EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

Populus nigra Network

EUFORGEN

Report of the first meeting
3-5 October 1994
Izmit, Turkey

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

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

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Contents

Introduction

The first meeting of the *Populus nigra* Network was held at Izmit, Turkey from 3 to 5 October 1994 in parallel with the 37th meeting of the Executive Committee of the International Poplar Commission (IPC).

On 3 and 4 October, the participants attended the meeting of the Breeding working party of IPC. On 4 October Dr E. Frison gave a presentation on the European Forest Genetic Resources Programme (EUFORGEN) and Dr F. Lefèvre presented the history of the creation of the *Populus nigra* Network. In the evening of the same day an information meeting was attended by 18 participants from 11 countries during which the functioning of EUFORGEN in general and the *Populus nigra* Network in particular were discussed.

On 5 October the Network meeting was attended by 12 participants from 10 countries (see list of participants). The meeting was opened by E. Frison who welcomed the participants on behalf of IPGRI. He stated that this first meeting of the *Populus nigra* Network was also the first meeting within the framework of EUFORGEN. He thanked the Turkish Government and the local organizers for having made it possible to hold this first meeting in Izmit and expressed his wishes for a successful meeting.

He further stressed the fact that this Network belonged to the participants and that its success would depend on the enthusiasm and dedication of the participants. IPGRI's role in providing a secretariat is mainly to act as a facilitator. Dr Frison also mentioned that the future of EUFORGEN will depend on the number of countries joining the programme, a minimum budget being required to make it viable. For this first meeting of the Network, the participants from countries which had not yet signed the Letter of Agreement to join EUFORGEN were exceptionally sponsored by the IPGRI budget in order to allow the Network to start its activities.

Dr Tunçtaner welcomed the participants to Turkey and expressed its satisfaction at seeing the first meeting of the Network held in Izmit.

Dr Frison also thanked FAO for having allowed this meeting to take place on the occasion of the meeting of the Executive Committee of the International Poplar Commission and thereby to ensure a close collaboration between the Network and IPC. He hoped that Mrs Palmberg-Lerche would have the opportunity to address the Network on behalf of FAO.

Following these introductory statements, Dr F. Lefèvre was unanimously elected as Chairman of the Network and Dr L. Cagelli as Vice-Chair.

The draft agenda was adopted and the participants briefly introduced themselves.

Synthesis of the national activities

Before the meeting, the participants were asked to prepare a brief, informal presentation of the national activities for the conservation of *P. nigra* resources, some of which were presented in greater detail during the session of the Breeding Working Party of IPC.

The situation of *P. nigra* is highly variable among the countries. Turkey has a unique situation where *P. nigra* commercial stands represent 50% of the annual poplar production. Dense plantations along the fields and roads are used for the production of small logs for construction; except for these plantations, native stands are not frequent in that country. Plantations of pure *P. nigra* selected clones are also found in Hungary and the Netherlands. In contrast, the occurrence of native stands or natural regeneration is particularly scarce in Belgium, Germany and in the Netherlands, because of intensive agriculture on the riparian sites. In Bulgaria, the native stands of *P. nigra* were overexploited because of particular patterns in the wood which make it attractive for veneer, and only a few stands remain on the islands of the Danube.

The national programmes for the conservation of *P. nigra* often started more than 20 years ago (Belgium, Bulgaria, France, Germany, Hungary, Netherlands, Turkey), and some countries have recently developed new programmes (Croatia, France, Italy, the Netherlands, Spain, Turkey). Inventory of the resource is often the first step, and repeated inventories of *P. nigra* give an insight of the threat to the species (more than 50% of the stands have disappeared in the middle part of the Ebro Valley, in Spain, during the last 40 years). Early inventories specific for *P. nigra* exist on a national scale in Germany and Hungary and have to be updated; in other countries this work is in process (France, Spain). Depending on the country, nonexperts are involved in the inventory (mainly nongovernmental nature conservation organizations); therefore, some guidelines are needed for the identification of species. This is difficult because of the risk of introgression from the cultivated interspecific varieties and the difficulty of distinguishing pure *P. nigra* and introgressed forms. An inventory is planned both at the single tree level in collections and at the stand level (pure stands or mixed stands).

Populus nigra is actively used in the breeding programmes, sometimes as a pure species but often as a parent for Euramerican hybrids, and therefore a lot has been done on *ex situ* conservation of the species in the breeding institutes. Three levels of *ex situ* conservation are generally used: networks of trial plantations, genebanks as stool-beds, genebanks as adult tree plots. Passport data, characterization data and evaluation data (mainly resistance to various diseases) are often available for this material and sometimes stored in computerized databases (France, Italy). Intraspecific crosses, within or among provenances, have often been achieved to enlarge the genetic diversity. A particular problem arises when it is necessary to renew old adult collections (cost in space, time and money). In such a situation, and when starting *ex situ* programmes, guidelines are needed for a safe and economic conservation strategy.

In situ conservation is also used (Bulgaria, Croatia, Hungary, the Netherlands, Spain). This directly concerns particular *P. nigra* stands or individual trees, or it concerns a particular site that includes *P. nigra* among other species. Protected areas are in public and private lands, with possible uncertainty about the status of this land in the future (Hungary). Many different legal and technical measures are taken for these protected areas, which have to be synthesized. The plantation of Euramerican hybrids is sometimes forbidden in the vicinity of the protected area. A further study of the introgressive process and its consequences might be needed since such measures may have important economic and social consequences. For the establishment of plantations for the purpose of *in situ* conservation, the objective of conservation excluding commercial production has to be officially recognized in order to be able to grow unregistered material in the nurseries.

Workplan

From the review of the national activities concerning the conservation of *P. nigra*, and after consideration of the draft workplan established in Viterbo in 1993, a series of practical objectives has been identified, and the coordination of the tasks was distributed among the Network members. The various tasks for members are highlighted in bold in the text.

1. A standardized descriptor list for *P. nigra* stands
   (Coordination: R. Schulzke)

   This is the first step required to initiate an inventory using a standardized language. It is proposed to base the development of such descriptors on those used for other species. The list does not have to be very long. **A preliminary list will be sent by Dr Schulzke to the network members before 1 December 1994 for revision. Revised lists and comments should be sent back to the coordinator by 15 January 1995. A revised list should be available by the spring of 1995** and will be discussed at the next meeting of the Network.

2. Identification sheet for *P. nigra*
   (Coordination: F. Lefèvre)

   It was agreed that a short (2 pages) identification sheet for *P. nigra* would be developed. Special emphasis will be placed on the distinction with Euramerican hybrids and possible introgressive forms.

   F. Lefèvre will coordinate the production. A preliminary list of characters and illustrations was distributed to Network members. It was agreed to include the following illustrations:
   - range of *P. nigra* tree shapes in the wild;
   - twigs and buds;
   - leaves from different types of branches;
   - bark designs.

   All members are required to send comments and illustration to F. Lefèvre by 1 December 1994. These identification sheets are meant for people making inventory, who are not necessarily *P. nigra* specialists.

   A similar information sheet will be developed for *P. nigra* at the nursery stage. This will mainly include leaf shapes in the nursery, buds and twigs (especially lenticelles).

   J. van Slycken offered the services of a professional artist to make drawings for the information sheets if required.

   Selected illustrations **will be sent to Belgium by F. Lefèvre before the end of December 1994.**

   The two types of identification sheets should be available in the spring of 1995.

3. Descriptor list for *P. nigra*
   (Coordination: J. van Slycken)

   It was agreed to develop a descriptor list for *P. nigra*. This list should be as

comprehensive as possible but the network will also agree on a minimum set of descriptors which everyone should apply.

The UPOV and FAO descriptor lists will be used as a base and several other descriptor lists for other species published by IPGRI will serve as a model. **E. Frison will send IPGRI descriptors for other species to all members by 31 October 1994** as a model. **J. van Slycken will circulate drafts for comments to all members by 31 May 1995.**

4. Reference clones for *P. nigra* characterization

The set of UPOV reference clones originating from the same collection (probably the German reference collection) will be sent to all participants. **J. van Slycken will arrange for this set of clones** (10 cuttings of each) **to be sent to the participants by February-March 1996.**

5. European database
(Coordination: L. Cagelli)

A questionnaire on *ex situ* collection was sent to different countries after the Viterbo meeting and a number of countries have replied. This information was compiled and those countries which have not yet replied should send the information to L. Cagelli.

It was agreed to develop a European database for *P. nigra*. Comments on the set of descriptors to be used for the database, which was developed by F. Lefèvre, **will be given by IPGRI to L. Cagelli by the end of November 1994.**

L. Cagelli will then send this list by mid-December 1994 to all countries requesting the information for inclusion in the database. **Network members should send the information before 1 February 1995 to L. Cagelli.**

L. Cagelli accepted the responsibility for establishing and maintaining the European database for *P. nigra*. It was recommended that L. Cagelli visit IPGRI Headquarters for a few days of interaction with the germplasm documentation specialists to be trained in documentation.

6. Synthesis of *in situ* conservation measures
(Coordination: S. de Vries)

After the meeting in Viterbo, information was requested from participants on what methods and regulations for *in situ* conservation exist in different countries. Some raw data in various languages were received but these were not very useful. **It was agreed that each participant would summarize** (in English) **the different regulations and methods** (maximum 2 pages, with possible annexes) **and send these to S. de Vries by 31 January 1995.**

7. Review of *P. nigra* literature
(Coordination: F. Lefèvre)

At the Viterbo meeting, it was agreed that a literature review would be compiled by F. Lefèvre. This was carried out successfully and this review was distributed to all members. A first update was distributed at the meeting (see Selected Bibliography).

It was agreed that participants would send all relevant literature references, with specification of the language and in the format of the first survey, **to F. Lefèvre who will make an updated list before each meeting of the Network.**

8. Study of genetic diversity of *P. nigra*

The Network agreed that it was very important to develop tools to study the distribution of the genetic diversity of *P. nigra*. This should also allow the study of genetic diversity of *P. nigra* in the wild: the structure of this diversity, geneflows, vegetative vs. sexual reproduction. It will also allow the study of dynamics of native stands in different environments and the introgression process of *P. nigra* from hybrids and other *Populus* species.

The results of such studies are essential for the development of appropriate collecting and *in situ* conservation strategies. Different levels of management for *in situ* conservation will be required depending on the outcome of such studies.

9. Guidelines for maintenance and duplication of *ex situ* field collection (**Coordination: S. de Vries**)

It was agreed to develop simple guidelines for the establishment of *ex situ* collections. **S. de Vries will send a draft for comments to participants by 15 January 1995 and comments should be sent to S. de Vries by 1 March 1995.** The guidelines should be presented at the next meeting.

The participants stressed the importance of safety duplication of unique material and **it was agreed** that attempts should be made to duplicate unique material.

10. Other *ex situ* conservation methods

The usefulness of other *ex situ* conservation methods was discussed and it was agreed that different alternatives should be investigated. **C. Maestro agreed to carry out** a review of the literature on storage of seeds, pollen and buds of species similar to poplar by 31 December 1994. **Relevant references should be sent by members to C. Maestro before 15 November 1994.**

The Network also recommended that research be undertaken on alternative *ex situ* conservation methods.

11. Core collection

It was agreed to postpone the discussion on the establishment of a core collection for *P. nigra* until the European database is established.

12. Development of joint proposals

It was agreed that the *Populus nigra* Network was an ideal forum for the development of joint proposals for research and conservation activities to be submitted to the EC and other donors.

13. Inventory of *P. nigra* genetic resources

Once the necessary tools have been developed (descriptors for individual trees and descriptors for stands; identification sheets) a European inventory of the remaining

resources of *P. nigra*, as individual trees and native stands, should be made for inclusion in the database. Further material should be collected and characterized.

14. Future of the Network

It was recommended that IPGRI invite the National Coordinator for EUFORGEN of Portugal to nominate a participant for the *P. nigra* network if interested.

The participants agreed to hold the second meeting of the *Populus nigra* Network in the autumn of 1995. J. van Slycken kindly offered to organize the meeting in Belgium and the participants welcomed the offer. After the end of the meeting, a suggestion was made to hold the next meeting in Casale-Monferrato, just before or after the final meeting of the current AIR project and therefore allowing some savings on travel costs. The two possibilities will be further examined in consultation with the Chair and Vice-Chair of the Network.

The participants thanked F. Lefèvre for his excellent and hard work since the meeting of the IPC in Spain in 1992. They also thanked Mr van Slycken for the simultaneous translation which he provided to Drs Toth and Tsanov.

The participants encouraged the countries which had not yet signed the Letter of Agreement to join EUFORGEN as soon as possible.

Agenda

1. Introduction
   1.1. Welcome address (*K. Tunçtaner*)
   1.2. Welcome address and presentation of the European Forest Genetic Resources programme (*E. Frison*)
   1.3. History of the black poplar conservation network (*F. Lefèvre*)
2. National activities for the conservation of *P. nigra* genetic resources - presentation of countries
3. Discussion of needs for a European inventory of native black poplar stands
4. Synthesis of the existing *in situ* protection systems (*S. de Vries*)
5. Development of descriptors and database for the black poplar *ex situ* collections (*L. Cagelli, J. van Slycken*)
6. Development of guidelines for *ex situ* conservation (*S. de Vries*)
7. Identification of common needs to study genetic diversity and the possibilities of joint project proposals
8. Literature survey (*F. Lefèvre*)
9. Agreement on network tasks and development of a workplan
10. Final session: Approval of the report

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Toward the conservation of Eurasian black poplar, *Populus nigra* L.

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Populus nigra L. is a tree species of social and economic interest. It belongs to the riparian ecosystem which contributes to the natural control of flooding and water quality, and which is characterized by a high level of diversity of the fauna and flora. *Populus nigra* plays a central role in poplar-breeding programmes: 63% of the poplar cultivars from the international register descend from that species, mainly as interspecific hybrids. It is used as a pure species for wood production in some countries, e.g. it represents 45% of the poplar plantations in Turkey. Linear plantations of *P. nigra* cultivars are also used as windbreaks for the protection of agricultural land. Finally, some *P. nigra* cultivars are used for ornamentation and landscaping, and belong to our cultural heritage.

There is an international mobilization for the preservation of *P. nigra* germplasm. It was recognized as an endangered species by the countries who signed Resolution 2 on the Protection of Forest in Europe. This situation is due to the artificialization of riverbanks, the destruction of the riparian forest, and the risk of decrease of genetic diversity following introgression by a reduced number of cultivars. National programmes devoted to the conservation of *P. nigra* exist in most European countries, generally under the responsibility of poplar-breeding institutes. International coordination of these programmes was a recommendation from the International Poplar Commission (FAO) in 1992, and the EUFORGEN network on *P. nigra* was born in 1993.

Populus nigra is a model tree species for conservation biology. The *Populus* genepool is characterized by frequent interspecific hybridization. In Europe we can define the introgression process between native *P. nigra* and the cultivars (pure species and interspecific exotics) as a wild/cultivated complex. *Populus nigra* is widespread in Europe and Asia, under various climatic conditions. It is a typical pioneer species, dioecious, heliophilous, and characterized by an efficient dissemination of seeds (wind and water) and pollen (wind), but also by a good ability for natural vegetative propagation. The species is easily monitored through biotechnology techniques.

Research is still needed for gene resource conservation. Our objective is to maintain and/or improve adaptability. That means monitoring sufficient genetic diversity as base material for any future use, not only breeding for adaptation to some particular condition today. The minimum is thus to identify and preserve an adequate sample of the diversity. Then, we need inventories and research on the amount and structure of the genetic diversity. The static *ex situ* conservation strategy is applicable in the short term, but it may not be sufficient in the long term. We also have to define a dynamic strategy which may be *in situ* or *ex situ*; then, we need research on the evolution of natural populations, on the effect of poplar cultivation on the native stands, and on theoretical conservation biology.

Poplar germplasm conservation: *ex situ* conservation methods under controlled conditions

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Summary
Among the approaches for conservation of forest tree germplasm, the *ex situ* measures involve preservation away from the site of the original stand. This aim can be achieved under either field or controlled conditions. This latter category includes storage of seed, pollen, plants, shoots, buds, meristems and cells at low temperatures. Temperatures for storage range from +5 to −196°C (liquid nitrogen).

Seed storage
Seed is an important and convenient form of germplasm preservation. Seeds of forest tree species are generally stored at low temperatures from +4 to −20°C (Ahuja 1993). At these low temperatures, the seeds can be stored, in a majority of cases, only for a limited time.

According to seed storage, poplar is included in the suborthodox group proposed by Bonner (1990). This kind of seed can be stored at subfreezing temperatures and low moisture content (under 10%) no longer than 1 to 6 years.

Long-term storage (longer than one rotation of culture) is possible for many species and it could become a strategy for conservation of many other species if storage technology could be improved (Bonner 1990).

The following factors are involved in seed preservation:

Dehydration
- The moisture content of seeds at collection must be reduced. Two ways can be considered: oven drying or freeze-drying;
- Experiences with poplar seeds (Table 1) show that a seed moisture content of 6-8% is suitable for a good germination value after 1 to 6 years of storage at different temperatures;
- Although freeze-drying reduces seed moisture content quickly, the best germination value in poplar seeds (*P. deltoides, P. nigra*) was obtained from oven dehydration at 35°C (Cagelli 1992);
- Vacuum storage and sealed containers seems to improve seed longevity and can be a useful way to exchange seed.

Storage temperature
The +5 to −196°C range has been tested in poplar (Table 1). Specific trials made with *P. nigra* seeds by Cagelli (1994) show that an identical germination rate can be obtained after storage at −18 or −40°C.

The cryopreservation of poplar seeds in liquid nitrogen (−196°C) has been tested by Ahuja (1986). There was no loss of viability in seeds of *Populus tremula* × *P. tremuloides* after 1 and 6 days of storage at −196°C.

Table 1. Storage conditions for poplar seed conservation

Reference	Moisture content (%)	Temperature (°C)	Time
Zsuffa (1974)	6	−3, −8	—
Tauer (1979)	6-10	+5, −20	6 years
Muller and Teissier du Cros (1982)	7-8	+4	5 years
Ahuja (1986)	—	−196	1 and 6 days
Bonner (1990)	10	0 to −15	1-6 years
Catalan (1991)	6	0	1-2 years
Cagelli (1994)	6-8	−18, −40	4-5 years

Cryogenic storage offers a great promise for long-term storage of some forest trees seeds (Jörgensen 1990). It would appear that relatively small seeds such as those of *Populus* are less prone to injury by freezing-thawing than large seeds (beech, oak), according to Ahuja (1986) and Jörgensen (1990).

Two clones of *P. nigra* stored at −40°C for 4 years showed a different rate of germination (38% and 71% respectively), according to Cagelli (1994).

Pollen storage

As with seeds, moisture content and storage temperature are the most important factors for good conservation of tree pollen (Wang 1975).

Specific information on poplar pollen storage has not been found, although Cagelli (1994) studied the optimal conditions for long-term storage of pollen (moisture content, storage temperature, rehydration, viability tests after storage) on some genotypes of *P. deltoides* and *P. nigra*. So, it seems that the moisture content of poplar pollen when collected can vary with the genotype and the date of collection. It can be reduced to 7-10% after 2 hours of dehydration on silica gel at +4°C. Storage temperatures of +4°C and −18°C are being tested.

Moreover, pollen of *P. deltoides* stored, not dehydrated, at −40°C was used for crossing after 4 years of storage and seed set occurred (Cagelli, pers. comm.).

Cryopreservation seems a succesful method for long-term pollen storage of fruit trees (Polito and Luza 1988) as well as for forest trees (Jörgensen 1990; Lanteri *et al.* 1993). Cryopreservation of poplar pollen should be evaluated.

Storage of vegetative material

Storage of dormant buds

Dormant buds from adult trees could be used for poplar germplasm preservation. Such material offers prospects for long-term storage of germplasm if the dormant state can be prolonged without loss of viability and differentiation potential.

The potential of dormant buds for conservation of poplar germplasm has been tested by Ahuja (1988) with 14 aspen clones and hybrids. This study shows that storage potential of a clone is determined by the genotype and the conditions of storage. Five

storage temperatures were tested (0, –5, –8, –18 and –80°C) and –80°C seemed to be the best temperature for long-term storage (up to 2 years) of dormant buds.

After storage, viability and regeneration potential of buds must be tested by culturing bud explants on a regeneration medium and rooting of microshoots to yield whole plants. So, storage of dormant buds is a suitable way of conservation for these species where an effective protocol of *in vitro* micropropagation has been developed. This is the case for *P. nigra* (Whitehead and Giles 1977; Douglas 1984).

Cryopreservation (–196°C) of dormant buds of aspen also has been tested (Ahuja 1991). The growth and differentation of microshoots from buds after cryogenic storage can be achieved for all the clones tested only when cryopreservation is applied by a slow-freezing method (buds are first cooled to –40°C in a cryostat at –1°C/minute).

As pointed out by Ahuja (1991), cryopreservation of dormant buds is relatively simple compared with meristems or cells which require *in vitro* culture along with cryoprotectants and further preparation before storage in liquid nitrogen.

These studies should be extended to dormant buds of other species of *Populus*.

In vitro *culture storage*

Tissue culture techniques can be applied for storing germplasm over long periods. This system can be very useful for plants that are normally propagated vegetatively. With regard to the kind of explants, shoot cultures appear to be the most widely used for long-term storage.

In vitro shoot cultures can be maintained for several years with minimal growth if they are stored at low temperature (+2 to +4°C) combined with weak light or darkness (George and Sherrington 1984).

Other factors, such as the increase of sucrose concentration in the media, chemical treatments (abscisic acid) or culture under low partial oxygen pressure, have an inhibitory effect on plant cell growth (Bridgen and Staby 1983).

Shoot cultures of the hybrid *P. alba* × *P. grandidentata* 'Crandon' have been stored at +4°C in the dark for 5 years (Son *et al.* 1991). The survival was 70% and 25% after 2 and 5 years respectively. Color variations (0.25% albinos and 12.8% red pigmented) were observed in plants regenerated from long-term stored shoots (5 years). This suggests that long-term cold storage can induce genetic variations.

Storage conditions of poplar shoot cultures are being researched by Gaspar and coworkers (Universite de Lige). After 12 months of storage at +4°C in darkness, 6 of 8 clones tested had regenerated normal shoots when transfered to the normal *in vitro* culture conditions (Gaspar, pers. comm.).

Callus cultures also can be used as explants for long-term storage. It has been shown that after long storage of *P. nigra* callus cultures, the regeneration in whole plants is very difficult (Sumiya *et al.* 1988).

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Proposal for passport data for *Populus nigra*

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1. Clone name/number

Name or number assigned when a clone is entered into the collection.

Name or number that serves as a unique identifier and is assigned whenever a clone is entered into the collection.

Letters should be used before the name/number to identify genebank or national system.

In this case it could be:
ISP_N001 for the material maintained at ISP (Casale Monferrato)
ISP Brisighella for the material maintained at ISP (Casale Monferrato)
INRA_71002 for the material maintained at INRA (Orléans).

In this way codes used by the different institutes involved in *P. nigra* conservation (N001 for ISP Casale, 71002 for INRA Orléans, etc.) can be preserved with letters before the names/numbers to identify institutes that maintain a clone.

It is important to use letters that identify **the Institute** and not just the country, because in some countries there might be more than one institute involved in genetic conservation.

2. Country where maintained

Country in which clone is maintained.

3. Institutions where maintained

Name of institution in which clone is maintained.

4. Original clone name/number

Codes assigned from the institution of origin.

This is very important when the clone was obtained from another institution. It could be useful to identify duplicates held in different collections and should always accompany samples wherever they are sent.

5. Collecting number

Original number assigned by collector of the sample.

This item could be useful to identify duplicates held in different collections and should always accompany samples wherever they are sent (this number could be different from the "original clone number").

6. Other name associated with the name/clone
Any other identification number known for this clone. Another number can be added such as 6.3, etc.
6.1 Other number 1
6.2 Other number 2

7. Type of maintenance
1. Nursery stool-bed
2. Adult plantation
   2.1 Commercial plantation
   2.2 Conservation plantation
3. Pollen
4. More than one type (e.g. 1, 2)
5. None

8. Notes
Specify here any additional information.

9. Collecting institution
Institution which collected the sample.

10. Country of origin
Country in which the sample was collected.

11. Province/State
Name of the primary administrative subdivision of the country in which the sample was collected.

12. Department/Country
Name of the secondary administrative subdivision (within a Province/State) of the country in which the sample was collected.

13. Location of collecting site
1. Nearest town or village.
2. Unknown

14. Latitude of collecting site
Degrees and minutes followed by N (North)
(e.g. Casale Monferrato 45°07′ North: 04507N)

15. Longitude of collecting site
Degrees and minutes followed by E (East) or W (West)
(e.g. Casale Monferrato 8°30′ East: 00830E)

16. Elevation of collecting site (m)
Altitude above sea level.

17. Type of sample
Form of sample collected
1. Vegetative
2. Seed

18. Sex

1. Female
2. Male
3. Unknown

19. Pedigree

1. Female parent
2. Male parent

Specify original clone name/number (codes assigned from the institution of origin), country of origin and, if known, geographic coordinates (latitude, longitude and altitude) of the collecting site. Include the parents in the database even if they are not currently present in stool-beds (the Type of Maintenance should then be "none").

Country reports

Programme for the conservation of *Populus nigra* in France

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The programme for the conservation of *P. nigra* genetic resources started in the 1970s, with financial support from the ministry in charge of forests. Activities are at the national level and involve several institutions, mainly INRA, CEMAGREF and AFOCEL.

The first inventories and collections of native material were made in 1971-82, mainly in the upper valleys where poplar cultivation is almost absent. A second phase of prospecting started in 1991 along the main rivers, even in the vicinity of cultivated stands. These accessions only come from cuttings taken on presumed 'pure' individuals. The current *ex situ* collection comprises more than 300 accessions, vegetative copies from individual clones and progenies, from 108 sampling stations all over the country. This material is kept in a stool-bed; the establishment of conservation stands as adult trees is in progress. We also have 101 clones of various origins. Passport data are stored in a computerized database from the Forest Department of INRA.

The collection was first evaluated for selective traits, mainly resistance to rusts, and intraspecific crosses were made for the study of inheritance of quantitative traits. Characterization of the material, based on morphological and biochemical traits (isozymes), is in progress. Part of this material is used in the breeding programme, by crossings with *P. deltoides* and *P. trichocarpa*.

Research activities have three main objectives:

1. Describe the genetic diversity within the *ex situ* collection in order to have more efficient conservation stands.
2. Study the structure of the diversity in the wild to determine strategies for future collecting.
3. Set up a dynamic conservation strategy.

The genetic diversity (350 genotypes) is studied at different scales, between and within stands. The phylogeny of *Populus* species is studied through molecular markers and hybridization compatibility among species.

Populus nigra was elected this year as a pilot species by our National Technical Commission for Forest Genetic Resources Conservation, and a programme is being set up for the next 4 years. The main points are:

- inventory of the resources, both at the level of individual trees, and of native stands;
- survey of the pathogen populations;
- complementation of the *ex situ* collection, up to 500 clones;
- study of the genetic diversity between and within stands;
- development of managed *in situ* conservation strategies;
- protection of particular sites.

To achieve such a programme, we identified some urgent needs for which the network might be useful:

- guidelines for the identification of the species for the inventories;
- tentative typology of the native stands, according to demographic characteristics;
- reference material for the characterization of the collection;
- further research activities in the fields previously mentioned.

Populus nigra genetic resources in Italy

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Existing *in situ* protection system

No protection programmes exist in Italy for the preservation of *Populus nigra* L. *in situ* reserves. Although *P. nigra* can be found in some Regional Natural Parks or Natural Reserves, there are no specific programmes for its preservation.

In every region, protected areas are regulated by specific legislation. There is a general law in which the different kinds of protected areas are described (natural parks, natural reserves, natural monuments or other areas of particular interest), and the formalities for their institution, their managing board, the instruments for management and the administrative sanctions are indicated. The law also contains a list of the existing protected areas (e.g. for the Lombardy Region: Legge Regionale 30 novembre 1983, n° 86).

For the management of such protected areas, every park or reserve is to prepare a plan indicating the different areas included in the park (integral reserves, oriented reserves, agricultural-forest natural park, special protected areas, agricultural areas) and the general criteria for their management. One of these is the 'Piano territoriale di Coordinamento' for the Ticino Park, which is the most important riparian protected area in northern Italy.

Every park is also supposed to prepare a management plan, and in some cases also a more detailed plan taking into consideration all the aspects involved in the management of single areas. One of these is the 'Piano di settore boschi del parco lombardo della Valle del Ticino', which is a plan regulating forest management within the park. However, not even such a specific plan makes any reference to the situation of *P. nigra*. The only reference to it (though indirect) is to the cultivation of poplar hybrids in the park and to the relative limitations (Funaioli 1989; Borelli 1994).

The situation is rather complicated. From the establishment of a protected area to the drawing up of the plan for management a very long time can elapse. Many are the small protected areas, many are the institutions in charge of their management, different are the status of preservation and the information about the presence of *P. nigra* in these areas (many papers talk about *P. nigra* but often they are only hybrids) so it is very difficult to do something in a short time for *in situ* reserves of this species.

Establishment of *ex situ* genetic resources

The first collection of spontaneous *P. nigra* started before 1980 in central Italy. Some years later, in 1981-83, a large collection was made, covering the whole national territory. About 350 individuals were identified and vegetatively propagated. A form was compiled for each tree with measurement data (height, diameter, estimated age), description of the genotype (sex, habit, stem form, branchiness), health conditions and geographic coordinates. Distance from areas of large-scale poplar cultivation, old age, safety distance between sampled trees and ratio between sexes were the criteria adopted during sampling (Bisoffi *et al.* 1987).

In 1988-91 the Lombardy Region charged ISP with carrying out a study on the distribution of spontaneous poplars (*P. nigra* and *P. alba* L.) in the Lombardy plain (Anonymous 1989; Malinverno 1992); 270 genotypes of *P. nigra* and 130 of *P. alba* were located: 171 of the former and 75 of the latter were considered to be representative of the natural population. Every individual was described and photographed. The

sampling criteria were the same as those adopted in previous collections. Up to now only 43 of these genotypes have been collected. In this study we also considered the areas that could be preserved for the establishment of primary genetic resources. We found only small and fragmentary areas mainly in the valley of the Ticino Park and in some small areas in the Ogli and Adda Park and in some areas, still unprotected, along the Po River.

The ISP collection also includes some *P. nigra* clones provided by foreign countries. All the genotypes (about 450) are now in a collection in stool-beds, clonal banks and in arboreta (Table 1). A dBASE archive stores all the information available about the genotypes: code, synonyms, sex, geographic coordinates (latitude, longitude and altitude for native clones), institution of origin (for the clones obtained from other institutes), means of conservation (stool-beds, clonal banks and/or arboreta), behaviour toward main diseases and insects, use in crossing programmes and genotypes selected as best parents in the programme of the *P.* × *euramericana* breeding.

Evaluation

Nursery evaluation

To evaluate the variability existing in the ISP collection 426 genotypes were tested in nurseries established in three different localities (Casale Monferrato, AL; Palazzolo dello Stella, near Udine, and Bagni di Tivoli, near Rome) in 1992. Measurement data (height, diameter), morphological traits (leaf shape, stem form, branchiness), phenology and resistance to the main diseases (*Marssonina, Melampsora*) were considered.

As for the behaviour toward diseases, susceptibility to rusts was observed at Casale and Palazzolo (first year), and susceptibility to *Marssonina brunnea* was rated at Casale (second year). The variables obtained and the number of plants that survived at the end of the second year were then used to perform Principal Component Analysis.

As susceptibility to *M. brunnea* and *Melampsora* spp. had little relationship to any of the major components, they were removed from the analysis. Susceptibility to *Melampsora* spp. was then used only as an individual selection criterion. The analysis was then carried out on the first two components, which accounted for 54% of the total variation. The first one is linked with growth, stem straightness and survival at the end of the second year, the second one to the branch index.

Forty-eight individuals characterized by fast growth and a low branch index were selected (Table 2). They will go through further selection in plantations to be established in three different localities (Casale, Palazzolo and Spello). These genotypes were selected on the grounds of their behaviour toward rusts and *Phloeomyzus passerinii* Sign. (on the basis of lab-test results), although a few valuable genotypes were not eliminated.

It is worth noting that a high correlation exists among the three localities considered, as far as morphological characters and growth-related traits are concerned: diameter (0.7), height (0.6), branch index (0.6) and stem straightness (0.7). This will enable us to select, in one locality only, genotypes suitable for different environments. As to the origin of the genotypes selected, it is worth noting that most of them came from northern Italy (32), while only a few came from central-south Italy (5), Hungary (5), France (4) and Turkey (2).

Behaviour toward diseases

In addition to collecting information in the nurseries for the evaluation of *P. nigra* genotypes, observations were carried out at Casale on 570 individuals belonging to 114 *P. nigra* families, in a nursery established for the evaluation of existing correlation between breeding value estimated with intra- and interspecific crosses. The following information was obtained:

- *Marssonina brunnea* (Ell. & Ev.) P. Magn. — all the genotypes proved to be fairly susceptible; variability was low;
- *Melampsora* spp. — all the genotypes proved to be fairly susceptible, though there was some variability;
- *Venturia populina* (Vuill) Fabr. — there was some variability in behaviour;
- *Discosporium populeum* (Sacc.) Sutton — high susceptibility;
- Poplar Mosaic Virus — *P. nigra* showed resistance to PMV.

Behaviour toward insects

- *Phloeomyzus passerinii* Sign. — a large number of genotypes (about 250) have been evaluated in laboratory tests (in one or more trials) for resistance to *P. passerinii*. Variability is very high and not influenced by the growing locality. Resistance seems to be correlated with the latitude of origin; resistance is higher in the genotypes coming from the warmer areas of southern Italy (Allegro and Cagelli, unpublished).
- *Hyphantria cunea* Drury — *P. nigra* is avoided by females in the field and its leaves hinder larval growth (Allegro 1994).

Correlation between resistance and the phenolic content of the bark

About 30 genotypes are at present being investigated for possible correlations between resistance to *D. populeum* and *P. passerinii* and the phenolic content of the bark.

Correlation between resistance and isozymes

About 400 genotypes have been sent to CNR-Porano for isozyme analysis. The same genotypes are evaluated for resistance to *Marssonina* and *Melampsora* to investigate possible correlations.

Use in the breeding programme

P. × euramericana

Populus nigra is largely used in breeding for the production of *P. × euramericana* hybrids. Male and female *P. nigra* have been tested in progeny trials in the nursery within the breeding programme of *P. × euramericana*: 139 males were tested by crossing with 4-6 *P. deltoides* Bartr. females ('common tester' method); 41 females were tested by intraspecific crossings ('poly-cross' method).

The General Combining Ability of the parents was evaluated for early growth, stem straightness, branch index and susceptibility to *M. brunnea*.

The progeny tests in the nursery enabled the selection of the best parents: 39 males and 15 female. Within the *P. nigra* female progeny 71 clones have been selected.

Correlation between Breeding Value estimated with intra- and interspecific crosses

To estimate the General Combining Ability (Breeding Value) and the Specific Combining Ability in intra- and interspecific crosses, nursery tests have been established in three different localities with:

114 families *P. nigra* × *P. nigra* (570 individuals)
76 families *P. deltoides* × *P. nigra* (380 individuals)
81 families *P. deltoides* × *P. deltoides* (405 individuals).

*Crossings between Aigeiros section and Tacamahaca (***P. simonii** *and* **P. maximowiczii***) section*

Exploratory crossings with selected parents of *P. nigra* and *P. deltoides* are underway.

Commercial breeding

The best parents of *P. nigra* (8) and *P. deltoides* (7) selected in the progeny tests are used in a crossing programme to evaluate Specific Combining Ability and to obtain a large number of clones to be utilized in clonal selection.

Table 1. *Populus nigra* L.: origin of ISP genetic resources

Country	Nursery test[1]	Clonal bank[2]	Stands	Pollen stored	Tested in progeny trials		Selected from progeny trials	
					M	F	M	F
Northern Italy	176	242	150	18	60	28	19	12
Central Italy	77	77	75	17	32	7	10	3
Southern Italy	78	81	81	11	36	6	9	—
Afghanistan	1	1	1	—	—	—	—	—
Austria	—	1	1	—	—	—	—	—
Bulgaria	1	1	1	—	—	—	—	—
Czechoslovakia	1	1	1	—	—	—	—	—
France	39	41	41	2	2	1	—	—
Germany	2	4	4	—	—	—	—	—
Greece	6	6	5	1	1	—	—	—
Hungary	9	9	9	—	—	—	—	—
Iran	3	3	3	1	—	—	—	—
Iraq	1	1	1	—	—	—	—	—
The Netherlands	2	2	2	—	—	—	—	—
Poland	1	2	2	—	1	—	—	—
Portugal	1	1	1	—	—	—	—	—
Romania	—	1	—	—	—	—	—	—
Spain	3	3	3	3	3	—	—	—
Syria	1	1	2	—	—	—	—	—
Turkey	13	18	19	3	3	—	—	—
United Kingdom	1	1	1	—	—	—	—	—
ex-Yugoslavia	4	5	3	—	—	—	—	—
Selected by CSAF, Italy	5	5	5	1	1	—	—	—
Selected by NE Expt. Stn., Philadelphia, USA	1	2	1	—	—	—	—	—
Total	426	509	412	56	139	41	39	15

Table 2. Genotypes selected in nursery tests

Identification no.		Sex	Lat. N	Long. E	Alt.	Country	Prov.	Locality
N002	C 070	F	46°22′	13°04′	296	Italy	UD	Amaro
N009	C 074	M	46°11′	12°46′	30	Italy	PN	Cavasso Nuovo
N010	C 075	M	46°11′	12°46′	30	Italy	PN	Cavasso Nuovo
N011	C 081	M	46°10′	12°14′	383	Italy	BL	Belluno
N020	C 082	M	46°05′	12°01′	308	Italy	BL	Santa Giustina
N033	C 092	M	45°52′	11°02′	204	Italy	TN	Rovereto
N040	C 102	M	45°48′	9°50′	514	Italy	BG	Casnigo
N043	C 101	F	45°47′	9°49′	396	Italy	BG	Fiorano
N045	C 174	F	44°45′	11°52′	3	Italy	FE	Rovereto
N047	C 100	F	45°45′	11°25′	279	Italy	VI	Piovene Rocchette
N049	C 056	M	45°40′	8°09′	500	Italy	VC	Trivero
N058	C 094	M	45°33′	11°09′	470	Italy	VR	Badia Calavena
N071	C 053	M	45°06′	8°05′	428	Italy	AT	Robella
N094	C 012	M	44°59′	9°41′	89	Italy	PC	Turro
N122	C 017	F	44°46′	8°21′	138	Italy	AT	Nizza
N133	C 165	M	44°41′	10°06′	158	Italy	PR	Fornoro di Taro
N139	C 038	M	44°34′	8°56′	350	Italy	GE	Ronco Scrivia
N150	C 159	F	44°28′	10°29′	562	Italy	RE	Carpineti
N164	C 163	M	44°12′	9°58′	64	Italy	SP	Aulla
N224	40	F	41°45′	13°16′	543	Italy	FR	Fiuggi
N324	139	M	42°19′	14°03′	49	Italy	PE	Rosciano
N356	TR-56-73 (56/73)	M				Turkey		
N357	JEAN_ POURTET	M				France		
N365	VERT_DE_ GARONNE	M				France		
N378	C 182	F	45°32′	8°25′	204	Italy	VC	Carpignano S.
N379	C 183	-	45°33′	10°13′	218	Italy	BS	Concesio
N380	C 184	F	45°43′	10°13′	386	Italy	BS	Marcheno
N385	C 189	F	45°42′	10°23′	300	Italy	BS	Idro
N388	C 192	M	46°02′	10°43′	761	Italy	TN	Tione
N400	LP 1	F	42°33′	12°46′	367	Italy	TR	Lago di Piediluco
N413	MC 18	F	43°11′	12°56′	600	Italy	MC	Fiuminata
N430	BRISI-GHELLA	-	44°10′	11°38′		Italy	RA	Brisighella
N449	62/4	M				Turkey		
N476	PE 4-66	F				Hungary		
N477	PE 5-66	F				Hungary		
N478	PE 6-66	F				Hungary		
N479	PE 7-66	-				Hungary		
N480	PE 8-66	-				Hungary		
N507	AFO 76403	-				France		Drôme
N514	AFO 77012	-				France		Gironde
N527	1 NT 88	-	43°48′	10°33′	19	Italy	LU	Lunata
N529	3 NT 88	-	45°05′	11°36′	9	Italy	PD	Lendinara
N531	5 NT 88	-	45°06′	11°30′	11	Italy	RO	Badia Polesine
N533	7 NT 88	-	45°14′	11°39′	15	Italy	PD	Este
N534	8 NT 88	-	45°24′	11°53′	12	Italy	PD	Padova
N538	12 NT 88	-	45°14′	11°39′	15	Italy	PD	Este
N543	19 NT 88	-	45°02′	8°51′	76	Italy	AL	Isola S.Antonio
N547	23 NT 88	-	44°45′	7°36′	260	Italy	CN	Murello

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Conservation of forest genetic resources of *Populus* in Spain

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The conservation of forest genetic resources is closely related to the activities of forestry breeders. A clonal bank of *Populus nigra* has been established in Zaragoza and a collection of seeds of some families of *Populus alba* has been made by CIFOR-INIA. A procedure for genetic conservation in *P. alba* and *P. nigra* includes the basic phases in the maintenance of the genetic variation: exploration, collection, conservation and characterization.

The procedures of Spanish forest genetic conservation are now closely related to the endeavors of forestry breeders to capture the genetic variability of the forestry population.

In general, all work related to the study of genetic parameters is of relevance to genetic conservation, because this work has passed through the different phases established for genetic conservation — exploration, collection and characterization — leaving conservation to be dealt with.

Work on provenance of the main Spanish forestry species should facilitate the drafting of conservation plans.

A program to conserve and use the phytogenetic resources (ORD.: 23 april 1993 published B.O.E. 7 may) has been approved, in response to current concern about genetic resources. A program for the genetic conservation of *P. alba* and *P. nigra* was approved by the Ministry of Agriculture.

Measures for the conservation of autochthonous *Populus* resources

Genetic resources in the riparian communities are under threat. In Spain, as in the rest of Europe, riparian communities are being threatened by development prejudicial to conservation.

Procedures for *Populus* resource conservation are underway. Thus the Unit for Forestry Resources (SIA-Zaragoza) has begun some activities aimed at the preservation of *P. nigra* genetic resources. Some of the work of CIFOR-INIA has been devoted to the collection of seeds in some families of *P. alba* and to carrying out progeny trials.

The basic phases for the maintenance of the genetic variation (namely exploration, collection, conservation and characterization) are included in the proposal scheme for *Populus* genetic resource conservation.

Exploration

In recent years a Forestry Map was made, so stands of *P. alba* and *P. nigra* were identified and located. This map will be of great help in developing exploration work; the information included in the map (geological, climatic and botanic) is also very useful.

Exploration work has been classified into several stages:

1. Location of stands on the map, search for information and outlining of itineraries.
2. Visit to the stands to check and to evaluate the need to preserve them. Data to record are: other species, estimated area, pests and diseases, level of conservation. This information will give us a resume of stand peculiarities.
3. We will complete the information about the selected stands with data from the field and the forestry map. Then the stands for collection will be selected according to evaluation.

Collection

After exploration, at least two trees of different sex will be selected, if the sexual composition of the stand allows it. We have to consider the sexual and clonal distribution within the stands in order to guarantee genetic differences among clones selected.

During collection we will take the passport data: registration number, collector, date, location, province, latitude, longitude and elevation. Other data could be: dendrometric data (height, diameter), straightness and other geographic parameters.

Conservation

Vegetative propagation is the method chosen for maintenance of the genotype and genetic variability of the two species, since the ease of handling and the long collection period of the cuttings make it easier than collecting seeds. The conservation of *Populus* seeds is difficult and could produce anomalous seedlings. Furthermore, the propensity of both black poplar (*P. nigra*) and white poplar (*P. alba*) to interspecific hybridization could produce genetic contamination.

On the other hand, vegetative reproduction is an inconvenience in some cases. When the tree is old, the quality of cuttings is low and they have difficulties producing roots, so the cuttings collected should be placed in S-L pots in a greenhouse until rooting is sure. In some cases *in vitro* culture or hormone application could be used in order to ensure propagation.

Two different conservation situations ('collection' and 'stool') have been established:

Collection: its purpose is to take data for characterization of material, and to obtain catkins for breeding purposes.

Stools: material to make easier the work of conservation, propagation and interchange. We estimate our future collection will consist of 350 clones.

Characterization

Characterization of the material collected will be based on easily observed traits: phenology, growth straightness and branching.

We estimate that the program will take 4 years to complete. A catalogue of the collection with elementary data about *P. alba* and *P. nigra* will be published.

Black poplar (*Populus nigra*): the situation in Hungary

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Populus nigra is an indigenous species in Hungary; its reproduction is assured both by seeds and by coppice shoots. In most cases it regenerates successfully along rivers but often can be found in drier sites too. From a historical perspective, *P. nigra* was to a great extent artificially planted in order to fix moving sands on the Great Hungarian Plain at the beginning of the 18th century. Growing of this species was then gradually replaced by the introduction of Euramerican poplars (first *Populus* x *euramericana* var. 'Marilandica' and later var. 'Robusta') at the beginning of the 20th century.

The cultivation of native black poplar was more difficult because of the species' vulnerability to bark diseases, which is one of the major causes for high mortality rates.

Attention was again drawn to *P. nigra* in the 1950s, when the Forestry Research Institute started the selection of parental material for hybridization breeding of poplars. The selection criteria included: good base shape, vigorous growth and high tolerance to diseases (especially to *Dothichiza populea*, the most threatening poplar disease in Hungary).

Until 1960, Hungarian scientists had selected a total of 39+ trees. The first genetic collection of *P. nigra* was thus created at the Forestry Research Institute. This collection still exists (as a stool-bed orchard, *ex situ*).

Meanwhile experts started to select clones of black poplar along the Danube for wood production. Most of the selected trees still grow *in situ*. In most state-owned forest enterprises, stool-bed orchards were established for practical propagation purposes as well.

However, the cultivation of *P. nigra* in Hungary became insignificant because of the improved performance of hybrids with introduced poplar species. This process was reinforced by Hungarian scientists, who obtained Euramerican clones with excellent growth performance using selected clones of *P. nigra* of Hungarian origin. These hybrid clones utilized in forestry practice have already been cultivated to a large extent (*Populus* x *euramericana* var. 'Pannonia', 'Kaperky' and 'Kaltay').

Experts from several Danubian countries (Hungary, former Yugoslavia, Romania, Czech Republic, Slovakia and Bulgaria) met in Budapest in 1985 and decided to establish a common programme of action aiming at the preservation of the genepool of *P. nigra*.

The importance of black poplar in Hungary has increased in the 1980s. Several fluvial valleys were set under nature protection, where it is allowed to reforest only with indigenous species, including *P. nigra*. New selection activities concerning *P. nigra* were thus started. In 1992, Hungarian experts created a special committee for the genetic conservation of *P. nigra*. The Committee has a fundamental task to identify production sites (individuals, groups of trees, subpopulations and populations), to consider their sustainable utilization and *in situ* conservation, as well as the establishment of *ex situ* collections (single trees and stool-bed orchards) and further development of relevant methods. Hungarian experts therefore wish to participate in the international network on genetic conservation of *P. nigra* and agree on the tasks defined at the working group meeting in Izmit. We believe that it is of high importance to publish a homogeneous identification methodology.

It is the identification of pure *P. nigra* species that is of great concern. The reason for that is the frequent occurrence of spontaneous hybridization with Euramerican poplars.

Table 1 shows habitats and identified collections (the note "not controlled" means lack of identification) of *P. nigra*. There is evidence that the number of the provenances

shown in the table will decrease considerably due to subsequent identification. According to a forest inventory showing groups of trees and populations of *P. nigra* in Hungary, the share of black poplar in the total area of poplar plantations decreased as follows (after successive data inventories):

1953 20.0%
1957 12.8% (4452 ha)
1973 2.9% (4477 ha).

Table 1. Genetic conservation in *Populus nigra* in Hungary, 1994

Institute **Locality or region of conservation**	*Ex situ* **collections, samples**	*In situ* **units**	**Notes**
Forestry Research Station (ERTI), Püspökladány			
Bajti	43		by trees; controlled
Bajti	46		stool-bed orchards; controlled
Balotaszállás	18		by trees; controlled
Mosonmagyaróvár		46	selected trees; controlled
Ujrónafö		6	selected trees; controlled
Dunaszentbenedek		18	selected trees; controlled
Baja		22	selected trees; controlled
Middle part of the Tisza river valley		3	selected trees; controlled
Upper Tisza valley		155	individual trees, groups of trees or subpopulations; not controlled
Upper Tisza valley		42	groups of trees in various mixed stands (562 ha in total); not controlled
Company for Forest Action "Gemenc"		55	individual trees, groups of trees or subpopulations; controlled
Institute for Agriculture, Budapest			
Area of Hungary		1060	individual trees or groups of trees in various stands; not controlled
Total	107	1407	

Conservation of poplar and arborescent willow genetic resources in Croatia

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Introduction

The conservation of genetic reserves of particular tree species, especially the endangered ones, must be given the highest priority in any extensive program of forest resource management. It is necessary to conserve the existing genetic diversity of forest stands and to preserve it over the following generations in order to maintain the stability of any forest community ecosystem. The conservation of genetic diversity of each particular forest tree species makes possible the optimal utilization of all ecological niches for artificial reforestation in forest range expansion (Schütz 1990; Ahuja and Libby 1993).

The genetic diversity conservation of some species of poplars and arborescent willows is particularly important for several reasons, the most important being:

- the growth of human population results in the increased demand for products made of soft deciduous wood;
- the consumption of soft deciduous wood has a positive correlation with the increase in level of living;
- the forest range expansion and the establishment of pioneer plantations in regions of lowland forests require facilitation of the renewal of the common oak and the narrow-leafed ash, and the availability of clones specifically adaptable to atypical poplar and willow stands;
- in our tree improvement work, collections of clones produced by hybridization and having divergent genetic constitution will enable us to create new progeny;
- clones of superior quality, compared with the existing ones, in terms of adaptation as well as tree trunk and wood quality. It is important to produce genotypes reacting well by modifications to environmental factors and adaptable to optimal environments, as well as clones with a specific adaptability to environmental stress. Selections of poplar and willow clones with specific adaptability to the environmental stress will be particularly suitable for growing in marginal sites and will play an important role also in operations of forest range expansion in stands which proved to be unfavourable for food production. Possibilities based on the selection for GCA and SCA in forest trees being very pronounced, it is to be expected that the improvement of poplar and willow trees by combined methods of selection and hybridization will provide new genotypes which will meet the requirements of ecology, genetics and economy.

In operations intended for conservation of poplar and willow tree genetic diversity, care must be taken to preserve variability, namely the population variability, at the level of provenances and local populations, the family variability and the individual variability within the family (Zsuffa 1974; Vidaković 1992; Vidaković and Graćan 1994).

The selections produced are to be tested in a carefully planned network of field experiments. These experiments must include a large number of selections and must be located in sites where the (potential) cultivation of a given selection is planned. Only in this way is it possible to classify selections in groups for their cultivation in a given stand or a group of similar stands in the multiclonal plantations (Bisoffi 1992). Thus it is possible to use successfully all potential ecological niches of a given species and as well to extend the forest range to such stands which are less favourable for the given species. From the clonal tests and the comparative clonal plantations (archives) established on various sites, it is necessary, through a periodical selection, to make permanent

introduction of new clones into the reproduction, which will considerably improve silviculture of the given species.

The multiclonal plantations of poplar trees and arborescent willows, together with the existence of genetic divergency among clones, make the established plantations very similar to natural ecosystems, i.e. to stable ecosystems as well (Krstinić *et al.* 1992).

The conservation of poplar tree and willow tree genetic reserves by means of clones using the *ex situ* method is justified for several reasons:

- poplar and willow seeds cannot stand long storage since they soon lose their germinability;
- by cloning superior genotypes in natural stands, the most valuable genetic material from our forests will be kept unchanged, thus enabling the total genetic variance to be preserved and the average genetic gain to be obtained;
- it should be pointed out also that only by cloning selected individuals in natural poplar and arborescent willow populations is it possible with a given genotype to maintain positive effects of recombinations of this transgression which is often present, for instance, in forest stands of the European black poplar and the white willow.

Poplar and arborescent willow clones which represent interspecific hybrids can be very useful in our future work on improvement by transgression, backcross with autochtonous species, and in the production of trispecies hybrids.

Conservation using the *in situ* method

For preserving genetic reserves of the European black poplar, the white poplar and the white willow using the *in situ* method in the territory of Croatia, in total 10 654 ha of forest stands have been selected (Table 1). The larger areas of these stands are located along the Danube and Drava Rivers, while the smallest ones are along the Mura and Sava Rivers. The age of these stands ranges between 1 and 67 years. As far as the age structure of the European black poplar is concerned it is not satisfactory from the aspect of genetic reserves conservation. It is known that, owing to the spontaneous hybridization of female hybrid clones *P.* x *euramericana* with the local black poplar resulting in the introgression of the Eastern cottonwood, *P. deltoides* (Bisoffi *et al.* 1987), younger forest stands of European black poplar do not meet fully the requirements of conservation. On the other hand, with regard to the white poplar (*Populus alba*) and white willow (*Salix alba*) populations, the selected stands meet fully the requirements of the conservation of genetic resources.

The abovementioned three species do not occur in pure stands but in mixed ones, with the exception of the white willow which in lower positions forms pure stands. The best known phytocoenosae are: *Populetum nigro-albae* Slav. 1962, *Salici-Populetum nigrae* Tx. 31, Meijer-Drees 36 *rubetosum caesii* Rauš 1973 and *Galio-Salicetum albae* Rauš 1973. Together with the white poplar (*P. alba*), hybrids between the white poplar and the aspen (*P. tremula*) can often be found. Grey poplar (*P.* × *canescens*) is relatively easy to distinguish morphologically from white poplar (*P. alba*).

The selected forest stands of these species are spread discontinuously and longitudinally near the Danube and Drava Rivers. Thus, together with the smaller areas selected along the Mura and Sava Rivers, the conservation of genetic diversity of these species in the territory of Croatia has been ensured.

In these selected forest stands, regular silvicultural operations such as care, cleaning and thinning should be carried out. Cleaning should be done when trees are 4 and 7 years old (Majer 1994) so that the best trees (phenotypically) grow tall. Thinning should start at the age of 10 years and be continued until felling. The resulting stands would be of mixed type, as similar to the natural structure as possible, which would ensure their stability, productivity and long life (Rauš and Matić 1990).

Table 1. Forest stands of poplars and arborescent willows in the Republic of Croatia

No.	Provenance	*Populus nigra*		*Populus alba, P. tremula, P. x canescens*		*Salix alba*	
		Area (ha)	Age (yrs)	Area (ha)	Age (yrs)	Area (ha)	Age (yrs)
1	Danube River	3689.00	1-56	47.00	40	3713.80	1-67
2	Upper part of Drava River	31.00	1-30	—	—	884.32	1-48
3	Lower part of Drava River	613.59	1-40	426.12	3-40	838.13	3-35
4	Upper part of Sava River	113.11	21-56	—	—	—	—
5	Middle part of Sava River	—	—	3.37	30-60	—	—
6	Mura River	295.00	17	—	—	—	—
	Total	4741.70	1-56	476.49	3-60	5436.25	1-67

Using the ex situ method for poplars

An important collection of poplar clones from various Sections is available, mostly from Section *Aigeiros* (Table 2), representing a good basis for further activities on improvement of the poplars (Krstinić and Kajba 1994). The prerequisite for the successful improvement of poplar trees from Section *Aigeiros* is also the existence of an important collection of Eastern cottonwood clones (*P. deltoides*), in addition to the European black poplar (*P. nigra*). The collection of Eastern cottonwood and *P.* × *euramericana* clones in clonal archives, clonal tests and plantations (in total 13 331.76 ha) provides the genetic diversity of the Eastern cottonwood (Ying and Bagley 1976), as one of the partners in the improvement of the European black poplar by hybridization. By providing a sufficient number of European black poplar clones with the divergent genetic constitution, it is possible to prepare meticulously conceived improved programs for these species based on GCA, SCA and RRS (Bisoffi 1990). Clones of the interspecific hybrids *P.* × *euramericana*, with divergent genetic constitution, make it possible for us to use a method of improving Eastern cottonwood and European black poplar by transgression. This method has given good results in improvement of arborescent willow trees.

Clones *P.* × *interamericana*, as well as clones and families of pure species, *P. simonii* and *P. trichocarpa*, enable the production of trispecies hybrids. By selection and cloning the plus variant from such hybrid combinations, it is possible on the basis of recombinations to take out genotypes which also will have positive economic properties (Frison and Bisoffi 1988).

White poplar (*P. alba*) is an interesting species for cultivation on heavy common oak soil in Posavina, and on poorer soil along other rivers in Croatia. By creating collections of clones of this species that propagate autovegetatively, breeding of this species will be considerably improved.

Table 2. Poplar clones in clonal tests, archives and plantations

No.	Species	No. clones	Progeny	No. cultivation localities	Origin
Section *Aigeiros*[1]					
1	*Populus nigra*	18	—	1	Croatia
2	*Populus deltoides*	72	—	19	USA
3	*Populus* × *euramericana*	20	—	14	Italy, Hungary, Spain
4	*Populus* × *interamericana*	3	—	5	Belgium
Section *Tacamahaca*					
5	*Populus trichocarpa*	—	300	1	seed obtained by Dr Steenackers
6	*Populus simonii*	1	—	1	
Section *Leuce*					
7	*Populus alba*	2	—	2	Italy

[1] Area = 13 331.76 ha.

The activities on conservation of the European black poplar (*P. nigra*) genetic reserves started in 1993 (Table 3). In the nursery we have produced 36 clones, but our intentions are to go on with our work in the region of the Mura, Sava and Drava Rivers in 1994/95; unfortunately, any such activities in the region of the Danube River are impossible owing to the occupation of the eastern part of Croatia.

The European black poplar stands where the selection will be made are distributed longitudinally near the rivers; this ensures the genetic diversity in clonal collections of this species. This is also an endangered species in Croatia because of human actions, whose interventions in the environment destroy its original stands, and because of the presence of genes of the Eastern cottonwood (*P. deltoides*) in younger stands; thus, through the spontaneous hybridization with female clones *P.* × *euramericana*, especially I-214 (Bisoffi *et al.* 1987), we are confronted with the problem of the selection of pure European black poplar. The selection of the pure European black poplar is possible only by cloning very old individual trees or groups of trees of this species preserved in particular localities.

We think that the selection of plus variants from young forest stands of the European black poplar is also very interesting since in this way it is possible to select new genotypes which will be well adapted to specific, local sites in which the silviculture of the Eastern cottonwood and hybrid poplars *P.* × *euramericana* (Komlenović and Krstinić 1994) are problematic. In our opinion, the collection of 400-500 clones of the European black poplar from the territory of Croatia would meet the requirements of conservation of genetic resources of this species, provided that the selection is made in the way shown on the attached map of Croatia (Fig. 1). Of course, the requirements for the sex ratio in the selected material to be approximately 50:50 should be met.

Table 3. Selected clones of European black poplar

No.	Provenance	Selected clones	Age (yrs)	Planned clones	Remarks
1	Lower part of Drava River	10	100	100	
2	Lower part of Drava River	17	200	100	
3	Upper part of Sava River	9	10	50	Plus variants from the progeny of *P. nigra,* most probably the introgression with *P. deltoides*
4	Mura River	—	—	100	
5	Danube River	—	—	100	
	Total	36		450	

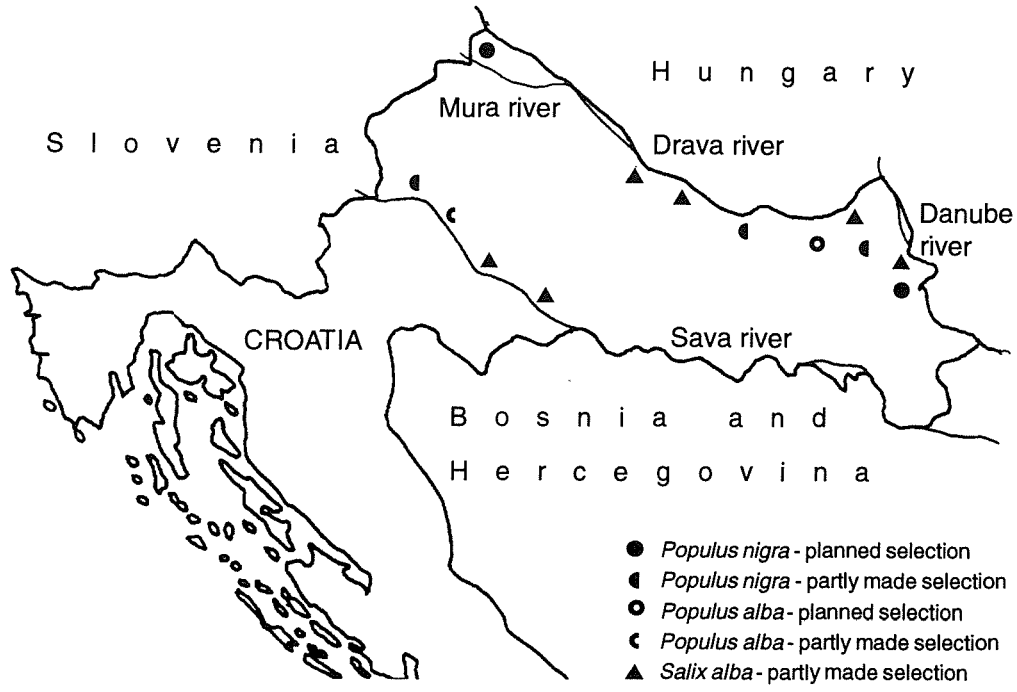

Fig. 1. Selected sites of selection of poplars for conservation in Croatia.

The production in the tree nursery comprises 43.4% of the Eastern cottonwood clones and 46.4% of *P.* × *euramericana* clones. Proportions of clones of other poplars are negligible in this production (Table 4).

Table 4. Poplar clones in nursery production

No.	Botanical name	Designation		Country of origin	Reproduction in 1993	
		Intern.[1]	Original		Pieces	%
1	*P. deltoides*	Lux; 618	I-69/55	Italy	16 427	12.8
2	*P. deltoides*	Begej; 725	S-179-1	Belgium	7 602	5.9
3	*P. deltoides*	Drina; 450	A-19	Germany	8 037	6.2
4	*P. deltoides*	Drava	55/65	Poplar	8 249	6.4
5	*P. deltoides*	Tisa	457	Institute,	10 540	8.1
6	*P. deltoides*	Dunav	S 1-8	Novi Sad	5 315	4.0
7	*P.* × *euramericana*	I-214	I-214	Italy	10 169	7.8
8	*P.* × *euramericana*	BL Constanzo	BL Constanzo	Italy	24 908	19.2
9	*P.* × *euramericana*	Cima; 270/81	Cima	Italy	8 825	6.8
10	*P.* × *euramericana*	Tiepolo; 275/81	Tiepolo	Italy	2 911	2.2
11	*P.* × *euramericana*	M 1	cl. 59/3	Hungary	13 365	10.4
12	*P. alba*	L 12	—	Italy	3 695	2.8
13	Other clones (11)				9 600	7.4
	Total				129 643	100.0

[1] Name according to international register.

Using the ex situ *method for arborescent willows*

Clonal archives and clonal tests comprise 256 clones (Table 5). The largest number of clones belong to the species *Salix alba*. The clones of pure species *S. alba* as well as clone hybrids *S.* × *viridis* and *S.* × *rubens* have been selected from local stands in the regions of the Rivers Danube, Sava and Drava. In addition to autochtonous clones we have a certain number of white willow clones of allochtonous origin, the whole representing a good basis for the genetic differentiation of reproduced plant materials. The existence of genetic divergence in the raised clones of both the white willow and its hybrids made possible the secondary selection of genotypes with specific adaptability to atypical willow sites, such as, for instance, oak tree sites in the region of lowland forest along the Sava river. Such genotypes are particularly important in establishing pioneer plantations on degradated and devastated areas inside the lowland forest range with the aim to facilitate the renewal of more valuable broadleaved trees, in the first place those of the common oak (*Quercus robur*) and the narrow-leafed ash (*Fraxinus angustifolia*).

We also have very valuable collections of clones of pure *S. matsudana* as well as clones of hybrids of *S. matsudana* × *S. alba*, with the divergent genetic constitution. These clones were supplied from China and New Zealand (by courtesy of Dr Zhu Zhaghua and Dr A.G. Wilkinson). This collection of clones is a good basis for the production of new genotypes suitable for production of biomass in short rotation, as well as for the improvement of arborescent willows by transgression, backcross, production of trispecies hybrids and self-fertilization (Krstinić 1971; Krstinić and Trinajstić 1992).

Furthermore, we have very rare interspecific hybrids of wide-leaved willows and the white willow which are verified triploids *S. alba* × *S. sitchensis* (Borzan *et al.* 1993), *S.* × *savensis* (Trinajstić and Krstinić 1989).

Table 5. Arborescent willow clones in clonal tests, archives and plantations

No.	Species	No. of clones	No. of cultivation localities[1]	Origin
1	*Salix alba*	183	18	Croatia, England
2	*Salix fragilis*	6	2	Croatia
3	*S.* × *rubens*, *S.* × *viridis*	20	8	Croatia
4	*S. alba* × *S. sitchensis*	5	10	Croatia, Alaska
5	*S.* × *savensis* Trinaj. et Krst.	1	1	Croatia
6	*Salix matsudana*	4	3	China, New
7	*S. matsudana* × *S. alba*	10	2	Zealand
8	*S. matsudana* × *S. fragilis*	1	1	China, New
9	Multispecies hybrids	27	—	Zealand, Croatia
	Total	257		China, Croatia

[1] Area = 4450.24 ha.

For establishment of classical willow plantations in the territory of Croatia, the clonal material of autochtonous origin and with the divergent genetic constitution is reproduced in tree nurseries (Table 6). Clonal plantations are multiclonal with the mosaic distribution of clones. So far, 4450 ha of arborescent willow plantations, between 1 and 20 years old, have been established in Croatia.

Table 6. Arborescent willow clones in nursery production

No.	Original designation	Botanical name	Country of origin	Reproduction in 1993	
				Pieces	%
1	107/65/6	*Salix alba*	Croatia	14 870	16.7
2	284	*Salix alba*	Hungary?	3 480	3.9
3	378	*Salix alba*	Hungary	2 150	2.4
4	V 158	*Salix alba*	Croatia	2 020	2.2
5	V 160	*Salix alba*	Croatia	5 250	5.9
6	B 44	*Salix alba*	Croatia	3 850	4.3
7	B 72	*Salix alba*	Croatia	3 820	4.3
8	79/64/2	*Salix alba*	Croatia	2 600	2.9
9	73/64/6	*Salix alba*	Croatia	8 480	9.5
10	73/64/8	*Salix alba*	Croatia	1 870	2.1
11	V 093	(*S. alba* × *S. a.* var. *vit.*) × *S. alba*	Croatia	4 630	5.2
12	V 052	*S. alba* var. *calva* × *S. alba*	England, Croatia	9 760	10.9
13	V 164	*S. alba* × *S. sitchensis*	Croatia, Alaska	2 600	2.9
14	V 0240	*S. alba* var. *calva* × *S. alba*	England, Croatia	2 180	2.4
15	11/1	*Salix alba*	Croatia	4 850	5.4
16	Other clones (58)			18 360	20.6
	Total			88 770	100.0

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Populus nigra in Germany: a case study

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Situation

From the EU Red Lists of species threatened by extinction in Germany, published by the nature conservation administration (LOLF 1970), it is obvious that *Populus nigra* is highly endangered.

Populus nigra is touched on the species level, whereas many other tree species suffer from loss of genetic information. With respect to tree species the degree of danger could be compared with that of *Ulmus* spp. This opinion about the German situation corresponds to a publication of M. Arbez and F. Lefèvre (1993) about black poplar in a European forest genetic resources programme.

Investigations on the reasons for the decline of *P. nigra* in Germany show remarkable parallels with other European countries. For Germany the main reasons might be characterized as follows (Fröhlich 1963; DDG 1985):

1. Natural distribution of *P. nigra* is concentrated on riparian sites with good water and nutrition supply under favourable climatic conditions. Most of these sites have been destroyed and transformed into agricultural or intensively used industrial areas.

2. Hydraulic engineering, e.g. building of dams, led to loss of suitable areas and to the disturbance of natural regeneration.

3. Increasing cultivation of vigorous *P.* × *euramericana* cultivars displaced *P. nigra.*

4. The surrounding of natural stands with cultivated interspecific hybrids might cause genetic pollution as pointed out by Arbez and Lefèvre (1993). Our own experience proved this phenomenon, when evaluating natural regeneration in an area dedicated for nature protection near the River Rhine (Fröhlich *et al.* 1988).

Strategy

With regard to these facts and the dramatic situation of this species, a strategy for conservation has to be derived that includes *in situ* and *ex situ* measures. The main objectives could be summarized as:

- protection of sites where natural stands could be observed or where *P. nigra* could be cultivated;
- protection of stands and individuals;
- collection of reproductive material for establishing genepools and exchange with other institutions.

Requirements

Before putting the above objectives into practice, some requirements have to be met. First, inventories of stands and single trees have to be made in order to start preservation programmes; then, methods for propagation and exchange of material have to be developed.

Previous results in Germany

In 1963 the former Research Institute for Poplar Cultivation was in charge of an inventory of poplars in the Federal Republic of Germany (Fröhlich 1963). With the assistance of the forestry administration of the federal states, poplars which were supposed to be *P. nigra* were selected and catalogued. Determination and cultivation in a nursery followed as far as possible with respect to restricted funds. As a result, the *ex situ* collection includes 59 clones of *P. nigra* only. Hundreds of trees proved to be hybrids with characteristics close to those of *P. nigra*.

A German statute for protecting nature puts emphasis on riparian areas. There are special protection programmes for these sites. But this could be looked upon as a global measure without specific focus on *P. nigra*. Functioning preservation will only be guaranteed when declaration of natural reserves takes place. This needs concrete reasons, of which the existence of *P. nigra* is one.

The propagation of section Aigeiros by cuttings is very old and traditional. The establishment of stool-beds could be realized with sufficient success.

Problems and need for further research

The most important problem may rise from the fact that the inventory is about 30 years old. At that time, the inventory was already estimated to be incomplete. This lack of detailed information was enlarged when including stands in eastern Germany. There are no data because forestry administration had neglected tree species that seemed to be without economic value for a long time. *Populus nigra* had been incorporated in a group with other deciduous trees.

Meanwhile the situation has changed. Programmes for the conservation of forest genetic resources have started in Germany.

Another very decisive aspect is having a method for the identification of pure *P. nigra*. There is an urgent need for an easy and precise procedure which allows outdoor definition. Some characteristics have been published in a leaflet (Forschungsinstitut 1963; Hoffmann 1976) but nevertheless morphological and phenological criteria have to be compared with physiological ones.

Activities

To attain the aim of maintaining or increasing the genetic resources of *P. nigra* with regard to the facts mentioned, the following activities should be planned:

1. The elaboration of a map showing stands and individuals as output of a recent inventory covering the whole of Germany. The first step could be a questionnaire sent to forestry and nature protection administrations and nongovernmental organizations. It is suggested that one institute should be in charge to coordinate. Experienced researchers may verify the definition in the course of field trips.

2. Using activity 1 as a base, proposals for *in situ* conservation should be submitted to administrations. Nature preservation areas have to be declared to protect natural stands. A conversion to other types of land use that might affect *P. nigra* (e.g. agriculture, forestry) should be prevented.

3. Existing clone archives should be completed. As further results of these activities plants should be propagated in specialized nurseries based on collections of seeds and cuttings for cultivation in areas suitable for *P. nigra*. It is necessary to coordinate these measures, especially with nongovernmental organizations taking care of nature preservation areas.

4. Methods for identification should be improved. This work might be carried out in relation to activities 1 and 3.

5. The programme is supposed to be much more effective when integrated in an international framework. A coordinator should be named by the network for each country to provide, e.g. IPGRI and the international coordinator of the network with information to make possible the management of data.

6. To overcome the problems linked with widespread isolated stands and small numbers of individuals as a result of development activities that disturb natural regeneration and migration, an exchange of material should not be focussed within countries. International cooperation is one possibility for enriching genetic diversity of *P. nigra*.

National programmes

As mentioned before, valuable information and experience exist upon which new programmes could be based. For the time being there are neither administrative activities nor public funding specifically concerning *P. nigra*. Several regional measures are run by nongovernmental organizations. A coordinating institution does not yet exist. To reactivate and carry on activities, it is necessary to spend funds for labour and travel.

Summary

For the situation of *P. nigra* in Germany, we have to ascertain that this species demands *in situ* and *ex situ* conservation which could be a model for other species. Circumstances in Germany show extreme similarities to many other European countries. Previous research developed knowledge and experience. Nevertheless, there is an urgent need for further activities. If financial support could be found there are good chances to reactivate the work.

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Short note about *Populus nigra* in Belgium

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In situ situation of *Populus nigra* in Belgium

Populus nigra is considered as the most endangered tree species in Belgium. The situation is comparable with that in the Netherlands. One reason for that situation is the impossibility of the species to regenerate in a natural way owing to the canalization of the main rivers, so that no favourable sites for germination of seeds are available. A second reason is the high level of intensification of agriculture on alluvial soils which has caused the removal of old trees in the fields. A third reason is the afforestation of alluvial soils by highly productive hydrid poplars.

Nevertheless *P. nigra* is still occurring in a few numbers especially in some regions with an extensive agriculture until recent times as pollarded trees together with willows and very rarely as trees.

Ex situ conservation activities

Conservation activities in the past

The Institute of Forestry and Game Management has, as the former Poplar Research Station, paid a lot of attention to the native *P. nigra* as a parent in the breeding programmes. Thus, in the early 1960s prospecting and collection of the last remnants of the species were undertaken and they were used as parents in *euramericana* crossings and crossings with *P. trichocarpa*. The clones were collected mainly in the Dender Valley, where extensive agriculture persisted until some years ago.

More recently, the last remainders of the species were also found in the Yzer Valley and some very few in the Schelde Valley. In the Meuse Valley, the largest river in Belgium, no *P. nigra* have yet been found.

Apart from their use in the breeding programme as parents for hybrid crossings, the collected clones were also used for intraspecific crosses to enlarge the genetic diversity of the species. Parents and offspring are conserved in *ex situ* plantations.

Other collections of the species are also available from other countries; collections of *P. nigra* from France, e.g. the Loire and the Garonne Valleys, from Italy and the Netherlands. These collections also were used for intraspecific crosses to enlarge the genetic diversity of the species.

Recent conservation activities

During recent years futher prospecting of the species was undertaken. Further enrichment of the *ex situ* collection has been achieved for the *P. nigra* population of the Schelde Valley.

As well, a new collection of *P. nigra* from Poland has been established.

Actions planned in the near future

Further prospecting of the relict population with the aid of the forest service and nature conservation organizations will start in the near future.

Rejuvenation of the existing collection and screening against leaf diseases is planned for the next few years, along with controlled intraspecific crosses with the new material.

Screening of the genetic diversity of the relict population by enzyme polymorphism and RAPDs will be done at the University of Brussels.

In situ conservation in the frame of nature development projects in the main river courses is being studied at the moment.

Conservation of genetic resources of *Populus nigra* in Turkey

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Status of *P. nigra* in Turkey

Populus nigra has considerable economic importance in Turkey. The cultivars and hybrids of this species have been largely used in plantations. In Turkey, two forms of cultivated black poplar with very characteristic narrow, pyramidal crowns occur not only in towns but also in small villages along rivers and streams. The first, mostly scattered in the west part of the country, is *P. nigra* 'Italica' (syn: *P. italica* (Duroi) Moench, *P. pyramidalis* Rozan). It is characterized by blackish bark on older trunks. This form is represented by male trees originating from Lombardy (Italy). The second form (female only), *P. usbekistanica komarov* 'Afghanica' syn: *P. thevestina* Dode, has smooth bark which is white or greyish white also on older trunks. This cultivar is much more widespread and common than the former, especially in inner Anatolia, and originates from Central Asia. Many forms and hybrids of black poplars occur in Anatolia.

Modern poplar cultivation techniques have been practised in most of the sites where *P.* × *euramericana* or *P. deltoides* clones are cultivated. But in some parts of the central, eastern and southeastern regions of Turkey, *P. nigra* cultivars are cultivated with traditional methods. Row plantations are also traditional practices of poplar cultivation in Anatolia. Riverside or streamside, field and roadside plantations have been established for centuries by the farmers in Anatolia. In some regions of Anatolia where cultivated land is limited for agriculture, wood demand is very high. Therefore, row plantations are an optimal solution for an ideal land-use system in these regions. They protect arable land and provide wood for rural needs. Turkey also has a great potential for gallery plantations. The main river basins of Turkey offer a great opportunity to make them more productive for rural people and the national economy. It is estimated that the potential site for gallery plantation is about 100 000 km along the river and stream banks. Some ongoing gallery plantation projects are under the supervision of the Ministry of Forestry.

Internationally registered clones — Gazi (TR. 56/52) and Anadolu (TR. 56/75) — have been cultivated in the Central Anatolia, East Anatolia and Southeast Anatolia regions of Turkey. According to the latest inventory data, approximately 130 000 ha of poplar plantations are standing of which 70 000 and 60 000 ha are hybrid poplars and black poplars, respectively. Almost 40% of black poplars are not in the form of block plantations but of line plantations along the water canals, streambanks and around irrigable fields.

Industries consuming poplar wood have developed very quickly in recent years. The emphasis of the development is mostly in the industries of furniture, packing, particle board, plywood, matches, etc., which basically use the wood of Euramerican hybrid poplars. Nearly all the wood production from hybrid poplars (1 505 000 m^3) is consumed by the industries mentioned above. More than 80% of black poplar wood (1 746 000 m^3) is utilized as roundwood for rural construction purposes and for daily needs of the rural people.

Conservation of genetic resources

Populus nigra cultivars have been traditionally cultivated by farmers on private lands in Anatolia for centuries. Therefore, *in situ* conservation is limited to some restricted areas, and emphasis has been given to *ex situ* conservation (Fig. 1). *Ex situ* conservation programmes of genetic resources of poplars and willows in Turkey have been carried out

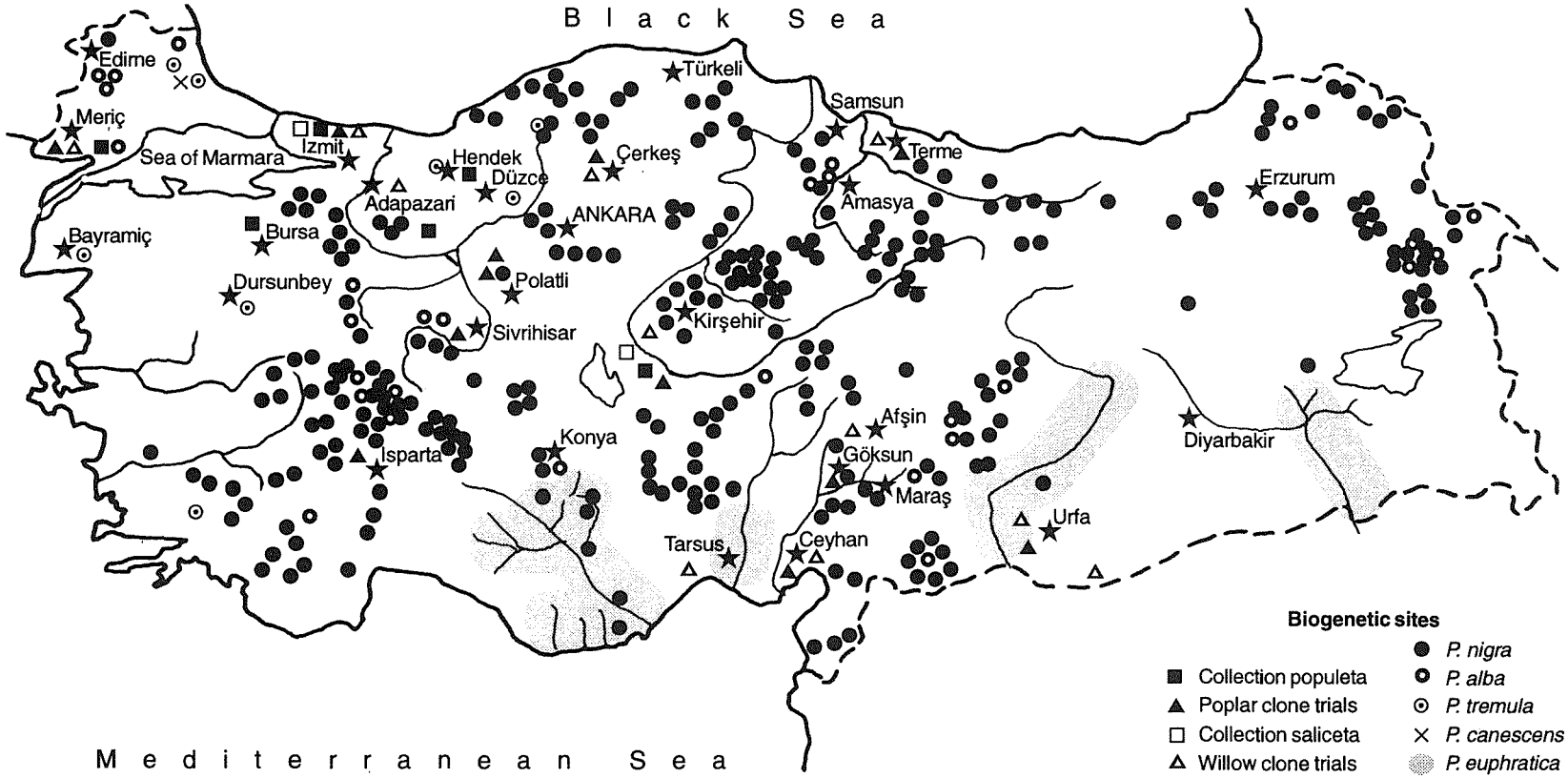

Fig. 1. Location of populeta and clone trials of *Populus* spp. in Turkey

by the Poplar Research Institute since 1962 (Tunçtaner 1993). Black poplars are scattered throughout the country, but the populations of this species have been threatened by urban extensions and rural management procedures. Therefore, a new conservation programme has been recently started to protect *P. nigra* in *ex situ* collections. So far, 297 clones have been collected in stool-beds in the Izmit nursery. Records kept on the clones include accession identity, name, institution of origin, original accession, genetic structure, latitude, longitude and altitude of origin, geographic zone, means of conservation, evaluation and location origin.

Various clone banks and clone trials (populeta) also have been established in different climatic regions of Turkey since 1962 (Fig. 1). The clones protected or tested in clone banks or trials have been selected in the country or introduced from outside of Turkey. Most of the trials are located in a temperate climatic region and contain *P.* × *euramericana* and *P. deltoides* clones. The trials established in continental regions contain mostly *P. nigra* clones, and emphasis is given to selection of indigenous black poplars resistant against frost in Central and Eastern Anatolia. The results obtained from these trials were published in Poplar Research Institute bulletins and magazines (Semizoglu 1967; Birler *et al.* 1978; Gökçe and Çetin 1978; Tunçtaner *et al.* 1983, 1987; Tunçtaner and Zengingönül 1988).

Improvement and selection

The programme of poplar improvement in Turkey is mainly concerned with *P. nigra*, *P. deltoides* and numerous hybrid clones of *P.* × *euramericana*. Domestic black poplar is included to a large extent in the breeding programme because of its adaptability to continental conditions. Production of *P. nigra* × *P. nigra* and *P. deltoides* × *P. nigra* hybrids is in progress in Turkey.

Useful traits of parents such as fast growth, desired wood quality and resistance to frost can be combined in a hybrid, and some clones can be selected from intra- or interspecific crossings. Artificial hybridization with poplars has been carried out in Turkey since 1967 (Ataizi 1967; Semizoglu *et al.* 1969; Ataizi *et al.* 1971) and satisfactory results were obtained from the crossings made with *P. nigra* and *P. deltoides* in greenhouse conditions. Therefore, the emphasis has been given to *P. nigra* × *P. nigra* and *P. deltoides* × *P. nigra* combinations in order to find new clones for Anatolian conditions. The registered *P. nigra* clone Gazi was used as the male parent for the intraspecific cross made in 1977. The hybrid clones 77/10 and 77/40 were selected from these crosses owing to their growth performance in the nursery. Apart from this, the clones 77/51, 77/10 and 80/4 were selected from the combinations of *P. deltoides* S.510-48 × Gazi (Zengingönül *et al.* 1982). These clones have been tested at different trial sites in Central Anatolia. Investigations on growth performance of the clones obtained from the intra- and interspecific crossings made in Izmit in 1986 and in the period of 1987-1990 (Tunçtaner *et al.* 1992) have been continued in the Marmara region.

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Populus nigra in the Netherlands

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The Netherlands find themselves in the outer range of the natural range of *Populus nigra*, which makes it even more interesting to conserve the still existing genetic material of this tree species.

Although scarce, there are still some possibilities for natural regeneration on our riverbanks. However, the main selection took place from 1950 to 1970, when the main reason for a thorough collection was the use of *P. nigra* in the crossing schemes with other species (mainly *P. deltoides*). This was at the same time the reason for collection on a major scale, because it was recognized that the *P. nigra* sooner or later was bound to disappear from Dutch landscapes. Farmers were using any square meter of the floodlands wherever they could, more productive hybrids were used for planting or replanting, dikes had to be enlarged, etc.

Population areas as such are not defined, but exact locations are recorded for every individual collected tree. The collected individuals altogether represent a large variability. Rather a large number of crosses are produced in order to finally select the perfect males to be crossed with the female *deltoides* clones available. Some of those progenies enabled us to select clones for direct use even when they were females and could therefore not be used for crossings.

An example of this is 'Schoorldam', clone nr. 1972, a result of a cross between two Dutch *P. nigra* from two different populations. This clone, together with other clones, is represented in our National Catalogue (de Rassenlijst van Bomen), which includes as much relevant information on individual clones as possible.

A relatively large number of trees from *P. nigra* are still being used in both landscape and to a smaller extent in forestry and city areas.

The rest of all this collected material is either evaluated in comparative trials or is stored in genebanks. One is at stool-bed level and a few are at tree level (now more than 20 years old). Foreign material is also present in either of these three forms.

These trial plots are usually forestry blocks, but also very often roadside plantations, along motorways or in cities. Evaluations and assessments (e.g. of treeform and branching habits) are better done along a road than in a dense stand. These trial plots and genebanks are so called *ex situ* collections, where we know every number and individual. For a few years, however, there has been at the national level consideration of a way to reintroduce *P. nigra* on the riverbanks and floodlands. In so-called 'Ooi-bossen' larger or smaller areas have been bought by nature conservancy organizations or are dedicated by the State for this purpose.

Some 50 male and 50 female clones from our genebanks are planted at every location in order to let nature do its work. These areas are nature reserves and are protected by law. The intention is to create more of these localities on the riverbanks of all major rivers in the Netherlands. Gene populations could be preserved this way without knowing the exact individuals.

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