. For health assessment, attention is paid to infection by *Chondroplea populea* Sacc. et Briard, *Marssonina brunnea* Ell. et Ev. and *Melampsora* sp. Calcium chlorosis and frost damage are also assessed. The shape of stems is the principal phenotypic trait.

The second stage of the selection of native black poplar is carried out on field experimental plots established in a range of site conditions, from the most humid to the driest forest types of the lowlands of Slovakia. The clones suitable for cultivation must show better growth than the standard clone 'Robusta'. They must also be resistant against bacteria *Erwinia cancerogena* Uroš., *Marssonina brunnea* Ell. et Ev., foliar pests and *Saperda carcharias* L.

Based on complex assessment in the field experiments over 15–20 years, the following clones meet the selection criteria:

- *Populus nigra* 5 is an ortet descending from a plus tree selected in the area adjacent to the Danube. It is a male tree, easily propagated by stem cuttings. The stem is slightly twisted. The crown width is medium. The clone tends to form strong branches that can be eliminated by early pruning from the bottom of the stem. The stem is initially slightly goosenecked but becomes straight with more intensive diameter growth in the pole stage. Rhytidome forms relatively early. The bark in the crown retains its typical light grey colour even at an older age. In spite of early flushing, damage by late frosts was not recorded. The leaf-shedding is early. The growth rate is comparable to 'Robusta' (see Table 2 and Fig. 1).
- *Populus nigra* I-1 is an ortet descending from a plus tree selected in the upper part of the Váh valley. It is a female tree easily propagated by stem cuttings. Its mean height is 15–20% higher than 'Robusta'. We recommend only saplings 1/2 or 2/2

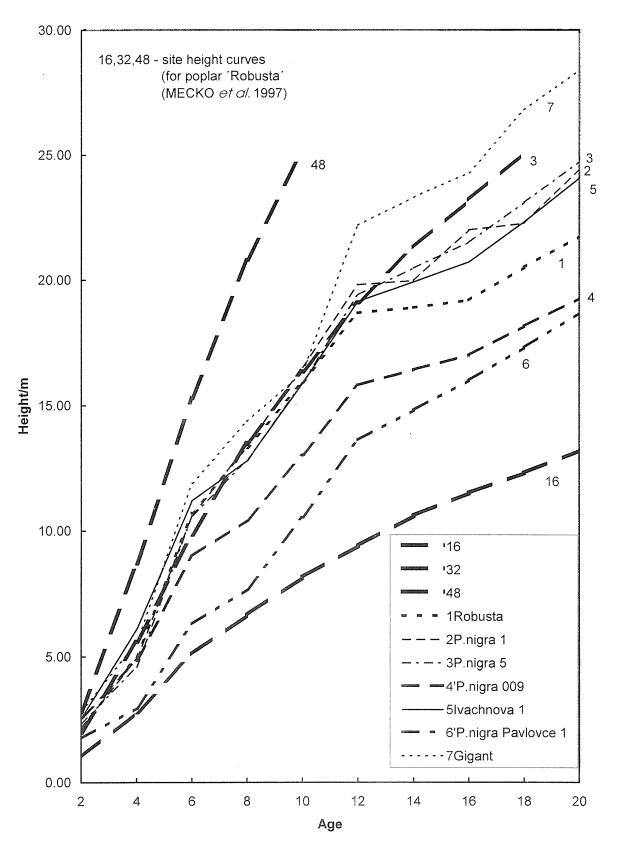
for plantation. The stem is straight and branches can be pruned easily. The crown is slender, later medium-wide. The crown retains smooth, light grey bark for a long time. The rhytidome in the lower part is dark grey, shallowly grooved. The time of flushing is moderate, leaf-shedding late.

• *Populus nigra* P-1 is an ortet of a plus tree selected at the River Uh in eastern Slovakia. It is a female tree easily propagated by cuttings. Only 2-year-old plants are suitable for planting because the early growth is slower than for the standard poplar 'Robusta'. The clone has a straight stem with medium-wide crown. It is typical of forming the bird-eye clusters. The cause of bird-eyes formation is not known exactly. It has frequently been ascribed to the activity of bacteria or strong pruning performed each year. New formations of bird-eyes on the stems of this clone appear between the fourth and sixth year. Their shape and size vary from case to case. With increasing height, their number drops abruptly and they occur only occasionally when the tree height is 8 m or more. With the aim to obtain top-quality wood, the bird-eye shoots must be regularly pruned in summer up to the height of 6 m.

		Height	t	Diame	ter	Mean	Deç	Degree of damage					
No.	Clone	Mean	Incr.	Mean	Incr.	vol. (m³)	Ср	Мр	Mb	Ec	с	Fp	Sc
1	'Robusta' (E)	22.4	1.1	24.5	1.2	0.382	3	1	1	3	0	1	2
2	'I-214' (E)	26.7	1.3	35.0	1.7	0.912	1	1	2	1	0	1	1
3	P. nigra 1 (A)	25.0	1.2	29.5	1.4	0.652	2	3	3	3	0	2	1
4	P. nigra 3 (A)	23.5	1.1	29.0	1.4	0.589	1	2	2	3	0	2	2
5	P. nigra 4 (A)	24.9	1.2	28.4	1.4	0.587	2	3	3	2	0	1	2
6	P. nigra 5 (A)	25.5	1.2	38.1	1.9	1.048	2	1	2	2	0	1	1
7	P. nigra 009 (A)	19.8	1.0	21.8	1.1	0.243	4	4	3	4	0	2	2
8	P. nigra 011 (A)	21.2	1.0	24.3	1.2	0.351	3	3	3	3	0	2	2
9	P. nigra	24.8	1.2	30.2	1.5	0.669	4	3	4	3	0	3	2
	'Ivachnova' (A)												
10	P. nigra 'Klin 1'	21.2	1.0	26.9	1.3	0.424	2	1	2	2	0	3	2
	(A)												
11	P. nigra	19.3	0.9	25.0	1.2	0.319	2	3	3	2	0	1	3
	'Pavlovce' (A)												
12	'Vojtus' (E)	23.0	1.0	30.0	1.5	0.587	1	1	2	2	0	1	1

 Table 2. Growth and health condition of black poplars tested on a relatively dry forest type, age 20 years

A, autochthonous black poplar; E, Euroamerican hybrid; Incr., mean increment at the age of rotation; Cp, *Chondroplea populea*; Mp, *Melampsora* sp.; Mb, *Marssonina brunnea*; Ec, *Erwinia cancerogena*; C, calciosis (calcium chlorosis); Fp, foliar pests; Sc, *Saperda carcharias*



AGE-HEIGHT CURVES OF POPLARS field test Asód

Fig. 1. Age-height curves of poplars: field test Asód.

Slovenia

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The Republic of Slovenia covers 2027 million ha. More than one-third of the area lies higher than 600 m above see level, which characterizes Slovenia as a mountainous country. Forests cover 53% of Slovenia. Less than 1% of the forest sites are occupied by poplars, willows and alders. The best known phytocoenological associations with poplars are *Salici-Populetum*, *Querco-Carpinetum* var. *Luzula*, *Querco-Fagetum* var. *Luzula*, and with willows *Salici-Populetum*, *Luzulo-Fagetum*, *Hacquetio-Fagetum* and *Enneaphyllo-Fagetum*. From the 1990 forest inventory it can be concluded that the share of poplars and willows in the total growing stock of lowland forests per hectare represent a maximum of 20%. Most often the share is below 10%, which means that poplars and willows in Slovenia's lowland forests grow only individually or in small groups.

Primarily poplar and willows groves in Slovenia were preserved only in small locations on alluvial sites along the Mura, Drava, Sava, Krka and Soča rivers. In 1959 the area of such sites was estimated at ca. 1300 ha, but has been continually reduced in the last decades. During the years of intensive poplar production (1960–1980) through intensive poplar plantations with several selected P. × *euramericana* clones, autochthonous tree species, including P. *nigra* and *Salix alba*, have been cleared from such sites. Similarly, the present distribution of the autochthonous P. *alba* has been reduced. Individual trees (solitaires) and small groups are present in wetlands along the rivers and in mixed lowland forests. A substantial contribution to the decline of the autochthonous vegetation in poplar and willow groves was due to the changes originating from river regulation and water accumulations.

Prior to the establishment of intensive poplar plantations, comprehensive studies have been undertaken on the compatibility of potential poplar plantation sites and poplar clone sources of autochthonous and foreign origin. The research programme of the Slovenian Forestry Institute, 'Biological and production silvo-technical studies of poplars' was carried out until recently. Initially the technological aspect of establishment and tending measures in poplar plantations of different planting methods and intensity dominated the research. Subsequently, different poplar clones studies prevailed. The first planting material for the establishment of poplar plantations derived from basic source trees of black and balsam poplars, selected in Slovenia. In forest nurseries planting material derived from tested poplar clones of autochthonous and imported origin. These were in particular clones from Italy (I-154, I-214, I-264, I-455, I-476, I-488, 45/51) and other European countries: Austria, Germany, Switzerland, France, The Netherlands and Belgium. Approximately 150 clones were tested, from which around one-third have been successfully accepted in production plantations. The professional control was provided by the Slovenian Forestry Institute, where the register of the basic material for vegetative propagation and production of reproductive material was also held.

Along with poplar wood plantations, clonal test plantations of chosen poplar trees have been established from the sections *Aigeiros*, *Tacamahaca* and their hybrids (Table 3), as well as hybrids from the section *Leuce* (Table 4). In total, 14 test plantations have been established in the years 1965 to 1980, covering an area of 8.6 ha.

Location	Year of plantation	No. of clones	No. of trees	Area (ha)
Vrbina (River Sava)	1977	4	192	0.27
Vrbina (River Sava)	1978	20	480	0.67
Vrbina (River Sava)	1978	8	128	0.18
Krška vas (River Krka)	1979	30	600	0.96
Krška vas (River Krka)	1979	8	640	1.02
Ljubljansko barje	1978	8	960	1.92
Ljubljansko barje	1978	15	240	0.48
Ljubljansko barje	1979	10	640	1.28
lakovci (River Mura)	1980	8	384	0.78
lakovci (River Mura)	1980	9	511	1.03

Table 3. List of clonal test plantations of poplar from the sections Aigeiros and Tacamahaca

Table 4.	List of clonal test	plantations of p	poplar from the	section Leuce

Location	Year of plantation	No. of clones	No. of trees	Area (ha)
Vrbina (River Sava)	1965	12	432	0.17
Dobrava (River Sava)	1966	5	168	0.08
Grnčarice (Kočevje)	1969	18	1554	0.60
Grnčarice (Kočevje)	1969	2	676	0.27

In ten plantations (sections *Aigeiros* and *Tacamahaca*) 51 clones were planted, including: *P.* × *euramericana* (6 clones), *P. deltoides* (41), *P. trichocarpa* (4). In the plantations of section *Leuce* 31 hybrid families were planted: *P. tremula* × *P. tremula* (9 families), *P. tremula* × *P. tremuloides* (7), *P. tremula* × *P. grandidentata* (3), *P. tremula* × *P. alba* (4), *P. alba* × *P. tremula* (1), *P. alba* × *P. grandidentata* (3), *P. alba* × *P. tremuloides* (1) and *P. alba* (3). Some of the mentioned plantations form the living clonal archive and serve as the source for collection of vegetative reproductive material. In order to prevent the loss of individual clones we have established the National Archive of poplar clones in the Institute's nursery Zadobrova near Ljubljana. In 1998 this archive contained 40 clones (Table 5).

There are at present no specific in situ or ex situ programmes for the conservation of autochthonous P. nigra or P. alba genetic resources. Their protection is merely included in the National Biodiversity Strategy as part of the Wetlands Protection Programme, as well as in the Forest Act, which prescribes natural regeneration in most areas (90% of all yearly regenerated areas in Slovenia). Although autochthonous poplars in Slovenia have no great porportion in forest management regarding their distribution area and economical importance, one of our future goals is also to protect the autochthonous poplar genetic resources, starting by presenting them in the IUCN vulnerable species category. On the other hand poplar, from its biological and ecological perspective, is an irreplaceable tree species, building wet-lowland river groves, occasionally flooded by high waters. Our future conservation activities will be oriented towards classification of autochthonous populations of *Populus* sp. as the prerequisite for development of the concepts for poplar genetic resource conservation strategy in Slovenia. Special attention should be paid to individual trees and small groups of these trees in small areas along the Mura, Drava, Sava, Krka and Soča rivers, along their streams, dead branches of rivers and on wetlands and occasionally flooded terrains.

		Desig	nation	
No.	Botanical name	Int. register ¹	Original	Country of origin
1	P. deltoides	S 1-3	S 1-3	Poplar Institute Novi Sad
		_	_	(FR Yugoslavia)
2	P. deltoides	S 1-5	S 1-5	Poplar Institute Novi Sad
-		0.0.1	0.0.1	(FR Yugoslavia)
3	P. deltoides	S 6-1	S 6-1	Poplar Institute Novi Sad
4	P. deltoides	S 6-7	S 6-7	(FR Yugoslavia) Poplar Institute Novi Sad
4	T. denoides	0.0-1	5.0-7	(FR Yugoslavia)
5	P. deltoides	S 6-20	S 6-20	Poplar Institute Novi Sad
Ũ				(FR Yugoslavia)
6	P. deltoides	S 6-36	S 6-36	Poplar Institute Novi Sad
				(FR Yugoslavia)
7	P. deltoides	S 11-8	S 11-8	Poplar Institute Novi Sad
	D <i>i i i i i</i>	55 (05	55 (05	(FR Yugoslavia)
8	P. deltoides	55/65	55/65	Poplar Institute Novi Sad
9	P. deltoides	709	709	(FR Yugoslavia) Poplar Institute Novi Sad
9	r. uenolues	109	703	(FR Yugoslavia)
10	P. deltoides	Lux, 618	I-69/55	Italy
11	P. × euramericana	12	cl. 45/51	Italy
12	P. × euramericana	17	cl. I-214	Italy
13	P. × euramericana	23	BL Constanzo	Italy
14	P. × euramericana	24	Gattoni	Italy
15	P. × euramericana	25	Triplo	Italy
16	P. × euramericana	Marilandica	Marilandica	Slovenia
17	P. × euramericana	Regenerata	Regenerata	Slovenia
18	P. × euramericana	Zelena Robusta	Robusta	Slovenia
19	P. × euramericana	M1	Panonnia	Hungary
20	P. trichocarpa	31	I-044/67	Italy
21	P. trichocarpa	Muhle Larsen, 47	. cl. 45/54	Germany
22	P. maximowiczii	41	cl. Max 1	Germany
23	P. maximowiczii	42	cl. Max 2	Germany
24	P. maximowiczii	43	cl. Max 3	Germany
25	P. maximowiczii	44	cl. Max 4	Germany
26	P. maximowiczii	45	cl. Max 5	Germany
27	P. androscoggin	46	Androscoggin	Germany
	Other clones (13)			Slovenia

Table 5. Poplar clones in the living archive of the Slovenian Forestry Institute in 1998

¹Name according to international register

We would also like to study the morphological characteristics/taxonomy of *P. nigra* according to the EUFORGEN Network recommendations, and our interests would include also *Salix* spp. taxonomical problems. However, no special research project exists at the moment in Slovenia, and one cannot be proposed to be of sufficient interest for funding by the Ministry of Science and Technology or by the Ministry of Agriculture, Forestry and Food, which are the principal sources of financing of our Institute. Only the management of the clonal archive forms part of the State Forest Service, provided by the Slovenian Forestry Institute. The perspectives might be in European collaboration and in collaboration in projects proposed by the Ministry of the Environment and Planning (section of water protection) and by the State Authority for Nature Conservation (section of the national strategy on Conservation of Biodiversity, concerning inland waters and wetlands).

Acknowledgements

We would like to thank Dr J. Božič for his critical reading of the report.

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Spain

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In Spain, the work related to the conservation of *P. nigra* genetic resources is being carried out by two institutions, the Centro de Investigación Forestal belonging to the Instituto Nacional de Investigaciones Agrarias (CIFOR-INIA), which deals with the whole country, and the Servicio de Investigación Agraria de Aragón (SIA-DGA), which deals with the Aragon autonomous community. Both institutions cooperate in joint projects concerned with conservation and research.

Research

Studies on variation are being carried out in natural populations in the Ebro Valley and in the whole Spanish collection. The study on the variation of the collection is being carried out on a total of 154 clones, using the EUROPOP methodology, with isoenzymes and morphological characteristics.

Practical conservation activities

The collecting of new clones has continued in the prospecting areas of the Pyrenees and the Alto Tajo. Of the 28 clones collected, 18 were propagated, six more with some difficulty, and four were lost altogether.

Other activities related to the maintenance of the already established collections were:

- Regeneration of the stool-beds in the SIA-DGA: two stools per genotype (110 genotypes) from the banks of the Ebro. This collection is kept in stool-beds and in the adult tree plot.
- The collection initially obtained by CIFOR-INIA, including clones from other regions, is being transferred to the Valsaín nursery in order to be kept in an adult tree plot. A total of 25 clones have already been installed in this nursery.

Field inventories

Work concerned with the inventorying of riparian forest on the banks of the Ebro has been ongoing throughout 1998 and 1999. Twenty populations were prospected, in a surface range of 2 to 126 ha. *P. nigra* was present in 19 of these, and natural regeneration was observed in seven.

Public awareness

For the second year running, activities related to *P. nigra* conservation have been reported in different meetings on poplar cultivation.

Ukraine

Igor Patlaj, Valentyna Rudenko and Roman Volosyanchuk

Ukrainian Research Institute of Forestry and Forest Melioration, Kharkiv, Ukraine

Since the last Network meeting the state of *P. nigra* conservation has not changed significantly. In the meantime, the following activities have been carried out.

In situ conservation

- inventory of poplar stands has been continued in the north-eastern part of the country;
- a catastrophic flooding damaged many natural poplar stands in the Transcarpathian province. Measures for their re-establishment are under development.

Ex situ conservation

- two new clones were added to the national core archive stool-bed;
- a programme for soil and river banks protection in the Chornobyl radioactive zone is under development. Anchoring the banks with planting poplars is a considerable part of the programme.

Core collection

Two clones were sent for the EUFORGEN Core Collection.

Reference clones

Eighteen reference clones were obtained from the Institute for Forestry and Game Management (Geraardsbergen, Belgium). The clones were distributed to three institutions: the Ukrainian Research Institute of Mountain Forestry (Ivano-Frankivsk, western Ukraine), the Central Botanical Gardens (Kyiv, northern part of central Ukraine), and the Ukrainian Research Institute of Forestry and Forest Melioration (Kharkiv, eastern Ukraine).

Introductory country information on Populus alba

Austria

Berthold Heinze Institute of Forest Genetics, Federal Forest Research Centre, Vienna, Austria

Distribution

Populus alba is found over almost the same distribution range as *P. nigra*, being only slightly more demanding as far as summer temperatures are concerned. Where it occurs in stands along the major rivers (mainly the Danube east of Krems), the forestry practice of cutting in relatively short intervals of 30–40 years has often resulted in vigorous regeneration from root suckers. This has created stands in which individual genotypes can be distinguished by their growth characteristics. On somewhat drier sites, the grey poplar (*P.* × *canescens*) is said to take over; however, taxonomy between those two is unclear and an open problem for botany in Austria.

Use in forestry

Populus alba is not a favourite species of foresters; however, in the floodplains of the Danube, slowly drying out, there are sites where there is hardly any alternative, and therefore the selection of good clones among the root suckers became an option. Timber of such stands is currently sold; more often, it is used as fuelwood. To our knowledge, however, there have not been substantial efforts to propagate superior clones *ex situ*, or to transfer them on to new sites. The main reason may be the cumbersome propagation by root cuttings.

Need for conservation

There are no genetic data on *P. alba* in Austria. Because there is no risk of hybrization, such as in *P. nigra*, it can be assumed that the threat for the species is not as high. However, the interesting thing about *P. alba* is the high incidence of vegetative regeneration, over many generations, compared to generative regeneration. There is no information whether this has had any effect on the genetics of the species. In general, propagation by seeds is also hampered by the lack of regular floodings in our river floodplains, but the extent of this effect is probably not as great as with the black poplar. White poplar seedlings can be found in a number of sites (it might be called a quite aggressive species) and in different floodplain forest associations; what is missing now compared to the times of undisturbed river flow are probably larger linear stands along the river edges of generative origin, i.e. higher genetic diversity. Again, whether these probable changes in fine-scale distribution (less continuous stands, more scattered trees) have had any effect is not known.

Management plans for the Danube Floodplains National Park east of Vienna consider *P. alba* as a native species that deserves attention, but does not need special consideration under present circumstances.

General forestry practice has not yet called for help in improving the species, or for the need to secure sources of supply. The margin that might be obtained through genetic improvement is probably substantially lower than the increase in profits by switching to other species (hardwoods) altogether.

Belgium

Jos Van Slycken and An Vanden Broeck

Institute for Forestry and Game Management, Geraardsbergen, Belgium

Populus alba is considered a native species only in the dunes near the coast, where the species mainly appears as root suckers. However, there is some discussion about the origin of the species in Belgium. No historical reports on the presence of the species are available.

Populus × *canescens*, on the other hand, was an important species in forestry. It had a very important economical value from the seventeenth to nineteenth centuries. Historical data from harvests report a share of 40-60% of *P. canescens* of the total harvested wood volume.

Nowadays neither *P. alba* nor *P. canescens* are used in forestry. Conservation of valuable clones of *P. canescens* started in Flanders.

France

François Lefèvre Unité de Recherches Forestières Mediterranéennes, INRA, Avignon, France

Populus alba is not a priority for the French National Commission on forest genetic resources. However, germplasm has been preserved from previous breeding programmes, and information on this resource has been published.

Hungary

Ernö Gabnai, István Bach and Sándor Bordács

National Institute for Agricultural Quality Control, Budapest, Hungary

According to the recent forest inventory, the area of white and grey poplars is as shown in Table 6.

				Age/ha				
Species	1–10	11-20	21-30	31-40	41–50	51–60	61+	Total
<i>P. alba</i> (white poplar)	1181	1831	994	1184	658	389	184	6421
<i>P. canescens</i> (grey poplar)	9355	9679	5541	5612	2562	888	471	34108

Table 6. Area of white and grey poplars

Some experts assign *P. canescens* to *P. alba*, because in Hungary (mainly on the Great Hungarian Plain, which is the largest occurence area of *P. canescens*) the stands of *P. tremula* are very few. This means that as a result of continuous backcrossing, these hybrids are putative white poplars.

Plus trees have been selected by scientists since the begining of the 1950s. The aim was to obtain more timber production by artificial crossing. According to the 1985 forest inventory there were 48 *P. alba* and 54 *P. canescens* plus trees. Since then, 35 additional *P. alba* plus trees have been selected.

Populus alba is included in the National Environment Protection Fund as *P. nigra*. This means that the gene conservation and breeding activities are supported by the Hungarian Government.

Some companies are interested in the establishment of seed orchards, but at present it is cheaper to gather seed from the large scale of natural *P. alba* and *P. canescens* forests.

An exact morphological description is required as soon as possible as for *P. nigra*. It is important to develop reliable DNA identification methods.

Italy

Luisa Cagelli, Stefano Bisoffi and Lorenzo Vietto Poplar Research Institute (ISP), Casale Monferrato, Italy

ISP collection

All *P. alba* material included in the ISP collection (115 genotypes) was propagated in stoolbed in 1999; about 50 cuttings per clone were planted.

Environmental restoration

A nursery with 51 genotypes of *P. alba* was established at the same time in order to produce plants for natural re-establishment and for ornamental use; eight male clones and 43 clones selected from a family obtained by open pollination of *P. alba* Villafranca (the only *P. alba* clone registered in Italy) were propagated.

Russian Federation

Ivan Popivshchy

Russian Tree Breeding Centre (CENTRLESSEM), Pushkino (Moscow), Russian Federation

Populus alba is a broadleaved tree, 30–35 m high, dioecious, with incomplete flowers, anemophilous, anemochorous and hydrochorous, tolerating light soil salinity. It is the autochthonous species of the first canopy layer in lowland forests, the so-called 'white-populeta', in Russia. *Populus alba* has no gum, its coppices have finger-lobate leaves, with dense white pubescence on the inferior side of the leaves. As an admixture, *P. alba* occurs often in black poplar stands and is heavily attacked by pests, fungi and bacterial diseases.

Nearly 80% of lowland forests in Russia are so-called populeta. The *P. alba* range occupies the central and southern regions of the European part of Russia (Table 7) and the southern part of western Siberia. It also occurs in Kazakstan, Central Asia, Iran, Afghanistan, China and north-west Africa. White poplar stands, jointly with black poplar, are represented by small plots in the lowlands of large Russian rivers (Volga, Don, Cuban, Ural, Ob, Irtysh etc.; see Fig. 2).

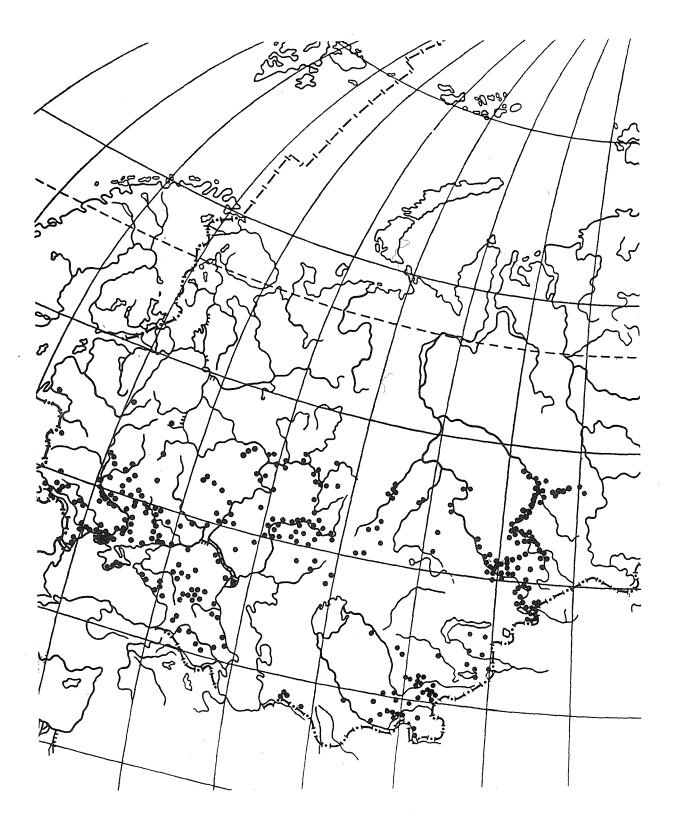


Fig 2. Distribution of *Populus alba* in Russia and the former USSR.

Table 7. Populus alba stands in the European part of Russ	sia (in 1999)
Republic, region, district	Area (ha)
Stavropol	2989
Karachaevo-Cherkesya	38
Kabardino-Balkarya	2600
Volgograd	28000
Saratov	888
Voronezh	356
Bashkortostan	588
Krasnodar	2993
Adigeya	1010
Rostov	2100
Total	41562

Populus alba participates in the initial stage of forests on young sandy-gravel deposits. Its stands develop in two ways:

- as a result of self-seeding on new territories; and
- as a result of *P. nigra* succession.

The latter is characteristic of the transition zone between the riverbed and central river valley. Here P. alba settles under the P. nigra canopy and replaces it gradually. Rubus caesius dominates under the canopy of *P. alba* with a large participation of *Bromus inermis*, *Convallaria majalis, Carex* spp. etc.

The extinction of the river accumulative activity on these plots leads to the formation of specific soils and Ulmus laevis and Quercus robur invade under the canopy of P. alba. Besides Rubus caesius, its composition includes Aristolochia clematitis and Convallaria majalis.

All types of 'white-populeta' are characterized by a high stand productivity, canopy closure (0.4-0.8), rich shrub layer, prevalence of forest and grassland species in the herbaceous layer. However, generative reproduction of *P. alba* is difficult because of a high herbaceous layer density, matted soil and insufficient light. Coppice regeneration of P. alba prevails.

In case of an active accumulative river activity and great alluvial layer of deposit at the site of young 'white-populeta', 'Aristolochia-white-populeta' develop. Rubus caesius dominates in the shrub layer, and Aristolochia clematitis in the herbaceous layer, with participation of grassland. Here also occur 'Bromus-white-populeta' with Bromus inermis, Glechoma hederacea and Glycyrrhiza glabra. For the higher sites there are characteristic 'Calamagrostis–white-populeta', with Calamagrostis epigeios, Bromus inermis, A. sibiricum and *Carex praecox* in the herbaceous layer, as well as many steppe species and halophytes.

The development of so-called 'black-white-populeta' may be considered as an example of anthropogenic succession. It is a derivative forest type where *P. alba* is represented by coppice individuals. In western Siberia river lowlands P. alba often forms mixed stands – the so-called 'urems' with participation of P. nigra, Salix spp., Betula spp., Padus racemosa, Cornus alba and Viburnum opulus.

Populus alba belongs to the group of fast-growing tree species, reaches its maximum size in 30–40 years, but is not a long-lived tree: its mean life span is 80 years. It is undemanding regarding soil conditions, but prefers light, well-drained alluvial soils with high moisture availability. Populus alba tolerates long floodings and excessive water supply. It develops a powerful root system, capable of producing abundant root sprouts, even at considerable distance from the mother stem. Such a system effectively resists soil erosion, drifts, and therefore *P. alba* may be used for fixing slopes. Due to these qualities and a high productivity and good wood properties, *P. alba* is considered one of the valuable tree species in Russian lowland forests.

As a rule, white-populeta exist for only one generation, and then, in the course of lowland development, they are succeeded by coniferous trees in the north and by broadleaved lowland stands (urems), consisting of *Padus*, *Sorbus*, *Betula* and other species in the south (in the steppe and forest-steppe zones).

The mean volume growth increment in white-populeta is $8-10 \text{ m}^3/\text{ha}/\text{year}$, and for the stands aged 80-90 years the growing stock may reach $500-700 \text{ m}^3/\text{ha}$. Because of the high susceptibility of *P. alba* stands to fungus diseases they reach their 'technical maturity' at 15–25 years in the European part of Russia, and at 25-35 years in Siberia.

Populus alba is a source of raw materials and fulfils various functions: soil protection, water regulation, etc. It is widely used in field-protective, roadside, recreative, decorative and other plantings, and in the breeding of fast-growing poplar hybrids. *Populus alba* is short-lived, although individual trees may reach 150–200 years. However, their most intensive growth is until 25–30 years of age.

At 25 years the white poplar with aspen hybrids forms trees with a mean stem diameter of 30.5 cm and mean height of 27.2 m. At a density of 907 trees/ha it supports a growing stock of 935 m³/ha compared with 468 m³/ha for aspen and 665 m³/ha for intraspecific *P. alba* hybrids. The triploid poplar variety 'Seyanets ES-38' was selected, vegetatively propagated and successfully cultivated in the district of Voronezh.

There are no-national strategies for *P. alba* and *P. nigra* gene resources conservation. In contrast to these poplars, gene resources of main forest species (pine, spruce etc.) are investigated, forest seed orchards are created, experimental plots are established and gene resources are conserved *in situ* and *ex situ*. However, in the present conditions of fast-changing environment, with geomorphological and hydrological disturbances and variations, and the expected global climate warming with unpredictable effects, it may be necessary to take some measures for the poplar gene resources conservation. A task list for *P. alba* gene resources conservation is as follows:

- 1. Gene resources inventory in particular regions, subregions, national parks, reserves, forestry enterprises, etc.
- 2. Understanding and preservation of the patterns of white poplar geographical diversity.
- 3. Maintenance of some interesting populations with considerable morphological diversity in the lowlands of the Volga, Don, Kuban, Ural, Ob, Irtysh and other rivers.
- 4. Training of collaborators for inventory and identification of white poplar stands.
- 5. Studies of introgression and its consequences for white poplar.
- 6. Revision of experimental trial plantations and genebanks as *ex situ* conservation units.
- 7. Renewal of old collections.
- 8. Establishment of protected areas, especially for *P. alba*; legal and technical measures to forbid the plantation of other poplar species and hybrids in the vicinity of such protected areas.
- 9. Investigation of white popular population genetic diversity structure, geneflow, vegetative vs. sexual reproduction, dynamics of native stands in different environments, etc.

However, the fulfilment of these tasks would be possible only through direct funding from interested sponsors, since the scarce funds of the Russian Forest Service are exhausted by the maintenance of major forest tree species.

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Spain

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Found all over Spain, except for Galicia and the Cantabrian seaboard in the north, *P. alba* is most frequent in the south, due to its thermophilous character. The development of the Forestry Map has revealed its distribution, with nearly 2000 sites identified. It is shown as part of floodplain vegetation, and elsewhere in isolated patches where edaphic conditions allow.

The timber characteristics of this species are not highly regarded, though it has occasionally been used, similar to *P. nigra*. It has also been subject to attempts to eliminate it where farmland has come under competition from its invading roots. It is, however, appreciated from the ornamental and ecological points of view, and has been for some time considered suitable for riverside planting.

In Spain, varieties of the species with different morphological characteristics, have been identified. These are: *P. alba* var. *denudata*, in mountainous areas of southern Spain, sometimes confused with *P.* × *canescens*; *P. alba* var. *nivea* in the central part of the peninsula; *P. alba* var. *hickeliana*, found throughout the east of the peninsula (New Castile, Aragon and Andalusia); and *P. alba* var. *integerrima*, in south-western Spain.

Its ability to stand certain conditions of hydric stress and salinity makes it very valuable in improvements, both as a species and for the possibilities it offers for interspecific hybridization.

At species level, work has focused on the study of variation in a collection made up of families gathered in different regions of Spain (Fig. 3), with particular attention to the Ebro basin and south-eastern Spain (Almería); tests have also been carried out for salinity tolerance. The collection obtained (Alba and Navalón 1992) consists of 24 families, originally with 40 individuals per family, though at present only about 30 per family remain.

Isoenzyme study of variation

In this study a total of ten systems and 14 families were analyzed. Because of analysis problems, an initial study only took four systems into account (ADH, GOT-1, SKDH, PGM). These analyses have shown a high genetic diversity, both within and between families. The variation study was carried out by estimating the allelic frequencies with the pollen frequencies (eliminating the maternal contribution within the families). The processed data allowed an estimation of the diversity within and between populations, arriving at higher values than those shown in other *Populus* varieties. They also show a within-population

structure with little genetic exchange, which may favour the fixing of certain rare alleles. In order to complete this study with the other systems still awaiting interpretation, controlled crossings were carried out, which in some cases have led to the solving of interpretation problems (Alba *et al.* 1997).

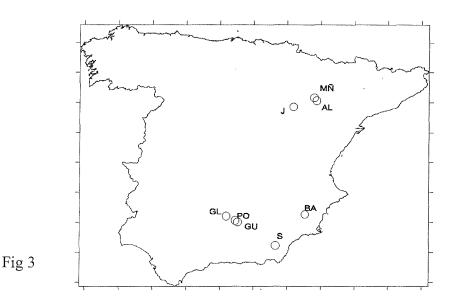


Fig. 3. Location of natural stands where *Populus alba* families were collected in Spain. AL, Ebro River; MÑ, Gállego River; J, Jalón River; PO-GU, Guadalquivir River; GL, Guadalimar River; BA, Segura River; S, Imanzora River.

Tolerance to salinity

Twelve *P. alba* clones were subjected to different irrigation doses, with conductivity of 7.89 dS/m and 14.15 dS/m. After 70 days of irrigation, neither growth nor survival of the clones considered the most resistant were affected, even in the case of the highest dosage (Cuevas *et al.* 1997). A new salinity resistance study has been started this year on seven *P. alba* clones, two *deltoides* x *alba* hybrids, two Euroamerican hybrids and one *P. euphratica* (propagated from mixed clones due to the problems in normal propagation of this species).

Interspecific hybrids

Another study carried out on this species has focused on the hybrids of *P. alba* and *P. deltoides*. Although crossbreeding was carried out in both directions, with *P. alba* as both male and female, the result was much better with *P. deltoides* used as the mother (Alba 1992). These clones are in process of evaluation in different plots.

The white poplar, like *P. nigra*, is considered useful for the recovery of riverbank sites, and there are different types of subsidy available for establishing populations. Thus in recent years there has been a slight increase in the planting of this species.

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In situ conservation of Populus nigra in the riparian ecosystem

Introduction

Long-term *in situ* management is a priority for the conservation of forest genetic resources. As one of their tasks, the EUFORGEN Networks develop technical guidelines for *in situ* conservation (e.g. Koski *et al.* 1997, for Norway spruce). For *Populus nigra*, an integrated *ex situ* and *in situ* strategy was recommended by the Network (Frison *et al.* 1995; Lefèvre *et al.* 1998).

Because black poplar populations are highly dependent on the dynamics of the riparian ecosystem, *in situ* conservation of genetic resources concerns both population and ecosystem management practices.

The following papers address two main topics concerning *in situ* conservation and restoration of *P. nigra* resources within the riparian ecosystem. Research on this species is more recent than for other forest tree species, and the management of riparian forests has not received much attention. Nevertheless, we tried to draw minimum requirements for *P. nigra* on the basis of current knowledge. These technical requirements will not all be immediately operational in every country. We also tried to identify key research points which are needed to implement such a strategy.

These papers were prepared by only a small number of authors, but they rely on the fruitful discussions among all Network members since 1994, lastly during the Fifth *P. nigra* Network meeting in Kyiv, Ukraine (see List of participants, at the end of this volume).

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Indicators for monitoring the evolution of *Populus nigra* genetic diversity *in situ*

François Lefèvre and D. Kajba

The definition of indicators for monitoring *in situ* conservation of the genetic diversity is becoming a focal question for research. Obviously, *in situ* conservation is primarily governed by the biological features of the target species, and general concepts have to be implemented on a specific basis. In this paper, we try to adapt the general concepts developed by Savolainen and Kärkkäinen (1992), Namkoong *et al.* (1996), Brown *et al.* (1997), Palmberg-Lerche (1998), and Thomson *et al.* (in preparation) for the case of *P. nigra*.

P. nigra is a strictly pioneer tree species, part of the dynamic and complex riparian ecosystem. Therefore, the dynamics of the species are closely linked with the dynamics of the ecosystem, which in fact means the dynamics of the river itself: many indicators of the genetic evolution of the species are expected to come out of ecological observations. Furthermore, dealing with a pioneer species which can only be maintained through perturbation of the ecosystem, the concepts of 'stability' and 'resilience' cannot be applied locally, but at a higher scale: we have to consider metapopulations rather than populations. Different scales for *in situ* management can be identified: firstly, the conservation unit, a local site which is often structured into different forest associations where *P. nigra* is represented, and secondly the network of *in situ* conservation sites. The overall objective of maintaining the genetic diversity refers to the whole network of *in situ* conservation sites, whereas monitoring is mainly, though not only, achieved within each site. Other important characteristics of the species are its dioecy (strict allogamy), the possibility for vegetative propagation and the geneflow with cultivated hybrids or *P. nigra* varieties (few clones, broadly distributed).

General concepts and application to *P. nigra*

We can follow the general approach in three steps proposed by Namkoong *et al.* (1996) to monitor *in situ* conservation and apply it to *P. nigra*.

Identify the processes which govern the evolution of genetic diversity

- Demographic processes: *local extinction* (needed for re-colonisation), *age structure* (interfering with drift, mating system).
- Genetic processes: *drift* (random effect on gene frequencies and differentiation in finite populations related to demographic aspects), *selection* (directional change of gene or genotype frequencies due to environmental conditions or human activities), *mating system* (inter-generation process of evolution, inbreeding), *migration* (exchange of seeds and pollen among populations or complex genepools). (Mutation is considered here as out of the scope of monitoring, as a first approximation.)

Identify the forest practices that can affect these processes

- Water management.
- Forestry and landscape management.
- Others.

Determine criteria that should be achieved for long-term conservation of genetic diversity in situ, determine indicators to follow these criteria, and verifiers of these indicators

- Ecological indicators.
- Demographic indicators.
- Genetic indicators.

Land managers need to deal with multiple objectives, and this is particularly true for riparian forests. At the European level, riparian habitats are preserved for their biodiversity (which includes poplar and other tree species, but also insects, mammals, birds, or other plants that may receive higher priority), for their role in water regulation and effects on water quality. Thus, it becomes evident that management practices cannot be governed by the single objective of conservation of *P. nigra* diversity. To implement a policy, several management practices are carried out, each of these having several impacts on the global environment, including multiple consequences for *P. nigra* diversity (Fig. 4).

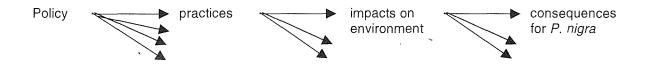


Fig. 4. Management of *P. nigra* conservation sites: from the decisions to their consequences.

An example of the possible impact of management practices is given in Table 8. Indicators should be used throughout the decision process for the evaluation of the impact of practices on *P. nigra* diversity. It has so far only been possible to identify possible indicator variables. Further research is therefore needed to define the scale of measurement for each variable, and, even more complex, determine the threshold values.

Choice of indicators

Ecological, demographic and genetic indicators are proposed that could be relevant for monitoring *in situ* conservation of *P. nigra* genetic resources (Table 9). These indicators are related to, and provide information on the processes of evolution of the genetic diversity. As stated by Brown *et al.* (1997), a single indicator is generally related to several processes, and the study of individual processes requires the use of a set of indicators. Different indicators may require different levels of technical and financial investment; however, all indicators listed here are considered to be realistic. These indicators concern the monitoring of each conservation site in the long term. Other specific indicators would be needed for the monitoring of the network of conservation sites as a whole (structure of diversity among conservation sites etc.).

impacts		
Practice	Impact on ecosystem	Impact on <i>P. nigra</i>
<i>Water management</i> Stabilization of the water regime in rivers	Stabilized riverside Decreased water table Changed soil texture	Colonization Health conditions
Prevention of flooding	Stabilized riverside Exceptional floodings avoided	Colonization Demographic structure
<i>Extraction</i> Extraction of solid material inside riverbed	River erosion Stabilized riverside	Colonization
Extraction of solid material outside riverbed	Bare soil exposed	Colonization
Extraction of groundwater	Decreased water table	Health conditions
<i>Ecological engineering</i> Extraction of the organic soil layer	Bare soil exposed Nature development supported	Colonization
<i>Forestry and landscape management</i> Timber logging <i>P. nigra</i>		Demographic structure Selection Introgression
Timber logging cultivated poplars (<i>P. nigra</i> and hybrids)		Introgression
Planting wild <i>P. nigra</i> (restoration)		Population size Geneflow
Planting cultivated poplars		Introgression
Coppicing <i>P. nigra</i>		Flowering Health conditions
Thinnings in riparian forests	Stand density	Flowering Selection
Other uses		
Grazing	Herbaceous layer	Colonization Competition
Game management	Herbaceous layer	Colonization Competition
Recreation	Anthropic damages	Colonization Health conditions

Table 8. Examples of management practices in the riparian ecosystem and their multiple possible impacts

Ecological indicators

Ecological indicators are obtained by looking at the whole ecosystem. They provide information about long-term perspectives on a large scale (e.g. evolution tendencies of the whole riparian ecosystem) and about demographic and genetic processes (e.g. suitability of the site for generative regeneration of poplar). Possible indicators may include:

- The frequency of flooding provides information on potential local extinction, potential seedling recruitment and the possibility for young cohorts to reach the adult flowering stage (ageing). Compared with exceptional flooding, annual flooding events do not allow the seedlings to reach adult stage.
- The soil texture, interacting with water regime, provides information about the possibility for seedling recruitment (Barsoum and Hughes 1998).
- Indicator species may be related to the long-term dynamics of the ecosystem; they can also provide information about ageing.
- The forest associations where *P. nigra* is present, related to the phases of the dynamics of the ecosystem, are also indicative of demographic aspects (different age classes in different associations) and mating system (variation of flowering intensity in different associations).
- Aggressive species may represent strong competition for colonisation even when ecological conditions are suitable for seedling recruitment (e.g. *Robinia pseudoacacia*).
- The amount of cultivated varieties (hybrids and pure *P. nigra*) in the vicinity provides information about potential geneflow with cultivated genepool and possible impact on effective population size (mating system).
- The populations of major parasites may have significant impact on the natural selection and the potential seedling recruitment.
- The exploitation of the riparian zone (logging, grazing, etc.) may be related to demographic structure, natural selection, mating system and potential seedling recruitment.

Demographic indicators

Demographic indicators are obtained after specific observation of the *P. nigra* population. They provide information about current demography and tendencies. They can also be related to the genetic processes and may include:

- The occurrence, quantity and spatial distribution of seedlings directly provide information about the possibility of effective recruitment (without vegetative propagation).
- The demographic structure in the conservation site (age classes) and its spatial organization, related to the population size, provide information about mating system and potential drift effect.
- The number of flowering trees, their investment in flowers (related to tree size and local density), the sex ratio and variation in flowering phenology directly provide information on the effective population size, subsequent mating system and potential drift.
- Die-back and sanitary conditions are clear indicators of the long-term dynamics of the *P. nigra* population.

Genetic indicators

Genetic indicators require the observation of *P. nigra* genome. They are directly related to the genetic processes of evolution. They require specific technical aids, but most technology is now available for *P. nigra*. They include:

• The level of diversity can be assessed on genetic markers (gene/genotype frequencies) or adaptive traits (additive variance), and it provides information on the effect of genetic drift, mating system (including the potential effect of vegetative propagation on the effective population size) and selection. (Direct

observation of vegetative propagation is only possible at a very juvenile stage, when it has no effect on the mating system, therefore it was not included in the list of demographic indicators; the detection of vegetative copies among adult trees, which has an effect on the mating system, generally requires the use of fingerprints.)

- Differentiation among age classes provides information on drift and possible recent bottleneck effects, and on selection and migration processes.
- Differentiation among stands provides information on drift, geneflow and selection at another spatial scale.
- Introgression, which could be defined in this case as gene exchanges with the cultivated genepool (either *P. nigra* cultivated clones, or interspecific hybrids), is related to geneflow and to the effective population size.

Recommendations for *in situ* conservation of *P. nigra* and questions for research

At present it is only possible to give some general recommendations on the monitoring of the whole network of *in situ* conservation sites. As individual countries are responsible for the conservation and use of the genetic resources in their territory, diversity should be sampled at the national level. An international network composed of priority conservation sites identified by the countries could then be promoted. Each conservation site should be self-sustainable in the long term, although – in theory – this is not needed because some sites may disappear as long as the diversity is maintained at the level of the network. This is, however, not realistic since land management objectives cannot be changed easily, and once a site is dedicated to *P. nigra* conservation we would like to preserve it. For selecting such sites, even a preliminary evaluation of the *ecological* and *demographic* indicators on a large scale can easily provide valuable information on the potential processes of evolution in *P. nigra.* Such information can be obtained from the list of stand descriptors established by the Network (Alba 1999, in press). Concerning the size of each conservation site, it should be large enough to include all demographic stages for the species (adult trees and seed progeny), and large enough to be able to absorb geneflow from outside without major impact on the genetic diversity. Due to the particular riparian ecosystem, conservation sites will never be totally 'closed'. The question of 'what is large enough?' is still left to research.

In a further step, monitoring *in situ* conservation sites relies on a precise evaluation of the possible impact of the management practices that are planned (in a multiple objective policy) on the follow-up of present genetic diversity and possible changes in the processes of its evolution. This requires a more intensive use of indicators: evaluation of *ecological* and *demographic* indicators on a smaller spatial scale, and use of *genetic* indicators. Defining measurement scales and threshold values for each indicator becomes crucial in order to optimize their efficiency and minimize the management cost. As stated earlier, research is needed to determine these values, although some key features of black poplar population biology and its relation to the riparian ecosystem have already been established (see Bibliography Addendum at the end of this volume).

Different national and international research programmes are currently dealing with *P. nigra*. Among these, the EU/FAIR-EUROPOP project mainly addresses questions from the genetic point of view (van Dam and de Vries 1999; http://www.ibn.dlo.nl/europop). The following questions at least need to be addressed:

- What is the structure of genetic diversity at species range level?
- What is the scale of geneflow along a drainage (size of the metapopulation)?
- What is the effective population size of a *P. nigra* stand?
- What is the effective impact of introgression with cultivated poplars (hybrids or *P. nigra* varieties)?

	· · · · · · · · · · · · · · · · · · ·	Related process of evolution				tion	
Indicator	Directly related phenomenon	EX	AS	DR	MS	MI	SE
Ecological indicators	<i>Dynamics of the ecosystem, demographic and genetic proceses</i>						
Frequency of floodings	Long-term dynamics, potential seedling recruitment, potential ageing	Х	Х			Х	
Soil texture	Potential seedling recruitment					Х	
Indicator species	Long-term dynamics, potential ageing	Х	Х				
Aggressive species	Competition					Х	
Cultivated varieties	Potential introgression				Х	Х	
Major parasites	Potential seedling recruitment, selection					Х	Х
Forest exploitation	Potential seedling recruitment, age structure, selection		Х		Х	Х	Х
Demographic indicators	Demography, demographic and genetic processes						
Seed regeneration	Seedling recruitment					Х	
Age classes	Population size, ageing		Х	Х	Х		
Flowering trees	Population size (number, sex ratio, phenology)				Х		
Sanitary conditions	Long-term dynamics, selection	Х					Х
Genetic indicators	Genetic diversity, genetic processes						
Level of diversity				Х	Х		Х
Differentiation among age classes within stand				Х		Х	Х
Differentiation among stands				Х		Х	Х
Amount of introgression					Х	Х	

Table 9.	Indicators	and their inforr	nation value	related to	processes	of evolution	of P.	nigra diversity	y
									-

EX, extinction; AS, age structure; DR, drift; MS, mating system; MI, migration; SE, selection

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In situ restoration genetics of riparian populations of P. nigra

Berthold Heinze and François Lefèvre

Populus nigra is not an endangered species in the sense that only a few individuals are left and might disappear soon. The situation is rather that the number of populations is decreasing, that they are more and more fragmented, and that the turnover of generations is slowing down, all because of threats to the ecosystem. As a pioneer species, a high migration rate is probable, and the scale of its adaptation to local conditions is as yet unknown. As a dioecious outbreeding species, a certain level of genetic load is expected which could make it sensitive to a sudden increase of inbreeding.

Restoring populations of the species where riparian areas or indeed the riparian ecosystem have disappeared is worth consideration because there are large genebanks and long-term breeding populations available, the species is easily handled, and restored popular populations would generally contribute towards the restoration of an ecosystem.

Theoretical approaches

The concept of the minimum viable population size (MVP) for trees considers a population relatively safe from the risks of extinction because of genetic and demographic, environmental, etc. reasons if it has a minimum size that keeps those risks at an acceptable low level over a certain period of time. For example, a standard model population of 50 flowering trees (in monoecious species under panmixia), and their direct descendants in a population of constant size, is likely to retain 99% of their original genetic variation in terms of allelic richness over the next 100 years, and therefore is likely to survive if only genetic causes are considered (Lawrence and Marshall 1997). General recommendations for long-term minimum viable population sizes in trees which are based on such theoretical considerations suggest numbers of at least 500–2000 trees (Geburek 1992; Lynch 1996).

Genetic drift - the random loss of genetic information which is most obvious in small populations – also affects quantitative genetic variation. Young et al. (1996) point out that, on the one hand, the additive component of variance decreases as the effective population size decreases. A loss of 1% of additive genetic variance, considered safe on the basis of animal breeding experience, would make an effective population size of 50 seem suitable. On the other hand, however, due to fixation of alleles in small populations, genetic interactions contributing to non-additive variance will decrease, and hence additive variance may actually increase. Total prevention of the loss of any genetic variation would require infinite population sizes. In tree species in general, the population size may fluctuate (expand or contract) in the foreseeable future (i.e., it is unclear whether there will be suitable conditions for seedling establishment and survival in the long term), while most models in population genetics deal with constant population sizes. Seen over the course of several nonoverlapping generations (which is again a non-realistic assumption for many tree species), the inbreeding effective population size for example is determined by the smallest generation (harmonic mean over generations). All these considerations disregard mutation as a mechanism for creating new variance, the effect of which is difficult to estimate.

More specifically, these numbers are influenced by the following factors:

- the sexual system of dioecy;
- the discrepancy between genetically effective population size, i.e., successfully flowering trees and census size;
- populations vary in their respective degrees of genetic relatedness of the plants (important to minimize future inbreeding);
- the generation time, compared to other tree species, is relatively short.

While most of these factors increase estimates for MVP, the existence of genetic back-ups (*ex situ* collections of clones and seed sources) will make the effects of the loss of trees at a restoration site less dramatic.

The concept of the Multiple Population Breeding System (MPBS; Eriksson *et al.* 1993, 1995) develops arguments in favour of subdividing larger populations into a number of smaller subpopulations. A minimum of 50 unrelated clones in each of at least 20 subpopulations is recommended for most monoecious tree species (for many parts of Europe, this requirement is already difficult to put into practice). The subpopulations are managed for rapid adaptation to different environmental conditions and/or selection regimes. This increases among-population variation, gives more emphasis on low-frequency alleles and usually speeds up evolution. Similar effects can also be achieved in larger *in situ* reserves harbouring patchworks of environmentally contrasting sites.

A more practical approach is to look for apparently isolated populations, count the number of clones, and assess the viability of their offspring (Mosseler 1998). Populations on the verge of genetic problems will produce offspring of poor viability, compared to populations safely above the threshold size. Gliddon and Goudet (1994) point out that there are actually a number of different 'effective population sizes': those affecting inbreeding, variance and extinction. They also argue that in the short term, demographic criteria are the most relevant for conservation, as catastrophes may strike long before the effects of inbreeding become visible after a number of generations. Roberds and Bishir (1997) calculated that in clonal forestry, in the short term, genetic risk can be minimized by employing more than 30–40 clones (regardless of rotation regime). Such a number of unrelated clones safeguards against catastrophic diseases to a similar degree as do much larger populations.

From all these considerations, the following points deserve attention:

- Genetic variation includes additive components (gene diversity), and nonadditive components (gene interactions, co-adapted gene complexes).
- Variation *per se* is not the objective, but rather adaptability, i.e. the potential for adaptation, which is related to potential genetic variation (the ability to recombine the genetic information that is present in the parental population into a very large number of different genotypes in the offspring).
- As a dioecious species, *P. nigra* probably has an important genetic load of deleterious alleles. In large populations and with a balanced mating system, this high level of genetic load can be sustained, especially by the immense number of offspring that is produced and offered to the action of natural selection. In small populations and populations and with unbalanced mating systems, the species could thus be susceptible to a rapid and drastic increase of inbreeding (the so-called 'vortex of extinction': the reduced effective population size increases inbreeding, which reduces mean fitness of the population, which may lead to further reduction of population size, etc.).
- Non-additive components may be more important for strictly allogamous species, 'buffering' a little the reduction of genetic variation after reduction of gene diversity.

On the basis of these premises, the following priorities can be proposed:

- 1. Avoid the risk of catastrophic destruction (demographic stochasticity).
- 2. Limit the risk of reduction of fitness, generally by avoiding inbreeding in the restored populations (therefore, one should focus on the inbreeding effective population size, rather than others).
- 3. Avoid a too drastic reduction of diversity.

Examples from breeding of captured wild animals in zoos give quite a good outlook for sustaining populations against the effects of inbreeding. Animals are also 'dioecious' and breeding programmes often start with very small numbers. Designing proper mating strategies is very important. In the case of *P. nigra*, this means careful selection of clones and design of the planting at the sites of restoration.

It should always be remembered that in *P. nigra* these numbers mean *clones*, not trees. Populations sometimes consist of considerably less clones than trees. While some of the numbers introduced above seem extremely high, it should be noted that the adverse effects of small populations only strike in totally isolated populations, and only after a great number of generations. Nevertheless, once loss of genetic variation has started in the first generations, it cannot be reversed without mutation, which is not considered by the models.

Risks for restored populations

Genetic risks for isolated populations include inbreeding and loss of genetic variation, and 'flooding' by hybrid poplar and var. 'Italica' pollen or seed. For example, loss of genes or alleles might predispose the plants in a restored population towards certain disease races. Non-genetic risks include catastrophes like severe flooding, drought and other climatic abnormalities, game browsing, spread of diseases against which there is no resistance, and so on. Comparing the latter group of risks to the genetic ones, it seems much more likely that we lose a population through such catastrophes, than on genetic grounds alone (see above). However, risks are always associated with probabilities, so even a low risk means that the adverse event may take place. It is certainly prudent to prepare for both types of risk, genetic and non-genetic, with due consideration in a given restoration project.

The most effective way to counter such genetic risks is to allow for 'migration', i.e. the exchange of pollen and seed with neighbouring *P. nigra* populations. In a less obvious sense, migration also takes place if additional clones are planted at the site, or if nearby younger stands come into flowering age. It follows that given a certain number of plants available for a restoration project, it may be wiser to plant them over a number of places, for example, along the same river, so that they are still in 'genetic contact' via pollen and seed transport, but less vulnerable to catastrophic events.

This is mimicking to some extent what seems to be the strategy of poplars to fight off such adverse stochastic effects: to produce very large seed crops and disperse them widely. To assess risks for a restored population of *P. nigra*, we therefore also need to consider:

- the degree of isolation of a given population (pollen and seed export and import of other true *P. nigra*); and
- the likelihood of losing a whole population because of a single catastrophic event.

With respect to 'isolation', the degree of genetically effective isolation from hybrid poplar and var. 'Italica' introgression is also important.

Practical approach: what material is available and how is it best employed?

It is obvious that numbers for MVPs in trees are extremely high, probably higher than we can at present put into practice in *P. nigra*. However, the assumptions on which the theoretical considerations are based may deviate substantially from what we plan to do. It is not very sensible to restore riverside populations where they will have no more contact with neighbouring stands of the species, where they cannot spread or increase their numbers at least in the seedling generation, and where nobody will care for them. Factors for consideration in this respect include:

- the origin of the material (adaptation to local climate etc.);
- the genetic variation in the material (full-sib family, open pollinated families of how many mother trees, how many potential pollen donors, or presumably unrelated clones, etc.);
- the degree of 'tender loving care': will the planting be monitored, and whether there will be management measures should anything go wrong; and
- over- and under-representation of individual clones in partially clonal stands.

Considering relatedness among clones, it is important to have variation at different levels:

- clones (their nuclear genes; as discussed above); but also
- cytoplasm: cell organelles and their genetic provide information are only passed on through seed, not through pollen. Certain genetic factors, as yet unknown, may be transmitted by cytoplasm only. Disease resistance factors in maize are an example taken from plants. In this example all clones that are descendants of a susceptible maternal line will also be susceptible; and
- families: interrelated families harbour less variation than families obtained by breeding independent parents. An extreme example of related families is a topcross (pollination of many females with pollen from the same male). The inbreeding effective population size in topcross equals 4, approximately.

Available options for sources of plant material in restoration projects fit into a scheme (see Table 10), as discussed at the Network meeting.

Source	Local adaptation*	Unrelatedness (presence of parents plus offspring or siblings)	Number of genotypes available
National collection	+/		++
Local collections	++	<u> </u>	+/*
Seed collections	÷	_	+
Controlled crossings of <i>P. nigra</i>	÷ .	_	_
Clones from neighbouring regions	+/	+	+
Range-wide collections		++	++

Table 10. Comparison of reproductive material sources for the restoration of P. nigra populations

+ and - indicate advantageous and disadvantageous points, respectively

*Research needs were identified in these areas.

For example, national collections, which typically consist of 100–500 clones, are mostly 'unrelated' and 'diverse', but not always suitable for all sites in a country. Clones from a neighbouring region of another country might be more suitable (quite often, rivers form borders between European countries, and clones from each side of a given river are suitable for restoration on either side). Introduction of clones from further afield, such as through the EUFORGEN core collection of clones, brings with it the risk of the breakdown of co-adapted traits encoded by more than several genes working together (e.g. bud flush and winter hardiness), should they exist, in later generations (Lynch 1996). This would be especially true for species exhibiting outbreeding depression. There is currently not sufficient data available in the literature, to assess whether there is outbreeding depression, especially in later generations in *P. nigra*. This type of data may, however, be present in the files of national breeding programs (e.g. Pichot and Teissier du Cros 1988).

Unfortunately, many question marks remain. Higher clone numbers safeguard better against unwanted effects; therefore, for a given area to be planted, use the highest number of clones reasonably possible. On the other hand, if the number of clones is limited, but plenty of space available, planting many trees from each clone will minimize the risk of losing a clone altogether. The pattern of mixture is also important. Intimate mixtures of clones will give competitive clones an advantage, while mosaics of monoclonal plots will give slower growing clones a better chance to survive and reproduce effectively.

A further point for consideration is how many plants should be produced from a single clone. A geneticist's answer is simply as few as possible. It is advisable to turn over clones in the base collection from which plants for restoration projects are produced quickly. The clones should not be used for too long in an increasing number of restoration projects. Of course it is tempting to use the same stool-beds as long as possible. If several sites are to be planted with material from the same source of clones, the use of different proportions of plants from individual clones should be considered. This gives clones with reduced juvenile growth and later onset of flowering a greater chance to pass on their genes. One may argue that such clones, being less fit, should perish anyway, because in the case of in situ conservation, natural evolution is more important than the conservation of favourable forestry-related traits (such as enhanced vegetative growth as opposed to the production of many offspring). A question in this respect is whether natural selection is too harsh to allow the population to survive. By slowing down its impact, the potential to form a great diversity of new genotypes is retained, and tolerant genotypes might evolve over time, especially in very small populations where, as an example, the late-flowering genotypes considered above might also, by chance, harbour unlinked genes important for other traits. These hypothetical genes would then be lost before being presented to selection (in the form of, for example, a pest that only appears sporadically in a stand's lifetime). The number of new genotypes that can be formed depends on the effective number of gametes that is formed. A genetically more reasonable strategy is to substitute the original clones by seedlings from established restoration sites by and by.

Mixing different plant sources for restoration projects will sometimes be an option, for instance because there are not enough local clones available. The only real risk of mixing provenances is the unwanted breakdown of adaptive traits (see above). The general recommendation is not to transfer material over steep climatic gradients or boundaries (e.g., across major watersheds, over a wide latitudinal range), and not transfer over too long distances in a given climate. Depending on local geographic and climatic conditions, this distance could be between 100 and 500 km (on the basis of general findings by Farmer 1996; Pichot and Teissier du Cros 1988). If seed transfer zones are established, these may give a good indication. Considering these points, clones can be added from appropriate sources, but possibly only to a low percentage, and with lower representation of such clones compared with local ones. Such a strategy would mimic clinal variation which is typical for many long-lived, widespread tree species. Results of the EUROPOP research project will hopefully will provide more exact estimates for *P. nigra*.

Numbers

On the basis of all these considerations, a few very broadly defined recommendations need to be given – very tentatively, but there has to be a point at which to start.

For isolated restoration projects, use at least 100 unrelated clones (no full or half-sibs), with both sexes (if known) in equal proportions (Lefevre et al. 1998). If full or half-sibs are present, such families should not 'count' equally as unrelated clones – the variance effective population size is much lower. However, the probability of losing parental alleles is smaller in larger families. In other words, if such families of related clones are the only reasonably diverse source of plant material, many family members should be planted to conserve a high proportion of the parental alleles. They could be employed in numbers according to the scheme in Table 11, inspired by a similar approach to that of Lefevre et al. (1998) in sampling clones from residual populations, and the reduction in additive genetic variance (Young et al. 1996), as 'rules of thumb':

Table 11. Scheme for numb	er of clones required		
Plant source	Proposed calculation of corresponding number of unrelated clones	Numerical example: number of family member clones (x)	Corresponding number of unrelated clones (y)
Half-sib family harvested from a female among many males (1)	$y = [ln(x)]^{2.5}$	15 50	12.07 30.27
Half-sib family harvested from a female in an isolated small group of trees (2)	$y = [ln(x)]^2$	15 50	7.33 15.30
Full-sib family (3)	$y = \sqrt{x}$	15 50	3.87 7.07

The unrelated parents of 100 clones constitute a genetically effective population size [N(e,p)] of 200. They will conserve 99% of the additive variance [v(a)] in the 100 clones offspring (when compared to 100 parental clones).

- 1. More than 10 males. N(e,p) is approx. 4 (Geburek 1992), loss of v(a) is around 12.5 % (Young *et al.* 1996)
- 2. 3–10 males. N(e,p) = 3-4, loss of v(a) = 12.5-17%
- 3. N(e,p) = 2, loss of v(a) = 25%

Fig. 5 shows how these numbers develop with increasing numbers of related clones.

As pointed out before, there should be a range of clones, cytoplasms, and families present at any isolated restoration site. If there is a fair chance for the restored population for genetic contact with other populations of *P. nigra* trees, the overall minimum number of clones could be reduced to 50. If the chances for genetic contact are considered low to moderate, a number of around 75 clones will probably be enough. In a situation of high genetic contact among previously established and newly planted trees, these together form a breeding unit (population). It follows that restoration plantings can be successful if they 'top up' the numbers of already existing trees to a level above the ones recommended above. In other situations, it may be possible to 'link' existing trees genetically by planting new clones in between, for instance in line plantations along roads or rivers.

The situation is more difficult where not enough local or national clones are available for such a restoration planting. Here, the existing *ex situ* collections become even more important and should be closely interlinked with restored populations. The populations to be planted could be composed of:

- the available local/national clones in varying proportions at different sites; or
- mixtures of local/national clones and introduced clones of neighbouring regions with comparable climatic conditions.

Genetic exchange ('artificial contact') among the newly established populations and the *ex situ* collections could be achieved by waiting for the onset of flowering and then exchanging seedlings among the individual populations, and with the *ex situ* clone banks. Poorly flowering clones could be replanted to give them a fair chance of passing their genes on to a new generation. By constantly monitoring, increasing and supplementing the genetic make-up of such populations, it may be possible to counteract the deleterious effects of gene loss. Theoretically, the only way to increase genetic diversity is to allow for 'migration' (genetic exchange) and mutation, while at the same time keeping 'genetic drift' (random loss of genetic variants due to stochastic processes) at a minimum.

It is always important for future generations of *P. nigra* trees, when near the thresholds in population size, to be able to 'increase and multiply'. Production and establishment of a dense seedling population is the most effective way to keep a high number of genetic variants 'alive'. Further monitoring and active management of the restoration projects is certainly a key to the long-term success. A 'plant and walk away' or 'leave it all to Nature's inherent wisdom' strategy is probably very romantic, but inappropriate where we are already below the levels of population sizes considered safe for a tree species. Management measures should include replanting of poorly flowering clones, corrective thinning, new additions to and from the genebanks, and cutting down clones that turn out to be genetically unsuitable because of introgression or insufficient adaptation.

All this could be done in a concept of 'constantly decreasing intervention', that is, reducing active silvicultural management if monitoring indicates that survival and reproduction, and especially the quality of the offspring, is acceptable. It can only be hoped that over the years, our knowledge of the parameters and processes that influence the long-term survival of tree species accumulate, and that our efforts today suffice to keep *P. nigra* 'alive and kicking' for that time.

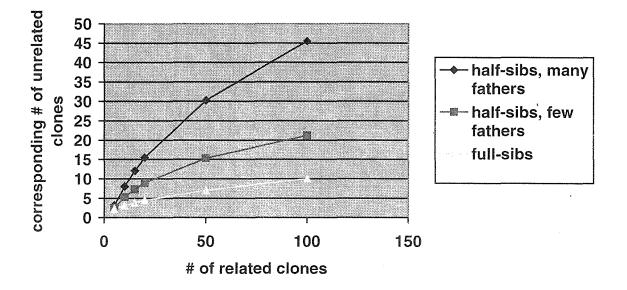


Fig. 5. Relationship between related and unrelated clones.

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Case study: Inventory and characterization of *Populus nigra* resources within nature reserves in France

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Introduction

The French programme for the conservation of black poplar combines *ex situ* and *in situ* strategies. The management of the national clone collection is fully operational; it follows the recommendations of the EUFORGEN Network. The National Commission for Forest Genetic Resources is currently setting up the *in situ* approach.

The methods for *in situ* conservation of a pioneer tree species such as *Populus nigra* differ fundamentally from those used for climacic species like beech or fir: the scale of management is the ecosystem in its whole complexity, rather than a self-regenerating forest stand only. Moreover, the few remaining riparian forests which include black poplar are rarely managed by the Forest Service. For these two reasons we developed a new approach to *in situ* conservation, based on the idea that a significant resource of *P. nigra* might already be preserved indirectly within protected areas. We therefore undertook this survey of *P. nigra* resources in the Nature Reserves and Voluntary Nature Reserves, which both have a perennial status. This is also a first link in our country between the programmes of conservation of sites and conservation of gene resources.

A first network of *in situ* populations will be formally established from this inventory. The *in situ* network can then be enlarged in order to have a better representation of the diversity; for that next step other protection zones could be involved.

The results of this survey are presented here in some detail as they represent the first use of the EUFORGEN list of stand descriptors on a wide scale, rather than by specialists, in France.

Methodology

The standardized minimum list of descriptors for *P. nigra* stands established by the EUFORGEN Network (Alba 1998) was sent to 130 Nature Reserves and 116 Voluntary Nature Reserves throughout the country. Two questions were added concerning forest associations where *P. nigra* and accompanying species are found; additional information was also recorded concerning the situation along the river drainage, and along transverse transects. The EUFORGEN identification sheet and an illustrated leaflet were attached to the questionnaire in order to inform site managers about the aims and perspectives of the project.

The site managers were asked to fill in only one file per reserve (some questions had a multiple response as poplar can be found in various conditions on a single riparian site). If the reserve area itself did not contain *P. nigra* but the species was found in the vicinity, the managers were also asked to provide information on these areas. The questionnaire was sent in June 1998, and a reminder was sent in October 1998. Most responses were obtained by December 1998. Answers to the questionnaire were stored in a database and analyzed.

Results

Answers to the questionnaire

A total of 99 answers were received: 84 from Nature Reserves (65%), 13 from Voluntary Nature Reserves (10%), and two from Biotope Protection Decrees.

According to what is known of these reserves, we are confident that most of the managers of sites containing *P. nigra* did respond; most of the non-responders were managers who were not concerned by the species and did not take the time to return the questionnaire.

Presence of Populus nigra within reserve areas (Fig. 6)

Of the 99 responses received, 29 indicated the presence of *P. nigra*, and 70 that poplar was totally absent. It should be noted that 90% of the areas containing black poplar are found in the south-eastern half of the country, mainly along the great drainage (Rhône, Loire-Allier, Rhin, Doubs, Garonne). This survey concerns mainly the occurrence of poplar stands, even small. However, when looking for isolated trees to enlarge the *ex situ* collection, we also noticed the rarity of the species in the north and west of the territory.

Population structure

Note: in the following tables, the figures indicate the number of sites which gave the particular answer to the questionnaire (total of 29, unless specified when multiple answers were accepted).

Structure of the riparian forest where poplar is found (Fig. 7)

In most cases (65%), *P. nigra* is found in mixed stands. Poplars are mainly found as scattered trees or in lineal forests (< 50m) (Table 12).

Forest type	No. of sites	Species purity	No. of sites
Scattered trees	_ 17	Pure stand	4
Lineal forest	10	Mixed stand	19
Riparian forest	8	NA	6
Not available (NA)	4		

Table 12. Forest type and species purity in the riparian forest

On the maps showing either the area of the riparian forest, or the approximate number of adult flowering poplar trees, we can identify a small number of major sites for the species (Loire and Allier, Rhône, Rhin) (Table 13).

Table 13.Major sites for P. nigra

Area of riparian forest	No. of sites
<10 ha	3
10–20 ha	2
20–50 ha	4
50–100 ha	1
>100 ha	4
NA	15

No. adult trees	No. of sites
1–10	2
10–100	7
>100	6
NA	14

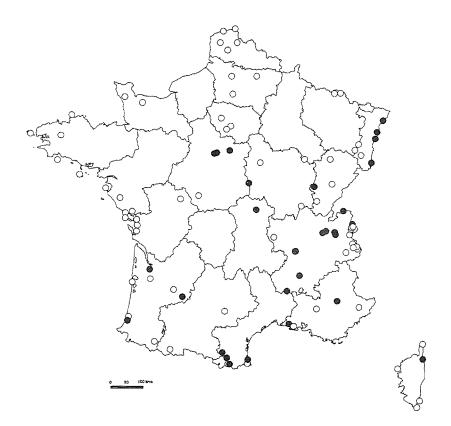


Fig. 6. Presence of *P. nigra* within Nature Reserves [$\bullet = P$. *nigra* present (29); O = P. *nigra* absent (70)].

Concerning the typology of riparian forests in these reserve areas where *P. nigra* is represented, the main associations were *Salicion albae* and various forms of *Ulmenion minoris* (Table 14).

Table 14.	Typology	of riparian	forests

	Associations	No. of sites where found*
Willow coppices	Salicion eleagni, Salicion triandrae	3
White willow forests	Salicion albae	15
Black poplar forests	Populion nigrae	3
Alder forests	Alnenion glutonoso-incanae	4
White poplar forests	Populion albae	1
Hardwood riparian forests	Ulmenion minoris	14
Edge of alluvial dry meadow	Festuco brometea, Geranion sanguinei	3
NA	<u> </u>	7

*Multiple answer allowed

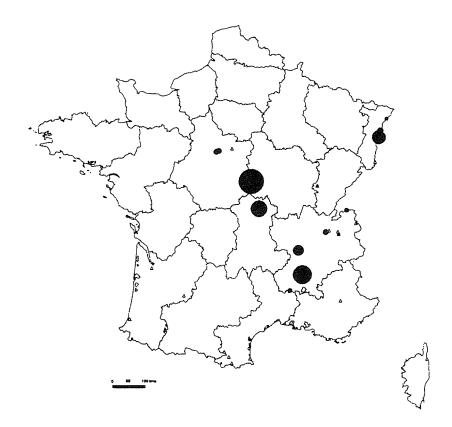


Fig. 7. Protected areas including *P. nigra* (\bullet = 20 ha; \bullet = 100 ha; \bullet = 200 ha; Δ = no answer available).

Characterization of the *Populus nigra* population (Figs. 8 and 9)

Concerning the demographic structure of *P. nigra* populations within these riparian sites, information was more difficult to obtain. In all cases when information was provided about sex (10 reserves out of 29), both male and female trees were mentioned (Table 15).

No. of cohorts	No. of sites	When >1 cohort*	No. of sites
1	1	Mixed cohorts	7
2	6	Spatial structure	3
4	1		
NA	21		

Table 15. Demographic structure of the P. nigra populations

*Multiple answer allowed

The presence of remarkable trees was mentioned in 38% of the sites: these are not always 'plus trees' but more generally can be remarkable for their age or any singularity. The size of dominant trees is approximately estimated in Table 16.

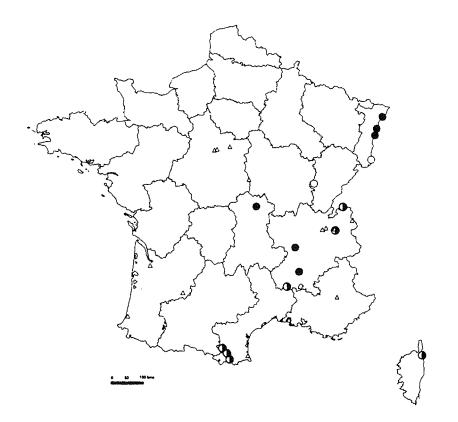


Fig. 8. Number of adult *P. nigra* trees (open circles = 1–10 flowering trees; half-shaded circles = 10–100 flowering trees; closed circles = more than 100 flowering trees; Δ = no answer available).

Table 16. Size of dominant to	rees
-------------------------------	------

Diameter (cm)	No. of sites	Height (m)	No. of sites
<50	1	≤15	4
50–100	10	20	4
≥100	2	≥25	6
NA	16	NA	15

The occurrence of regeneration, either through sexual or vegetative propagation, was mentioned in 48% of cases. Moreover, the questionnaire asked for information on the presence of 'favourable conditions for regeneration', without definition of these conditions, simply based on the manager's own experience (see Table 17 and Fig. 9).

Table 17. Occurrence of regeneration

Natural regeneration	No. of sites
Sexual regeneration alone	7
Vegetative regeneration alone	2
Both sexual and vegetative	6
No regeneration or NA	14

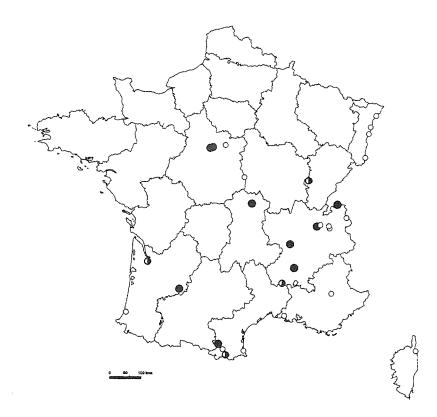


Fig. 9. Conditions for natural regeneration through seedlings (half-shaded circles = suitable conditions but no seedlings; closed circles = suitable conditions and seedlings; open circles = no suitable conditions).

Health status and disturbances

Concerning sanitary conditions, information was scarce, probably due to the fact that little attention has been paid to *P. nigra* until now in these reserves. However, defoliation was mentioned, and decrease of water table was proposed as a possible explanation. On the two sites where significant damage is mentioned, natural regeneration also occurs (sexual and vegetative) (Table 18).

Status*	No. of sites
Significant damage	2
Discoloration	1
Defoliation	5
Galls	1
Dead trees	1
Beaver attacks	1

Possible damaging agent	No. of sites
Water table decrease	4
Beavers	1
Flooding, exploitation	1

*Multiple answer allowed

Damages could be mentioned as non-significant.

The presence of cultivated poplars in the vicinity was not always mentioned (Table 19). In particular, one might expect a greater occurrence of *P. nigra* varieties (var. Italica' for example): are they really absent or are they just not mentioned because they are too common?

Visible cultivated hybrids	No. of sites	Visible planted <i>P. nigra</i>	No. of sites
None	12	None	15
Occasional	8	Occasional	8
Many	8	Many	3
NA	1	NA	3

Table 19. Presence of cultivated/planted poplars

Environment (Fig. 9)

Populus nigra is found in very diverse situations along the river drainage. The river drainage was split into three zones: collecting, carriage, and sedimentation zones. However, none of the reserves which include collecting parts of the rivers mentioned the species: in fact these protected areas are located at high elevation, and there may be a lack of protected areas just below. Within each riparian forest the location of black poplars was also described along a transversal transect (Table 20).

 Table 20.
 Location of black poplars

No. of sites	transversal transect*	No. of sites		
0	Within river bed			
9	Gravel or sand bank	7		
11	River bank	24		
11	Floodplain	6		
	0 9 11	No. of sitestransect*0Within river bed9Gravel or sand bank11River bank		

*Multiple answer allowed

Concerning soil texture, multiple answers were generally obtained, but no more than two answers per site were kept for the analysis. *Populus nigra* is mainly found on fine soil structures (loam, sand) (Fig. 10), and the species is rather located at the limit of the 'active' part of the riparian ecosystem. This can be partly explained by the fact that most of the alluvial sites investigated either have lost their river dynamics, or, at least, these dynamics have been drastically altered by the management of the river. The pioneer stages of poplar stands are no longer renewed, and the softwood forest evolves towards hardwood forest. This is also noticeable from the information about tree species most commonly associated with black poplar (>1% citation) (Fig. 11).

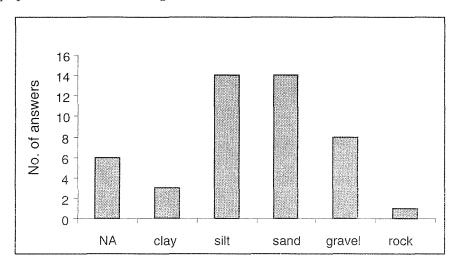


Fig. 10. Soil texture.

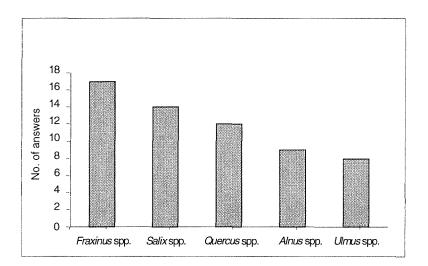


Fig. 11. Most common associated species (multiple answers allowed).

Soil pH is generally basic or neutral; only one situation in acidic soil was cited (Table 21).

Table 21. Soil pH	
Soil pH	No. of sites
Acid	1
Basic	9
Neutral	8
NA	11

Thirteen reserves mentioned occurrence of suitable conditions for regeneration (Table 22). It would have been useful to quantify this information as favourable conditions might be found in very restricted areas. In some cases, seedlings could be mentioned in the absence of favourable conditions, on a very restricted scale (but mentioned): such a situation is generally due to exceptional events (flooding etc.). This also means that exceptional events are important for the maintenance of the species.

 Table 22.
 Occurrence of suitable conditions for regeneration

	No. of sites
Favourable conditions and regeneration observed	9
Favourable conditions but no regeneration observed	4

Concerning the river dynamics, only one answer per site was kept: the most frequent one (e.g. if 'annual flooding' and 'exceptional flooding' were mentioned, 'annual' was kept). However, some reserves might include various *P. nigra* populations submitted to various water regimes (Table 23).

Table 23. Water regime

Water regime	No. of sites			
Annual flooding	12			
Occasional flooding	4			
Control of the river	5			
NA	8			

Ongoing management of the forest

The riparian forest which includes *P. nigra* is generally not exploited, possibly due to the small size of the stands, the protection status, and the weak productivity (Table 24).

 Table 24.
 Forest management type

	No. of sites
Exploitation	2
Plantations	5

Conclusion

In the French Nature Reserve areas, black poplar is found in three different situations:

- Large alluvial plains of the Rivers Loire, Rhône and Rhine, where huge stands can be found (see Table 13, area of the riparian forest, number of adult trees). These areas are also submitted to a strong pressure of agriculture, urban development, industry, communication routes, etc. Therefore natural reserves should play a significant role for the preservation of *P. nigra* in these sites.
- Small lineal riparian forest stands along dynamic mountain rivers (the Pyrenees, the Alps).
- Littoral zones where scattered trees can be observed on fine soil textures (clay, silt).

Clearly, today, *P. nigra* is mainly found in advanced stages of the sylvigenesis, rather postpioneer than pioneer associations. Forest associations, accompanying species, and soil texture show that black poplar is mainly present beyond the active part of the riverside where conditions would be more suitable for regeneration. In fact, most pioneer associations including *Salix*, typical regeneration zones for poplar, were only mentioned three times.

Thus, the current status of *P. nigra* should be considered with care:

- In the large alluvial plains, stands mostly consist of adult trees, inherited from a previously more dynamic situation of the ecosystem. Thus, Nature Reserves where the river dynamics has been preserved (Drôme, Allier, Loire) play a major role for the conservation of the species. However, even in these places, the possibility for recent colonizing cohorts to reach the adult stage should be checked: this can only occur if the lateral movement of the riverbed is still allowed. If this is not the case, current poplar stands will definitely evolve towards hardwood alluvial forests: this is already the case along canalized rivers like Rhône and Rhine. In these situations, the preservation of black poplar would require active management to support regeneration.
- Along mountain rivers, natural regeneration is favoured by exceptional natural floods which destroy previous adult stands and open new areas for colonization where poplars can then grow for a while. If no alteration of the water regime occurs (control of the river, erosion of the riverbed), and in the absence of over-exploitation of the riparian forest itself, the *P. nigra* resource is supposed to be naturally preserved in such conditions.
- In littoral zones, the ecology of black poplar still needs to be specified in order to evaluate its sustainability.

From a genetic point of view, large alluvial plains might also differ from narrow mountain rivers regarding scale of the geneflow (ecosystem more widely open for seeds and pollen) and amount of cultivated hybrids.

For the conservation of genetic resources, we focused on three options as follows:

- Where the riparian ecosystem is dynamic enough to ensure regeneration of *P. nigra* (some large alluvial plains, most mountain rivers), priority is given to the preservation of these dynamics, and to the limitation of exotic introductions.
- Large alluvial plains where river dynamics have been lost probably represent the highest priority for conservation as populations are still large, and probably contain important genetic variation, but threatened in the medium or long term. Moreover, the remaining old trees have been exposed to selection over a long period of time.
- In the littoral zones, due to the limited number of isolated trees, priority is given to *ex situ* conservation.

Of course, due to the number of Nature Reserves involved in this survey, it was not possible to meet all reserve managers. This is considered to be a first step to identify a preliminary list of potentially interesting sites for *in situ* conservation. From the answers, 14 sites were identified *a priori*:

- Five reserves with dynamic rivers, where *P. nigra* could be self-maintained if the ecosystem is preserved in its present status.
- Four reserves in large alluvial plains, where active management would be required to achieve regeneration.
- Five reserves which only provided partial answers to the questionnaire but are supposed to be relevant for the conservation of the species.

The next step will be based on further meeting and exchange of information with the managers of these reserves: they have the responsibility to decide whether the reserve should or should not enter a network for the *in situ* conservation of black poplar. The resource included in these areas will be further characterized in detail. The diverse experience from the whole group will serve the definition of management rules for *in situ* conservation of *P. nigra*, and a close collaboration with the EUFORGEN Network and the FAIR-EUROPOP research project is expected. Management rules for *P. nigra* should be compatible with the other objectives of the reserves.

A first phase of the network for *in situ* conservation should then be formalized in the coming months. Later on, the network can be extended to other areas under different protection status (for example, the south-eastern part of France could be more represented).

It should also be noticed that such riparian areas often include other tree species for which *in situ* conservation is recommended: *Ulmus, Malus, Pyrus,* etc., and a particular link should be established with the EUFORGEN Noble Hardwoods Network.

Acknowledgement

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Overview of *in situ* gene conservation activities on *Populus nigra* (status May 1999)

	AUT	BEL	HRV	CZE	FRA	DEU	HUN	ITA	NLD
Specific legislation for the protection of different aspects and at different levels									
(a) <i>P. nigra</i> as species	Ν	Ν	Ν	Ν	N	N	N	Ν	N
(b) Forest communities	Y	Y*	N	Y	Ν	Y	Y	Ν	Y*
(c) Areas/habitats	Y*	Y*	Y*	Y	Y*	Υ	Y*	Y*	Y*
(d) Movement of all planting material controlled	Ν	Y	Υ.	Ν	Ν	Y	Y		N
(e) Implemented at the regional level by government (g) or by private owners (p)	g/p	g/p	g	g	g/p	g/p	g	g	g/p
(f) <i>In situ</i> conservation of individual trees (i) or trees in mixed stands (m) or trees in pure stands (p)	i/m	i	m	i/m	i/m/p	i/m	i/m	i/m	i/m
Reintroduction carried out or planned (p)	Y	р	р	Y	Ν	Y	Y		Y
Economic importance of <i>P. nigra</i> for wood production	Ν	Ν	Y	Y	Ν	Ν	Y		Ν
Regulations on planting poplar hybrids in vicinity	N	Ν	N	Ν	Ν	Ν	Ν		Ν
Occurrence of natural regeneration	Y	Ν	Y	Ν	Y	Y	Y		Y
Inventories carried out at a national (n) or regional (r) level, or are planned (p)	n	n/r	n/r	n	n/r	n/r	n/r	r	n/r
Availability of maps for the <i>in situ</i> conservation areas	N	Y	Y	Y	Y	Y	Y	Y	Y

*With management rule

Y = yes, available; N = no, not available

AUT = Austria; BEL = Belgium; HRV = Croatia; CZE = Czech Republic; FRA = France; DEU = Germany; HUN = Hungary; ITA = Italy; NLD = Netherlands; ESP = Spain; SVK = Slovak Republic; TUR = Turkey; GBR = United Kingdom; YUG = FR Yugoslavia (Serbia and Montenegro); UKR = Ukraine; POL = Poland; SLO = Slovenia

	ESP	SVK	TUR	GBR	YUG	UKR	POL	SLO
Specific legislation for the protection			•	ts and				NI
(a) <i>P. nigra</i> as species	N	N	N	-	Ν	Ν	Y	Ν
(b) Forest communities	Y	Y	Ν	Ν	Ν		N	Y
(c) Areas/habitats	Y	Y	Y	Ν	Ν	Y*	Y*	Y*
(d) Movement of all planting material controlled		Y		N ·	N	Y	Ν	Y
(e) Implemented at the regional level by government (g) or by private owners (p)	g	g/p		g	Ν	g	g	р
(f) <i>In situ</i> conservation of individual trees (i) or trees in mixed stands (m) or trees in pure stands (p)	i/m/p	i/m		i		m	i/m	m
Reintroduction carried out or planned (p)	Y	Y		р	р	Y	Ν	Ν
Economic importance of <i>P. nigra</i> for wood production	N	Y	Y		Ν	Ν	Ν	
Regulations on planting poplar hybrids in vicinity	Ν	Ν			Ν	Ν	Ν	N
Occurrence of natural regeneration	Y	Y			Ν	Y	Y	Y
Inventories carried out at a national (n) or regional (r) level, or are planned (p)	p/r	n				n	r	р
Availability of maps for the <i>in situ</i> conservation areas	Y	Y			N	Y*		Ν

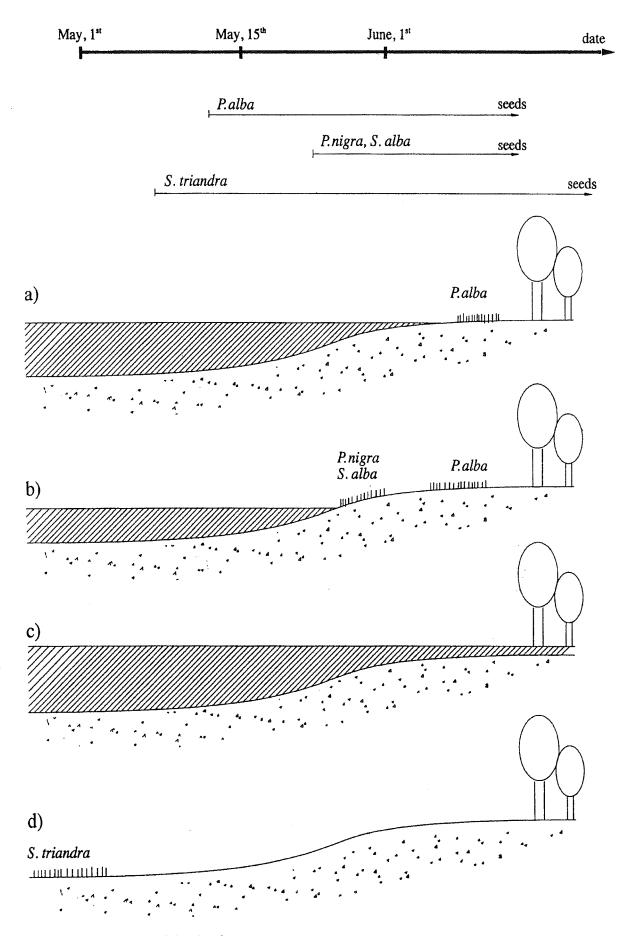


Fig. 12. Syndynamics of riparian forests.

Initial stage

This stage occurs most frequently in favourable ecological conditions on newly formed deposits on river banks and islands, when these are conquered by the seeds of purple osier (*Salix purpurea*), white willow (*Salix alba*) and almond-leaved willow (*Salix triandra*). The growth of willow shrubwood and the development of phytocoenoses are dependent on favourable water levels over several years. Extremely high water levels lasting for several days may choke the shrubwood in the very first year.

The most important pioneer associations of the initial stage are thicket of purple osier (*Salicetum purpureae* Wend. Zel. 1952) and that of *Salicetum triandrae* (Malc 1929). They reach their optimum between four and eight years of age, while at the age of ten they decline, leaving new sediments of sand and other materials, and forming conditions for the growth of white willow, which suppresses them. White willow forms the best distributed monodominant transitional association in the Croatian Danube region, *Galio-salicetum albae* Rauš 1973 (white willow forest with madder), which may last for several generations, depending on the intensity of syndynamic changes.

In the initial stage of riparian forest development, all associations are rich in hydrophytes, of which *Myosotis palustris*, *Galium palustre*, *Calystegia sepium*, *Solanum dulcamnara*, *Carex elata*, *Carex riparia*, *Rubus caesius*, *Gliceria fluitans*, *Iris pseudocorus*, and other species are especially prominent.

Optimal stage

This stage is marked with the occurrence of mixed forests of white willow and black poplar (*Salici albae-Populetum nigrae* Tx. 1931), and forests of black and white poplar (*Populetum nigro-albae* Slav. 1952). Unlike pure forests of white willow, they develop under conditions of rare, short-lasting floods on elevated parts of the Danube islands with recent alluvial loamy-sandy soils, in which intensive humus-forming processes are underway. Some white poplars with breast diameters of up to 2 m and heights of over 30 m can be found here.

The floral composition of the shrub layer consists of *Frangula alnus*, *Cornus sanguinea*, *Crataegus pentagyna*, *Viburnum opulus* and *Crataegus nigra*, while the ground layer, along with swampy species from the initial associations, is composed of several mesophylous species, such as *Lycopus europaeus*, *Scrophularia elata*, *Agrostis alba*, *Carex remota* and others.

The stage with forests of black and white poplars can last from one to several generations, depending on changes in synecological factors. Therefore, these associations are often viewed as a permanent stage. As pedunculate oak frequently occurs in mixed poplar forests in numerous localities, such stands represent the upper boundary of forest vegetation.

Terminal stage

This stage is marked with forests of spreading elm and narrow-leaved ash (*Fraxino-Ulmetum laevis* Slav. 1952). In the Croatian Danube region fragments of these forests inhabit older and well-developed soils in the highest positions, where floods are rare and short. Apart from the spreading elm and narrow-leaved ash, there are also species of fresh sites, such as *Acer campestre*, *Crataegus nigra*, *Rumex sanguineus*, *Festuca gigantea*, and others. In the past, this phytocoenosis was distributed over a much wider area along the Danube, but the land has been given over for agricultural purposes.

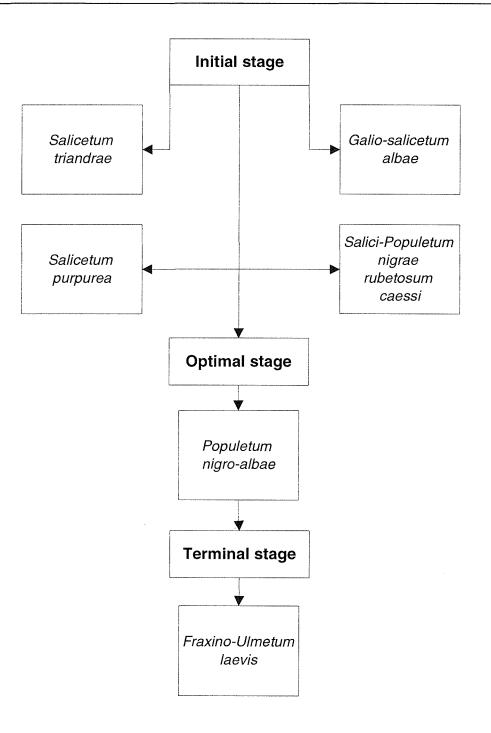


Fig. 13. Succession stages of riparian forests in the Croatian Danube region.

Main conclusions

- 1. The floral structure and distribution of plant communities in riparian forests depend on the duration and height of floods, the elevation of the terrain, the distance from a riverbed, and the degree of parent substrate and soil genesis. Syndynamic processes are very intensive, and the manner and rate of changes depend primarily on the water regime, i.e. flood water.
- 2. The succession of riparian forests in the Danube region of Croatia from riparian shrubwood of almond-leaved willow (*Salix triandra*) or purple osier (*S. purpurea*), over white willow (*Salix alba*) and poplars (*Populus nigra*, *P. alba*) to climatogenous forests of *Ulmus laevis*, *Fraxinus angustifolia* and *Quercus robur* can be followed in three stages: initial, optimal and terminal.
- 3. The importance of riparian forests in Croatia is seen in their relatively well preserved state, their natural origin, and the fact that they provide important biotopes for the preservation of endangered plants and animals.

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Ex situ conservation: update on the EUFORGEN core collection and the database of clones

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The EUFORGEN core collection

All the clones received from Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, The Netherlands, Poland, Romania, Slovakia, Spain, Turkey, United Kingdom, Ukraine and FR Yugoslavia for the constitution of the core collection, were propagated in stool-beds in 1998 and 1999. The reference clones (150–200 cuttings each clone) were also propagated.

The stool-beds established in 1996 and 1997 were maintained so as to produce as many cuttings a possible. Unfortunately some clones failed completely (KAE_N.92.145 and POL_TORUN_25) and others are in a bad condition (including the Italian ISP_351, the southernmost clone existing in the European database).

To duplicate the core collection in another locality, all available clones were sent to The Netherlands; some material was supplied to the University of Milan (Italy) and to INRA (France) for molecular analysis, and to IBW (Belgium) for isoenzyme analysis.

Some *P. nigra* genotypes collected from adult trees in natural forests along the River Ticino, in the framework of the EUROPOP project, were propagated in stool-beds in Casale and Tromello (Pavia). Sixty-four genotypes collected from the natural forest 'Bosco Negri' and 66 genotypes from the natural forest 'Nuova Zelata' were planted.

The European Populus nigra database

Information regarding the *ex situ* collections in the Czech Republic (181 clones) and in Ukraine (nine clones) were included in the *P. nigra* database during 1998; data concerning the Italian, French and Spanish collections were also updated.

In addition to the data available for the clones from Bulgaria, Germany, Poland, Romania, Slovakia and Ukraina included in the core collection, information about the clones maintained in the *ex situ* collections in Austria, Belgium, Croatia, Czech Republic, France, Hungary, Italy, The Netherlands, Spain, Turkey, United Kingdom, FR Yugoslavia, is included in the database (2257 entries as of April 1999; see summary of the meeting).

It should be noted that although an effort was made to complete the information about the origin, data regarding latitude and longitude of the collecting site of about 300 clones are still missing, whereas information is available on the parents of about 100 clones obtained from artificial crossing.

		Cuttings					
		Planted	in stool-b	ed	Sent to		
Code	Country	1998	1999	IBN-DLO	IBW	INRA	UNI-MI
FBVA_LH35	AUT	-	68				_
FBVA_LH_HL55	AUT	2	8	2	_	-	
IBW_N004	BEL	52	160	25	_	5	5
IBW_N009	BEL	91	200	25		5	5
SEEFAR_PAZARDZIK_N1	BGR	39	31	27	5	7	5
	BGR	39	83	15			
SEEFAR_SVICHTOV_N2					10		5
VULHM_880044	CZE	14	73	-	10	5	5
VULHM_880045	CZE	28	144	14	10	5	5
FBS_215/63_JUGENHEIM _1	DEU	42	150	25	10	-	5
FBS_87/65_OFFENBURG _1	DEU	112	154	25	10	5	5
- SIA_LUCENI'_2	ESP	75	86	18	5	7	5
SIA_PASTRIZ_1	ESP	56	66	30	5	7	5
INRA_71017-401	FRA	61	160	15	5	, 7	5
NRA_92510-1	FRA	30	78	10	10	-	5
FCRA_HUNTINGDON	GBR	39	129	25	10		
FCRA_HOBSONS_COND						_	5
JIT	GBR	40	70	20	10	2	5
F_V336	HRV	17	100	10	5		_
F_V408	HRV	41	138	14	10	_	5
ERTI_33-3-1	HUN	19	64	25	5	_	-
ERTI_33-3-2	HUN	3	25	-	-		
SP_N068	ITA	46	175	_ 25		_	-
					10	_	5
SP_N351	ITA	8		8		-	-
SP_N347	ITA		33	_		-	-
BN_1238	NLD	89	200	25	10	5	5
BN_1792	NLD	65	200	25	10	5	5
POL_TORUN_B	POL	25	60	10	5	2	5
POL_TORUN_25	POL	-	-			_	_
	POL	6	19	8		2	_
CAS_3	ROM	6	31	6	_	-	_
CAS_4	ROM	6	48	6			
CAS_5	ROM	7	40	9	_	2	_
							_
CAS_6	ROM	2	21	2	-	-	-
LVU_BAKA	SVK	251	200	30	10	5	5
_VU_IVACHNOVA	SVK	326	200	30	10	5	5
KAE_N.90.013	TUR	44	45	30	5	7	
KAE_N.92.145	TUR	-		-			_
JKR_B11	UKR		6	_		_	
JKR_B12	UKR	3	16	3			_
ZT_NS001	YUG	134	200	25		5	5
ZT_NS002	YUG	174	200	25		5	5
		1/4			-	5	5
JFRI_HRADIZKY	UKR		6	-		-	-
JFRI_KELIBERDYNSKY	UKR	-	6		-		-

Table 25. The EUFORGEN core collection

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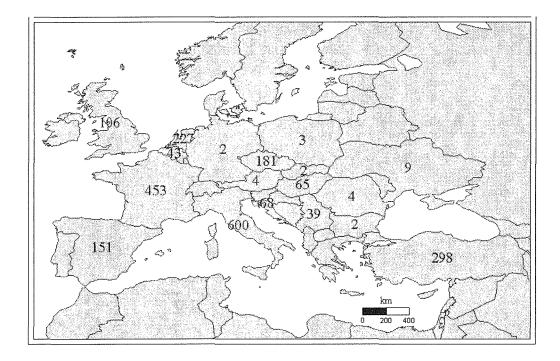


Fig. 14. The European Populus nigra database: number of clones from each country.

Discriminant analysis of leaf morphological characters of the European black poplar (*Populus nigra* L.) in natural populations in Croatia

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Summary

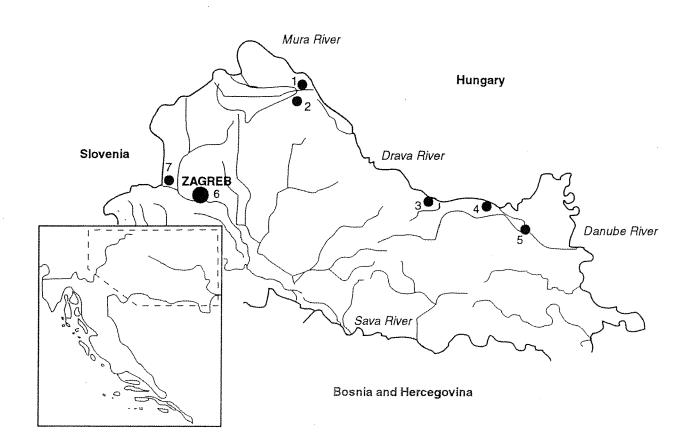
The European black poplar is a typical species of alluvial soils near large rivers. In Croatia it grows along the Mura, Sava, Drava and Danube rivers. The black poplar stands have been considerably reduced by human activities, either directly by felling or indirectly by modification of ecological conditions in their habitats. Although today we have very well-preserved riparian forests in the Baranja and Danube regions, as well as partly along the River Drava, in most other habitats the European black poplar has been reduced to smaller stands or individual trees.

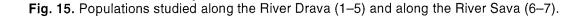
In 1925, the establishment of the first plantations with the so-called Canadian poplars ('Robusta' and 'Serotina' clones) started in Croatia along the Drava and Danube rivers in the riparian forest areas. Since 1960, new hybrid clones P. × euramericana have been introduced progressively in the cultures with a mass establishment of monocultures, mainly with the 'I-214' clone. Later, the *P. deltoides* clones were introduced in the plantations; however, in the 1980s, due to lack of alluvial soils, diseases and the price decrease in the wood market, the afforestation volume was reduced. Some of the introduced clones were female, with their flowering time synchronized with that of the acclimatized poplar trees and the autochthonous black poplar trees. Nowadays, the European black poplar progeny, in which the existence of introgression with the Eastern cottonwood (*P. deltoides*) is questionable, or which was developed as the progeny from the female 'I-214' hybrid, is found along the above mentioned rivers. For these reasons, based on the morphological characters, we wished to determine the phenotypical manifestation of some properties characteristic of the Eastern cottonwood in natural populations of the European black poplar. The determination of introgression variability would indicate the hybrid character of the European black poplar younger populations. Based on the leaf morphometrical research in the European black poplar carried out so far in Croatia, the presence of contamination by the Eastern cottonwood genes has been determined for some characters (Krstinić et al. 1998).

Leaf variability in the European black poplar was studied in young stands and adult trees by morphometrical analysis of leaves from short shoots. As within one tree the leaf dimorphism has been determined, for the leaf variability analysis in the generative progeny, leaves were taken from the short shoots only. This confirms the literature data (Rehder 1940, according to Zsuffa 1974) describing a rhombic-oval leaf shape on the long shoots and a rhombic leaf shape on the short ones. Seasonal heterophylly in poplars was also found. Preformed and neoformed leaves also often differ considerably in texture, shape and toothing. Preformed leaves generally provide a better taxonomical diagnostic than neoformed leaves and tend to differ more among major sections of poplars than among species within sections (Eckenwalder 1996).

The analyzed material from populations was compared with the leaf measurements on the short fertile shoots from two European black poplar trees (*Populus nigra* L.) of about 200 years, one Eastern cottonwood (*Populus deltoides* Bartr.), one '618' ('Lux') clone, and one 'I-214' hybrid clone ($P. \times euramericana$ (Dode) Guinier) as well as with the measurements concerning the Lombardy poplar clone P. nigra 'Italica'.

Leaf samples for the European black poplar generative progeny were taken from a 3–4year-old generative progeny from seven locations. Five of the populations studied were close to the River Drava (Zirovnjak, Tršćana, Bobrovac, Šućurica and Topolje), while two of them were near the River Sava (Jarun, Zaprešić), as shown in Fig. 15.





The analysis included only sound, fully-developed leaves, collected in mid-July. From each individual adult tree, 30 to 95 leaves were analyzed, while the generative progeny sample represented 300 leaves taken from 60 plants per population. The properties measured were:

- maximum leaf blade length;
- maximum leaf blade width;
- distance between the leaf base and the leaf widest part;
- petiole length;
- leaf blade width at 1 cm from the leaf tip; and
- α angle between the first lateral vein and the horizontal, as shown in earlier research (Krstinić *et al.* 1998).

The possibility of mutual discrimination of the groups on the basis of the six characters analyzed was determined using discriminant analysis (Mardia *et al.* 1982; Kachigan 1991). The variables for grouping are the populations (*Populus deltoides*, *P. nigra*, *P. nigra* 'Italica' and 'I-214'), and the independent variables are the six morphological characters analyzed listed above. The independent variables were introduced in the model by the forward stepwise method. The tolerance limit for all analyses was 0.01.

Each of the seven populations observed was compared with *P. deltoides*, *P. nigra*, *P. nigra* 'Italica' and 'I-214' in seven separate analyses. Detailed results of this study can be obtained from the authors and will be published elsewhere.

On the basis of the studied leaf morphological properties in seven natural young populations of the European black poplar in the Drava and Sava river areas, the existence of individuals which, according to the discriminant classification, can be attributed to the Eastern cottonwood, was found. Their presence can be explained by the recombinations and the transgression which occurred through crossing with the introduced representatives of the Eastern cottonwood or through the widespread hybrid clone 'I-214' which is female.

Of six morphological properties studied, the discrimination of the Eastern cottonwood from the European black poplar natural populations is influenced mostly by the property of the angle between the first lateral vein and the horizontal. This property might be indicative for the determination of the proportion of hybrids in the European black poplar populations. For these reasons, in works aiming at European black poplar genetic resources preservation, the selection should be made on old trees only, which have not been influenced by the Eastern cottonwood genes.

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(Note: these are new references to be added to the previous lists; see Reports of the First, Third and Fourth Network meetings. The full, compiled list will be published on the Internet at http://www.cgiar.org/ipgri/euforgen).

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- Cao, F.L. and W.H. Conner. 1999. Selection of flood-tolerant *Populus deltoides* clones for reforestation projects in China. Forest Ecology and Management 117(1–3):211–220. (English)
- Steenackers, V. 1996. Towards a global management of poplar genetic resources. Proceedings of the 20th Session of the International Poplar Commission (FAO), Budapest, Hungary, 1-4 October 1996. (English)

Appendix I. Black poplar (Populus nigra) and the WWF European Freshwater Programme



WWF International Danube-Carpathian Programme

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Brief outline

Black poplar (Populus nigra) and the WWF European Freshwater Programme

The WWF European Freshwater Programme is currently carrying out a species project. The aim of this project is the development of a strategy for the European Freshwater Team including the selection of a number of freshwater species. This selection of species should help the European Freshwater Programme to meet its main objectives:

- Conservation of biodiversity
- Maintenance of the ecological integrity and functions of the freshwater ecosystems
- Reduction of pollution of freshwater systems
- Restoration of damaged freshwater systems

Not all the species chosen will be used by WWF in the same way. The European Freshwater Programme plans to initiate several projects focusing on certain species (e.g. Lutra lutra or Acipenser sp.). Other species would be used to communicate complex ecological issues in international awareness campaigns (e.g. cranes or riverine dragonflies).

Black poplar has been chosen by the team as it represents particularly well the habitat of natural floodplains throughout Europe, it is attractive and historically mankind have always had a strong relationship to it. Nevertheless, today this tree is to be seen as highly threatened for several reasons. Therefore, actions for its conservation have been taken on an international level (Ministerial Conference on the Protection of Forests in Europe, Resolution 2, Strasbourg 1990).

WWF European Freshwater Programme would like to initiate the following projects on an international and national level:

- Awareness raising campaign for natural wetlands using Black poplar as a flagship within the "Living Rivers Campaign" of the WWF European Freshwater Programme:
 - 0 This campaign should focus on the general public, but also on water authorities and foresters
- Awareness raising campaign for the species itself:
 - Production of information material in the local language about the ecological, social and historical importance of Black poplar, distributed throughout over Europe
 - Develop a map indicating the historical Black Poplar sites
- Lobbying for the European-wide protection of the existing Black poplar sites (at least as Natura 2000 sites).

The WWF European Freshwater Programme is strongly committed to realising some of the above mentioned projects in partnership with strong European organisations, including research units, local NGOs and others, in order to promote natural, floodplain forests focusing on Black poplar.

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Registered as:	President	: Syad Babar Ali
WWF - Fondo Mondiale per la Natura	Vice President &	
WWF - Fondo Mundial para la Naturaleza	Hon. Treasurer	: Rodney Wagner
WWF - Fonds Mondial pour la Nature	President Emeritus	: HRH The Duke
WWF - Welt Natur Fonds		of Edinburgh
WWF - World Wide Fund for Nature	Director General	: Claude Martin
(Formerly World Wildlife Fund)		

Programme

6 May

- 1. Opening of the meeting
- 1.1 Welcome (Host country and Chair of the Network)
- 1.2 Introduction (EUFORGEN Coordinator)
- 1.3 Adoption of the agenda and nomination of rapporteurs
- 2. Joint research
- 2.1 Update on the progress made in EUROPOP (S. de Vries)
- 2.2 Discussion
- Country updates on the progress made (Austria, Belgium, Croatia, France, Germany, Hungary, Italy, The Netherlands, Poland, Russian Federation, Slovakia, Spain and Ukraine)
- 3.1 Introductory reports from newly attending countries: Portugal and Slovenia
- 4. Synthesis of *in situ* conservation measures and activities (S. de Vries)
- 5. List of descriptors for inventories of stands review (N. Alba)
- 6. Network discussion: Coordinating action on *Populus alba* genetic resources in Europe
- 7. In situ conservation strategies
- 7.1 Effects of ecosystem management on dynamic processes in *P. nigra* populations (S. de Vries and I. Popivshchy)
- 7.2 Restoration of riparian ecosystem (B. Heinze)
- 7.3 Indicators for monitoring the evolution of diversity in riparian ecosystem (F. Lefèvre and D. Kajba)
- 7.4 Discussion
- 7 May
- 7. *In situ* conservation strategies (Discussion continued and recommendations)
- 8. Presentation of Ukraine's national programme on forest genetic resources (R. Volosyanchuk)
- 9. Field trip to *P. nigra* stands on the river Dnipro

8 May

- 10. Core collection of clones update
- 11. European Database update
- 12. Public awareness activities of the Network (S. de Vries/J. Turok)
- 12.1 Review of the slide collection
- 12.2 Production of the CD-ROM version
- 12.3 Other public awareness initiatives
- 13. Bibliography update (F. Lefèvre)
- 14. Overview of a study on leaf morphology (D. Kajba)
- 15. Miscellaneous
- 15.1 Election of Chair and Vice-Chair
- 15.2 Date and place of next meeting
- 16. Conclusions

List of participants

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