

# **IPGRI** *Populus nigra* Network

Report of the seventh (25–27 October 2001, Osijek, Croatia) and eighth (22–24 May 2003, Treppeln, Germany) meetings

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## Summaries

## Seventh EUFORGEN Populus nigra Network meeting

#### Opening of the meeting

Davorin Kajba, Head of the Department of Forest Genetics and Dendrology, and Vice-Chair of the Network, welcomed participants to Osijek on behalf of the Forestry Faculty of Zagreb University and wished everybody a pleasant stay and a successful meeting.

Mr Pavle Vrataric, Director of the Osijek Forest District Office, welcomed the participants. He expressed his satisfaction for hosting the meeting and wished the Network a fruitful time in Osijek. He gave a brief description of the area in which the meeting was held and underlined that almost half of the area is covered by riparian forest. This area is also very important for studies on biodiversity and conservation, which were started over 40 years ago.

Mr Joso Gračan, Director of the Forestry Institute and EUFORGEN National Coordinator for Croatia, again welcomed the participants and highlighted the role of Croatia in the Network and its active participation in all EUFORGEN Networks. He also thanked Simone Borelli for his contribution to the programme and wished him luck in his new job.

Sven de Vries, Chair of the Network, welcomed the participants and thanked the local organizers for all their efforts. He thanked Joso Gračan and Pavle Vrataric for their kind words. He encouraged members to actively participate in the meeting and in all activities of the Network.

Simone Borelli welcomed the participants on behalf of the EUFORGEN Secretariat and gave a brief overview of the recent developments in the programme, including recent and upcoming meetings and outputs.

In order to facilitate discussion, all participants briefly introduced themselves for the benefit of newly attending members. Melita Mihaljevic, Director of the Kopacki Rit Nature Park, was also present.

The agenda was adopted with some minor amendments.

#### Update on progress in gene conservation

Eighteen countries (Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Malta, Moldova, the Netherlands, Portugal, Russian Federation, Slovenia, Spain, Switzerland and Turkey) participated in the meeting. All participants presented brief updates on the progress made since the last Network meeting held in February 2000. Country updates will be provided to the Secretariat in electronic format by 15 November 2001.

Highlights of the country reports will be summarized in table format and included in the final report. The full text of the reports will be made available on the EUFORGEN Web site. Some highlights from the country updates are provided in Annex I below.

#### Report on the International Poplar Commission (IPC)

Davorin Kajba provided a summary of the paper on the activities of the Network that was presented at the IPC meeting held in September 2000.

Sven de Vries underlined the fact that gene conservation was a main concern of all participating countries present at the IPC and that it is important to keep this point on the political and technical agenda of individual countries. The next meeting of the IPC will be held in Chile and Argentina in 2004.

#### **Technical bulletin**

A draft copy of the '*In situ* Conservation of *Populus nigra*' technical bulletin was distributed to the participants and they were asked to mark any mistakes/changes on a master copy to be returned to the printers for finalization.

In order to facilitate dissemination, it was suggested that an extended version of the summary, including some of the tables and graphs, should be prepared for translation into different languages. François Lefèvre, Peter Rotach and Berthold Heinze will prepare this document and provide it to the Secretariat for distribution by 31 January 2002.

It was also agreed to produce a technical sheet (4 pages) in the format already agreed upon by the Noble Hardwoods and Conifers Networks. This would be translated into national languages for the use of forest practitioners in the field. A space will be left at the back of the sheet for national information. The idea was discussed and accepted by the Network. An Vanden Broeck, Georg von Wühlisch and Peter Zhelev will prepare a draft and circulate it to the Network before 31 March 2002.

The production of a joint technical publication based on the recommendations of the project EUROPOP was discussed. The paper would have a 'question and answer' format and be based on typical questions asked by managers. All Network members will collect relevant questions and send them to the Secretariat for compilation and distribution one month before the next Network meeting.

#### In situ conservation

#### Introgression of P. × euramericana and P. nigra

An Vanden Broeck presented a study on introgression into *P. nigra* from other *Populus* species in Belgium. The main conclusions are that there are no phenological barriers to introgression; that there is no random mating, rather it is preferential and unbalanced; and that restoration of natural populations should not start with single trees or small groups because of the higher risk of introgression. This is particularly true in countries where no natural populations exist (Belgium, UK) and restoration of riparian forests is being carried out. In a mixed pollen cloud (*P.* × *euramericana* + *P. nigra*), pollen from *P. nigra* may be more successful than pollen from *P.* × *euramericana* in pollinating females of *P. nigra*.

Future activities will include controlled crossings with pollen mixes to test the hypothesis of pollen competition. A summary of the study will be included in the meeting report.

#### EUROPOP

François Lefèvre summarized the main results of the EUROPOP project and the recommendations that can be derived from these. A full synthesis will be published in the proceedings of the EUROPOP final meeting.

A preliminary conclusion was that genetic diversity is not evenly distributed, so conservation sites should be distributed over the entire range of the species and should include more than one site per river. It is also useful to have a preliminary estimate on genetic diversity among adult trees.

Another result is that gene flow with cultivated poplars is scarce but not absent. As a consequence, there is no need to forbid poplar plantations around conservation units. However, attention should be paid to small populations and single trees. Introgression should be checked among reproductive trees. Introgression with var. 'italica' is usually not a problem, except possibly in southern Europe.

It also appears that mating is not random but preferential, and very few pollinators are involved in reproduction. Gene flow follows the model of isolation by distance and is symmetrical, upstream and downstream. Thus, the number and distribution of female trees is critical. Also, establishment of seedlings should be favoured, and assisted regeneration, if needed, should be carried out on small plots.

Finally, genetic drift is observed within stands. It is therefore important to monitor all practices that impact on flowering and regeneration. The main indicators for this are hydroperiod, structure, and number and distribution of female trees.

#### Ex situ conservation

#### Core collection of P. nigra clones

Lorenzo Vietto gave a presentation on the current situation of the core collection. The collection was flooded in the year 2000 and sand was deposited on the clones. Luckily, it was possible to salvage all the clones, but it was not possible to provide reproductive material during this period. Currently, 19 countries are represented, with 37 clones. In fact, a new clone was provided by Portugal, possibly a female. The 15 reference clones were propagated.

Duplicates of the collection are currently present in five different institutes. The conditions of the clones in the different locations vary in terms of health. However, in general, the quantity and quality of clones is good. A duplicate of the collection will be planted next year in the vicinity of Mantova, Italy. Another duplicate will be planted in Spain. During the last year, some of the clones had to be replaced for health reasons or propagation difficulties and others might also need to be replaced in the near future. Nevertheless, it was agreed to keep to the original collection as much as possible.

It was suggested that records should be kept of where cuttings from the collection are sent. A field will be added to the database, and information on material sent in the past will be provided to Lorenzo Vietto by 30 November 2001. Datasets on molecular characterization of individual clones will also be included in the database after publication of EUROPOP papers.

Problems to be solved include, among others, the inclusion of missing countries in the range of distribution. A first letter was sent by the EUFORGEN Secretariat to these countries and replies are expected in the coming months. Network members offered to contact Algeria, Greece, Morocco and Iran. To facilitate collaboration with non-attending and non-European countries on the core collection and other activities, the Secretariat will circulate an updated list of country contacts for *P. nigra* for comments/suggestions by 31 December 2001.

#### Core collection of P. alba clones

István Bach provided an update on the establishment of this core collection. Two suitable sites have been selected in Hungary: Sárvár and Tolna. Two clones have already been received from Italy and propagated. However, planting in the field was not very successful. After some discussion, it was decided to send material (either as rooted plants or as root suckers) to two locations: Germany and Hungary. Interested countries will provide two clones (ideally 10 cuttings per clone) of *P. alba* typical for their country by 28 February 2002.

#### Sharing responsibilities for the conservation of genetic resources in Europe

A brief summary of the discussion that took place during the Inter-Network Group meeting in Antalya in October 2000 was provided. The objective of this group is to harmonize thematic priorities and coordinate activities among the five Networks. One of the important themes that emerged was that of sharing responsibilities for *in situ* conservation of forest genetic resources. This could be achieved through carefully designed and implemented networks of gene conservation units for selected target species of highest common priority within their entire distribution areas.

Berthold Heinze pointed out that the *P. nigra* Network has a lot of information on the conservation of the species and it is time to take some more practical action. Breeding

institutions are present in many countries, natural stands are still available and a lot of knowledge exists. So far, work has been carried out on a country-by-country basis. However, this approach has many limitations in terms of resources and it should be possible to increase collaborative efforts. Many borders in Europe are set by rivers, and direct transboundary collaboration would help in the tasks of the Networks. Of course, any activities should be carried out on a voluntary basis. The creation of a Europe-wide *In Situ* Conservation Network would provide an ideal framework for such activities and would also have public awareness value. It could also be used as a motivational tool for encouraging countries to share the conservation tasks.

This 'masterplan' would of course apply to all species and it will be necessary to coordinate with other Networks. A suggestion was made that the Inter-Network Group would prepare a concept note for the attention of the Steering Committee. The document would be also discussed through the listserver and could include potential practical applications. Due consideration should be given to species with limited economical importance. The Steering Committee would then bring the document to the attention of the Ministerial Conference on the Protection of Forests in Europe (MCPFE).

As a contribution from this Network, the document would be backed up by a practical case study on *P. nigra* that will be prepared by Berthold Heinze on the basis of his original document. Network members will provide him with comments by 9 November 2001.

After some discussion, it was proposed to hold a meeting of the Inter-Network Group in February or March 2002 to discuss this document, possibly in Vienna. The Secretariat will verify financial and logistic possibilities for organizing such a meeting and will inform potential participants in due course.

#### Links with non-attending members or non-European countries

The Network agreed to continue attempting to create links with all countries within the distribution range (see above).

#### In situ conservation

#### **Biodiversity**

Peter Rotach presented a paper on poplars and biodiversity (pp. 78 of this publication). He highlighted the importance of poplar for supporting biodiversity. Over 700 species of insects feed on poplar, even though only about 80–100 of them are specialized. Butterflies, moths and beetles are the most frequent. In general, insects seem to make no distinction among species in the genus, so there is no proof that hybrids are less valuable or have negative effects. However, there is some evidence that resistance traits may have negative effects on biodiversity.

In terms of structure, natural populations are much richer in biodiversity than plantations. Plantations might also change the natural vegetation composition and are less rich in bird life. On the whole knowledge is still scarce, incomplete and biased towards species that cause damage to the stands. Thus, further information is needed.

Several Network members (Croatia, France, Germany, Malta, Portugal and Spain) indicated that they could provide additional sources of information. In Belgium, a new study was started looking at plant species associated with poplar stands. Some preliminary results are available and will be provided.

All information and comments will be provided to Peter Rotach by 30 November 2001. A final version of the paper will be sent to the Secretariat for inclusion in the meeting report by 31 January 2002.

#### Research

Sven de Vries mentioned the issue of introgression of *P. tremuloides* into *P. tremula* in Estonia and Finland that had been discussed in the past months by some of the Network members, following a request for assistance. It was decided that in similar cases, it would be easier to provide input on an individual basis.

The issue of genetically modified organisms (GMOs) as a possible research item was raised. In France, for example, scientific discussion is currently centred upon introgression processes and gene flow and also includes GMOs. A literature review on GMOs in forestry was prepared for a recent meeting and will be circulated to Network members.

An Vanden Broeck has also made a preliminary literature search on introgression in *P. nigra* and she is willing to act as a focal point for reviewing literature on introgression and gene flow for the entire *Populus* genus. Georg von Wühlisch will assist her in this task. All Network members will provide literature and references by 31 March 2002. The results of this work will be presented at the next Network meeting.

The idea of carrying out a genetic resources collection of *P. nigra*, aimed at studying variation of adaptive traits, was also raised. A nursery trial was carried out in the framework of EUROPOP and the plants could be used for a collection of provenances. EUROPOP participants will be contacted to verify their availability to provide the material to initiate the collection. Additional material could be collected following the same sampling strategy. Hungary offered to host the collection. Objectives of the collection and general instructions on how to provide material are included in Annex II below.

#### Documentation

#### Update on P. nigra clone database

Lorenzo Vietto gave a report on the current status of the database, which currently contains approximately 3000 entries, with some duplicates. Currently 13 countries participate, but in fact 28 are represented in the collections, covering almost the entire distribution range. Current problems include data format and missing or incomplete information in the national databases.

Recent activities included an update of the database and the preparation of new software for data input. This new software was demonstrated and all Network members expressed their satisfaction with this new tool. Countries that are already contributing information received a personalized copy of the programme to be used for future updates. Updates or new information should be provided to Lorenzo Vietto by 31 January 2002. A further update will be carried out one month before the next meeting.

#### Update on P. alba clone database

National collections of *P. alba* are currently only found in Spain, Italy, Hungary, Czech Republic and possibly Bulgaria and Germany. It was agreed that the format of the *P. alba* database, hosted by Spain, would be regularized to the one proposed for *P. nigra*. Lorenzo Vietto will adapt the format and send it to relevant countries by 30 June 2002.

#### PGR information platform

Simone Borelli briefly introduced the new format of the IPGRI Regional Office for Europe Web site and presented the idea of using this as a gateway to information on plant genetic resources throughout Europe. Participants were encouraged to visit the Web site, provide comments and also information on their national programmes (i.e. focal persons, Web sites, etc.) for inclusion in their respective national pages.

#### **EUFORGEN Bibliography**

Simone Borelli provided an update on the current status of the EUFORGEN Bibliography Database. The database went online in January 2001 and has become one of the most frequently visited pages of the IPGRI Web site. However, most of the references currently found in the database (about 1700 items) are dated 1999 or earlier, and all Network members were encouraged to continue providing references on a regular basis, in order to keep this tool valuable. Participants were also reminded of the importance of following the format and instructions provided by the Secretariat.

The contents of the database were also discussed and there was general agreement that new additions should refer only to grey literature. In this respect, the issue of access to the original literature was also discussed and it was agreed that, even though it would not be advisable to name a contact person directly on the database, it would be useful to have a full list of Network members on the *Contacts* page of theWeb site. The Secretariat will take the necessary action as soon as possible.

#### Public awareness

#### **CD-ROM**

Sven de Vries distributed a copy of the slide collection to all relevant participants. A new version will be provided as soon as slides become available, possibly using the database system developed for the Social Broadleaves Network. Dominique Jacques, the person responsible, will be contacted accordingly.

However, some of the themes that were decided upon in the last Network meeting have not yet been covered and relevant network members have agreed to provide slides by 30 June 2002 as follows: mixed riparian ecosystem (István Bach, Davorin Kajba and François Lefèvre), western China (Georg von Wühlisch), biodiversity (Peter Rotach and François Lefèvre), north African landscape (François Lefèvre and Carmen Maestro), vine–poplar systems (Maria Carolina Varela), and restoration techniques (Georg von Wühlisch).

A slide collection for *P. alba* will also be created under the responsibility of István Bach. All participants were encouraged to send slides as soon as possible. Moldova and Croatia had already provided the first slides. The images will be catalogued and included in a database format (see above). For this collection high-resolution scans are also acceptable.

#### Poster

Sven de Vries briefly illustrated the process of preparing the Social Broadleaves poster and circulated a copy of the latest draft. The *P. nigra* Network did not feel that this was currently a priority. However, it was suggested that existing posters developed in different countries should be sent to the Secretariat, and that some should be brought to the next meeting for inspiration. The idea of a general EUFORGEN poster was also put forward.

#### Listserver

There was general agreement that it is a useful tool, although so far its use has been quite limited. It is expected that use will increase if the 'masterplan' and other issues of general concern are discussed over the next few months.

#### Other public awareness initiatives

Simone Borelli presented the new EUFORGEN brochure, which is intended to contain a leaflet for each of the five Networks and to be used mainly for public awareness purposes.

It was agreed that Peter Zhelev, Eman Calleja and Sven de Vries will prepare text for a *P. nigra* leaflet by 31 December 2001 and send it to the Secretariat for final production.

Lorenzo Vietto supplied copies of a poster produced for an IUFRO meeting in Rome that

could be used as an example for future presentations.

Sven de Vries described the presentation that he recently made in China on the activities of the Network.

In Croatia, the current meeting was extensively covered by national and local press and two interviews were given to Croatian national television.

In Hungary, a TV special was devoted to the Gemenc Region, and a substantial part (5–6 minutes) of it focuses on gene preservation of *P. nigra*.

In Moldova, an ecological trip for students was organized by a national NGO and part of the trip was devoted to conservation of biodiversity in riparian ecosystems. The trip was followed by a press conference, and news of the tour was broadcast on TV as well as in the specialized press.

In Malta, the Department of Agriculture provides school representatives with tree seeds (including *P. alba*) that are then grown in schools, and prizes are awarded to the schools with best results. In January, a full week is devoted to visits in nature reserves and tree planting.

Network members highlighted the importance of having a standard presentation on EUFORGEN to be used for different purposes. The Secretariat will make this available to all Networks through the listserver.

An identification sheet for *P. alba* will be prepared by István Bach. Carmen Maestro will contact Nuria Alba (Spain) to verify whether she is willing to assist in this task. Jos Van Slycken (Belgium) will be contacted in due course with regard to illustrations.

The possibility of collaborating with WWF and other environmental NGOs was mentioned and the Chair encouraged participants to foster contacts that might be useful for Network activities.

Additional public awareness activities can be found in the individual country reports (see Annex I).

#### Adoption of the report

The report was adopted.

#### Date and place of the next meeting

Georg von Wühlisch offered to host the eighth EUFORGEN *P. nigra* Network meeting on behalf of Germany. The Network accepted this kind offer. The meeting will be held in spring 2003. Further information will be provided in due course.

#### Closure

The Chair thanked the organizers on behalf of the Network for the excellent organization of the meeting and for their warm hospitality. He also thanked Network members for their contribution to all tasks. He also thanked Simone Borelli for his excellent contribution and wished him luck in his new position. The Chair declared the meeting closed.

#### Annex I: Highlights of progress made in individual countries

In **Austria**, the new inventory that is currently under way will attempt, for the first time, to distinguish natural *P. nigra* from hybrids. This will provide useful information for the elaboration of gene conservation strategies.

In **Belgium**, many new relict individuals had been identified and characterized. The database currently contains a total of 216 individuals. However, it appears that many of these are in fact the same clone. Forty-nine genotypes have been identified so far, but work is ongoing.

Bulgaria has launched a new project on mapping individuals and small populations of P.

*nigra*. The project is multidisciplinary and also looks at biodiversity in these stands. A clone collection will be established in the framework of the project.

The area of **Croatia** in which the meeting was held was occupied during the war and was reintegrated in 1997. A clonal archive, established in 1995, is present in the area and also includes clones from Bosnia-Herzegovina. Some protected areas have been established at the confluence of the Drava and Danube and are ideal for *in situ* conservation of *P. nigra*. However, mines still pose a major problem.

In the **Czech Republic**, the Forestry and Game Management Research Institute currently holds several clones of *P. nigra*, *P. alba* and other poplar species. Demonstration fields have been established for technical and public awareness purposes. An inventory of *P. alba* was started recently and 17 clones have been collected.

In **France**, an inventory of rust-resistant clones is in progress. The collection of clones has been completed with 37 clones from southeastern France. An adult plot of the core collection will be established in 2003. A three-day science event was held in the Drome, directed at both visitors and local people. A meeting of the 12 reserves belonging to the *P. nigra* conservation network was held and the managers highlighted the need for technical guidelines.

In **Germany**, inventories continued and over 4640 trees have been identified. These trees have also been propagated and *ex situ* plantations have been established. Some restoration projects are underway on the Rhine, the Elbe and the Oder. Rolf Schulzke organized a large public event for environmental education in Kassel, which included a stand with information on *P. nigra*. Work on identification of *P. alba* was also carried out.

In **Hungary**, additional clones have been collected. There are now over 600 registered genotypes. The EUROPOP congress was held in Hungary and it was a very good opportunity for raising public awareness through newspapers and TV.

In **Italy**, inventories were carried out in Piemonte and Emilia Romagna through remote sensing and field checks. Further inventories will take place in Lombardia and Veneto. The inventories included both cultivated plots and natural forest. A Hydrogeological Management Plan for the Po river, which includes incentives for the re-establishment of natural vegetation, was recently approved. The Rural Development Plan was also approved and includes incentive for biomass plantation and arboriculture on marginal agricultural lands. A pool of *P. nigra* will be registered for these plantations. Two *P. alba* clones, Marte and Saturno, were also registered. A new research proposal on stress tolerance in *P. nigra* was submitted to the EU. A new project funded by the Branca Foundation was started for inventories, creation of genebanks and restoration. In Lombardia, 100 hectares of rice paddies were converted to *P. alba* plantation.

In **Malta** there is a new law for protection of native species, which includes *P. alba*. Most of the trees present are male and they mostly come from the same parent trees. There is an ongoing campaign for growing trees (including *P. alba*) around schools, and prizes are awarded every year.

In **Moldova**, most of the poplar forests are made up of *P. alba*. Mother plantations were established seven years ago and are now being used for plantings throughout the country.

In **the Netherlands**, a new threat for *P. nigra* appeared in the last year—beavers. They seem to love big poplars! Two articles on the ID sheet appeared in a magazine and a lot of requests were received. The Dutch version of the sheet is much appreciated.

In **Portugal**, there is some collaborative research with Austria and Italy. An agricultural school is willing to establish a duplicate of the core collection.

In **Russia**, a large number of plus trees and plus stands have been selected in different regions. Conservation is underway in nature reserve and national park. However, there is limited attention to *P. nigra* and the area of poplar has been steadily declining. Its health

conditions are also worsening.

In Slovenia, there is limited activity on poplar. However, there are plans for future work.

In **Spain**, the inventory of the Ebro valley was finished and 190 individuals were collected for genetic studies. A new plan for management of the Ebro valley was presented and it includes measures for protection of natural forest formations. Two institutions are currently working on *P. nigra* and *P. alba* in Spain and the national collection of clones is maintained in both Zaragoza and Madrid. Clones from the collection are being multiplied for use in restoration. Two new publications are being prepared.

In **Switzerland**, progress was made thanks to regional collaboration. There are now 98 identified pure individuals. Two clones will be provided to the core collection. Unfortunately, funds for the project on rare species, now entering its second phase, were reduced and it will not be possible to included floodplain species.

In **Turkey**, 29 new clones were selected. Nurseries and field trials were established in different regions. Informative material was distributed to poplar growers.

#### Annex II: Objective of the provenance collection and instructions for sending material

The Network stressed the need for the establishment of an international collection of provenances. Considering the dioecious nature of the species and the results obtained in the EUROPOP project on mating systems, i.e. preferential mating with very few males effectively pollinating each female tree, it was recommended to use a sampling strategy based on clones rather than half-sib progenies as is usually done for other tree species.

The EUROPOP material available in the nurseries of different countries provides an opportunity for starting this work. This includes approximately 10 populations represented by 60 clones each that were compared to the EUFORGEN reference clones and to the core collection in nursery trials. The following steps are proposed:

- In order to conserve the EUROPOP material, Hungary offered to maintain and propagate this collection. France will contact EUROPOP partners and ask them to send 3 cuttings per clone to Hungary before 1 March 2002 (10 populations × 60 clones + 15 reference clones + 35 clones of the core collection = 640 clones).
- Several countries (including Russia and Turkey) offered to collect new provenances following the sampling strategy of EUROPOP: in each population 30 adults and 30 juvenile trees are sampled. Populations should be at least 50 km apart. A more detailed set of instructions will be provided. The cuttings will be sent to Hungary for initial propagation before 1 March 2002.
- Hungary will maintain the EUROPOP material and propagate the new material in order to establish secondary propagation in 2003.

## Eighth EUFORGEN Populus nigra Network meeting

### Opening of the meeting

Thorsten Hinrichs welcomed participants to Treppeln on behalf of the Federal Ministry for Consumer Protection, Food and Agriculture, and the National Coordinator of Germany. He emphasized that the *Populus nigra* Network clearly gains an advantage from focusing on only two species in its work. He also highlighted that EUFORGEN has achieved a great deal and mentioned the point that a third phase largely depends on the work carried out by the five Networks.

Sven de Vries, Chair of the Network, welcomed the participants and thanked Thorsten Hinrichs for his kind words. He also introduced new Network participants from Bosnia Herzegovina (Dalibor Ballian), Ireland (Kevin Keary), Bulgaria (Denista Pandeva), France (Marc Villar) and the new EUFORGEN Coordinator (Jarkko Koskela).

The agenda was adopted with some minor amendments and Maria Carolina Varela and Kevin Keary were appointed as rapporteurs.

#### EUFORGEN update

Jarkko Koskela welcomed the participants on behalf of the EUFORGEN Secretariat and gave a brief overview of the recent developments in the programme, including recent and upcoming meetings, outputs and other relevant involvements of the Secretariat to promote EUFORGEN. He also mentioned that Emile Frison, who was very much involved in the establishment of EUFORGEN, has been selected as the new Director General of IPGRI and that he will take up his new duties by 1 August 2003.

The EUFORGEN strategy paper, developed by the Steering Committee, was submitted to the expert level preparatory process of the fourth Ministerial Conference on the Protection of Forests in Europe (MCPFE) which was held in Vienna in April 2003. As a result, forest genetic resources are now mentioned in the Vienna Resolution 4 on forest biological diversity which 'promotes the conservation of forest genetic resources as an integral part of sustainable forest management and continue the pan-European collaboration in this area'.

He also updated the Network regarding the new regulation of the European Commission (EC) on genetic resources in agriculture (including forest genetic resources) which has an emphasis on the practical implementation of gene conservation. The new regulation is currently being finalized and there will be a first call for proposals at the end of 2003 or early 2004 and a second call in 2005.

Reference was made to the Network of Excellence proposal 'Evolution and Management of Diversity in European Forest Trees (EVOLTREE)' which was submitted to the EC Sixth Framework Programme. The proposal focuses on developing a genomic approach for European forest trees involving 32 partners from 14 countries and is coordinated by INRA (Antoine Kremer). One of the 14 Work Packages is proposed to be coordinated by IPGRI, covering the dissemination of the results through the EUFORGEN Networks.

#### Update on progress in gene conservation

Seventeen countries (Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Malta, Moldova, the Netherlands, Portugal, Serbia and Montenegro, Spain and Switzerland) participated in the meeting. Bosnia and Herzegovina and Ireland were participating in a *Populus nigra* Network meeting for the first time, and introductory country reports were presented by Dalibor Ballian and Kevin Keary, respectively. Other participants presented brief updates on the progress made since the last Network meeting held in Croatia in October 2001. The country updates of the seventh and

eighth *P. nigra* Network meetings will be combined and printed as a joint report. A short note on *P. nigra* in Romania was also delivered by Berthold Heinze. The note was developed by a Romanian student who is currently studying in Austria.

Those participants who did not sent their reports to the Secretariat before the meeting were asked to provide contributions in electronic format to Jarkko Koskela during the meeting. All participants are requested to merge the country reports of the seventh and eighth Network meetings and send them to the Secretariat by 30 June 2003.

The full text of the reports will also be made available on the EUFORGEN Web site.

#### Technical guidelines and leaflet

The target audience of the technical guidelines are practical forest managers. The leaflet will be aimed at the general public. An Vanden Broeck developed a draft of technical guidelines for *P. nigra* which is similar to those recently published by the Noble Hardwoods and Conifers Networks. It was decided that this draft should be developed in the same format that the other EUFORGEN Networks have already used for their technical guidelines. Eastern parts of the existing distribution map for *P. nigra* need to be revised on the basis of Weisgerber's map (1999) and other existing maps on the eastern parts of the distribution area. Ion Palancean and Marc Villar will send information to the Secretariat for further development of the map by 30 June 2003. The deadline for providing comments to An Vanden Broeck is 30 June 2003 and she will then send the final version to the Secretariat before 31 July 2003.

It was also discussed that separate technical guidelines for *P. alba* are needed and it was suggested that Ion Palancean and Nuria Alba could develop these. Ion Palancean will circulate the draft text among the Network members by 1 July 2003. Responses are to be sent to the authors by 31 July 2003. A draft distribution map for *P. alba* will be circulated to the Network for inputs on a country-by-country basis.

The final version of the technical guidelines for *P. alba* should be submitted to the Secretariat by 30 September 2003.

Each country can translate technical guidelines into their national languages and print them at their own costs. The format is available from the Secretariat. The original authors should be kept while translators and national contacts can be added to the last page.

A leaflet intended for the general public, will be developed for the Network, including information on both *P. nigra* and *P. alba*. Sven de Vries, Eman Calleja and Peter Rotach will develop the draft text for the leaflet based on the DYGEN poster and circulate the draft to the Network by 31 December 2003. The deadline for providing comments to the leaflet is 31 January 2004 and the authors will then send the final version to the Secretariat before 28 February 2004.

#### Common Action Plan for Populus nigra

Berthold Heinze gave a presentation on the Common Action Plan for *in situ* conservation of *P. nigra* genetic resources. The objective of this Common Action Plan is the sharing of responsibilities for the *in situ* conservation of the genetic resources. In general, this could be achieved through carefully designed and implemented networks of gene conservation units for selected target species of highest common priority within their entire distribution areas. This could be initiated by way of developing bilateral projects between countries, such as ongoing initiatives between Croatia and Hungary along the Drava river. After discussions, the Network strongly supported this approach for the practical implementation of gene conservation in *P. nigra*. Further discussion took place regarding funding opportunities and incorporating these activities into national forest programmes that are in place in many

countries or are being developed. Cooperation with NGOs was also stressed as a positive influence towards reaching common environmental goals.

As a first step for further development of the Common Action Plan, it was decided to create a density map for *P. nigra* within its distribution area. The idea is to locate precisely the occurrence of *P. nigra* along the European rivers. The density map can also be used for other purposes, such as research planning and public awareness.

Marc Villar, Ion Palancean, Lorenzo Vietto and Eman Calleja were given the task of developing a proposal for the density map during the meeting, and Marc Villar presented the proposal during the wrap-up session. According to this proposal, river sections where *P. nigra* is not present will be indicated by a red band and rivers where the species is present will be indicated by a green band. A continuous green band will be used to indicate large continuous populations along a river. A broken green band will indicate fragmented distribution of *P. nigra* along a river and the degree of fragmentation will be indicated by the degree to which the green band is broken. Single green dots will be used for isolated trees and these can also be used for indicating trees outside river systems. An asterisk will be used on or beside the green band to indicate natural regeneration. Areas with no information on *P. nigra* will be left uncoloured to indicate 'white spots' or gaps in the existing information on the occurrence of the species along the river systems in different countries.

The participants agreed with this proposal. It was pointed out that the proposed density map is highly relevant for the EUFORGEN Steering Committee while it discusses and develops a proposal for the Phase III of EUFORGEN. Therefore, it was agreed that the first version of the density map should be available before the next Steering Committee meeting, which is scheduled for May/June 2004. The density map will be presented at the Steering Committee meeting as well as during the next *P. nigra* Network meeting.

Marc Villar will act as a focal point for the density map and will send out a survey for the Network participants to collect information for the map by 30 June 2003. The participants are requested to provide relevant information and send it to Marc Villar by 31 October 2003. He will then prepare the density map and send it to the Secretariat by 31 December 2003. The first draft of the map will be prepared in hard copy format and later it will be made available in electronic format.

#### Public awareness and communication

#### **Slide collection**

Sven de Vries provided an update on the slide collection and mentioned that some requested items were received after the previous Network meeting. Missing slides, i.e. North African landscapes (Marc Villar and Carmen Maestro), West China (Marc Villar) and vine–poplar systems (Maria Carolina Varela), and restoration techniques (Rolf Schulzke), should be sent to Sven de Vries by 31 August 2003. The third CD-ROM will be issued at the next Network meeting. Contributions should be sent as slides only. Sven de Vries also gave copies of the CD-ROMs to the newly attending participants

A slide collection for *P. alba* is being developed by István Bach. All participants were encouraged to send more slides as soon as possible. The images will be catalogued and included in a database format. For this collection high-resolution scans are also accepted.

#### EUFORGEN Web site

Jarkko Koskela informed the Network that the EUFORGEN Web site is currently being revised and will be launched by 2 June 2003. He demonstrated the new structure and the way the EUFORGEN Network products will be made available through the Web site. The new address is *www.euforgen.org*.

## Posters

Sven de Vries presented a *P. nigra* Network poster that was presented at the DYGEN Conference in Strasbourg in December 2002. Copies of this poster in A4 size were distributed to the participants. A EUFORGEN poster was also displayed. The poster was developed for the fourth Ministerial Conference on the Protection of Forests in Europe (MCPFE), held in Vienna at the end of April 2003.

## Identification sheet for P. alba

István Bach presented an advanced draft of the ID sheet for *P. alba* containing six pages with drawings. The draft was distributed amongst the participants for comments. Corrections should be sent to István Bach by 30 June 2003. He will send out a second draft to the Network by 31 July 2003. Comments on the final draft should be sent to István Bach by 30 September 2003 after which he will send the final version, including the original drawings, to the Secretariat by 31 October 2003.

## Other public awareness efforts

The excellent public awareness activities developed by Davorin Kajba during the seventh Network meeting held in Croatia in October 2001 were reviewed. He distributed photocopies of newspaper articles and brought a copy of the interview that was broadcast by the national TV news in Croatia.

Additional public awareness activities can be found in the individual country updates.

## Ex situ conservation

## Core collection of P. nigra clones

Lorenzo Vietto gave a presentation on the current situation of the *P. nigra* core collection. At present 20 countries are represented in the core collection with a total of 39 clones and 15 reference clones. Duplications of the core collection are present in eight countries (Austria, Belgium, France, the Netherlands, Portugal, Spain, Turkey and Ukraine). Since this is an ongoing process, none of these collections is fully complete. New entries are expected from European countries, China and Algeria. Ireland and Bosnia and Herzegovina will send their entries as appropriate, and Moldova will first discuss this internally. Slovenia will be contacted by Davorin Kajba and Carmen Maestro will try again to contact Algeria. Marc Villar and Jos Van Slycken will do the same for China and Greece. These new entries will then be sent to Lorenzo Vietto to be included in the core collection and later duplicated in other countries.

The *P. nigra* core collection is available through the Internet, and information on these new entries will be added to the database. It was discussed that new fields should be added to the core collection database to provide information on the original source of a clone. The new fields could be morphological descriptions, river names, soil type and climate. Also, new fields should be added for clonal information. These could contain haplotypes and morphological descriptions like those derived from the EUROPOP project. Jos Van Slycken, Carmen Maestro and Lorenzo Vietto will develop a proposal for these new fields and send it to the Network participants by 30 September 2003. Comments from the participants should be returned to Lorenzo Vietto by 31 October 2003. Lorenzo Vietto will circulate the updated database one month before the next Network meeting.

## Core collection of P. alba clones

During the previous meeting it was decided to send material (rooted plants, cuttings or root suckers) to two locations: Hungary and Germany. Interested countries should provide two

clones (ideally 10 cuttings per clone) of *P. alba* typical for their country.

István Bach provided an update on the establishment of this core collection which is still in its initial phase. So far only Italian and Hungarian material has been rooted in Hungary and Italian, Spanish and Turkish material has been rooted in Germany. All participants are invited to send cuttings following the guidelines to Hungary and Germany by 28 February 2004.

#### Population collection of the EUROPOP project

István Bach has received material from five countries (France, Germany, Italy, The Netherlands and Spain). Non-rooted clones within these populations should be re-sent and Istvan Bach will inform the relevant countries about the clone numbers. Berthold Heinze will investigate about the missing material from Austria. The material should arrive in Hungary before 28 February 2004.

#### **Research facilitation**

Marc Villar introduced a EU-funded POPYOMICS project which focuses on ecophysiology, growth studies and molecular markers in poplars. In the near future, natural populations of *P. nigra* will be used within the project and the *P. nigra* Network offered its material and information for this purpose.

On behalf of An Vanden Broeck, Jos Van Slycken presented a draft review paper on introgression and gene flow in *Populus* spp. The participants discussed the draft paper and provided some feedback to Jos Van Slycken who will forward the comments directly to An Vanden Broeck. The participants were requested to send their detailed comments to An Vanden Broeck by the end of June 2003. She will then provide the final version to the Secretariat by 30 September 2003 to be included in the combined printed report (pp. 00–00 of this publication).

Marc Villar asked whether Network outputs, such as this review paper, could be published in scientific journals instead of EUFORGEN reports. After some discussion, the majority of the participants agreed that papers developed by a Network member(s) for the Network and reviewed by other Network participants should be first published in the Network reports. These papers improve the quality of the Network meeting reports. The Network encourages the Secretariat to print the reports in due course.

#### Documentation

#### Update on P. nigra clone database

Lorenzo Vietto gave a report on the current status of the database, which at present contains 3105 entries, with some duplicates. Currently 20 countries (seven countries only through the *P. nigra* core collection) have provided their data to the database. The database is accessible at *www.populus.it* (click 'banche dati') and an English version of the Web site will be online within one month from now. Network members have received a password and they can update their national data while online to the database.

It was proposed that new fields should be added regarding the name of the river system, whether the purity of the material has been checked, what testing methods have been used and whether the material was obtained from seed or by vegetative propagation.

On behalf of the Network, Sven de Vries thanked Lorenzo Vietto and his institute ISP in Casale Monferrato in Italy for all the work done for this database with special thanks to his colleague Gaetano Castro.

#### Update on P. alba clone database

During the previous meeting, it was agreed that the format of the *P. alba* database, hosted by

Spain, would be regularized to the one already in place for *P. nigra*. Before this meeting Nuria Alba circulated a questionnaire to which the following countries had responded positively: Austria, Czech Republic, Hungary, Italy, Malta, Portugal, Slovakia, Spain and Turkey.

#### **EUFORGEN Bibliography**

Jarkko Koskela provided an update on the current status of the EUFORGEN Bibliography Database. After clearing out some duplicates, the database contains 1975 entries altogether including 261 on '*Populus*'. However, the database lacks recent publications and all Network members were encouraged to continue providing references on a regular basis. Very recent updates with new grey literature are specifically requested and Network participants can send their contributions to the Secretariat. It was suggested that a national contact person (participant of the *P. nigra* Network) should be indicated on the Web site so that hard copies can be obtained when necessary.

#### Seminar on in situ conservation of black poplar in Germany

Rolf Schulzke gave a presentation on *in situ* conservation of black poplar along the River Eder in Germany (pp. 115 of this publication). Georg von Wühlisch presented the contributions of Harald Koss (River Elbe) and Manfred Weidner (River Rhine) who were unable to attend and deliver their papers. The papers include some overlapping information so it was proposed that the authors and the Secretariat should discuss the possibility of presenting these contributions as a single paper in the meeting report.

#### Wrap-up session

#### Any other business

Georg von Wühlisch highlighted the problems in natural regeneration of *P. nigra* that were demonstrated during the field excursions of this meeting. The participants then discussed possible solutions. The problems relate to the overall hydrology and dynamics of the river systems and it seems to be difficult to provide a simple and straightforward solution. The participants discussed various approaches to overcoming the problems for natural regeneration and genetic conservation of *P. nigra* in this region. The recommendations based on the discussion are summarized by Georg von Wühlisch in the following paragraph.

It became clear that natural regeneration is only possible if the hydraulic situation of the Oder river is changed. However, since this is unrealistic, other options have to be envisaged. The aim is to raise the size of the present population which is considered to be too small for permanent existence. The size of the population can be increased by increasing the number of genotypes. This means that generative propagation is necessary. This may be achieved by collection of seed on open-pollinated female trees. Another possibility would be to obtain seed from controlled crosses. The seedlings from the seeds should be grown in a nursery and planted at the age of 2–3 years at suitable spots on the island. For satisfactory monitoring of the genetic structure of the black poplar population the implementation of molecular markers would be advisable.

Hans Muhs mentioned that the International Poplar Commission (IPC) will organize a conference in Chile in October 2004 and suggested that the *P. nigra* Network should be represented at this conference.

Marc Villar presented a request from a French scientist, Pascal Frey, who is interested in collecting *P. nigra* leaves infected with rust for a pathological study. Marc Villar will provide the contact details of the participants to Pascal Frey who will then contact participants as appropriate.

Sven de Vries informed that the European Forest Institute (EFI) had contacted all five Chairs of the EUFORGEN Networks and requested information on countries which have been participating in EUFORGEN activities over the years. EFI is conducting a study on indicators for biodiversity and will later contact participating countries to collect information for this purpose. The list of countries which have been involved in the activities of the *P. nigra* Network was reviewed and the participants indicated that a few more countries should be added to the list. Sven de Vries will forward the updated list to EFI.

Davorin Kajba gave a short presentation on the hairy type of *P. nigra* that is found in the Balkan region. This update was based on his presentation on the same topic during the DYGEN Conference in Strasbourg in December 2002. It was suggested that this hairy type should be included into the core collection.

## Date and place of the next meeting

Peter Rotach indicated Switzerland's interest in organizing the next *P. nigra* Network meeting at the end of 2004. Berthold Heinze also indicated Austria's interest in organizing the next meeting, as an alternative option.

## Adoption of the summary report

The report was adopted.

## Closure of the meeting

The Chair, Sven de Vries, thanked the local organizers on behalf of the Network for the meeting and excursion arrangements and for their warm hospitality. He also thanked Network members for their contributions to the network tasks and discussions during the meeting. The Chair declared the meeting closed.

## Introductory country reports

## The status of black and white poplars (*Populus nigra* L. and *P. alba* L.) in Bosnia and Herzegovina

#### Dalibor Ballian

Faculty of Forestry, University of Sarajevo, Bosnia and Herzegovina

Bosnia and Herzegovina is located in the northwest area of the Balkan Peninsula, between 42° 26′ and 42° 15′ N and 15° 44′ and 19° 41′ E. It is mostly a mountainous country; mountains take up 42% of its territory, hilly regions 24%, plains 5% and rocky terrain 29%. The average altitude above sea level (a.s.l.) is 500 m, and the highest peak is Mount Maglić (2387 m a.s.l.).

The total forest area is 2 501 000 ha, consisting of 1 130 000 ha of commercial forests, 841 000 ha of degraded forests and 530 000 ha of barren land (Matić *et al.* 1971). According to data from the 1964–1968 forest inventory, the natural poplar forests cover approximately 712 ha and plantations 480 ha. The situation has not changed much since then.

The overall length of rivers and creeks in Bosnia and Herzegovina is around 11 000 km (Alikalfić 1998), but the habitat areas of black and white poplars are limited only to valleys of the bigger rivers, such as the Drina, Bosna, Vrbas, Una, Sana and Neretva (Figure 1), with the total length around 1200 km. Black and white poplars in Bosnia and Herzegovina grow at the alluvial, sandy soils along the rivers, plains and lower hilly regions up to approximately 300 m a.s.l. (Šilić 1983). However, the two species reach 530 m a.s.l. in the Sarajevo Valley.

#### Area of distribution

In the Drina river valley, black poplar (Populus nigra) and white poplar (P. alba) grow along the upper Drina from the city of Foča to the city of Višegrad, and along the lower Drina from Tegare to Rača where the river joins the river Sava. All along the river Bosna to the place where it enters the river Sava, black poplar grows singly or in small groups. In the valley of the river Vrbas there are two separate groups of black poplars, one on the upper Vrbas in the Uskoplje valley, from the city of Gornji Vakuf to the city of Bugojno, and the other from the city of Banja Luka downstream along the river Vrbas. The reason for this isolation is that the river Vrbas flows through a canyon which provides no suitable habitats for black poplar. Along the river Una, populations of black and white poplar occur on the middle Una, which is in the Bihać valley, and downstream to where it enters the river Sava. In the Sana river valley, the habitats of black and white poplars are on the lower Sana from the city of Sanski Most to the place where it flows into the river Una. Along the river Sava, in Bosnia and Herzegovina, there are also black and white poplars. Along the river Neretva, a special type of black poplar can be found, called hairy poplar (Populus nigra subsp. caudina). White poplars also occur on the lower Neretva from the village of Žitomislići to the border with Croatia (the city of Metković) with a small and specific black poplar population at the lower Trešanica creek.

Because the vegetation associations of broadleaves in Bosnia and Herzegovina have not been scientifically researched, we usually use results from Croatia and Serbia, specifying these types of forests as *Populetum nigro-albae* Slavn. or *Salicetum albae* Issl. (Stefanović 1986). Based on these facts, it is easy to see that we still have research to do in this field.



Figure 1. The distribution of Populus nigra along major rivers in Bosnia and Herzegovina.

#### Systematics of poplars in Bosnia and Herzegovina

For a long period, the literature of Bosnia and Herzegovina reported only three native poplar species: aspen (*Populus tremula*), black poplar and white poplar (Beck-Mannagetta 1906) until the investigations of Fukarek (1959) and Janjić (1983, 1984, 1990, 1992–1996) when classification of poplar into lower systematic units began. These units show great diversity in forms within each of three species in Bosnia and Herzegovina (see Table 1).

Besides this, Bosnia and Herzegovina did not avoid the introduction of cultivars of Canadian poplars ( $P \times canadensis$  Moench), as mentioned in the papers of Fukarek (1959) and Janjić (1992–1996). In addition to Canadian poplars, the balsam poplars were also introduced. The first introduction of the exotic poplar species was connected with the Austro-Hungarian occupation of Bosnia and Herzegovina when poplars were planted along the railway stations.

However, we can still say that the genetic resources of black poplars in Bosnia and Herzegovina are still in good condition, because intensive establishment of poplar plantations has never taken place. There were some attempts to establish poplar plantations but these were quickly forgotten, especially after the occurrence of some diseases and pathogens. There is, however, a small possibility of introgression of allochthonous genes into genetic reserves of autochthonous species. The main threat to the genetic resources of autochthonous poplars is non-planned land use along the rivers, regulation of river flows, gravel pits, dumps and a lack of legislative regulations.

#### Conservation of poplar genetic resources

In 1997, as a result of cooperation with colleagues of the Faculty of Forestry, University of Zagreb, 54 black poplar trees were selected in the areas of Sarajevo, Kakanj and Zenica. From those trees, 50 were successfully cloned (Krstinić and Kajba 1997). The current situation in the field is that one third of the selected trees have been destroyed or died because—except for six young trees from an old coal mine in the city of Kakanj—they are all old. Only two of ten selected trees in the Sarajevo area are still alive, but they will be cut very soon because of their age. In February 2003, ten hairy black poplar trees were selected at the Čapljina area

#### Table 1. Taxonomy of poplars in Bosnia and Herzegovina

Gen. Populus L. Subgen. Balsamifera Bugala (syn. Subgen. Eupopulus Dode) Sect. 1. Aigeiros Duby P. nigra L.-Black poplar subsp. nigra-Typical black poplar (syn. P. nigra L. apud. Jov. et. Tuc. 1972) var. nigra f. nigra f. vistulensis (Dode) Janjić, stat. nov. (syn. P. vistulensis Dode) f. truncata Jov. et Tuc. f. maserica (Jov. et Tuc.) Janjić stat. nov. (syn. P. nigra var. maserica Jov. et Tuc.) + cv. 'Italica'-Lombardy black poplar (syn. P. nigra var. italica Muenchh.; P. pyramidalis Rozier) subsp. caudina (Ten.) Bugala-Hairy black poplar (syn. P. caudina Ten.; P. nigra var. pubescens Parl.) var. grandifolia (Džekov) Janjić (syn. P. nigra var. pubescens f. grandifolia Džekov) f. grandifolia f. pseudocaudina Janjić f. torulosa Janjić (syn. P. pubescens var. maserica Jov. et Tuc.) f. hispidula (Bornm.) Janjić (syn. P. nigra f. hispidula Bornm.; P. nigra var calvescens Džekov) var. narentana (Jov. et Tuc.) Janjić-Hairy black poplar f. Narentana - hairy black poplar P. nigra subsp. nigra × P. cv. 'Italica' (syn. P. pannonica Kit.; P. nigra var media Schur) +P. afghanica (Aitch. et Helmsl.) Schneid. cv. 'Afghanica'-white bark pyramidal poplar (syn. P. thevestina Dode; P. nigra L. cv 'Hamoui') P. × neapolitana Ten. nm. kakanjensis Janjić-Kakanj poplar (= P. afghanica cv. 'Afghanica' × P. nigra subsp. caudina) P. afghanica cv. 'Afghanica' × P. nigra subsp. nigra (syn. P. × pannonica Kit. nm. ilidshensis Janjić; = P. × charkowiensis Schroed. vel affine) +P. deltoides Marshall-Eastern cottonwood (svn. P. monilifera Ait.) +P. wislizenii (S. Wats.) Sarg. (syn. P. fremontii var. wislizenii S. Wats.)

+P. × canadensis Moench—Canadian poplar (syn. P. × euramericana Guin.; = P. deltoides or P. angulata × P. nigra) cv. 'Serotina' (syn. P. serotina Hartig; = P. nigra × P. deltoides var. monilifera) cv. 'Marilandica' (syn. P. marilandica Bosc; = P. nigra × P. serotina) cv. 'Robusta' (syn. P. robusta Schneid.; = P. angulata × P. nigra cv. 'Plantierensis') cv. 'Regenerata' (= P. × re (= P. angulata generata Henry) cv. 'I-214' or P. deltoides × P. nigra) cv. 'I-154' (= P. deltoides var. monilifera × P. nigra) cv. 'I-455' (= P. deltoides var. monilifera × P. nigra cv. 'Italica') Sect. 2. Tacamahaca Spach +P. simonii Carr.—Simon poplar (syn. P. przewalskii Maxim.) cv. 'Fastigiata' +P. yunnanensis Dode +P. cathayana Rehd. +P. trichocarpa Torrey et Gray +P. × gileadensis Rouleau—Ontaro poplar (syn. P. canadicans Ait.; = P. balsamifera × P. angulata) +P. × berolinensis (K. Koch) Dipp-Berlin poplar (= P. laurifolia × P. nigra cv. 'Italica') Gen. Populus L. Subgen. Populus (syn. Sect. Leuce Duby) Sect. 1. Populus (syn. Sect. Albidae Dode) P. alba L.-White poplar f. macrophylla (Bugala) Janjić (syn. P. alba var. macrophylla Bugala) f. chenopodiifolia Gombocz f. insignis Gombocz f. gercifolia Janjić f. stenophylla Janjić f. brevifolia Janiić f. ovatifolia Janjić f. australis Janjić + cv. 'Nivea' (syn. P. nivea Willd.) + cv.'Pyramidalis' (syn. P. bolleana Lauche; P. alba L. cv 'Roumi')

(+ denotes introduced species)

and we hope that their vegetative reproduction at the Faculty of Forestry of the University of Zagreb will be successful so that they will be added to a clone archive and thus find their way to the European archives of black poplars.

## Future work on conservation of poplar genetic resources

- Research on intra- and interpopulation variation in morphological characteristics (flowers and leaves)
- Research on intra- and interpopulation variation at molecular level (isozymes and DNA markers)
- In situ conservation
- Ex situ conservation with establishment of a national poplar archive
- Legislative regulations

#### References

- Alikalfić, F. 1998. Johe uz obale naših vodotoka i njihov značaj za kvalitet životne sredine [Alders growing along our river banks and their role for the quality of the environment]. Works of Faculty of Forestry University of Sarajevo 1: 1–17.
- Beck-Mannagetta, G. 1906. Flora Bosne i Hercegovine i Novopazarskog sandžaka [Flora from Bosnia and Herzegovina and sandžak of Novi Pazar], II(1) dio. Glas. Zem. Muz., 18. Sarajevo.
- Fukarek, P. 1959. Pregled dendroflore Bosne i Hercegovine [Review of dendroflora in Bosnia and Herzegovina]. Narodni šumar., Sarajevo 13(5/6):263–286.
- Janjić, N. 1983. Dve nove hibridne polupiramidalne crne topole iz centralne Bosne [Two new semipyramidal hybrid black poplars from central Bosnia]. Biosistematika, Beograd 9(1):15–27.
- Janjić, N. 1984. Prilog poznavanju dlakavih svojti crne topole (*P. nigra* L.) u Bosni i Hercegovini [A contribution to the knowledge of hairy-type forms of black poplars in Bosnia and Herzegovina]. Glas. Zem. Muz. BiH, Prir. Nauke, Sarajevo, 23: 255–278.
- Janjić, N. 1990. Prilog sistematici bele topole (*Populus alba* L.) u Bosni i Hercegovini [A contribution to the systematics of *Populus alba* L. in Bosnia and Herzegovina]. God. Biol. Inst. Univ., Sarajevo 42:45–56.
- Janjić, N. 1992–1996. Pregled prirodnih i gajenih topola Bosne i Hercegovine [Conspectus of native and introduced poplars of Bosnia and Herzegovina]. ANU BiH, Radovi LXXXIX, odjelj. prir.-mat. Nauka, Sarajevo, 26: 109–117.
- Krstinić, A. and D. Kajba. 1997. Ex situ conservation of the european black poplar (*Populus nigra* L.) in Croatia. In *Populus nigra* Network. Report of the fourth meeting, 3–5 October 1997, Geraardsbergen, Belgium. IPGRI, Rome, Italy.
- Matić, V., P. Drinić, V. Stefanović, M. Čirić *et al.* 1971. Stanje šuma u SR Bosni i Hercegovini, prema inventuri na velikim površinama u 1964–1968 godini [The condition of forests in the Socialist Republic of Bosnia-Herzegovina, according to the inventory of large areas in 1962–1968.]. Šum. fak. i inst. za šum. posebna izdanja br. 7, Sarajevo, Bosna i Hercegovina. 639 pp.
- Stefanović, V. 1986. Fitocenologija sa pregledom šumskih fitocenoza Jugoslavije [Phytocenology with review of forest phytocenosis of Yugoslavia], II dopunjeno izdanje, Sarajevo, Bosna i Hercegovina. 269 pp.
- Šilić, Č. 1983. Atlas drveća i grmlja [Encyclopaedia of trees and shrubs]. Sarajevo, Bosna i Hercegovina. 218 pp.

## Black poplar (Populus nigra L.) resources in the Republic of Ireland

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Black poplar (*Populus nigra* L.) is mainly distributed in a band stretching from Co. Dublin and moving in a southwesterly direction through counties Kildare, Offaly, Tipperary and Limerick. Across the river Shannon into southeast Galway, many trees have been found especially along the banks of the Cappagh and Kilcrowe rivers, which feed into the Shannon at Lough Derg. In past surveys, concentrations of black poplar individuals have been found as far north as Lough Allen, also on the river Shannon (Figure 1).





#### Distribution

Various surveys have been carried out over recent decades with a view to mapping the distribution and quantifying the number of black poplar individuals in Ireland. However, a complete national survey of the species has yet to be completed. A survey carried out in 1989 recorded 210 trees (Hobson 1991). A later survey in 1990 revealed the total number of trees as 373 (Hobson 1993). Mainstream opinion at the time advocated that black poplar was a very rare introduced species planted from English stock. Indeed, even as early as the late 1890s, when the species may have been more widespread, it was not considered native to Ireland (Colgan and Scully 1898, cited by Hobson 1991). Hobson's findings led him to suspect that black poplar was native to Ireland due to the unbossed stem form found in Ireland as opposed to the bossed stems commonly found in Wales and England. This theory was also reinforced by the close link the species has with river valleys and floodplains, especially along parts of the river Shannon. Ever since, there has been widespread debate among ecologists, botanists and foresters whether the species is native or introduced in Ireland.

A more recent survey, funded in part by the National Council for Forest Research and Development (COFORD) and carried out by the author in August 2000, used the data originally collected by Hobson in 1989. This survey used a global positioning system to log

Irish national grid northing and easting coordinates of each tree, and covered counties Dublin, Kildare, Offaly, Tipperary and some of Galway. Other counties that are known to have black poplars but have not been surveyed since Hobson's verification include counties Leitrim, Limerick, Clare, Cork and Wexford.

## Economic importance for forestry sector

Black poplar has no current economic importance for the forestry sector in Ireland.

## Conservation

Of the counties that have been surveyed, about 90 trees had their geographical coordinates recorded. These trees, along with an additional 24 others, had cuttings taken from them in the spring of 2003. A further 25 trees were recorded in Co. Galway for which no geographic coordinates or cuttings were procured. These trees were in small groups and lone individuals. In the counties that were not surveyed in August 2000, a further 31 trees were recorded by Hobson in 1989.

## Status of black poplar in Ireland

In Ireland black poplar survives mainly as small scattered collections of trees or single trees in hedgerows. This is the case especially in counties Kildare and Offaly, where land is suitable for agriculture and the only uncultivated areas are non-commercial forest or field boundaries. Occasionally only one or two trees might be present in a 20 km radius, while in other instances there might be groups of three or four trees. However, relatively larger groups of trees do exist in counties Tipperary and Galway close to the northern shores of Lough Derg. One group of 15 trees exists in Ballinderry in north Co. Tipperary, beside a small pond. Other important groups exist west of Portumna, Co. Galway on the banks of the Kilcrowe river. A common feature of these collections is that they are linear in structure, constituting field boundaries between agricultural land. In the Ballinderry group, a small plot of land has been fenced off and is left uncultivated but it is unlikely that natural regeneration occurs there. West of Portumna on the northern shores of Lough Derg, approximately 30 trees exist within a radius of 2 km. These trees are grouped into three collections of approximately 6–8 trees each and the remainder are scattered. One of these, the Sawnagh townland group, is growing on a floodplain on the banks of the Killcrowe river. The existence of such a relatively large collection of trees in a habitat typically associated with black poplar adds strength to the suggestion that the species is indeed native to Ireland.

## Ex situ conservation

In the spring of 2003, cuttings from 114 individuals of black poplar were sent to the Forestry Commission Northern Research Station at Roslin, Scotland for genetic fingerprinting. These cuttings are being analysed with five microsatellite loci in order to determine the number of different genotypes in the sampled trees. Their DNA will be saved. In time it is hoped to sample much more of the Irish population in order to gain knowledge about the number of different clones in existence.

The results of the microsatellite analyses will be useful in prioritizing and optimizing the efficiency of collecting more cuttings in the future. A stool bed containing the entire range of genotypes should then be established and material supplied to the EUFORGEN core collection.

Furthermore, once the genetic diversity of the Irish population is known, it is desirable to carry out an analysis of the cpDNA to ascertain whether the Irish population is from a different origin from the Welsh and English populations. While there are no definite plans to

carry out a cpDNA analysis at the moment, it is hoped to do so in due course.

#### In situ conservation

*In situ* conservation of black poplar in Ireland presents a much larger challenge to Irish foresters and land managers, because many of the landowners who have black poplar on their holdings are not aware of the rarity of the species. Furthermore, many of the existing trees have large, old, unwieldy crowns and in the eyes of the farmers constitute a threat in the face of strong winds, with the result that they are felled.

Stakeholders like the farming public and national organizations such as Duchas, the Heritage Council, the Regional Inland Fisheries Boards and county councils must be made aware of the importance of black poplar as a species under threat in Ireland. From a planning perspective it is also important that these stakeholder organizations be kept abreast of developments occurring in the general area of black poplar *in situ* conservation.

Tree preservation orders should be used in order to retain healthy trees. Placing such preservation orders on the trees would be a positive step but it will only serve static conservation purposes. Black poplar should be included in various county council heritage plans. Each county council holds a County Heritage Forum that involves staff from different organizations e.g. the Forest Service, Teagasc, Coillte and Duchas.

There is also a need to protect the area where the trees live in order to give black poplar a chance for reproduction and long-term survival. This will require a multidisciplinary team of experts on river management, forestry, planning and genetics in order to plan and manage such a project. This is further complicated by the fact that black poplar requires new sites for regeneration where no mature trees usually exist. *In situ* conservation will be most effective in areas where the floodplain ecosystem has been or will be restored, or where the river is managed to allow episodic flooding (Rotach 2001). A possible solution to this requirement may be the designation of certain stretches of tributary rivers of the Shannon as dedicated riparian vegetation areas and classified as Natural Heritage Areas with an agreed riparian vegetation management plan. Natural Heritage Areas in Ireland have a legislative basis under the Wildlife Act 1976, the Wildlife (Amendment) Act 2000 and the European Communities (Natural Habitats) Regulations 1997.

A new, more complete and up-to-date survey of the current distribution of black poplar is required in order to get an accurate estimate of the extent of the tree. Data from Hobson's 1990 survey would be very useful for this work.

In their present state, it is unlikely that any of the scattered groups of black poplar trees will be able to reproduce in the future and achieve long-term survival because the populations are too small and the sex ratio of females to males is approximately 2:100. Any establishment of new black poplar forests will probably require imported planting material from other locations.

#### Conclusions

The present status of black poplar in Ireland is undefined and may be unsustainable. This is because of the small number of trees and in particular the lack of female trees. Pressure arising from agriculture and unsuitable river management are also responsible for its decline. Tree preservation orders are required immediately to prevent the genetic base declining further. This should be followed by *in situ* conservation strategies that will rely on current and future knowledge of the populations from DNA analyses and of practical black poplar management techniques. Financially resourced multidisciplinary efforts will be needed between staff in different organizations if *ex situ* and long-term *in situ* conservation is to succeed in Ireland.

#### References

Hobson, D.D. 1991. The status of *Populus nigra* L. in the Republic of Ireland. Watsonia 18: 303–305.

Hobson, D.D. 1993. Populus nigra L. in Ireland—an indigenous species? Ir. Nat. J. 25(6): 244–247.

Rotach, P. 2001. General considerations and basic strategies. Pp. 8–15 *in In situ* Conservation of *Populus nigra* (F. Lefèvre, N. Barsoum, B. Heinze, D. Kajba, P. Rotach, S.M.G. de Vries and J.Turok, eds.). IPGRI, Rome Italy.

## **Country reports**

## Progress on national activities on gene conservation of *Populus nigra* and *P. alba* in Austria 2001–2003

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Currently, floodplain forests with *P. nigra* and *P. alba* play a minor role in Austria. The two species used to be more common but especially *P. nigra* is declining. Austria joined the EUFORGEN *Populus nigra* Network in 1996 at the third meeting in Sárvár, Hungary. This update covers the period from October 2001 to May 2003.

#### Field inventories

No dedicated poplar inventories are running or planned. However, the Forest Inventory Department of our Research Centre is currently analysing the field data from the periodic National Forest Inventory (2000–2002). They are trying to distinguish the different poplar species (and hybrids) in the field. We hope that with the result of this assessment, which will become available in the course of 2004, we will have a better basis for estimating the situation of *P. nigra* (and *P. alba*).

## Policy and legislation

There are no changes in policies or legislation to report. However, over the years, more and more of the provincial governments are becoming aware of the problems in poplar conservation and some of them actively take part in activities. Forestry is governed by federal laws in Austria, while nature protection is a matter of the provincial legislations.

An open dialogue with all relevant stakeholders in society has been initiated recently by the Federal Ministry in order to draft a National Forest Programme (more information can be found at *www.walddialog.at*).

#### Research

Results from the Austrian participation in the EUROPOP project have been welcomed with great interest (Krystufek 2001). A new chapter on *Populus* for the revision of the Flora of Austria is currently being drafted by B. Heinze and M.A. Fischer. We are also trying to get funding for a genomics research projects dealing with *P. alba, P. × canescens,* and *P. tremula,* in order to find genes related to flood tolerance (C. Lexer, J. Gloessl, B. Heinze). Preliminary laboratory investigations into hybridization in this species complex are currently under way (C. Lexer, M.-S. Nica, B. Heinze).

#### **Practical implementation**

No new *in situ* conservation measures took place during the reporting period. Our next targets are the Salzach and Drau/Drava rivers. For the river Salzach, the provincial government is supporting floodplain conservation and we hope to make a case for *P. nigra* there.

For *ex situ* conservation, new genebank accessions are added sporadically (last year, a population was discovered in the city of Salzburg). Our own collection of about 180 *P. nigra* genotypes was defended from being axed under general austerity measures and we could add most of the EUFORGEN core collection genotypes this year. However, we have not yet

been able to extend the collection to *P. alba*.

Regarding forest rehabilitation and afforestation efforts, a few river restoration schemes are running or planned. However, these are focused on river dynamics and fish conservation, and we will have to convince managers that such projects could also promote genetic conservation of tree species. For example, practically under my office window, *P. nigra* is reclaiming its natural habitat in a recently remodelled side-channel of the river Wien.

The opening up of further side arms of the river Danube in the national park of the Danube floodplains is attracting international attention. A hitherto unreported rehabilitation scheme was conducted by the city of Linz a few years ago, which can be directly traced back to EUFORGEN influence. Native *P. nigra* clones have been planted at a recreation site on the river Danube by the city authorities, with support by the provincial government.

#### Coordination at national level

The floods of the summer of 2002 have once more highlighted the need for larger flood retention areas in river floodplains. Although there was much public debate on this issue for a short time afterwards, no national plans have yet been initiated.

#### Public awareness

Slowly but steadily, poplars are drawing public attention. The national park of the Danube floodplains (*www.donauauen.at*) provides information about black poplar conservation in their excursion programmes. Occasional articles in local journals show reactions from the readership (Heinze 2002). Unfortunately, none of the 45 or so ministers responsible for forests who gathered for the fourth Ministerial Conference for the Protection of Forests in Europe (MCPFE) planted a black or white poplar when they gathered in Vienna in April 2003 (each of them planted a tree typical of their country in a memorial forest). However, they made a field excursion to the Danube floodplains national park where they, and the public via media coverage, could see some of the problems in restoration of poplars.

#### References

- Heinze, B. 2002. Schwarzpappeln in Salzburg—eine Baumart an der Verbreitungsgrenze. NaturLand Salzburg 3: 35–38 (*www.salzburg.gv.at/fachbeitraege302.pdf*)
- Krystufek, V. 2001. Population genetic analysis of *Populus nigra* in Austria using nuclear and chloroplast DNA markers. Dissertation, University of Vienna, Austria.

## Progress on national activities on gene conservation of *P. nigra* in Belgium

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Black poplar (*Populus nigra* L.) is considered to be one of Belgium's rarest native trees. A detailed survey carried out during the past five years estimates that there are at least 360 surviving relict trees. Belgium has a long history of poplar breeding, which is still going on. In the context of the poplar breeding programme and of the conservation of indigenous tree species, the conservation of black poplar has been an important research activity since 1960. Here we report on the Belgian activities on gene conservation of *P. nigra* from January 2000 till May 2003.

## Field inventories

Most relict trees were found in the Dender valley in mid-west Belgium (the province of Hainault). The species is extremely rare in the Meuse valley (only five locations detected) and the Scheldt valley (two locations detected). A detailed inventory carried out near the river IJzer in the northwest of Flanders (the province of West-Vlaanderen), near the French border, revealed 99 old black poplar trees. Presumably, all of them were propagated vegetatively and planted by man. Based on the sex of the trees (all of them are female) and on a preliminary study, they probably represent only two different genotypes. A detailed DNA analysis (with AFLP markers) of all 99 trees is under way.

No natural regeneration of black poplar is observed in Belgium. However, introgressive hybridization with *P.* × *canadensis* is reported along the river Meuse. A DNA analysis revealed that seedlings originated from hybrid × hybrid crosses and that *P. nigra* × hybrid crosses colonize the riverbanks of the Meuse. These seedlings seem to be well adapted to the river dynamics and the local climate.

## **Policy and legislation**

An increasing interest and availability of funds for conservation activities is observed.

## Research

#### **Finalized research projects**

A research project running from December 2000 to November 2001, financed by the Flemish Government, was concerned with the restoration of black poplar along the river Meuse on the Dutch–Belgian border (Vanden Broeck *et al.* 2002). The aim of this project was to study whether natural regeneration of black poplar occurs in the study area and to investigate the amount of introgression of foreign genes in the offspring of female black poplars.

#### Mating system and introgression

A study was conducted to investigate whether *Populus* × *euramericana* (Dode) Guinier (syn.: *P.* × *canadensis* Moench.) and the fastigiated Lombardy poplar (*Populus nigra* L. var. *italica* Moench.) hybridize spontaneously with native black poplar. In the presence of *P. nigra* pollen, *P.* × *euramericana* was at a selective disadvantage in fertilizing black poplar females. The mating system of *P. nigra* in the orchard under study deviated strongly from panmixis. This research was carried out in collaboration with the Flanders Interuniversity Institute for Biotechnology (the Department of Molecular Genetics, Department of Plant Genetics, Ghent) within the EUROPOP project. Data analysis and publication of the results is under way.

Controlled cross experiments carried out with heterospecific pollen mixtures confirmed the presence of cospecific mating advantage.

## Genetic, morphological and phenological diversity in the EUFORGEN *P. nigra* core collection and the Belgian *P. nigra* genebank

A field trial was established in winter 2000 using cuttings of 100 Belgian *P. nigra* individuals, 30 genotypes of the EUFORGEN *P. nigra* core collection and 20 clones of the EUFORGEN reference collection. Morphological leaf characteristics and phenological data were recorded during the first and second growing season. The genetic diversity is currently being studied with microsatellite markers. DNA fingerprinting based on AFLP markers of the whole Belgian *ex situ* collection is also carried out in order to identify identical genotypes.

#### New projects and proposals

A proposal for a national project on influences of exotic species on the recovery of endangered native tree species, with poplar as a model tree, was submitted to the Flemish Government.

## Practical implementation

#### In situ conservation measurements

There are no natural populations of black poplar in Belgium.

### Ex situ conservation measurements

The complete collection of 360 trees is screened for clonal duplicates and genetic diversity with AFLP and microsatellite markers. A total of 12 field plantations were established in April 2002 with 970 black poplars representing the different genotypes of the Belgian *ex situ* collection. The EUFORGEN core collection was also planted together with the Belgium *ex situ* collection on two different sites (Limburg and Vlaams-Brabant).

#### Rehabilitation and afforestation efforts

As the relict individuals along the river Meuse (the Dutch–Belgian border) are too sparse to act as a seed source for recolonization, reforestation efforts were started. At this site, a nature development project aims to restore the river dynamics and the floodplain habitats over a distance of 45 km and in an integral nature reserve area of 3000 ha. A total of 400 two-year old trees originating from cuttings representing 20 indigenous genotypes were planted on the riverbank in April 2002. In order to avoid genetic and non-genetic risks, 150 new genotypes from natural populations from the river Rhine, located within 200 km of the restoration site, are propagated in the nursery. They will be planted in winter 2004 on different locations in the area in order to form meta-populations that can act as seed sources for the natural colonization of the river banks.

## **Public awareness**

A press report was circulated concerning the restoration activities of black poplar along the river Meuse and the current new findings concerning introgression. Based on this press report, two daily papers published an article on black poplar with picture on their regional pages. The restoration of black poplar was also on the local radio news (Radio-2 Limburg).

#### References

Vanden Broeck A., H. Jochems, V. Storme, and K. Van Looy. 2002. Mogelijkheden tot herstel van levensvatbare populaties Zwarte populier (*Populus nigra* L.) langsheen de Grensmaas. [Strategies for restoration of natural populations of Black poplar (*Populus nigra* L.) along the Maas valley]. Vlaams Impulsprogramma Natuurontwikkeling. Eindrapport 0010. 62 pp.

## Black poplar (*Populus nigra L.*) gene conservation activities in Bulgaria

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Black poplar (*Populus nigra* L.) is a comparatively widely spread species especially in northern Bulgaria, along the river Danube and its tributaries. The biology, ecology and habitats of the species are well studied; the seeds show very good germination and the natural regeneration on suitable sites is successful. However, some negative developments regarding the poplar habitats have been observed during the last few years. These include changes in the starting dates and the duration of flooding periods as well as anthropogenic effects such as uncontrolled cutting, draining of land around the rivers and the uprooting of poplar plantations for establishment of agricultural fields.

Spontaneous hybridization with the highly productive clones of American black poplar (or eastern cottonwood) (*Populus deltoides* W. Bartram ex Marshall) also influences the conservation of black poplar genetic resources. Although black poplar is not included in the Bulgarian Red Book as a threatened species, some preliminary measures for its conservation are planned.

This report covers activities on black poplar in Bulgaria from October 2001 to May 2003.

#### Inventories

A register of centuries-year old trees, growing mainly in southern Bulgaria, has been created with the help of specialists from the Forest Research Institute and the University of Forestry. So far 53 individuals were found, accounting for 2.4% of all registered old trees in Bulgaria (Alexandrov *et al.* 1998). Geographical coordinates and altitude for each individual tree were determined by a team of the University of Forestry using the global position system (GPS). The work on this register is ongoing.

New populations and solitary trees of black poplar were found and mapped during the last two years on the islands and along the river Danube, as well as in the valleys of the inner rivers Maritsa and Mesta. Variability in some morphological and phenological traits was observed within these populations.

There is special interest in individual trees with curly-grained wood, which is highly valued by furniture industry and sought for handmade souvenirs. The formation of swellings mainly in the lower part of the stems is result of abnormal cambial activity, which may be due to injuries or infections. Trees with curly-grained wood are very rare, and the reasons for the appearance of this phenomenon have not been properly studied.

#### Measures for conservation of black poplar and its habitats

#### In situ measures

At present there are no special regulations foreseeing special measures for protection of black poplar. There are solitary trees growing on the territories of several national reserves, such as Persina, Kamchiya, Baltata and Ropotamo. However, none of them has been established especially for black poplar.

#### Ex situ measures

Shoot cuttings from some old black poplars have been collected and used for the creation of a stool bed in Pazardjic Poplar Station. The clones will be investigated by the University of

Forestry for determination of their sex, based on international experience. This will enable faster sex identification in the future.

The experimental station in Svishtov has done some ecophysiological experiments with different clones of *Populus nigra* × *Populus deltoides* hybrids in order to determine their ecological requirements and tolerance towards extreme environmental factors. The effects of soil nutrition conditions and water availability were studied with potted plants. Two-year old saplings of the same hybrids have also been planted in an open field for further studies of the effects of soil water availability. Along the river Tundja, near Yuanbol, a four-year old plantation was created with 16 hybrid clones and their adaptation to the existing ecological conditions has been investigated. The first results will be published by the end of 2003.

## Legislation

Regulations are being developed for protection of the centuries-year old trees. Some of the representative habitats of the species should also be put under special control.

## **Public awareness**

A catalogue of poplars, containing information about their botanical classification, geographical distribution and economic importance, as well as a full list of the poplar hybrids and clones bred in Bulgaria, has been published (Tsanov and Mikov 1997). The University of Forestry plans to publish brochures and leaflets providing information about the significance of black poplar and the measures necessary for its preservation. Seminars will be also organized in order to increase public awareness of the biology and ecology of this species and the importance of its genetic resources conservation.

### References

Alexandrov, A., E. Popov and G. Hinkov. 1998. The centuries-old trees as a natural phenomenon and as genetic resource. Proceedings of the Jubilee Scientific Conference with International Participation: '70th Anniversary of the Forest Research Institute', 6–7 October, vol II, pp. 50–54.

Tsanov, Ts. and M. Mikov. 1997. Catalogue of Poplars. National Forestry Board, Sofia.

## Natural poplar stands and gene conservation of *Populus nigra* in Croatia

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Riparian poplar forests typically occupy forest lands which are not suitable for other plant species because of a higher risk of flood and a higher proportion of sand in the soil. Thanks to the richness of its hydrographic network, Croatia has a relatively large area of alluvial soils which provide opportunities for the development of natural poplar and willow stands within the areas of large forest complexes.

Alluvial soils extend along the rivers over a surface area of about 400 000 ha or 7% of the territory of the Republic of Croatia. Larger areas of alluvial soils are found on flooded alluvial terraces of major rivers, i.e. the Drava, the Sava and the Danube. In terms of pedotaxonomy, alluvial soils (fluvisols) belong to a group of hydromorphous soils characterized by temporary or permanent saturation of all soil pores with water which is neither salty nor alkaline, as well as by reduction processes throughout the whole profile or part of it. Within the group of hydromorphous soils, alluvial soils are further classified into undeveloped soils with poorly marked pedogenetic processes, either because the deposits are very recent or because sedimentation prevails over pedogenesis. The origin of the name fluvisol (from *fluvis*, the Latin word for river) indicates that the formation and properties of these soils are conditioned mostly by the characteristics of the river water regime and the materials deposited by the water. The anthropogenic impact on rivers through the construction of infrastructure facilities (dykes, channels, storage reservoirs) results in radical changes of their natural water regimes, which directly affects the alluvial soil water-air regime and, consequently, the intensity and direction of pedogenetic processes. Moisture sources and mechanical composition play a very important role in the water-air regime of alluvial soils. There are three sources of moisture: rainwater, groundwater and floodwater. Mechanical composition is important for the reception, displacement, storage and use of soil humidity.

#### Natural regeneration

Abundant occurrence of young seedlings as a basic prerequisite for natural regeneration is a typical natural phenomenon. Generally, young seedlings occur in large numbers on new alluvium deposits along the river banks, as well as on the sediments in the riverbed (river islets) in the years when water levels and climatic conditions favour germination during and after the period of seed dispersal. The survival of young seedlings and the subsequent development of young stands, especially in the first years of their life, depend on the dynamics of water level. It often happens that young seedlings from earlier years die in floods and make a kind of reinforcement for the sedimentation of alluvial matter, creating conditions for the occurrence of a new generation of seedlings. Larger complexes of such stands are formed when the main stream is displaced: river islets and riparian alluvial deposits are linked together as the old riverbed fills up. Because stands are heterogeneous, plant associations making poplar and willow stands rarely cover coherent surfaces; rather, they occur as more or less large fragments within each other.

Parts of riparian forest areas proclaimed to be special-purpose forests are intended to ensure the conservation of the genetic resources of black and white poplar, as well as to enable scientific research and monitoring of the ecosystem succession in the floodplain of the river Drava.

The formation of river islets, the development of young willow and poplar germ (*malat*), natural erosion and the filling in of riverbeds and biotopes, as well as forest vegetation at various stages of development, is a rarity in Europe. All the stands in the studied area are well preserved and are not affected by large-scale desiccation. The natural structure of these stands under current conditions ranks them as being very rare and valuable in Europe (Vukelić *et al.* 1999).

Currently, natural stands contain species which were imported during the establishment of intensive poplar plantations. These are widely spread owing to a successful natural regeneration from seeds. The growth of these natural stands and the formation of vegetation in the entire area are closely connected with the water level dynamics.

Dynamic changes involving the disappearance (landslips) and formation (deposits) of the soil are a permanent feature. The mainstream is often displaced as parts of the old riverbed are being gradually filled in and new riverbeds are made. Such changes are especially intensive during high water levels and floods. In accordance with these constant changes, pedogenetic processes in alluvial soils are permanently in their initial phase. They do not display any truly formed horizons but only deposited layers. These soils often contain layers of pure sterile sand.

As a natural stand matures for felling, the soil frequently rises and the sand settles, resulting in a substantial change in site conditions compared to those prevailing at the time of the stand's formation. Such raised sand surfaces offer poor conditions for the occurrence of young seedlings in large coherent areas, which is a serious obstacle to normal natural regeneration (Sikora 1995). This point marks a critical moment during which a natural stand undergoing progressive development reverts to regression or succession to other forms of vegetation.

The difficulties manifested in the natural regeneration of these stands, as well as the poor quality of coppiced stands, are certainly among the reasons for a mass introduction of selected clonal material into natural stands after felling. The succession towards the more valuable and permanent forms of forest vegetation (common oak, ash) is marked by the occurrence of individual trees and groups in micro-locations with favourable conditions for their development. Natural succession is an evolutionary process in which barriers must be overcome. These barriers result from a very heterogeneous pedological soil pattern with distinct micro-relief and the occurrence of high water levels, which can happen at any time of the year.

Generally, periodicity in the occurrence of high water levels, to which flood area vegetation has been adapting through evolution, has been considerably disturbed by human operations intended to improve water flow. Riverbeds are transformed so that the flow of water is accelerated. However, this process reduces the formation of alluvial deposits and sites for new generations of young seedlings.

Another problem for the maintenance of natural poplar and willow stands is that before these newly formed areas are included into forest land water-management plans, companies exercise their legal right to use the materials to hand for the necessary river engineering operations. This exploitation of brushwood and thin sticks adversely affects future development of young stands. Although poplar and willow forests in the flood area constitute pioneer vegetation, the function of which is to ensure the conditions for the development of other, more stable vegetation forms, they are also permanently present there through their regeneration on new alluvial deposits formed by the meandering riverbed.

#### Gene conservation

Ex situ conservation of the European black poplar started in Croatia in 1993 with the
selection and vegetative propagation of old trees in the regions along the rivers Sava, Drava and Mura and the establishment of two clonal archives. A poplar and arborescent willow clonal archive (Salicetum) was established in 1995 covering 3 ha along the river Mura (Podturen) in the north of Croatia. It contains 83 clones of black poplar. Another clonal archive of black poplar, established in 1998 in Darda (the region of Baranja in eastern Croatia), already contains 76 clones (Kajba and Bogdan 2001).

For nursery reproduction, there are 37 black poplar clones from Croatia and Bosnia and Herzegovina. With the assistance of our colleagues from the Faculty of Forestry in Sarajevo, a total of 54 trees were selected in the area of Bosnia, and 50 trees were propagated vegetatively in our nurseries. The most valuable riparian forests with the most beautiful and oldest black poplar trees are found in the Danube basin. During the winter of 2001, the selection of trees was made impossible by high levels of floodwater. In 2002, nine selected trees from the river Danube were successfully cloned and are now in nursery reproduction.

Apart from this, in cooperation with colleagues from the Faculty of Forestry in Sarajevo, cuttings from 10 *Populus nigra* var. *caudina* trees (hairy black poplar) from Bosnia and Herzegovina were taken in the winter of 2003 and are now in nursery beds. During the spring of 2002, one-year-old cuttings were taken from 10 white poplar (*Populus alba*) trees and the cuttings from six trees have been successfully rooted.

Under the Nature Protection Act of the Republic of Croatia, floodplain forest ecosystems are protected and they are of particular importance for *in situ* conservation of black poplar. One such ecosystem is Kopački Rit Nature Park, which was included in the list of Wetlands of International Importance on the grounds of its outstanding importance and well-preserved wetland biotopes (Ramsar Convention 1993). This nature park, covering an area of 17 770 ha, is bounded by the rivers Danube and Drava, where the occasionally flooded terrain favours the development of diverse vegetation, such as mixed stands of black and white poplar with willows.

Any planting of hybrid poplars is banned in a protected natural area. For this reason, the selection of black poplars and nursery selection and reproduction have to be intensified.

According to the Nature Protection Act of the Republic of Croatia, parts of nature can be protected as special reserves of forest vegetation. Such reserves are the Vukovar Danube eyots, which cover 115 ha of very valuable poplar and willow stands. Moreover, 560 ha of natural mixed stands of black poplar, white poplar and white willow in the lowland forest in the lower stream of the river Drava (Donji Miholjac) were protected and excluded from regular forest management in 2003. Earlier, in 1996, an area of 705 ha of natural black poplar stands in the central stream of the river Drava (Slatina) was also protected and excluded from regular forest management. In all, 1265 ha supporting natural poplar stands are protected along the river Drava. Apart from this, there are also 6000 ha of commercial natural poplar forests in Croatia, while 83 ha of the forests are intended for special purposes (scientific or recreational).

Croatia has well-preserved floodplain forest ecosystem, primarily thanks to the sustainable management method and permanent legal protection. This is a significant biological and ecological asset of national and international importance.

#### References

- Kajba, D. and S. Bogdan. 2001: Očuvanje genetičkih izvora europske crne topole (*Populus nigra* L.). [Conservation of genetic resources of the European black poplar (*Populus nigra* L.)]. Pp. 69–76 in Znanost u potrajnom gospodarenju hrvatskim šumama (S. Matić et al. eds.), Šumarski fakultet Sveučilišta u Zagrebu and Šumarski Institut, Jastrebarsko, Zagreb, Croatia.
- Sikora, J. 1995. Potrajnost i prirodno obnavljanje šumske vegetacije poplavnog područja. [Sustainability and natural reforestation of forest vegetation in a flooded terrain]. Šum. list 9–10:323–328.

Vukelić, J., D. Baričević and Z. Perković. 1999. Vegetacijske i druge značajke zaštićenog dijela 'Slatinskih podravskih šuma'. [Vegetational and other characteristics of the protected part of 'Slatinske podravske šume']. Šum. list 7–8:287–299.

# Populus nigra and P. alba genetic resources in the Czech Republic

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The surface area of alluvial forest in the Czech Republic is about 33 000 ha (Vasicek 2000). Most of this area falls within production forests without any special conservation. Review of the genetic conservation of black poplar reveals an unfavourable situation, as a result of changed hydrological conditions with less flooding. Human activities have also altered riparian ecosystems, by lowering the groundwater table and replacing poplar stands by agricultural land. The present composition of tree species favours economically important deciduous species or faster growing hybrid poplars.

The native stands of black and white poplars have been reduced to several tens of populations in the Czech Republic. *Populus alba* and *P. nigra* occur as old individuals or small groups growing in the oldest vegetation units. Natural regeneration is insufficient, where it occurs at all, and in many cases it is completely absent.

In afforestation projects, mainly hybrid poplars are planted for timber production. Poplar wood is used in many ways in the Czech Republic— for roof frames and boxes, and primarily for plywood production. However, plywood manufacturers face a lack of high-quality large logs appropriate for processing, and import raw material from Slovakia, Hungary or Croatia. Also the foresters who manage the riparian forests have noticed that large old black and white poplar trees are slowly disappearing without any reproduction while the demand for poplar wood is increasing in the market.

Conservation of biodiversity in protected areas generally falls under a law dating from 1992. There is a ban on managing these areas in such a way that it affects biological diversity or the structure and function of the ecosystem. Another ordinance direct that forest regeneration must be carried out in a way which maintains forest biodiversity, resilience, production and regeneration capacity, which are vital to meet the economic, ecological and social functions both at present and in the future.

A national strategy for the conservation and sustainable use of biological diversity is being prepared according to the Convention on Biological Diversity (CBD).

#### Research and conservation efforts

Two institutions are engaged in gene conservation of *P. nigra* in the Czech Republic, independently of each other: Silva Tarouca Research Institute for Landscape and Ornamental Gardening (RILOG) and Forestry and Game Management Research Institute (FGMRI). The recent research on black poplar at RILOG has focused on the following topics:

- support for black poplar in the Litovelské Pomoraví Protected Landscape Area (PLA);
- assessment of genetic diversity in several black poplar populations by microsatellite analysis; and
- breeding of fast-growing clones.

The support programme for original black poplar in the Litovelské Pomoraví PLA started in cooperation with some other state organizations in 2000. In the spring of 2000, about 1000 2-year-old seedlings, arising from controlled crossings of selected trees from the PLA, were planted. These seedlings were planted in four places, three of which represented a typical inundated zone of the river Morava. In September 2001, a total of 700 plants survived.

The controlled crossings of trees originating from this area were continued in 2000 and 2001. These crossings resulted in 30 successful combinations including about 1500

individuals which were planted in 2002 and 2003.

Nine microsatellite loci developed for black poplar at CPRO–DLO Wageningen (van der Schoot *et al.* 2000) and one locus adopted from the Poplar Molecular Genetics Cooperative SSR Database (*http://poplar2.cfr.washington.edu/pmgc*) were used in the RILOG laboratory. These loci (WPMS03, WPMS04, WPMS05, WPMS07, WPMS09, WPMS11, WPMS16, WPMS18, WPMS20 and PMGC14) are highly polymorphic and the quality of patterns is very good.

At first, a population of 66 individuals from the central Morava river basin and 52 old trees from the same localities were analysed, together with 200 seedlings from crossing of 5 males and 5 females (8 crossing combinations with 25 seedlings in each combination). All parental trees are included in the EUFORGEN Database. The laboratory analyses have been performed successfully but the results have not been yet processed.

Furthermore, DNA samples have been collected from 30 very young seedlings established by natural regeneration in the Litovelské Pomoraví PLA. These individuals were found on the gravel bank of the river Morava in 2000. Microsatellite analyses for these seedlings were carried out in 2002 to compare genetic diversity within this population with other populations in the Czech Republic.

The black poplar gene pool from Czech and Moravian regions was used in a breeding programme for fast-growing woody plants under a short-rotation system to produce biomass as a renewable energy source. The aim is to obtain clones with high biomass production that could be used particularly in areas with management restrictions (e.g. PLA and nature reserves).

At FGMRI, the inventory of *Populus nigra* and *P. alba* stands was started in the late 1980s as a separate programme for the conservation and reproduction of the poplar gene pool, and finished about 5 years later. The most comprehensive inventory was made mainly in the upper reaches of the river Morava. All trees found were classified on the basis of their morphological traits. Individual trees of *P. nigra* were identified and added to the clone collection. Plus trees were identified from the oldest stands in which autochthonous trees are more likely to occur. A total of 145 *P. nigra* clones and 50 *P. alba* clones have been collected and are now maintained in clonal archives at the Uherske Hradiste research station. Moreover, another 70 clones of *P. alba* were registered and some flower branches were also taken for artificial crossings. Seed obtained from the crossings will be used to produce planting material for reintroduction.

In addition to other species, such as *Sorbus domestica, Sorbus torminalis, Pyrus pyraster, Malus sylvestris* and *Cerasus avium*, black and white poplars were included in a project for the preservation and reproduction of gene resources of marginal and endangered forest tree species, which ran between 1995 and 1999. Since 2000, identification of plus trees for the poplars has been carried out within a programme on the breeding of fast-growing tree species. The conservation of *P. nigra* and *P. alba* is included as a project in the research proposal for 2004–2008.

The clonal archives at the Uherske Hradiste research station are maintained as a resource of high-quality genetic material, especially for breeding projects and *ex situ* conservation. Tree improvement projects focus on *P. nigra*, *P. alba*, *P. deltoides*, *P. × euramericana*, *P. tremula*, and *P. tremuloides*. Several trial plots with new poplar hybrids of sections *Aigeiros* and *Leuce* with *P. tremula* and *P. tremuloides* were established recently. The evaluation of the previous aspen trial plots is also continuing. In cooperation with RILOG, the evaluation of *P. nigra* hybridization has been carried out and the first DNA analysis was done for selecting parents for further hybridization.

# **Research needs**

In the future, the main objectives are to collect more material for further DNA analyses to study and describe the structure of the poplar genetic diversity in the Czech Republic. For this reason, a complete inventory is necessary.

The general public, and often foresters too, are not fully aware of the problems in gene conservation of poplars and thus there is a need to increase awareness in this regard. *In situ* conservation is supported by natural regeneration in places where residual populations can contribute to rehabilitation of their surroundings. Several rehabilitation projects, including restoration of the original water regime in floodplain forest or sites, have already been implemented at the Forestry Enterprise of Zidlochovice in Southern Moravia. These efforts should be continued in areas which are not accessible for intensive forest management.

#### References

- van der Schoot, M., M. Pospiskova, B. Vosman and M.J.M. Smulders. 2000. Development and characterization of microsatellite markers in Black Poplar (*Populus nigra* L.). Theoret. Appl. Genet. 101:317–322.
- Vasicek, J. 2000. Progress and evaluation of alluvial forests revitalization performed by the forestry enterprise of Zidlochovice. Pp. 11–13 *in* Management of Floodplain Forests in Southern Moravia, Ministry of Agriculture, Czech Republic.

# Conservation of *Populus nigra* genetic resources in France, 2000–2003

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# Research

The EU-funded project EUROPOP ended in 2001 and the final symposium was held in Sekszard, Hungary. The French research activities carried out by INRA-Avignon focused on gene flow along an entire river system (Drôme) and a description of the genetic diversity within the country using different markers.

A survey of *Melampsora* rust races in the native *Populus nigra* populations is carried out by INRA-Nancy every year. An ecological study of the riparian populations, including some genetic aspects for *P. nigra*, was conducted as part of the EU-funded project FLOBAR by N. Barsoum and E. Muller (CNRS-Toulouse). They are currently studying the effect of hydrological disturbances along the Drôme and Garonne rivers on asexual or sexual regeneration of black poplar.

In the spring of 2003, a phenological survey of 95 sexual mature trees was carried out along a 2.3-km strip in a natural reserve near Orleans (INRA-Orleans). The idea is to evaluate genetic diversity via easily observable characters, such as sex, floral phenology and bud burst. Correlation of such phenotypic diversity with genetic variation, measured using molecular markers, will be analysed. Vegetative propagation and possible introgression (with *P. nigra* var. italica and/or *P. × euramericana* hybrids) will also be checked. Preliminary results were summarized in a poster displayed at a scientific meeting on the conservation of biodiversity, held in Lyon in April 2003. This phenological survey will be extended to 11 other natural reserves involved in the *in situ* conservation of *P. nigra*.

# Ex situ conservation

A total of 47 new clones were collected in south-eastern France and they are currently being multiplied (not yet included in the database). A duplication of the EUFORGEN *P. nigra* core collection is now maintained together with our national collection. Copies of the 120 French genotypes in the EUROPOP collection were sent to Hungary in March/April 2003. Due to some problems at the customs, a similar transfer will be made again in winter 2003/2004.

#### In situ conservation

A group of 12 nature reserve managers, involved in the *in situ* conservation of *P. nigra*, met and expressed a strong need for a French version of the EUFORGEN technical bulletin. The group also discussed the recommendations of the EUROPOP project.

# **Public awareness**

Each year, there is a three-day 'Sciences en fête' period, during which schools and the general public are invited to visit research laboratories. Genetic conservation of *P. nigra* was presented in October 2001 in the nature reserve of Les Ramières, and this presentation was quite successful. The EUFORGEN *P. nigra* Network visited the same site in 2000. Local people were interested to see that the native poplars, which are common in their own area, are rare everywhere else in Europe and that there is a European concern for this species.

In October 2002, a video presentation was shown at the fourth National Symposium on diversity of genetic resources in La Chatre. The meeting was organized by Bureau des

Ressources Génétiques (BRG) and at the 'Sciences en fête' at the University of Orleans. An English version of a booklet presenting the national activities on the conservation of forest genetic resource was produced and disseminated to EUFORGEN contacts.

#### Publications

- Guilloy-Froget, H. 2002. Evaluation des conditions favorables à l'etablissement de *Populus nigra* et *Salix alba* en milieu riverain. Diplôme de Doctorat, Université de Toulouse. 140 pp.
- Barsoum, N. 2002. Relative contributions of sexual and asexual regeneration strategies in *Populus nigra* and *Salix alba* during the first years of establishment on a braided gravel bed river. *Evol. Ecol.* 15:255–279.
- Guilloy-Froget, H., E. Muller, N. Barsoum and F.M.R.Hughes. 2002. Dispersal, germination and survival of *Populus nigra* L. (Salicaceae) in changing hydrologic conditions. *Wetlands* 22(3):478–488.
- Imbert E. and F. Lefevre 2003. Dispersal and gene flow of *Populus nigra* (Salicaceae) along a dynamic river system. J. Ecol. 91:447–456

# Progress in gene conservation of black poplar (*Populus nigra* L.) in Germany

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# Field inventories

The search for specimens of pure black poplar (*Populus nigra* L.) continued, and around 1100 additional trees were identified as pure specimens by morphological and biochemical means. Of the trees identified earlier, some had died or been removed. The total number of black poplar trees in Germany is estimated to be about 5000. Most of the trees are located along the rivers Elbe, Rhine, Oder, Danube, and their tributaries. This species is highly endangered in Germany, especially since there is almost no natural regeneration as a result of the intensive use of rivers for shipping, which has caused damming of the larger rivers since the time of early industrialization.

# Policy and legislation

There are no specific laws to protect *P. nigra*. However, the European Directive 1999/105/EC on the marketing of forest reproductive material was implemented in Germany in 2003 through the Act on Forest Reproductive Material (Forstvermehrungsgutgesetz, FoVG) and three national regulations. The conservation of genetic resources is now explicitly stated as one of the objectives of the law.

# Research

A study by Manfred Weidner at the University of Cologne investigated genetic structures of the remnant black poplar individuals found in Nordrhein-Westfalia along the river Rhine and its tributaries. He found that many of the identified trees belong to vegetatively propagated groups of trees. A total of 464 black poplar individuals were identified and the number of clones (genotypes) was 253 (van Schyndel 2001; Hellenbrand 2001)., This means that almost every second tree is statistically a vegetative propagule of another one, so the number of black poplar genotypes in Germany is estimated to be only about 2700.

Ralf Kaetzel at the Forestry Research Centre of Brandenburg in Eberswalde is presently carrying out isozyme investigations on the genetic structure of the largest black poplar population in the federal Land of Brandenburg.

Two German institutions have participated in the EU-funded EUROPOP project. Research is being funded in the context of the restoration of riparian ecosystems. Work on identification of *P. alba* was also carried out.

# Practical implementation

- There are numerous *in situ* conservation activities: a major one on the river Elbe in Saxony Anhalt where a long stretch of riparian forest is being re-established in a joint effort of several state and non-governmental institutions, including the WWF.
- *Ex situ* conservation measures: extensive stool beds for black poplar have been established at seven locations along the Rhine (Arnsberg), the Rhine and Weser (Hannoversch-Münden Hesse), the Elbe (Flechtingen) and the Oder (Eberswalde).
- Rehabilitation measures for different rivers are planned after the heavy floods of recent years, especially along about 60 km of the central part of the Elbe in Saxony-Anhalt.

# Coordination at the national level

A permanent federal and state working group 'Conservation of Forest Genetic Resources and Forest Seed Law' has been established and it meets twice every year to coordinate actions at the national level.

# **Public awareness**

Although fiscal problems in Germany have caused cutbacks in some good conservation projects, public awareness is generally high as far as general nature protection is concerned. Also, there is much concern about the protection of riparian forests and their value in preventing or at least reducing floods since several heavy floods have occurred along the Rhine, Elbe and Oder rivers in recent years. However, the public is not aware of the serious threat of extinction of *P. nigra* in Germany.

#### Publications

- Hoffmann, H. 1999 Morphologische Beschreibung von Baumschulpflanzen zur Artbestimmung von Populus nigra L. – Vortrag [Description of morphological characters of nursery plants for species identification of Populus nigra L. Symposium paper.] Die Holzzucht 52(5):6–7.
- Hofmann, M. 1999 Die Bedeutung von Populus nigra als Genressource für den praktischen Pappelanbau. Vortrag. [The importance of *Populus nigra* as a genetic resource in practical cultivation of poplars. Symposium paper] Die Holzzucht 52(5):17–18.
- Joachim, H.F. 1999 Verbreitung, Artbestimmung und Inventur von Reliktvorkommen der Schwarzpappel—*Populus nigra* L.—im Bereich von Oder und Elbe. Vortrag. [Distribution, species identification and inventory of relics of black poplar—*Populus nigra* L.—along Oder and Elbe river. Symposium paper] Die Holzzucht 52(5):7–9.
- Joachim, H.F. 2000. Die natürliche Verbreitung der Schwarzpappel (*Populus nigra* L.) im Bereich von Oder und Elbe [The natural distribution of *Populus nigra* L. between Oder and Elbe] Beiträge für Forstwirtschaft und Landschaftsökologie 34(4):145–148.
- Joachim, H.F. 2000 Die Schwarzpappel (*Populus nigra* L.) in Brandenburg [Black poplar in Brandenburg]. Schriftenreihe des Ministeriums für Landwirtschaft, Umweltschutz und Raumordnung/Eberswalder Forstliche Schriftenreihe, Eberswalde, Germany. 66 pp.
- Joachim, H.F. and K.D. Gandert (eds). 2001. Zur Schwarzpappel (*Populus nigra* L.)—Hinweise zur Kenntnis und zum Erhalt der bedrohten Baumart [Regarding Black Poplar (Populus nigra L.)—remarks on the recognition and conservation of this endangered tree species]. Pp. 38–48 *in* Beiträge zur Gehölzkunde, Gartenbild Hansmann, Rinteln, Germany.
- Kausch-Blecken von Schmeling, W., H.P. Schmitt, W. Maurer *et al.* 2002. Seltene Bäume in unseren Wäldern. Erkennen—Erhalten—Nutzen[Rare tree species in our forests. Identification—Conservation—Utilization] Stiftung Wald in Not 13, Bonn, Germany. 37 pp.
- Volk, H. 1999. Sanierung und Wiederaufbau von Auewäldern—Waldbäche in den Mittelgebirgen und Auewälder am Oberrhein als Beispiele. Vortrag [Restoration and rehabilitation of riparian forests forest streams in the lower mountain ranges and riparian forests on the Upper Rhine as examples. Symposium paper] Die Holzzucht 52(5):10–13.
- Weisgerber, H. 1999 Vorkommen, Wuchsverhalten und Gefährdungen der Schwarzpappel *Populus nigra* L. Vortrag [Range, growth characteristic and endangerment of the black poplar *Populus nigra* L. Symposium paper]. Die Holzzucht 52(5): 1–5

#### References

Hellenbrand, B. 2001. Vergleichende gelelektrophoretische Analysen der Isoenzymmuster mutmaßlich autochthoner Schwarzpappelbestände in Westfalen zwecks Untersuchung der Reinrassigkeit und Klonabgrenzung bei *Populus nigra* L. Masters' thesis, University of Köln.

van Schyndel, P. 2001. Untersuchungen zur Reinrassigkeit und Klonabgrenzung bei der Schwarzpappel.

Vergleichende gelelektrophoretische Analysen der Isoenzymmuster mutmaßlich autochthoner *Populus nigra* L. Bestände im Rheinland und im Münsterland. [Investigation on species purity and clonal identity of black poplar. Comparative gelelectrophoretic analyses of isoenzymatic patterns of putatively autochthonous *Populus nigra* stands in Rhineland and Muensterland]. Master's Thesis, University of Köln. 188 pp.

# Progress in gene conservation of black poplar (*Populus nigra* L.) in Hungary

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*Populus nigra, P. alba, P. × canescens* and *P. tremula* are native poplar species in Hungary. The first three species are found in favourable site conditions in the lowlands where there were formerly rich above-ground water sources and large floodplains of unregulated rivers. Conservation of the genepools of native poplars is a very important task for Hungarian poplar growers and scientists, requiring information on the natural distribution of populations of *P. alba* and *P. nigra*. This update covers the period between November 2001 and May 2003.

# Field inventories

Between 2001 and 2003, the Fund for Environmental Protection financed an active gene preservation programme which provided *ex situ* preservation for 144 genotypes and tested 96 genotypes by DNA analysis. A total of 758 genotypes are available, of which 614 are DNA-tested.

Because of limited financial resources, the work of collecting and archiving had to be done first. The description of genotypes is still mainly in the form of handwritten sheets, but all data will be transferred into a computerized database during 2003.

# Production of reproductive material

Law N° LIV 1996 on nature conservation requires that forest owners must use indigenous species for reforestation in the protected areas of floodplains. Therefore, the demand for both generative and vegetative propagated material is increasing steadily.

	1998/1999	1999/2000	2000/2001	2001/2002	2002/2003
P. nigra					
Generative propagating material	710	905	340	402	660
(seedlings)					
Vegetative propagating material	14	66	95	88	81
(rooted cuttings)					
P. alba					
Generative propagating material	8 655	6 544	8 064	6 592	11 932
(seedlings)					
Vegetative propagating material	103	46	65	79	76
(rooted cuttings, 'Villafranca')					

Fable 1. Production of P	. nigra and P.	alba planting	materials	(thousands)
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# **EUROPOP** population collection

The idea of preserving the population samples collected during the EUROPOP project was developed at the seventh Network meeting. The population samples cover most of Europe, providing a basis for provenance trials. Hungary undertook the responsibility of preserving the collections. Project partners sent the material in the form of cuttings, which were then rooted to establish stool beds to provide planting stock. The collections maintained in this form provide the possibility of raising experimental planting material for the establishment of provenance trials. Hardwood cuttings were received from Spain and the Netherlands

during spring 2002, and from France, Germany and Italy in 2003 (Table 2).

Country	Year	Number of received	Rooted	Proportion of
		genotypes (pieces)	genotypes	rooted genotypes
			(pieces)	(%)
France	2003	128	90	70
Germany	2003	39	30	77
Italy	2003	85	85	100
Spain	2002	119	103	95
The Netherlands	2002	134	127	95
Total		505	435	

Table 2. Summary c	f the EUROPOP	population	collection
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The results of the rooting and establishment of Spanish, Dutch and Italian cuttings were good, meaning in practice that at least one cutting rooted per genotype. The German material arrived unpacked and thus its establishment can be expected to be poor. The French cuttings were held up for 40 days in a customs warehouse for unknown administrative reasons, so their rooting and establishment doubtful and the samples will probably need to be sent again. The other EUROPOP participants have not yet sent material.

So far the preservation work has been financed from a national fund, which will be either terminated or greatly modified with Hungary's EU membership (accession May 2004). Material which will be sent in spring 2004 will be archived but a funding source needs to be found for further processing.

# Progress on national activities on gene conservation of black poplar (*Populus nigra* L.) and white poplar (*Populus alba* L.) in Italy

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European black poplar (*Populus nigra* L.) is probably the most typical tree species of the ancient floodplain ecosystems of Italy. Even though this species is not of any direct commercial interest, it has always been important for its environmental role and for breeding purposes. The remarkable reproductive ability of black poplar, by means of cuttings, has always conditioned the development of poplar cultivation and breeding programmes in Italy. Since the beginning of poplar cultivation, poplar growers often selected empirical genotypes among the best spontaneous individuals found along the riverbanks in the Po valley with great success. During the early decades of the twentieth century, *P. nigra* was one of the most used native tree species. Subsequently, natural hybrids with *P. deltoides* or intermediate forms, which are not quite clear and are probably produced by spontaneous backcrossing, were cultivated.

Black poplar is one of the most important parents for the production of poplar hybrids because of its adaptability to different environments and soil, its excellent rooting capacity from stem cuttings and its resistance to some of the most important diseases such as bacterial canker, brown spots and, above all, viruses. In the early 1980s, the ISP started long-term breeding programmes using the best *P. deltoides* females in open pollination or controlled crossing with a large number of native *P. nigra*.

During the past 10 years, black poplar has acquired considerable importance for reasons other than timber production. This is due to growing environmental awareness, a greater emphasis on restoration of degraded areas and the need to re-establish natural forests. Black poplar, together with willows and white poplar, is among the best pioneer species for this purpose.

In Italy, the distribution of black poplar is sparse and the scattered trees are often of inferior quality. With expanding agricultural activities, the species' habitats have been reduced drastically and its genetic resources have gradually been eroded. Between 1981 and 1983, the ISP carried out surveys all over Italy to locate spontaneous black poplar populations along rivers. Currently, the germplasm collection in Italy is one of the largest in Europe and includes over 700 genotypes from 26 countries. Most of this material is characterized on the basis of morphologic and genetic traits.

The ISP has always participated actively in the Network activities. A European database on black poplar was proposed by the Institute and was unanimously accepted by all Network members. The Institute created the database and is now responsible for its maintenance. Furthermore, the Institute is also responsible for the maintenance of the EUFORGEN *P. nigra* core collection.

This report covers the period between November 2001 and May 2003.

#### Field inventories

- Inventories of line plantations were carried out using black and white digital aerial photographs with 1-m pixel size in the plains of Piedmont and Emilia Romagna. The results showed scattered shrubs and trees in rows (11 m/ha) which is lower than the ideal value to ensure the constant presence of avifauna (70 m/ha).
- The effects of floods in 1994 on agricultural fields and forests were investigated in areas

adjacent to the river Po for about 60 km in Piedmont. The results show that poplar stands and natural forests reduce soil erosion more effectively than agricultural crops. The same conclusions were confirmed after analysing the effects of floods that occurred in 2000.

- Inventories of poplar plantations and natural forests in the plains of Lombardy are being carried out using digital aerial photography. The results of these inventories, together with those acquired in Piedmont and Emilia Romagna, can be used for germplasm collection.
- Techniques for the interpretation of digital colour photographs on a PC monitor were tried out for the classification of single plants or small groups of *P. alba*, with encouraging results. Satellite images were also used to study the spectral signature, to test semi-automatic classification techniques. The early results are very promising.

# Policy and legislation

The Institutional Committee of the river Po has adopted a Hydrogeological Settlement Plan. This plan was implemented after feedback was received from interested stakeholders. The Po, which is the longest and most important river in Italy (652 km long, with 141 tributaries), is now considered as an integral unit; what happens upstream has impacts further down. An organization set up to administer water resources will cooperate with another organization responsible for the planning of territories. The Po flows through seven regions and drains a basin of 70 000 km<sup>2</sup> (58% hills or mountains and 42% plains). The agricultural surface covers over 400 000 ha and the population residing at this territory is about 16 million.

The Hydrogeological Settlement Plan is an important and strategic instrument, which will eventually form the basis for a new territorial administration and will respond to the current security demands and the environmental needs of the largest hydrographical basin in Italy. This could serve as a model to develop plans for other watersheds. The core of the plan is environmental restoration, expressed as a number of operations capable of restoring environmental characteristics or ecological functions, which can be implemented in the mainly state-owned river banks. Effective education at all levels (regional executives, managers of natural parks, employees of the interested public offices) is necessary to ensure participation of different interest groups and to introduce new procedures for implementing the new policy.

The Italian Centre for River Restoration (CIRF) could play an important role in this field. It is a recently established non-profit organization, with links to the European Centre for River Restoration (ECRR). The main objective of this organization is to analyse and disseminate information on river restoration and to set up practical projects for the restoration and protection of watercourses.

After systematic and extensive elimination of vegetation on the river banks, which lasted for several decades and impoverished the habitats, the new management efforts are beginning to bring back arboreal and shrubby vegetation along the rivers. A few pilot projects have been carried out on a small scale. The ISP has recently started collaboration with the CIRF and this will create opportunities to re-establish black poplar forests in fluvial areas.

Among the activities scheduled by the Rural Development Projects, following the recent EC agricultural policies (EC regulation 1257/99), many regions continue to promote afforestation of agricultural land. In order to reduce the surplus of crop production, financial support will be given for establishment of tree plantations during the period 2000–2006. These steps have been taken to diversify the economic activities of farms and to reduce soil erosion and land degradation. Farmers often favour native tree species, especially *Salicaceae*, while establishing pure plantations (white poplar) and mixed plantations (black poplar,

white poplar and other broad-leaved trees and shrubs).

After a period of experimentation aimed at seeking cultural models and more suitable species, the regions of Lombardia, Veneto and Friuli Venezia Giulia started to finance coppice plantations for short-rotation forestry. In a few years' time, wood production for energy could mark the beginning of a new type of agro-industrial production line and might occupy hundreds of hectares, especially in areas that benefit from EC subsidies (e.g. buffer strips, ecological interconnection network, controlled flooding of rivers, carbon sink plantations).

The re-establishment of forests in lowland areas is currently a priority in Italy, and both white and black poplars can play a major role in meeting this goal. Many provinces and local state-owned institutions are preparing afforestation projects: the province of Lombardia, for example, has already approved a project to create new lowland forests over the next 10 years.

The problem of environmental certification of poplar plantations was discussed during a pilot project coordinated by the ISP. The proper management of poplar stands can be achieved by adopting management systems such as ISO 14 000 and EMAS or forest certification schemes such as the Forest Stewardship Council (FSC) and Pan European Forest Certification (PEFC). The main objectives of these two certification systems include the protection of biodiversity and the partial conversion of agricultural areas of certified farms into natural areas. Forest certification can bring positive outcomes for nature protection and help to create new forest areas not only along rivers but also in typical agricultural areas.

The requirements of the directive on forest reproductive material (99/105/EC) will be included in a Legislative Decree and this will replace the 269/73 Law, which has regulated production and trading of this material for the last 30 years. The utilization of native species and local provenances will be favoured in reforestation efforts to minimize the use of material coming from outside local areas. The number of regulated species will also increase from 13 to 46, including the *Populus* genus as a whole.

#### Research

The Department of Forest Resources and Environment (University of Tuscia, Viterbo *http://www.unitus.it/dipartimenti/disafri/disafri.html*) is involved in a new EC project 'Integrated European Scientific Infrastructure for global change studies on forest and agro-forest ecosystems utilizing the FACE technology' (EUROFACE). This research will provide more information on the response of the ecosystem to increasing atmospheric concentrations of carbon dioxoide. Atmospheric carbon dioxide enrichment experiments will be carried out on poplar plantations (*P.* × *canadensis, P. alba* and *P. nigra*) in central Italy.

Investigations on the genetic diversity of two *P. nigra* populations growing along the river Ticino in northern Italy (morphological characteristics and molecular analysis) were concluded by the Biology Department of the University of Milan within the EUROPOP project. Four marker systems—isoenzymes, microsatellites, amplified fragment length polymorphisms (AFLPs) and cleaved amplified polymorphic sequences (CAPS)—gave similar results and revealed a high level of gene flow between the two populations, as a very low genetic distance between them was detected.

Breeding and selection activities continued at the ISP. In two locations in northern and central Italy, 25 *P. nigra* clones are being observed. These clones are considered to have good adaptation to environmental conditions and resistance to bark necrosis. These clones were selected from among progenies of *P. nigra* females (a polycross test of 39 females crossed with pollen mix from 15 males) for their growth, number of branches, straight stem and rust tolerance. A pool of 20 Italian wild genotypes has also been selected from three experimental

stands of *P. nigra* planted in the Po basin. These genotypes will be proposed for registration in the National Register of Forest Clones.

The ISP, in collaboration with the University of Milan, the University of Pavia and the National Institute of Forestry of Beijing, is involved in a project on the biodiversity of natural and cultivated poplars, funded by the private Bussolera-Branca foundation. Inventories and characterization of *P. nigra*, *P. alba* and *P. canescens* populations (river Ticino), fingerprinting, establishment of genebanks and reforestation activities in degraded areas are being carried out within the project. Several other projects are being developed for regional or ministerial funding on topics such as monitoring of fluvial environments for germplam collection, morphologic and molecular characteristics of *P. nigra* and *P. alba* genotypes, and dynamic *ex situ* conservation. A study on introgression of genes from *P. deltoids* and *P. nigra* var. *italica* into *P. nigra* is also planned.

A pilot project will be conducted on the restoration, using *Salicaceae*, of urban areas polluted by solid waste material. This project, to be developed by the University of Pavia together with the ISP, will evaluate the absorbing capacity and the accumulation of toxic metals by *P. nigra*, *P. alba* and *Salix alba*. The ISP will also collaborate in a second phytoremediation project, proposed by the Universities of Pavia, Milan, Bologna and Alessandria to the Ministry of Agriculture. In this project, transgenic black and white poplar plants will be produced to test their ability to clean polluted areas of heavy metals (especially zinc and copper).

Reliable data on the fate of alien genes once released into the environment is of crucial importance for objective risk/benefit analyses prior to commercial application of genetically modified (GM) poplars. The possible flow of alien genes from cultivated clones to naturally occurring interfertile relatives will be investigated in a MERAGEN project, which has been proposed to the EC for funding (Fifth Framework Programme). The reproductive biology of *P. nigra* and *P. alba* will be studied to obtain data for reproduction modelling. Factors affecting the time trees reach maturity (density, fertilization, water stress, cultivation system) and flowering phenology in mixed stands (length, overlapping among individuals) will be studied. The development of large-scale operational methods for tracing effective pollen movements is also planned, together with seed dispersal studies in natural sites. Risk assessment studies will be carried out using transgenic lines of *P. alba* 'Villafranca' and *P. nigra* 'Jean Pourtet' as these cultivars are interfertile with native species and with exotic or interspecific hybrids. Methods for the detection of alien DNA in wood will be developed as well.

#### **Practical implementation**

The River Po Fluvial Park, in collaboration with the ISP, has begun some relevant rehabilitation trials at degraded sites in the Po basin. In too many cases, gravel pits are abandoned in a highly disturbed condition, with little or no rehabilitation treatment. The objectives of post-mining land use were selected on the basis of factors such as compatibility with other surrounding uses, community expectations, biodiversity protection and regulatory requirements. Gravel pit rehabilitation should not be a process of burying wastes, smoothing out the landscape and applying a green mantle of valueless vegetation. Early attempts at land rehabilitation were started in two gravel pits of about 80 ha (Casale Monferrato and Isola San Antonio), and equal shares of the area are designated for lakes, wetlands and mixed forests. Poplars and willows will be utilized as early successional trees. However, the implementation will probably not start until 2006.

A specific river restoration project was carried out recently in a typical floodplain area of the River Po Natural Park. This area (70 ha) regularly suffers from partial or total submersion

due to flooding of river Po. The surrounding areas are under intense agricultural pressure and a number of poplar stands are established, with hybrid poplar clones together with rice fields. The main aim of this pilot project was to restore a floodplain forest using environmentally friendly methods and selected wild poplar genotypes, shrubs and other broadleaves. Native male black poplar trees were planted in this site in close proximity to native females in order to re-establish a dynamic system of gene conservation and adaptation. Different types of planting methods and cultivation techniques were tested to improve diversity of flora and fauna on the topsoil in an area of 27 ha. The ISP and the Fluvial Park of River Po are involved in this project.

Another interesting case study on environmental restoration and tourism started in spring 2003. This project has multiple objectives (flood protection, land use change, nature conservation, and promotion of tourism) and will be implemented in a publicly owned floodplain (40 ha) included in the River Po Fluvial Park, which is situated in a quite densely populated area. Funds were obtained from the regional government. Some public institutions are also involved, e.g. the municipality of Valenza, the Fluvial Park Authority and the ISP. The project area will be completely reorganized as a natural environment, creating meadows, scattered trees or patches of trees and shrubs, hedges and rows of plants which are typical of floodplain environments. The forests will consist of broadleaves and native poplars. The area will also be laid out for tourists (picnic areas, grounds equipped for physical activities, cycle tracks, rustic roads with areas dedicated for the observation of wildlife, and informative noticeboards). The accomplishment of this project will create an opportunity for promoting black poplar conservation and for raising public awareness about EUFORGEN activities.

The ISP has also supplied appropriate planting stock of native poplars for other rehabilitation sites along the river Po and encouraged investigations on the best options for propagating known genotypes from clone banks.

# Ex situ conservation measures

#### Clone banks hosted at the University of Tuscia

A number of half-sib *P. nigra* and *P. alba* families from the most representative Italian river basins are maintained at the Department of Forest Resources and Environment (Viterbo). Studies on genetic variability among and within populations for rooting ability, phenology, pests and diseases resistance, morphological traits and plant growth rate are being carried out.

#### Clone banks hosted at the ISP

The *P. nigra* and *P. alba* genotypes maintained in the archives or stool beds and damaged by floods in October 2000 were propagated again. Cuttings of hundreds of *P. nigra* genotypes (destroyed or unidentified, as they were covered under up to 1 m of sand and gravel) need to be collected again from adult trees, but at the moment the human resources for this at the ISP are quite limited. Since there is a growing interest in native black poplars, the feasibility of duplicating the clone banks in other locations is being investigated.

With regard to the phytosanitary condition of clone banks, it is worth reporting the heavy damage caused by poplar leaf beetle (*Melasoma populi*) and leafhopper (*Empoasca decedens*) during the past two years.

At the previous Network meeting, the need to establish an international provenance trial with the EUROPOP material was stressed. Therefore, about 85 *P. nigra* genotypes, representing two natural populations growing along the river Ticino in northern Italy, were sent to Hungary which offered to maintain and propagate the population collection.

# The EUFORGEN Populus nigra core collection

The Network's core collection, hosted by the ISP, is complete (Figure 1). So far 20 countries have sent two *P. nigra* genotypes for the collection (Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Switzerland, Serbia and Montenegro, Turkey, United Kingdom and Ukraine).



Figure 1. Distribution area of Populus nigra and countries represented in the EUFORGEN core collection.

The availability of cuttings is good for most genotypes, including the 15 reference clones. In order to supply material for all genotypes, the replacement of some clones needs to be considered (SIA PASTRIZ 1, SEEFAR PAZARDIK N1 and KAE N.90.013 in particular). The countries of origin have been asked several times to re-supply new material, but the quantity and the quality of cuttings, after propagating them for years, is still very low. The clones are not adapted to the pedological and the environmental condition in other countries. Because of this, incomplete duplicates of the core collection are currently maintained in seven countries (Table 1): Austria (Federal office and Research Centre for Forests—FBVA), Belgium (Institute of Forestry and Game Management-IBW), France (National Institute for the Agronomic Research-INRA), the Netherlands (ALTERRA-Green World Research), Portugal (Escola Superior of Agrária de Coimbra), Spain (Servicio de Investigación Agroalimentaria-SIA DGA), Turkey (Poplar Research Institute-KAE) and Ukraine (Ukrainian Research Institute of Forestry and Forest Melioration-URIFFM). Recently, in Ukraine and Belgium (Flanders), most of the core collection genotypes have been utilized in poplar stands together with other native clones in order to observe the phenological differences and their adaptation to pedoclimatic conditions in different sites.

The ISP was requested to supply the INRA forest pathology laboratory at Nancy, France with *P. nigra* cuttings (core collection clones and other genotypes within the natural range of the species) to investigate if *Melampsora larici-populina* is locally adapted to *P. nigra*, which is its natural host. Cross-inoculation experiments, extending to all geographical origins of the species and the rust isolates, will be carried out.

Table 1.	The c	urrent	state	of the	e Popi	ulus nigra	EUF	ORGE	EN co	ore collecti	ion:	availat	bility	y of
cuttings	at the	ISP (	© =	good,	☺ =	medium,	⊗ =	low)	and	presence	of	clones	in	the
duplicatio	ons hos	sted in	other	· count	ries (v	()								

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Cione name/number	of origin	Countries hosting the core collection								
	orongin	ITA	NLD	BEL	FRA	TUR	ESP	UKR	PRT	AUT
FARCF LH HL 35	AUT	0		$\checkmark$			$\checkmark$		✓	✓
FARCF LH HL 55	AUT	8	$\checkmark$				$\checkmark$		$\checkmark$	$\checkmark$
IBW N004	BEL	$\odot$	$\checkmark$							
IBW N009	BEL	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
SEEFAR PAZARDZIK N1	BGR		$\checkmark$							
SEEFAR SVICHTOV N2	BGR	$\odot$	$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
ETHZ BIEL	CHE	$\odot$								
ETHZ AARGAU	CHE	$\odot$								
VULHM 88044	CZE	8	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$		
VULHM 88045	CZE	$\odot$	$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
FBS 215/63 JUGENHEIM	DEU	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
FBS 87/65 OFFENBURG	DEU	$\odot$	$\checkmark$							
SIA-DGA LUC2	ESP	$\otimes$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$
SIA-DGA PA1	ESP		$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$		
FRA 71017–401	FRA	$\odot$	$\checkmark$							
FRA 92510–1	FRA	$\odot$	$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
FCRA HUNTINGDON	GBR	$\odot$	$\checkmark$							
FCRA HOBSONS CONDUIT	GBR	٢	✓	✓	✓	✓	✓	~	~	✓
FF V336	HRV	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
FF V408	HRV	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
ERTI 33–3–1	HUN	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
ERTI 33–3–2	HUN	$\odot$	$\checkmark$			$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
ISP N068	ITA	$\odot$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
ISP N347	ITA	$\odot$					$\checkmark$		$\checkmark$	$\checkmark$
ALTERRA 1238	NLD	$\odot$	$\checkmark$							
ALTERRA 1792	NLD	$\odot$	$\checkmark$							
POL TORUN B	POL	$\odot$	$\checkmark$							
POL KORNIK	POL	$\odot$			$\checkmark$		$\checkmark$		$\checkmark$	$\checkmark$
EFN 1	PRT	$\odot$							$\checkmark$	$\checkmark$
ICAS 5	ROM	$\odot$	$\checkmark$		$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$	$\checkmark$
ICAS 6	ROM	$\odot$	$\checkmark$				$\checkmark$		$\checkmark$	$\checkmark$
LVU BAKA	SVK	$\odot$	$\checkmark$							
LVU IVACHNOVA	SVK	$\odot$	$\checkmark$							
KAE N.90.145	TUR	$\otimes$		$\checkmark$		$\checkmark$			$\checkmark$	$\checkmark$
KAE N.90.013	TUR	$\odot$	$\checkmark$		$\checkmark$	$\checkmark$				
URIFFM HRADIZKY	UKR	$\odot$					$\checkmark$		$\checkmark$	$\checkmark$
URIFFM KELIBERDYNSKY	UKR	٢		~			✓		✓	✓
IZT NS002	YUG	$\odot$	$\checkmark$							
IZT NS001	YUG	0	✓	✓	$\checkmark$	✓	$\checkmark$	$\checkmark$	✓	$\checkmark$

The EUFORGEN core collection will be utilized as a reference to characterize the species for studies on gene flow between exotic and local poplars in Canada (University of Laval). Françoise Lefevre (INRA, Avignon) was contacted and he proposed to send DNA samples of the collection's subset (30 clones) utilized for the EUROPOP project.

The passport data of the core collection clones is currently uploaded to the European *P. nigra* database. However, some relevant information is still missing for individual clones.

The database will be integrated with datasets on molecular, morphological and biological characterization (according to the EUROPOP project results, resistance/susceptibility to pests and diseases recorded at the ISP) and on the exchange of material activity.

The ISP provided two Italian native clones to Hungary and Germany as a contribution to the establishment of *P. alba* core collection.

### The EUFORGEN Populus nigra database

There have been no major changes in the database since the last update. A total of 13 countries have given information about their national *P. nigra* collections. An additional seven countries are included in the database because of the two genotypes included in the Network's core collection. The European database contains 3105 entries from 28 different countries (as at May 2003), a 6% increase since the last update. About 100 accessions are duplications of clones. The Turkish and Italian national collections have recently been updated, and the Spanish, Czech and Belgian genebanks are currently being updated. The number of accessions maintained in the Belgian collection will be reduced after revision work carried out using molecular markers. The Turkish and the French collections are the most representative one based on the number of accessions, while the Italian collection is the most representative one based on geographic origin of the genotypes (clones from 26 countries are represented).

The *Populus nigra* EUFORGEN database is now available from the ISP Web site (*http://kompass.italycom.net/isp/nigranet.php* or *http://populus.it*) with links from the EUFORGEN Web site (*http://www.euforgen.org*). The ISP continues to be responsible for the database management, implementation and supervision but each country can update the data for its national collection directly online at any time (a provisional password for each country has been issued for this purpose). Only the database manager is allowed to modify the data concerning the country details (contacts, addresses and institution codes) and any changes in these should be mailed to the ISP as usual. The passport data and the general rules have been revised. As it was agreed that the format of the *P. alba* database will be regularized with the *P. nigra* one, this solution could be used for the *P. alba* European database as well (Spain will be involved in its management and supervision).

# Coordination at the national level

The Italian representatives of different EUFORGEN Networks met with the Italian EUFORGEN National Coordinator in order to discuss the proposal of the *P. nigra* Network for a new approach to the conservation of forest genetic resources throughout the distribution ranges of the species (common action plans). The proposal was considered as an important contribution to EUFORGEN's mission in the coming years and the issue of defining actual actions in line with this goal was discussed. A series of activities, such as inventories of genetic resources, studies on micro-geographic distribution, genetic control of adaptive traits, was proposed.

# **Public awareness**

Key local partners and public officials interested in management and protection of the species are kept informed about the EUFORGEN programme and the Network activities by offering copies of leaflets and of the guidelines for *in situ* conservation of *P. nigra*, encouraging commercial nurseries to supply appropriate planting material. The nurseries have also been involved in identification of suitable sites for creating new floodplain forest with native black and white poplars.

An international conference on trees and forests in lowland areas will be held in Milan in

October 2003. As the economic significance of services and functions carried out by forest is not always sufficiently acknowledged, although the awareness of their importance is growing, the main functions of trees and forests in intensively used farmland will be highlighted. The ISP will attend the conference, and EUFORGEN activities and a case study on the restoration activities of floodplain woodlands with native poplars will be presented.

#### Publications

- Anselmi, N., A. Mazzaglia, A. Vannini, *et al.* 2002. Influence of the atmospheric carbon dioxide increase on poplar pathogens attacks. International Abstract 136, Poplar Symposium III, Uppsala, Sweden.
- Calfapietra, C., B.Gielen, A.N.J. Galema, M. Lukac *et al.* 2003. Free-air CO<sub>2</sub> enrichment (FACE) enhances biomass production in a short-rotation poplar plantation (POPFACE). Tree Physiology.23: 805–814
- Calfapietra, C., P. De Angelis, E. Kuzminsky and G. Scarascia Mugnozza. 2003. Impact of elevated CO<sub>2</sub> concentration on leaf development in *Populus nigra*. Free air carbon dioxide enrichment experiment. *In* Proceedings of the Third SISEF meeting, Viterbo 15–18 October 2001 (in press).
- Confalonieri, M., S. Reggi, ,L. Anfosso *et al.* 2002. Genetic transformation of white poplar with a *Vitreoscilla* hemoglobnin (VHb)-encoding gene and evaluation of growth rate and biomass production. Abstracts, 10th International Congress of Plant Tissue Culture and Biotechnology, Orlando, 23–28 June 2002.
- Confalonieri, M., A. Balestrazzi, S. Bisoffi and D. Carbonera. 2003. *In vitro* culture and genetic engineering of *Populus* spp.: synergy for forest tree. Plant Cell Tiss. Organ Cult. 73:109–138.
- Gielen, B., C. Calfapietra, A.Claus, M. Sabatti and R. Ceulemans. 2002. Crown architecture of *Populus* spp. is differentially modified by free-air CO<sub>2</sub> enrichment (POPFACE). New Phytologist 153: 91–99.
- Gielen, B., M. Liberloo, J. Bogaert *et al.* 2003. Three years of free-air CO<sub>2</sub> enrichment (POPFACE) only slightly affect profiles of light and leaf characteristics in closed canopies of *Populus*. Global Change Biology 9: 838–848
- Grassi, F., M. Winfield, T. Fossati, F. Sala and S. Castiglione. 2002. Molecular analysis of natural populations of *Populus nigra* L. growing along the river Ticino in Northern Italy. Pp. 107–116 *in* Proceedings of the International Symposium, Szekszárd, Hungary.
- Lukac, M., C. Calfapietra and D. Godbold. 2003. Production, turnover and mycorrhizal colonisation of root systems of three *Populus* species grown under elevated CO<sub>2</sub> (POPFACE). Global Change Biology 9:1022–1037
- Paolucci, I., M.A. Pagnotta, M. Sabatti *et al.* 2003. Analisi genetica della variabilità in pioppo bianco (*Populus alba L.*) mediante marcatori RFLP e RAPD [Genetic analysis of variability in white poplar (*Populus alba L.*) using RFLP and RAPD markers]. *In* Proceedings of the Third SISEF meeting, Viterbo 15–18 October 2001 (in press).
- Piazzai, M., I. Beritognolo, E.Kuzminsky *et al.* 2002. Different salt stress response of three *Populus alba* ecotypes coming from different latitude. Abstract 180, International Poplar Symposium III, Uppsala, Sweden.
- Sabatti, M., I. Paolucci, , R.Muleo *et al.* 2002. Poplar as a model forest tree: the choice of parents for a *Populus alba* pedigree. Abstract 110, International Poplar Symposium III, Uppsala, Sweden.
- Taylor, G., P.T. Tricker, F.Z. Zhang *et al.* 2003. Spatial and temporal effects of free-air CO<sub>2</sub> enrichment (POPFACE) on leaf growth, cell expansion, and cell production in a closed canopy of poplar. Plant Physiol. 131:177–185.
- Vietto, L. and B. Bianco 2003. La conservazione del pioppo nero [The conservation of black poplar]. Infofiume 14:12–13.

# Progress on national activities on gene conservation of white poplar (*Populus alba* L.) in Malta

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# Legislation

A new law, the Trees and Woodlands Protection Regulations, was passed in February 2001. This law provides protection for all native tree species in Malta and makes it illegal to destroy or damage white poplars or their habitats. A number of sites have also been listed as protected woodlands and a few of them include coppices of white poplar.

# **Public awareness**

Until 2002, a campaign was organized amongst schools to increase the awareness of native trees. Seedlings and cuttings of native species were given to schoolchildren who then were allowed to grow the plants in schools. One of the tree species grown was white poplar.

# Research and gene conservation

Two specimens of a white poplar clone in Malta were sent to Spain to be included in the *P. alba* core collection.

# Field inventories

No field inventories have been done yet for white poplar in Malta. Separate clones are not identified either.

# Practical implementation

The main *in situ* conservation activity was establishment of several woodland nature reserves which practically cover all the native woodlands of *P. alba* in Malta. A new project, which includes afforestation of several sites in the country, might include *P. alba* as one of the tree species to be used.

# Coordination at a national level

All matters dealing with white poplar are now handled by the Ministry of Rural Affairs and Environment which incorporates both environmental and agricultural sectors. Previously they were the responsibility of several different ministries.

# In situ and ex situ conservation of white poplar (Populus alba L.) in the Republic of Moldova

### Ion Palancean

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The area of white poplar forests in the Republic of Moldova is 2784 ha. In the floodplain of the river Prut, stands of white poplar make up 17% of the territory covered by forests. These poplar stands represent a transitional form between willow groves and oak groves, which as flooded for short periods of time. The white poplar stands of the river Prut floodplains are described in detail by Tkacenco (1979). Most of the poplar stands are found in Balatina, part of the Padurea Domneasca reserve. There white poplar forms mixed stands with white willow (Salix alba) and black poplar (P. nigra). The ground layer vegetation is dominated by red dogwood (Swida sanguinea), European cranberry bush (Viburnum opulus) and European spindle tree (Euonymus europaea).

In these stands, white poplar is a dominant tree species and it also forms mixtures with other species, such as pedunculate oak (Quercus robur), common ash (Fraxinus excelsior) and white elm (Ulmus laevis). Common ash is very frequent in the stands of the river Nistru floodplain. Species found in lower canopy layers include field maple (Acer campestre), Tatarian maple (Acer tataricum), wild pear (Pyrus pyraster), blackthorn (Prunus spinosa), hazelnut (Corylus avellana), glossy buckthorn (Frangula alnus) and wild vine (Vitis sylvestris) (e.g. Postolache 1995).

Part of the white poplar stands were put under state protection by the creation of the Padurea Domneasca scientific reserve (Figure 1) in the River Prut floodplain in 1993. The total area of the reserve is 6032 ha, and naturally established stands amount to 2481.2 ha (Table 1).

their share of the total reserve area (Postolache 1995)		
Type of stand	Area (ha)	%
Natural stands of oak (Quercus robur)	1017.7	17.4
Natural stands of white poplar ( <i>Populus alba</i> )	1046.1	17.8

**Table 1.** The area of naturally established stands in the Padurea Domneasca reserve and

Type of stand	Area (ha)	%
Natural stands of oak (Quercus robur)	1017.7	17.4
Natural stands of white poplar (Populus alba)	1046.1	17.8
Natural stands of white willow (Salix alba)	371.5	6.3
Natural stands of black poplar (Populus nigra)	34.8	0.5
Natural stands of aspen tree (Populus tremula)	0.7	0.1
Natural stands of osier willow (Salix viminalis)	5.5	0.1
Natural stands of sessile oak (Quercus petraea)	4.9	0.1



Figure 1. Distribution of white poplar genetic resources in the Republic of Moldova.

# Conservation of white poplar genetic resources

All potential areas of white poplar in Moldova have been explored, and all available data sources have been used for their identification. Field and other surveys have been made at the level of each forest district. The stands for conservation have been selected according to external characters, and during the first stage only forest stands of natural origin have been selected.

A database of white poplar resources has been created, including maps. In the floodplains of the Nistru and Prut rivers, 1402 sub-compartments of white poplar have been located with a total area of 2784 ha.

Sampling for the conservation of genetic resources has been done on the basis of the data obtained at the exploration stage. One of the main criteria, besides 'representativeness', was

stand productivity, and white poplar stands with higher than average productivity in each Forest District were selected. Only stands older than 40 years and with non-managed structure were selected for gene conservation.

Following international guidelines, gene conservation areas consisted of a nucleus with a buffer zone for isolation and protection. The size of the nucleus can vary. It is considered that a tree population can be maintained viable with 50–100 reproductive individuals. The dimensions of the buffer zone should be enough to fulfil its function and can vary from 100 to 1000 m.

A total of 15 sub-compartments were selected; eight on the river Nistru floodplain and seven on the river Prut floodplain. These sub-compartments are characterized by a large volume of biomass ranging from 350 to 500 m<sup>3</sup>/ha. Their age is between 45 and 80 years and the diameter of the trees can be up to 70 cm (Table 2). The distribution of these areas is shown in Figure 1.

Table 2.	A ge	eneral	characteris	stic c	of the	selected	white	poplar	gene	conservation	stands	in
the Repu	blic c	of Mold	lova									

No.	Forest district	Altitude (m)	Area (ha)	Age (a)	Height (m)	Diameter (cm)	Volume (m³/ha)
1	Vadul lui Voda	15	9.2	65	34	56	440
2	Vadul lui Voda	15	3.8	70	32	68	395
3	Anenii Noi	10	5.9	55	35	44	462
4	Anenii Noi	10	9.1	70	36	54	495
5	Talmaz	5	6.8	85	32	50	395
6	Talmaz	4	2.8	85	33	48	417
7	Olanesti	2	1.0	65	28	38	355
8	Olanesti	2	1.1	70	29	40	378
9	Lipcani	110	0.9	70	21	42	420
10	Lipcani	110	2.2	70	21	48	410
11	Ungheni	35	0.6	50	25	46	330
12	Ungheni	35	6.4	45	26	38	345
13	Sculeni	45	12.5	55	24	40	230
14	Leova	20	4.1	55	32	40	355
15	Leova	20	10.6	60	32	54	355

A detailed description of the stands will be made in summer 2003. The description of the gene conservation stands will be based on the standardized list of descriptors for inventories of black poplar stands developed by the EUFORGEN *P. nigra* Network. The borders of each conservation stand will also be marked in the field.

For *ex situ* conservation, the Botanical Garden of Chisinau has started to develop a collection of autochthonous white popular populations. So far, stock from sources in the river Nistru floodplain has been planted at the Botanical Garden. The creation of a collection of autochthonous clones has also begun.

# Research

During field visits, root and branch cuttings have been collected for caryologic analysis and paternity determination.

# Policy and legislation

For the conservation of white poplar genetic resources, nine protected areas have been established. All these areas are included in the annexes of the law on state-protected natural areas. It will be proposed to the Government and Parliament that all new conservation stands of great genetic value should be included in the above-mentioned law.

# **Public awareness**

Increasing public awareness of white poplar has been a part of the effort to promote awareness of biodiversity in general. In 2000, a comprehensive map of biodiversity conservation in Moldova was published. It contains information on the distribution of endangered plant and animal species that were included in the first and second editions of the Red Book of Moldova (the latter was published in 2001). The locations of nature conservation areas are also shown on this map.

#### References

- Postolache, Gh. 1995. Vegetatia Republicii Moldova [The vegetation of the Republic of Moldova]. Academy of Science of the Republic of Moldova, Institute of Botany, Chisinau, Republic of Moldova. 339 pp.
- Tkacenco, A. 1979. Tipy lesa poimy reki Prut v predelah Moldavscoi SSR [Type of forest on the Moldavian side of the flooplain of the river Prut]. Academia Nauk Moldavscoi SSR, Botaniceski Sad, Kisinev, Moldavscaia SSR. 83 pp.

# Progress report on national activities on gene conservation of black poplar (*Populus nigra* L.) in the Netherlands

#### Sven M.G. de Vries

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Black poplar (*Populus nigra* L.) has always been an important tree species along the many rivers in the Netherlands. The species was under particularly serious threat from the early 1920s until 1950 for a number of reasons. In those days, *P. nigra* individuals were selected and collected to serve as parent trees for the creation of Euramerican hybrid poplars (*P. deltoides*  $\times$  *P. nigra*). The collected individuals were then cloned and kept in a genebank. This genebank is still in operation, just as it was in those days, although at a different location. Results from the field inventories were and still are added to this collection of clones. Today it also serves as a major source for the re-introduction of *P. nigra* in the Netherlands. A number of restoration projects have been initiated during the last 20 years.

This report covers the period between February 2000 and May 2003.

# **Field inventories**

As a result of the recent field inventories, more individual trees have been identified as *P. nigra*. These black poplar clones were added to the genebank, which consists of stool beds. During the inventories, it turned out that new beaver populations (*Castor* sp.) showed greater interest in the bigger adult trees of *P. nigra* than in the more abundantly growing willow species. Beavers could thus become another threat to the local *P. nigra* populations.

# Policy and legislation

A national policy document of the Government of the Netherlands was introduced during the sixth meeting of the Conference of Parties (COP) of the Convention on Biological Diversity (CBD) in The Hague in April 2002. Entitled 'Sources of existence: conservation and the sustainable use of genetic diversity', it covers genetic resources of all kinds; agricultural and horticultural crops, animal genetic resources and forest genetic resources.

Following the introduction of the EC Directive on forest reproductive material (1999/105/EC), preparations were started to include the directive's requirements into Dutch national law. This Directive makes it possible for member States to include a new category, 'Source Identified', in their national catalogues. This opens new ways and possibilities of using autochthonous seed sources of *P. nigra* for restoration projects of riparian ecosystems and riverine forests.

# Research

The international, EU-funded EUROPOP project, which was coordinated by ALTERRA in the Netherlands, ended in 2001 after it had been extended into a fourth year of operation. The final meeting was held in May 2001 in Hungary. The proceedings of the meeting, published in 2002, include the final results of the project. Copies of the proceedings were distributed to the participants of the EUFORGEN *P. nigra* Network at the meeting in Treppeln in May 2003.

# **Practical implementations**

The genebank of black poplar has supplied cuttings for practical purposes. Nurseries use these cuttings in order to provide managers with rooted plants to be planted in restoration projects along some of the Dutch rivers.

Some new entries have been added to the EUFORGEN black poplar core collection in Wageningen.

#### Coordination at the national level

Plans for the establishment of large field genebanks of autochthonous trees and shrubs on state forestry land are in an advanced stage. Several organizations in the country (both private and governmental) cooperate in this plan, which will be financed by the national government. Unfortunately, a joint application with Flanders to the EU LIFE programme did not succeed.

At national level, the Centre for Genetic Resources the Netherlands (CGN) is responsible for the research and most of the implementation of policies on genetic resources.

### **Public awareness**

From 2001 to 2003, a relatively large number (>100) of the identification sheets for *P. nigra* (Dutch version) were supplied for to private individuals and organizations as well as to local governments, after articles about it were published in two forestry magazines.

# Progress on national activities on gene conservation of black poplar (*Populus nigra* L.) in Portugal

### Maria Carolina Varela

Estação Florestal Nacional, Oeiras, Portugal

Portugal joined the EUFORGEN *Populus nigra* Network at the fifth Network meeting, held in Kyiv, Ukraine in May 1998. The participation in Network activities has allowed Portugal to deepen and update its knowledge on the species. This has facilitated the contribution of Portugal to the European efforts for conservation of genetic resources of the species.

The present report refers to the period 1999–2003.

# Practical implementation

#### In situ conservation measures

No specific measures have been implemented in Portugal for *in situ* conservation of black poplar. Some individual trees, however, have been located by state institutions for conservation purposes; two trees at Coudelaria de Alter, four at Coudelaria Nacional and one in the municipal park of the village of Valada, for example. Recommendations will be developed to raise awareness on the conservation of these trees. Practical guidelines to promote the natural regeneration of the trees located at Coudelaria de Alter and Coudelaria Nacional will also be created. For the tree located in the Valada municipal park, clonal propagation is an appropriate option for regeneration.

#### Ex situ conservation measures

Between 2000 and 2003, seven black poplar trees were identified in Portugal on the basis of phenological characteristics. The identification was also confirmed by genetic markers in collaboration with Austrian and German colleagues (Dr B. Heinze, Federal Office and Research Centre for Forests, Vienna and Dr A. Janßen, Servicestelle FIV, Hann.Münden). Details of these trees can be found online at the European Database of *P. nigra* clones (*www.populus.it/nigranet.php*). Three clones (EFN-CA 1; EFN-MEV 1 and EFN-MS 2) were also selected for the *P. nigra* core collection and sent to the Istituto di Sperimentazione per la Pioppicoltura (ISP) in Italy and to ALTERRA, Green World Research, in the Netherlands.

Portugal offered to host a duplication of the EUFORGEN *P. nigra* core collection. The Escola Superior Agrária de Coimbra (ESAC) has been assigned to consider the valley of the river Mondego, in Coimbra, as a suitable site for maintaining the collection. Following the ESAC's agreement to host the duplication of the core collection, the ISP in Italy kindly sent cuttings of about 30 genotypes to Portugal in 2003. The sending of the cuttings was delayed from 2001 to 2003 because of the severe floods that nearly destroyed the ISP collection (see page 51).

# Perspectives

The lack of specific funds for the conservation of *P. nigra* is a constraint on systematic activities in Portugal. However, the aim is to select more trees from areas with different ecological conditions. Efforts will be made to establish a clone bank of all selected trees to conserve genetic resources of *P. nigra* in Portugal.

# Introductory note on black poplar (*Populus nigra* L.) in Romania<sup>1</sup>

#### Marius Sorin Nica

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The indigenous poplar species of Romania include white poplar (*Populus alba* L.), black poplar (*P. nigra* L.), aspen (*P. tremula* L.) and grey poplar (*P. canescens* (Aiton) Sm.). These trees form pure or mixed stands with other species, or occur as small groups. Isolated trees can also be found the main river floodplains (Danube, Jiu, Olt, Mures, Prut and Cris). Most of the natural poplar stands are found in the Danube Delta National Park.

As in many European countries, also in Romania large parts of the natural poplar forests have been replaced with much more productive poplar hybrids. According to the latest inventory data (1995), Romania has 80 000 ha of indigenous poplar forests and 65 000 ha of Euramerican poplar hybrid stands. In total, poplars cover approximately 2.4% of the total forest area in Romania.

*P. nigra* has been replaced with poplar hybrids because of their faster growth rates and other economic characteristics. The main clones now cultivated on a large scale in Romania are *P.* × *euramericana* 'Sacrau 79', *P.* × *euramericana* 'Italia I-214' and *P.* × *euramericana* 'Robusta RO16'. In the Romanian clone collections we have more than 50 clones, some of them maintained at the Poplar Research Centre Cornetu (ICAS). Because of the intensive cultivation of the Euramerican hybrids, *P. nigra* trees or stands are now found mainly on the Danube islands or other isolated sites in the Danube delta. These stands are usually not pure, and *P. nigra* is associated with other riparian species such as *P. alba, Salix* spp. and *Alnus* spp. Some of the hybrid poplar plantations are also in unsatisfactory conditions because of climatic changes and transformation of local sites by water.

For these reasons, *in situ* and *ex situ* conservation efforts for *P. nigra* and other riparian species need to be strengthened in Romania. As a first step to conserve the genetic resources of black poplar and to improve the stability of the riparian ecosystems, it is necessary to start a national monitoring and inventory programme.

<sup>&</sup>lt;sup>1</sup> This note was delivered by B. Heinze on behalf of the author who is a visiting student in Austria.

# Progress on national activities on gene conservation of black and white poplars (*Populus nigra* L. and *P. alba* L.) in the Russian Federation

# Ivan Popivshchy

Russian Tree Breeding Centre, Pushkino, Moscow, Russian Federation

Stands of black poplar (*Populus nigra* L.) can be found in 16 regions and white poplar (*P. alba* L.) in 10 regions of the nearly 50 regions of European part of Russia. In terms of area, these stands cover about 100 000 ha and 40 000 ha, respectively. The species are mainly associated with riverine lowland forests of which approximately 80% are the so-called populeta. By September 2001, 62 plus trees of black poplar and 21 ha of seed collection stands had been selected in the southern region of Astrakhan. In addition to this, 10 plus trees of black poplar and 0.8 ha plus stands have been selected in the Voronezh region.

# In situ conservation

*In situ* conservation of poplar genetic resources is carried out through nature and gene reserves and national parks (31) in which they are found. Currently, the state of natural populeta in Russia is deteriorating and their areas declining. During the last 40 years, the area of poplar stands has reduced by nearly 50% and their standing volume by 66%. The overall site quality of these stands has also declined. From the phytosanitary point of view the situation has deteriorated, and only the younger stands are considered healthy and disease-free.

# Ex situ conservation

*Ex situ* conservation of black and white poplar is carried out at stool beds and different forest plantations. For example, there are 14.2 ha of plantations for black poplar and 2.2 ha for white poplar in the Kursk region, and 5.2 ha of black poplar plantations in the Astrakhan region.

In addition, efforts are carried out for hybrid poplars. There are 5.1 ha of stool beds for hybrid poplars and, in the Belgorod region, 20.6 ha of plantations for Central Asian and Euro-American hybrids of black poplars.

There is no clonal collection of black or white poplars in Russia. Breeding collections were created in the 1930s and 1940s, but these have been lost. Some effort has been made to reestablish these collections, but there have been problems with unhealthy cutting material. In an arboretum in the Moscow region, three ecotypes of white poplar and three individuals of black poplar are represented but they have poor growth and sprout production. The poplar collections in the arboretums of lvanteevka and Voronezh consist mainly of different cultivars and hybrids and contain no pure trees of the two poplar species.

# State of poplar gene conservation

For most of the Russian regions with natural poplar stands, the following aspects are common:

- biological and economical properties of the two poplar species are not investigated;
- breeding valuation of the stands is not carried out;
- gene reserves, plus stands and plus trees are not selected;
- collections, plantations and stool beds are absent;
- artificial propagation has not been studied; and
- the objectives for gene conservation or tree breeding have not been identified.

This situation is due to the fact that the authorities responsible for regional forest management do not consider that the poplar gene pool to be in danger. In contrast to other forest tree species, breeding work for the poplars has not been planned since it is considered of minor importance and value in Russia. Subsequently, conservation of poplar genetic resources has also received little attention.

Phenotypic and genetic diversity of black and white poplars still remains practically unexplored in Russia, although ecotypes with high productivity are found sporadically. Along with decreasing populetum areas, there is a danger of losing valuable genetic resources.

# **Research needs**

Inventories of poplar stands must be initiated. With this in mind, we have translated the black poplar identification sheet into Russian. The conditions of populeta need to be explored, and there is some previous experience to build on. The phenotypic and genetic diversity of white and black poplars also needs further research before any selection of traits for breeding or establishment of networks of *in situ* conserved populations. Unfortunately, funding opportunities for the work remain rare. There is a need for a pilot project like EUROPOP in at least one region of Russia, with the perspective of extending the efforts to other regions in the future.

# Public awareness

In general, the Russian public, including foresters, is aware of the danger of biodiversity loss in the country's lowland forests. Based on the opinions of many leaders and managers of forestry enterprises, interest in carrying out inventories of white and black poplar stands is increasing as a first phase of conservation and exploration of the biodiversity they host. The difference between Western Europe and Russia is that although the need to undertake active measures to conserve poplars is understood, Russia has to struggle with more urgent problems, such as the survival of forestry enterprises and how to maintain the livelihood of people working in these enterprises.

# Conclusions

Conservation of the genetic resources of black and white poplars in Russia needs additional efforts and more financial and human resources. As the first step, inventories of poplar stands and investigations of their conditions should be carried out. The most valuable areas should then be selected for conservation.

# Conservation of black poplar (*Populus nigra* L.) and other poplars in Serbia and Montenegro

# Sasa Orlovic, Andrej Pilipovic and Leopold Poljakovic Pajnik

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The group of 'European black poplars' in Serbia and Montenegro includes several species, i.e. *Populus nigra* L., *P. pubescens* (Parl.) Jov. et Tuc., *P. pannonica* Kit. et Bess., and *P. metohiensis* Tuc. The occurrence of spontaneous, simple and complex hybrids has also been recorded. All these species occur naturally in Serbia and Montenegro; the first three species are found in the north (Vojvodina province) and the fourth one in the south. The distribution area of black poplars is smaller than that of aspen (*P. tremula* L.) and white poplar (*P. alba* L.). Black poplars form pure stands and they also grow mixed with the Slavonian oak, white poplar and white willow forest types, as well as with a large number of shrub willows in the pioneer stage of these communities (Herpka 1963; Jovanovic and Tucovic 1965).

The distribution of black poplar is connected with the distribution of sandy alluvial soils on which poplars have no competition. Pure young black poplar stands usually inhabit the higher reaches of sandbanks, while in the lower parts of alluvial deposits a mixture of young growth of black poplars, white poplar and willows is found (Herpka 1963).

In Serbia and Montenegro, only a small amount of European black poplar has been conserved. Good mature trees were found in stands until the 1970s. Today, the black poplar stands have been considerablly reduced in favour of Euramerican poplar. Natural black poplar populations have also been destroyed by river engineering and dam building. In addition, black poplar is slowly disappearing because of its susceptibility to a bark disease (*Dotichiza populea*) and leaf diseases (*Melampsora* spp. and *Marssonina brunnea*). Under natural conditions, the stands mainly reproduce from seeds, frequently resulting in the occurrence of spontaneous hybrids.

Conservation of the genetic resources of European black poplar is carried out parallel to the poplar breeding programme implemented by the Poplar Research Institute in Novi Sad. In this programme, genotypes of European black poplar were used as male partners in controlled crossings, mainly with the genotypes of *Populus deltoides*.

Year of collection	No. of selected genotypes	Included in clonal archive
1995	10	2
1996	10	2
1997	40	35
2002	20	18

The work on the selection and fixation of genotypes in the section *Leuce* has been performed less intensively than in the section *Aigeiros*. However, during the 40-year development of the Poplar Research Institute, we have selected several genotypes and half-sib progenies have been cultivated. We have also created the hybrids of *P. alba* × *P. grandidentata* and *P. tremula* × *P. tremuloides*. Recently we have selected salt-tolerant poplars for saline soils, which account about 200 000 ha in Serbia and Montenegro. The use of poplars in the section *Leuce* for urban environment is especially interesting, and we have chosen several genotypes with an attractive bark colour and crown form for this purpose. The genotypes are first reproduced by tissue culture and then by root or stem cuttings.

Year of collection	No. of selected genotypes	Included in clonal archive
1970	40	40*
1993	40	35
2001	2	2
2002	3	3

\*Hybrids P. alba × P. grandidentata, P. tremula × P.tremuloides

#### References

Herpka, I. 1963. Postanak i razvoj prirodnih vrba u Podunavlju i donjoj Posavini [The origin and development of natural willow along the Danube and Sava rivers]. Topola 36–37: 18–27.

Jovanovic, B. and A. Tucovic. 1965. Neke fitocenoze vrba u SR Srbiji [Some willow communities in Serbia]. Glasnik Prirodnjackog muzeja Serija B, Knjiga 20, Belgrade, Yugoslavia.

# Conservation of black poplar (*Populus nigra* L.) and white poplar (*Populus alba* L.) in Slovenia

#### Gregor Bozič

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Slovenia is a montainous country with a forest cover of about 55%, amounting to 1.11 million ha. In most cases, the forests are regenerated naturally. Most of the forests are beech (55%), fir-beech (15%) and beech-oak sites (11%). Less than 1% of forest sites are occupied by poplars, willows and alders. Poplars and willows are found in the lowland forests only individually or in small groups.

In 1959, the area of poplar sites was estimated to be about 1300 ha and it has gradually decreased during in the past decades. The decline of the autochtocthonous vegetation in poplar and willow groves is due to the changes in river dynamics and establishment of intensive poplar plantations between 1960 and 1980. Poplar and willow groves are conserved in Slovenia only in small areas on alluvial sites along rivers (Mura, Drava, Sava, Krka, Soča), wetlands and occasionally flooded terrains.

Currently, there are no specific *in situ* or *ex situ* conservation programmes for indigenous *P. nigra* and *P. alba* genetic resources, or even a specific research project in Slovenia. The EUFORGEN-related activities, such as collection of autochthonous poplar resources in 1999, were included as part of other activities run by the Slovenian Forestry Institute.

Although poplars have no great economic importance in forest management in Slovenia, one of our future goals is to carry out an inventory of the indigeneous poplar gene resources and increase their protection. This work is in accordance with plans of the State Agency for Nature Conservation as part of the national biodiversity strategy (concerning inland waters and wetlands) and with the Forest Act, which deals with natural regeneration in most areas.

During the past decades, a lot of work has been done on assessing forest genetic resorces in Slovenia and this is very important from the bio-ecological and economic point of view. Now we would like to extend this work to the indigenous poplar species as well. In this regard, we would like to take part in international collaboration and renew contacts with other research centres. It is expected that the research on poplar species will be a part of our institute's work and research programmes in the future. Despite the fact that the proportion of lowland forests is relatively small as compared to other areas, the conservation of indigenous *P. nigra* or *P. alba* genetic resources is also considered an important part of our work. Slovenia urgently needs to develop a gene conservation strategy for poplars to avoid further loss and damages to these resources.

# Progress on national activities on gene conservation of black poplar (*Populus nigra*) and white poplar (*P. a lba*) in Spain

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Spain has developed several activities for the genetic conservation of black poplar (*Populus nigra* L.) and white poplar (*P. alba* L.). Both poplar species are considered useful from an ecological point of view, as well as being model tree species for genetic and physiological studies.

# Field inventories

Spain lacks a national inventory of floodplain forests, but an inventory of *P. nigra* populations has been made in the middle Ebro valley as part of the National Programme on Phytogenetic Resources Conservation (1998–2001). The inventory covered 55 sites with different vegetation types. Natural *P. nigra* stands have been located and mapped in 39 of these sites, mainly forming mixed stands with *Salix alba* but also with *Tamarix gallica* and *P. alba*. The stands have been described following the EUFORGEN descriptors for *P. nigra* stands and a database is being completed.

Nine naturally regenerated areas of *P. nigra* have been located and patches of seedlings mapped for later studies. Some additional sites have also been catalogued as 'potential' areas for generative regeneration of *P. nigra*.

Based on phenotypic traits, 120 individuals from two populations were collected for the EUROPOP study and 70 *P. nigra* trees were sampled from the rest of the mapped stands.

# Policy and legislation

There is no specific legislation relating to the conservation of *P. nigra* in Spain. At regional level, the Management Plan of Natural Resources of the river Ebro has recently been developed and will be relevant to the conservation of *P. nigra* in this particular valley. The existence of a large area where the river can meander freely makes these riparian forests valuable for conservation. Until now, a restricted area (777 ha) has been under some kind of protection in the Alfranca Natural Reserve. The new regional plan includes an increase in the area to be protected (31.2 ha) and proposes restrictions on activities affecting the ecological conditions of natural resources (flora and fauna).

Recently, the Spanish Government has approved a law to implement a National Hydrological Plan (PHN), which includes transferring water from the Ebro basin to the eastern parts of the country. This transfer will certainly alter the natural river dynamic dramatically, and modify the ecological conditions of the floodplain forests.

The Directive 1999/105/EC on the trade of forest reproduction material (FRM) was incorporated into Spanish legislation in March 2003. From now on, clones of autochthonous poplars, maintained in *ex situ* collections, can be included in some of the different categories for the FRM (identified, selected and qualified). Previously, we were working to develop a National Catalogue of *P. nigra* and *P. alba* clones including some recommendations for their use.
#### Research

#### Projects

Most of the research carried out in 2000–2001 was part of the EUROPOP project. Genetic diversity of *P. nigra* in natural stands (Ebro valley) and in a genebank collection (Ebro, Duero and Tajo rivers) has been evaluated at morphological, biochemical and molecular levels. Detailed results are shown in the final report of the EUROPOP project (Alba *et al.* 2002).

Analysis of leaf morphology has shown no significant differences in leaf size among the Ebro valley natural populations, whereas high interclonal variation within the stands is present. Morphological analysis of the genebank has shown a clinal structure for leaf characters (southern genotypes have bigger leaves than northern ones). Leaf morphology seems to be a useful tool in studying genetic variation of *P. nigra* in Spain and has provided a good way of discriminating pure *P. nigra* individuals from hybrids.

Based on qualitative leaf characters (leaf blade shape), we cannot conclude that a specific shape of leaf is associated with a geographic origin of clones. The more frequent forms of leaf in Spanish *P. nigra* genotypes from the genebank collection are shown in Figure 1. Some intermediate forms of basal shape and tip shape (referred to the EUFORGEN descriptors for *P. nigra* leaves) have been observed.



**Figure 1**. Commonly found leaf classes in *P. nigra* clones from the Spanish genebank collection: (a) broadly wedge-shaped, straight basal shape; (b) narrow, short, acuminate tip shape; (c) a straight junction with the petiole.

Genetic diversity values in natural populations varied from one type of marker to another. Isozyme analysis revealed a low value of gene diversity (0.009). However, the results of DNA analysis showed a high diversity, especially for microsatellites (0.630) and cpDNA (0.382), while AFLP showed intermediate values (0.144).

The low differentiation between populations reveals gene flow through pollen and seeds. The unexpected omnipresence of repeated genotypes, as revealed by the microsatellites and AFLP markers, is a consequence of a low vegetative propagation. This situation could be related to the active dynamics within the stands. No hybrids were identified in the natural stands that were studied, even though plantations of *P*. × *euramericana* are close to these populations.

The Spanish genebank contains a high proportion of different genotypes; isozyme analysis has detected six different genotypes and one hybrid. Coinciding with the isozyme results, 48 cytotypes have been identified, one of them as a hybrid. A total of 87 different genotypes have been detected with AFLP markers and 95 with microsatellites.

One of the most relevant results of the EUROPOP study in Spain concerns the richness of *P. nigra* haplotypes. A total of 37 different haplotypes have been found in natural populations in the Ebro valley and 55 in the genebank. Eight haplotypes are common in the populations and the genebank. On the basis of these results, the studied populations seem be in good condition and they could be part of an *in situ* conservation network for *P. nigra*. The results of cpDNA analysis revealed a strong presence of different haplotypes, suggesting a possible refuge of *P. nigra* in Spain. This result should be taken into account in the

management of *P. nigra* genetic resources.

Presently, there are two new research projects, 'Establishment, conservation and characterization of a *Populus* collection' and 'Genetic diversity in *Populus*: adaptive traits and molecular markers: Implications for genetic conservation', both financed by the National Programme for Conservation of Phytogenetic Resources. There is also a third project, 'Genetic diversity in the Iberian forest ecosystems: Geographic patterns and variability using genetic markers', which focus on several forest species, including *P. alba*.

# Study on adaptive traits in P. alba

A total of 34 clones from different regions were tested for their salinity response (7 and 14 dS/m) under greenhouse conditions. The evaluation of salt tolerance was based on survival, growth and photosynthesis rates. The first results showed that 15 clones have a good performance in experimental conditions (Sixto *et al.* 2000; Sixto *et al.* 2001). Salt-tolerant clones have been propagated for further testing in the field.

### Genetic diversity studies in P. alba

Studies on genetic diversity in *P. alba* populations are being carried out using cpDNA microsatellites and PCR-RFLP. A total of 18 populations from 6 rivers in the Iberian Peninsula were sampled with 2–10 trees from each population (6 populations from Ebro, 5 from Duero, 2 from Tajo, 3 from Guadalquivir, 1 from Segura and 1 from Almanzora). The sampled material was analysed with 14 cpDNA microsatellites, of which 2 were polymorphic. In the 113 samples analysed, 10 haplotypes were identified. South-eastern populations (Segura and Almanzora rivers), which live under extreme conditions, showed the highest levels of diversity.

The PCR-RFLP methodology for *P. nigra*, developed by EUROPOP, was applied to *P. alba* populations. *P. alba* showed a lower level of polymorphism than *P. nigra*, and a different pattern. Different fragments were then amplified and a higher level of polymorphism was found.

# Morphological characterization

Many clones of both species are being evaluated for leaf morphology, growth, phenology and tree architecture in nursery trials lasting two years. A nursery trial of 47 *P. alba* clones from genebank collection was established in March 2003 in two locations (Zaragoza and Madrid) for this purpose.

#### Practical implementation

#### In situ conservation measures

In Spain, there are no specific *in situ* conservation measures for autochthonous poplars. Only natural populations and/or individuals located in protected areas (natural reserves) benefit from limited human activities in these areas.

#### Ex situ conservation measures

Until 2001, the Spanish genebank of *P. nigra* contained 150 clones sampled from the Ebro, Duero and Tajo rivers at different altitudes (from 67 m a.s.l. in the Ebro valley to 1300 m in the Tajo valley). This collection has recently been expanded as a result of the studies carried out on the natural populations of the Ebro valley between 1998 and 2001. A total of 179 new autochthonous clones and 94 foreign clones have been included in the genebank, which now contains 325 autochthonous *P. nigra* clones. The non-autochthonous clones were received from the EUFORGEN core collection (32 clones) and from natural stands in northern Italy (62

clones). All these clones are maintained as stool beds at SIA-DGA (Zaragoza).

### P. alba genebank

The Spanish *P. alba* collection includes 647 autochthonous clones from three different regions. One foreign clone, from Malta, is also included.

### **Maintenance of collections**

We apply different conservation strategies for maintaining the poplar collections: stool bed plantations and adult tree plantations (Table 1), or a mixture of these. The collections are partially duplicated by two institutions (SIA and CIFOR) to facilitate characterization studies.

Autochthor	Autochthonous clones		n clones
Stool beds	Plantations	Stool beds	Plantations
325*	263*	94*	17*
35**	37***	_	_
68*	19*	1*	_
641**	_	_	-
	Autochthor Stool beds 325* 35** 68* 641**	Autochthonous clonesStool bedsPlantations325*263*35**37***68*19*641**-	Autochthonous clonesForeignStool bedsPlantationsStool beds325*263*94*35**37***-68*19*1*641**

Table 1. Ex situ collections	maintained as	stool beds and	tree plantations
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\*SIA (Zaragoza), \*\*CIFOR (Madrid), \*\*\*CIFOR (Valsain).

A populetum, established in February 2003, represents a milestone in the overall activities on poplar conservation carried out by SIA-DGA. The populetum includes 620 clones belonging to different species and hybrids, with an objective of medium-term conservation. Each clone is represented by two trees. A total of 263 clones of *P. nigra* and 19 clones of *P. alba* are included in this plantation. A replication of this plantation will be established in another location near Zaragoza during next winter (2003/2004).

# Coordination at the national level

In Spain, two research institutions are involved in the conservation activities of poplar genetic resources: CIFOR-INIA in Madrid and SIA-DGA in Zaragoza. These institutes collaborate within each other in various national and international programmes.

# **Public awareness**

A new law on forest reproductive material was explained at the meeting of the Spanish group of poplar cultivation held in Guadalajara (Spain) in December 2002. There was great interest in the utilization of autochthonous poplars from *ex situ* collections in restoration programmes in different regions of Spain. The EUFORGEN leaflet for *P. nigra* identification and the technical bulletin on *in situ* conservation of *P. nigra* were distributed to the interested participants.

#### **Publications and presentations**

- Alba, N. 2000. Variabilidad genética de *Populus alba* L. mediante caracteres isoenzimáticos y fenotípicos. Aplicación a la selección y conservación de los recursos genéticos.[Genetic variability of *Populus alba* L. in terms of isoenzymatic and phenotypic characteristics. Application to the slection and conservation of genetic resources]. Tésis Doctoral, Universidad Politécnica de Madrid.
- Alba, N. 2000. Conservación de recursos genéticos del género *Populus* en España [Conservation of genetic resources of the genus *Populus* in Spain]. Invest. Agr. Sist.Recur.For. Fuera de Serie nº 2, 45–57.
- Alba, N. and D. Agúndez, 2000. Characterisation of *Populus alba* by isozymes. Invest. Agr.: Sist.Recur.For. 9(2): 305–315.

Alba, N., C. Maestro, D.Agúndez and E. Notivol. 2001. Advances in the preservation of genetic resources in

*Populus nigra* L. in Spain. Abstracts of the International Symposium on Genetic Diversity in river populations of European Black Poplar—Implications for riparian eco-system management. Szekszárd, Hungary 16–20 May.

- Alba, N., D.Agúndez, R.Alía and A. Padró. 2001. Estudio comparativo de diversidad genética entre caracteres isoenzimáticos y fenotípicos en *Populus alba* L.[Comparative study of the genetic diversity between isoenzymatic and phenotyptic characteristics in *Populus alba*]. Actas del III Congreso Forestal Español, Granada, 25–28 September, Vol. II, pp. 533–538.
- Alba, N., C. Maestro and D. Agúndez. 2001. La conservación de los recursos genéticos en la Populicultura [The conservation of genetic resources in the cultivation of poplars]. Actas del I Simposio del Chopo, Zamora, 9–11 May, pp. 391–400.
- Agúndez, D., S.Fluch, C. Maestro and N. Alba. 2001. Introgresión genética procedente de plantaciones de híbridos en rodales naturales de *Populus nigra* [Genetic introgression originating from hybrid plantations in natural stands of *Populus nigra*] Actas del III Congreso Forestal Español, Granada, 25–28 September, Vol. II, pp. 546–552.
- Maestro, C., N. Alba and D. Agúndez. 2001. Conservación ex situ de *Populus nigra* en España: caracterización morfológica e isoenzimática de las colecciones establecidas [Ex situ conservation of *Populus nigra* in Spain: morphological and isoenzymatic characterizatio of established collections]. Actas del I Simposio del Chopo, Zamora, 9–11 May, pp. 401–409.

#### References

- Alba, N., C.Maestro, D. Agúndez and E. Notivol, 2002. Advances in the preservation of genetic resources in *Populus nigra* L. in Spain. Pp. 125–136 *in* Genetic diversity in river populations of European black poplar. Implications for riparian eco-system management (B. C. van Dam and S. Bordács, eds.). Proceedings of international symposium, Szekszárd, Hungary, 16–20 May 2001. Csiszár Nyomda, Budapest, Hungary.
- Sixto, H., J.M. Grau., A. Ferrer and F. Gonzalez-Antoñanza. 2000. Response to salinity in *Populus*. P. 165 *in* 21st Session of IPC. Poplar and willow culture: Meeting the Needs of Society and Environment. Vancouver-Washington, 24–28 September.
- Sixto, H., J.M. Grau, J.L. Montoto and N. Alba. 2001. Comportamiento de diferentes clones de *Populus alba* y *P. deltoides* × *P. alba* a tratamientos salinos [Behaviour of different clones of *Populus alba* y *P. deltoides* × *P. alba* in response to saline treatments]. Pp. 335–341 *in* Actas del III Congreso Forestal Español, Granada, 25–28 September, Vol II.

# Progress on national activities on gene conservation of black poplar (*Populus nigra* L.) in Switzerland

#### Peter Rotach

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Black poplar (*Populus nigra* L.) is a rare and highly threatened species in Switzerland. It is quite clear that only a few old individuals remain, although the exact number is not known. Black poplar belongs to the group of most threatened tree species in our country. Of the 330 000 black poplars that exist according to the national forest inventory, less than 1000 individuals are estimated to be pure *P. nigra*. The current situation is mostly a consequence of the loss and degradation of floodplain forests during the last century.

A detailed analysis has been given in the introductory country report. This progress report covers the period between spring 2000 and spring 2003. It is the first progress report since the introductory country report presented in France in spring 2000.

#### Inventories

A regional inventory has been completed in the major floodplain forests of the canton of Aargau. So far, 80 pure *P. nigra* individuals (checked with three DNA markers – see research section) have been identified and mapped with GIS. An additional 223 individuals which are most likely pure *P. nigra* and 118 putative hybrids with a slight chance of turning out to be pure *P. nigra* have been found and mapped. Genetic analysis of these trees is currently under way. A sample of these clones has been conserved in a local collection in order to produce planting material used in the area. This regional inventory was the result of a political decision—initiated by the public and adopted by a public vote—to conserve and restore the remaining floodplain forest in the area.

In another regional inventory (Lake Biel), 51 pure *P. nigra* individuals were identified (genetically checked) and mapped. More potential *P. nigra* individuals have been located, but they are still awaiting genetic analysis. A sample of these pure clones has been collected and is conserved and propagated in the nursery operated by the canton of Bern.

During the reporting period, the number of localized and identified pure *P. nigra* individuals has thus increased from zero to about 130 and many more potentially pure trees are currently in the process of being checked for purity. Most of these individuals are old trees; these genotypes thus urgently need *ex situ* conservation. As mentioned, some of the clones have already been included in local stool beds. However, neither a national clone register nor a systematic national clone collection exists so far.

In 2002, two Swiss clones—one from each of the regions mentioned—were sent to Hungary and incorporated into the EUFORGEN core collection.

At the end of 2002, a project for a national inventory of pure *P. nigra* individuals has been accepted and will be funded by the Federal Administration in Bern. The inventory will start in winter 2003 after the methodology and several open questions have been worked out (see research section below) and should be completed by the end of 2005. The inventory will be based on intensive fieldwork and a genetic check of all putatively pure *P. nigra* individuals. Field work will focus on the registered floodplain forests of the Swiss floodplain inventory, and on lakeshore areas. However, available funds will not allow a complete survey of the potential distribution area. The national inventory is part of the programme 'Conservation and promotion of rare species in Switzerland'. It is run by the Swiss Federal Institute of Technology, Zürich (Chair of Silviculture, project leader Peter Rotach) in collaboration with the Swiss Federal Research Institute WSL, Birmensdorf (Section Genetic and Ecology, project

leader Rolf Holderegger) and funded by the Swiss Agency for the Environment, Forests and Landscape (SAEFEL), Bern (Swiss Forest Agency in collaboration with Nature and Landscape, project supervisor Markus Bolliger).

# Research

During the reporting period, the purity of several hundred individuals has been checked by the Swiss Federal Research Institute WSL in Birmensdorf, using isozyme and DNA markers. Meanwhile, the methodology has been improved so that the purity checks will rely on DNA markers only, omitting the isozymes. One chloroplast marker (cpDNA trnL-trnF intron) and two nuclear markers (win3 and POPX) are used, allowing the identification of hybrids and backcrosses in the first and subsequent generations (and *P. trichocarpa* × *P. deltoids* and *P. nigra* × *P. tremula* hybrids). Methodology is currently tested using cambium cells from wood cores. Because buds or leaves are often unavailable without climbing, expensive additional harvesting campaigns would be needed for genetic checking. Coring would allow cheap sampling during the field inventory and thus all mapped individuals could be checked for purity.

# Coordination at the national level

The planned inventory of *P. nigra* is a coordinated effort of the Federal Agency of Forests in collaboration with Nature Conservation and Landscape. It is an integrated part of floodplain conservation and restoration activities and biodiversity conservation measures.

# **Public awareness**

Several publications in journals have helped to improve public awareness, especially within the forest service. As the progress report shows, awareness and interest in the species has clearly increased.

# Progress on national activities on gene conservation of black poplar (*Populus nigra* L.) in Turkey

#### Ferit Toplu

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Black poplar (*Populus nigra* L.) has considerable economic importance in Turkey, and many forms and hybrids of black poplar occur in Anatolia. The cultivars and hybrids of this species have been widely used in plantations. The taxonomy of indigenous black poplars in Turkey is described by Yaltırık (1973): *P. nigra* L. subsp. *nigra*, *P. nigra* L. subsp. caudina (Ten) Bugala and *P. usbekistanica* Kom. subsp. *usbekistanica* cv. 'Afganica').

In Turkey, two cultivated forms of black poplar are characterized by very narrow, pyramidal crowns. These are found not only in towns but also in small villages along rivers and streams. One of these cultivars, mostly scattered in the western 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 originates from Lombardy, Italy. The second form, *P. usbekistanica* komarow 'Afganica' (Syn: *P. thevestina* Dode) has smooth bark which is white or greyish-white also on older trunks (Browicz and Yaltırık 1982). This cultivar is much more widespread and common than the former, especially in inner Anatolia, and it originates from Central Asia.

Cultivars of black poplar are grown by traditional methods in central, east and southeastern regions of Turkey. In Anatolia, where arable land is limited, the demand for wood is very high and row plantings are an optimal solution for a land use system. They protect arable land and increase agricultural production. In addition, they provide wood for rural needs. It is estimated that the potential for row plantations is about 100 000 km along the banks of rivers and streams (Semýzoglu 1981).

According to the 1994 inventories, annual poplar wood production in Turkey is 3.5 million m<sup>3</sup>. Of this, 43% (1.5 million m<sup>3</sup>) is black poplar. Industrial plantations with fast-growing species are the important sources that can fill the gap between wood production and demand. Therefore, high priority has been given to poplar as fast-growing species (Birler and Koçer 1992). Industries consuming poplar wood have developed very quickly in recent years. Black poplar wood is mostly used for furniture, packing, particle board, cellulose, fuelwood and rural constructions.

In Turkey, native populations of black poplar are threatened by urban development and rural management procedures. Therefore, *in situ* conservation is limited to protected areas and emphasis has been given to *ex situ* conservation. A new conservation programme has recently been started to protect genetic resources of black poplar in *ex situ* collections. So far, several black poplar clones have been collected to form stool beds at nurseries in Izmit, Ankara, Erzurum and Ceyhan. Some of these clones were collected from natural stands and some were obtained from artificial crossings and open-pollinated trees. During the selection, emphasis is given to frost-resistant individuals.

The poplar improvement programme in Turkey is mostly focused on *P. nigra* and *P. deltoides*. Domestic black poplar is included in the breeding programme because of its adaptability to continental conditions. For this reason, creation of *P. deltoides* × *P. nigra* and *P. nigra* × *P. nigra* hybrids are in progress in Turkey. Controlled hybridization work on poplars has been carried out in Turkey since 1967. Satisfactory results were obtained from the crosses mentioned above. In addition, selection work on *P. nigra* is also ongoing. So far, 60 individuals have been selected and included in a stool bed in the nurseries (Toplu 1996).

This report includes works carried out between October 2000 and May 2003.

### **Field inventories**

During the reporting period, we have selected 60 new spontaneous *P. nigra* individuals and 20 of *P. alba*. The material collected from these individuals has been transferred to the stool beds in the nurseries. Furthermore, five new natural distribution areas of *P. nigra* were discovered in eastern Anatolia (Melet, river Mesudiye; Kelkit, river Tokat; Munzur, river Tunceli; Karasu, river Erzincan; and Pülümür, river Tunceli). Field inventories are ongoing to find new locations, especially in eastern Anatolia.

# Research

#### New project and proposals

We established five nursery clone trials in Harran (1997), Tunceli (1998), Kırşehir (2001), Ankara (2001) and Kutahya (2001) using *P. nigra* clones from our collection. Two first collection clone trials were also established in Harran (1999) and Tunceli (2000) using selected clones following the results of nursery clone trials. The results of the nursery clone trial which was established in Harran were published and presented at the second International Poplar Symposium in Orléans, France in September 1999.

Two new nursery clone trials were established using 210 clones in central Anatolia (Seydisehir) and the Lakes district (Egirdir). Three additional clone trials (first selection) were established in central Anatolia (Kutahya, Ankara and Kırsehir) using 30 successful clones from the nursery clone trials established at the same sites. Collecting cuttings of *P. nigra* for provenance trials was postponed to 2004 because of security problems in certain regions within the distribution of the species in Turkey.

# Practical implementation

#### In situ conservation measures

Five new natural distribution areas of *P. nigra* were discovered in eastern Anatolia (Melet, river Mesudiye; Kelkit, river Tokat; Munzur, river Tunceli; Karasu, river Erzincan and Pülümür, river Tunceli). *In situ* and *ex situ* conservation activities will be started in the newly discovered areas.

#### Ex situ conservation measures

A new stool bed with more than 350 black poplar individuals was established in the Ankara nursery. In addition to this, 377 individuals were obtained from artificial crossing (*P. nigra*  $\times$  *P. nigra*) and transferred to a stool bed in the Izmit nursery. The total number of *P. nigra* individuals in the collection is 686.

Cutting material of two *P. alba* individuals was also sent to Germany and Hungary, but only one individual arrived alive in Germany. New cutting material of the second individual will be sent to Germany next year.

# **Public awareness**

The seventh meeting of the National Poplar Committee was held in Izmit in April 2003 with representatives of the government, various foundations, researchers, poplar growers, faculties, nurseries and end-users. At this meeting, the committee agreed on several priorities:

- The number of registered clones should be increased.
- Emphasis should be given to the identification of frost-resistant clones.
- Greater emphasis should be given to research on adaptation of the clones to marginal lands. In this respect, priority should be given to *Populus alba*.
- Taxonomic studies relating to the distribution of black poplar in Anatolia should be

begun.

- The natural distribution area of black poplar in Turkey should be included in an *in situ* conservation programme.
- Collaboration with the EUFORGEN *P. nigra* Network should be increased.

#### References

- Birler, A.S. and S. Kocer, 1992. Guneydogu Anadolu projesi (GAP) bölgesinde kavak yetistiriciliginin organizasyonu ve sosyo-ekonomik önemi [The organization and socio-economic importance of poplar development in the GAP project in southeastern Anatolia]. Kavak ve Hızlı Gelişen Orman Agacları Arastırma Enstitusu. Çesitli yayınlar serisi No. 1 Izmir.
- Browicz,K. and F. Yaltırık. 1982. Flora of Turkey. Volume 7 (P.H Davis, ed.) Edinburgh University Press, Edinburgh.
- Semýzoglu, M.A. 1981. Modern Poplar Development Handbook. Poplar and Fast Growing Forest Trees Research Institute, Izmit, Turkey.
- Toplu, F. 1996. Breeding and conservation of genetic resources of Black Poplar (*Populus nigra* L.) in Turkey. Proceedings of the 20th Session of International Poplar Commission Budapest,1–4 October 1996, Volume II (I. Bach, ed.)
- Yaltırık, F. 1973. Taxonomy of Black Poplars growing in Turkey. I.U.Orman Fakultesi Dergisi.Seri.B, Sayı.2, Istanbul.

# In situ conservation

### Poplars and biodiversity

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Floodplain forests, the natural habitat of indigenous black poplar (*Populus nigra L.*), are among the most diverse ecosystems in Europe (Gepp *et al.* 1985). In Austria, for example, it was estimated that at least 12 000 species of animals and plants regularly inhabit the floodplains of the Danube (Gepp *et al.* 1985). According to Gerken (1988) more than 1000 species of beetles, most of the indigenous amphibians, 400–500 species of large butterflies (more than one third of all existing species) and between 150 and 200 species of birds occur in different floodplain habitats. Table 1 shows the numbers of invertebrates that have been recorded in the floodplains of the Rhine.

**Table 1.** Number of species of invertebrates in the floodplains of the Rhine, according to Tittizer and Krebs (1996)

Order	Number of species
Mollusca (land snails)	>60
Mollusca (water snails and mussels)	30–40
Odonata (dragonflies)	50
Coleoptera (beetles)	>1000
Lepidoptera (butterflies)	1000
Arachnida (spiders)	>100

Many of the species are highly specialized and depend on alluvial habitats. For example, 29% of the amphibians, 27% of the carabids, 20% of the reptiles and 12% of the dragonfly species in Switzerland occur uniquely or primarily in alluvial habitats (Walter *et al.* 1998). Undisturbed floodplain ecosystems are not only very rich in species, but also provide a unique or very important habitat for numerous threatened species and thus play a crucial role for species conservation. For insects, mammals, birds, reptiles and amphibians in Switzerland, for example, 17.5% of extinct species, 27% of those that are nearly extinct, 19% of highly threatened species and 11% of threatened species live exclusively, or primarily, in alluvial habitats (Walter *et al.* 1998).

Floodplains support a high level of biodiversity because they themselves represent a broad range of habitats with very different structures which are temporally dynamic. The small-scale mosaic of various habitats, in combination with varying water levels and frequent disturbances, creates a diversity of vegetation types which vary across the habitat to provide horizontally as well as vertically structured forests in various stages of development. The various animal and plant species which are characteristic of each particular succession stage may thus be found in close proximity. Although there are constant changes within the floodplain ecosystem, the number and area of the different habitats are astonishingly constant over time. This continuity, in combination with the high diversity of habitats, different stages of maturation and a mosaic of different horizontal and vertical structures, permits the maintenance of high and stable species richness over time (Gepp *et al.* 1985).

In contrast, poplar plantations are often criticized as they are considered to be highly unnatural. The use of non-indigenous species, hybrids and clones which are not integrated into the natural ecosystems are thought to have detrimental effects on the native fauna (Blab and Kudrna 1982; Dickson and Whitham 1996; LFU 1996; Waltz and Whitham 1997). In addition, the unnatural stand structures of such plantations, which are commonly monospecific with little or no vertical structure as compared to natural floodplain forests, are also thought to have negative effects on fauna (Gerken 1980; Späth 1981; Handke and Handke 1982; Dorsch and Dorsch 1991; Twedt *et al.* 1999) and flora (Hügin 1981; Schuldes and Kübler 1991).

Poplars are often cultivated in alluvial habitats where site conditions are optimal for their growth. The natural floodplain forests are replaced by stands with a different, highly artificial structure and composition. Specialized and threatened species depending on alluvial forests may be negatively affected by such plantations.

This paper attempts to give a brief overview of the current state of knowledge regarding poplars and their role and importance for the biodiversity and conservation of associated plant and animal species. The paper primarily addresses three major topics: (1) the value of poplars as a food source or habitat for herbivores and other species, (2) the role and impacts of poplar hybrids ( $P. \times euramericana$ ) and (3) of poplar plantations on biodiversity.

#### Poplars and biodiversity

Black and white poplars are natural elements of a highly diverse ecosystem. The role and importance of poplar species in contributing to the high biodiversity of alluvial forests is, however, poorly understood for a variety of reasons. Firstly, associations between tree species and biodiversity are difficult to investigate because a number of factors other than the species itself affect biodiversity. Factors such as climate, soil and water conditions, quality and distribution of habitats, age, structure, abundance and quality of the host species, as well as the demography of a given herbivore, form a complicated web which is difficult to disentangle. Furthermore, information available on species-host relationships in the literature has not been collected systematically. Since certain species or groups of species have been studied in more detail than others, these species or groups are clearly overrepresented while others are completely lacking. This is especially true for poplars. The literature on species-host relationships concentrates almost exclusively on insects and fungi which cause economically significant losses in poplar plantations (for an overview, see FAO 1979 or Delplanque 1998). The general literature dealing with species-host relationships, on the other hand, is mostly based on field observations made by entomologists. Many of the observations are rather general and lack proper scientific verification. Information on poplars, for example, is generally available only at the level of the genus (Populus ssp.), including planted hybrids. Finally, a difficulty arises from the fact that monophagous herbivores are rather an exception. Many so-called monophagous herbivores feed on a whole genus and are thus oligophagous in a strict sense. Moreover, certain herbivores will use a given host plant in certain situations but abandon it as a food source under a different set of conditions. These limitations must be borne in mind when interpreting the following results and statistics.

According to Grechkin and Vorontsov (1962, cited in Georgiev and Beshkov 2000), more than 700 insect species have been recorded as being associated with the genus *Populus*. Delplanque (1998) lists more than 650 species of insects which are associated with poplars (Table 2). However, many of these species are polyphagous and feed on poplar species as well as a number of other hosts. Although poplars may play an important role for a high number of unspecialized (euryphagous) herbivores, these polyphagous herbivores are not part of the following overview. It concentrates on the faunal biodiversity which exclusively or primarily depends on poplar species as a food source.

Insect families	Number of species	Insect families	Number of species
	associated with poplars		associated with poplars
Scolytidae	11	Cephidae	1
Buprestidae	16	Cimbicidae	3
Cerambicidae	17	Tenthredinidae	22
Chrysomelidae	30	Vespoidea	1
Curculionidae	56	Apoidae	1
Lucanidae	2	Thysanopteridae	9
Melonthidae	5	Cicadoidae	3
Rutelidae	4	Flatidae	1
Meloidae	1	Cercopidae	3
Nymphalidae	5	Membracidae	2
Nepticulidae	10	Cicadelidae	24
Cossidae	2	Aphididae	28
Cochlididae	1	Ortheziidae	1
Lyonetidae	1	Pseudococcidae	1
Graclillariidae	8	Coccidae	6
Phyllocnistidae	3	Diaspididae	9
Oecophoridae	4	Pentatomidae	8
Coleophoridae	3	Coreidae	2
Gelechiidae	3	Tingidae	1
Yponomeutidae	2	Lygaeidae	5
Sesiidae	3	Miridae	6
Tortricidae	43	Tetranychidae	3
Cochylidae	1	Eriophyiidae	9
Pyralidae	3	Phytoseiidae	32
Lasiocampidae	6	Coccinellidae	10
Attacidae	1	Carabidae	10
Thyatiridae	4	Staphylinidae	1
Geometridae	27	Chrysopidae	1
Sphingidae	2	Hemerbiidae	1
Notodontidae	19	Syrphidae	4
Lymantriidae	7	Spheciade	64
Arctiidae	3	Formicidae	6
Noctuidae	32	Pentatomidae	2
Agromyzidae	7	Nabidae	3
Cecidomidae	24	Reduviidae	1
Panphiliidae	2	Anthocoridae	5
Xiphidridiidae	1	Miridae	4
Sicicidae	1	Total	663

Table 2. Insects associated with poplars, according to Delplanque (1998)

Heydemann (1982) has published the numbers of specialized (stenophagous) species of herbivores which are associated with the most important trees and shrubs, including *P. nigra*, *P. alba* and *P. tremula* as well as the genus *Populus* in Central Europe (specifically Schleswig Holstein) (Table 3).

The genus *Populus* appears in the upper fourth of the ordered table (in seventh position), being host to 88 stenophagous species of herbivores. This compares with *Salix* and *Quercus* which respectively host twice and three times the number of specialized species. While *P. tremula* hosts a comparatively high number of species, *P. alba* and *P. nigra* are situated in the lower half of the table with 25 and 18 species of associated herbivores, respectively.

Somewhat higher but comparable numbers of stenophagous species associated with the genus *Populus* were reported by Southwood (1961) for the United Kingdom and Sweden. In

**Table 3.** Number of species of stenophagous phytophagous insects associated with important tree and shrub species in Central Europe (Schleswig-Holstein), based on data published by Heydemann (1982)

Tree/shrub species									Ins	sect	arour	os							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Quercus spp	12	12	5	3	0	4	0	75	10	16	70	15	2	16	8	22	24	4	298
Salix spp.	17	9	6	1	1	7	õ	0	33	16	38	6	3	16	6	23	25	11	218
Betula spp.	8	10	4	3	1	3	1	Õ	18	11	27	10	2	17	6	30	q	4	164
Pinus sylvestris	22	5	4	3	Ö	2	2	õ	1	16	42	54	0	1	1	6	1	2	162
Picea abies	17	4	12	1	Õ	1	0	Õ	1	10	44	44	Õ	1	1	11	2	1	150
Fagus sylvatica	6	2	1	2	õ	1	õ	Õ	0	6	38	19	Õ	7	3	7	4	4	100
Populus spp.	7	0	4	2	0	3	0	0	15	0	24	15	0	7	4	0	6	1	88
Ulmus spp.	2	4	6	4	0	2	0	0	0	3	28	19	2	0	2	1	5	1	79
Corvlus avellana	6	3	1	0	1	2	0	0	15	7	25	10	1	1	0	3	0	1	76
Populus tremula	2	4	1	0	0	0	0	0	0	17	0	9	2	12	0	0	12	8	67
Prunus spinosa	2	0	5	3	0	0	0	0	0	9	15	0	2	5	0	16	7	3	67
Alnus spp.	0	0	0	4	0	5	0	0	0	3	24	10	0	5	2	0	5	3	61
Crataequs spp.	2	1	7	0	1	0	0	0	5	6	10	2	1	4	0	12	6	3	60
Carpinus betulus	0	5	1	4	2	1	0	0	0	3	23	14	0	2	1	0	0	3	59
Abies alba	1	0	9	1	0	0	0	0	0	0	19	28	0	0	0	0	0	0	58
<i>Tilia</i> spp.	6	3	1	1	0	4	2	0	0	3	18	4	0	3	2	1	4	5	57
Alnus glutinosa	9	10	2	2	0	0	0	0	7	10	0	0	0	0	0	14	0	0	54
Larix spp.	3	0	1	0	0	2	0	0	1	2	9	31	0	0	0	1	0	0	50
Fraxinus excelsior	6	0	1	3	1	2	0	0	0	2	10	12	0	1	2	2	2	3	47
Malus sylvestris	5	0	5	3	0	0	0	0	0	10	0	8	1	4	1	4	3	1	45
Pyrus piraster	3	0	6	1	1	0	1	0	0	9	9	5	1	0	0	6	1	2	45
Vaccinium myrt.	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	18	16	2	40
<i>Rosa</i> spp.	0	1	9	2	1	0	1	10	1	3	8	0	0	0	0	0	1	1	38
Prunus padus	0	0	3	3	0	0	0	0	0	4	15	2	2	4	0	3	0	0	36
Salix alba	0	6	7	0	0	0	0	0	0	19	0	0	0	0	0	0	0	3	35
Salix aurita	0	67	3	0	0	0	0	0	0	16	0	0	0	0	0	0	0	9	35
Salix cinerea	0	2	7	0	0	0	0	0	0	14	0	0	0	0	0	0	0	11	34
Salix caprea	0	2	6	0	0	0	0	0	0	17	0	0	0	1	0	0	0	7	33
<i>Rubus</i> spp.	0	2	3	0	1	0	0	1	1	2	2	1	0	0	2	5	10	2	32
Prunus avium	0	2	3	3	0	0	0	0	0	10	0	7	2	4	0	0	0	0	31
Salix viminalis	0	3	5	0	0	0	0	0	0	18	0	0	0	0	0	0	0	3	29
Lonicera spp.	0	0	3	2	2	0	0	0	0	0	2	0	1	0	2	6	5	3	26
Sorbus aucuparia	1	1	3	3	0	0	0	0	1	0	2	6	0	0	1	5	1	2	26
Populus alba	0	4	2	0	0	0	0	0	0	11	0	4	0	1	0	0	0	3	25
Acer spp.	0	3	0	0	2	0	1	1	0	1	0	4	0	0	0	7	2	3	24
Ribes spp.	2	0	8	3	0	0	0	0	0	0	0	0	1	0	1	3	0	1	19
Populus nigra	2	0	5	0	0	0	0	0	0	9	0	0	0	0	0	0	2	0	18
Salix repens	0	3	2	0	0	0	0	0	0	2	0	0	0	1	0	0	0	9	17
Frangula alnus	1	0	1	0	0	0	0	0	0	0	6	4	3	1	0	0	0	1	17
Acer campestre	0	1	4	0	1	0	0	0	0	0	0	1	0	0	0	0	0	3	16
Rnamnus catnart.	0	0	1	0	0	0	0	0	0	0	6	4	0	0	0	4	0	0	15
Euonymus erop.	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	11
Salix pentandra	0	0	2	0	0	0	0	0	0	3	0	1	0	0	0	4	0	4	9
Sambucus spp.	0	0	3 ⊿	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	9
Viburnum opulus	0	1	4	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	/ 6
Vaccinium uliginas	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	2	2	0	0 5
l igustrum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<u>^</u>	∠ 3	1	5
Svringia vulgaris	0	0	0	0	0	0	0	0	0	0	0	о 3	0	0	1	0	1	0	5
Hedera heliv	ñ	ñ	ñ	ñ	n	n	n	0	ñ	ñ	3	1	ñ	ñ	0	1	0	ñ	5
Hinnonhae rhamn	õ	õ	1	ñ	0	n	0	n	õ	õ	õ	0	õ	õ	õ	0	õ	õ	1
llex aquifolium	õ	Õ	0	õ	0	0	0	Õ	Õ	Õ	Õ	õ	Õ	Õ	Õ	1	Õ	õ	1

Key to insect groups: 1 = Heteroptera (SH); 2 = Cicadina (SH); 3 = Aphidina; 4 = Coccoidea; 5 = Aleurodina; 6 = Thyanoptera; 7 = Copeognatha; 8 = Cynipidae; 9 = Chrysomelidae; 10 = Curculionidae; 11 = Cerambicidae; 12 = Scolytidae/Platypodiale; 13 = Rhopalocera (SH); 14 = Bombycoidea; 15 = Arctiidea, Sphingidea und small Lepidoptera families; 16 = Geometridea (SH); 17 = Noctuidea (SH); 18 = Cecidomyidea.
SH = data from Schleswig-Holstein only.

the United Kingdom, 97 species of insects were found to live primarily on poplars (fifth position), among them 78 species of Lepidoptera and Coleoptera, while 114 species were reported for Sweden. Similar results for the United Kingdom were published by Carter *et al.* (1979). Their results are summarized in Table 4.

**Table 4**. Number of phytophagous insects feeding on the most important tree species in the United Kingdom, according to Carter *et al.* (1979)

Tree/shrub species	Insect groups						
	Heteroptera	Homoptera	Makrolepidoptera	Mikrolepidoptera	Coleptera	Total	
Quercus spp.	37	10	106	81	50	284	
Salix spp.	22	20	100	73	51	266	
<i>Betula</i> spp.	12	4	94	84	35	229	
Crataegus spp.	17	1	64	53	14	149	
Prunus spinosa	4	2	48	43	12	109	
<i>Populus</i> spp.	8	11	33	26	19	97	
Malus domestica	18	3	21	42	9	93	
Pinus sylvestris	15	3	10	28	35	91	
Alnus glutinosa	14	8	28	27	13	90	
<i>Ulmus</i> spp.	11	4	33	26	10	82	
Corylus avellana	16	2	18	28	9	73	
Fagus sylvatica	4	3	24	16	17	64	
Fraxinus excelsior	10	2	16	9	4	41	
Picea abies	9	1	6	13	8	32	
<i>Tilia</i> spp.	7	2	15	5	2	31	
Carpinus betulus	1	0	7	16	4	28	
Acer campestre	2	2	8	12	2	26	
Juniperus comm.	6	0	4	8	2	20	
Larix spp.	3	0	6	6	2	17	

In terms of the number of associated species, the genus *Populus* is situated in the upper third (sixth position) of the tree and shrub species listed, although it supports less than half the species richness found on *Quercus, Salix* or *Betula* (Table 4).

The best and most complete overview of associations of host trees and herbivores, including poplar species, is given by Hondong (1994). Unfortunately, this very valuable compilation has not been published and is available only as a manuscript. For this reason, the results are reported here in detail. Hondong's work is especially valuable because it compiles all information on species–host associations available in the German literature on phytophagous insects. Moreover, herbivorous insects were classified on the basis of how endangered they are, thus providing information on the importance of host species for the conservation of endangered species of herbivores. Although most information sources relate to Germany, Hondong's compilation provides very valuable and unique information on species–host associations which in most cases should closely reflect the situation in Central Europe. It is interesting to note here that poplar–biodiversity relationships as well as possible impacts of poplar cultivation on biodiversity have mainly been studied in Germany. These topics have been of special interest because of the criticism that poplar plantations have encountered from nature conservation organizations in this country.

For the higher butterflies (Rhopalocera), skippers (Grypocera) and burnets (Zygaenidae), the following results are based on data from Baden-Württemberg (Ebert and Rennwald 1991), Central Europe (Weidemann 1986, 1988), Switzerland (SBN 1991) and Germany (Blab and Kudrna 1982). The larvae of these insects generally feed on buds, leaves and needles. A total of 177 species have been recorded in Germany, of which 91 (or more than half) are classified as threatened and 2 are now extinct. The importance of the most common tree and shrub species as a food source for the mentioned groups is presented in Table 5.

**Table 5.** Importance of tree and shrub genera as hosts for the larvae of higher butterfly (Rhopalocera), skipper (Grypocera) and burnet (Zygaenidae) species (after Hondong 1994). The numbers indicate how many species feed on each host

Geographic area	Baden-Württ	temberg <sup>1</sup>	Germ	nany <sup>2</sup>	Switzerland <sup>4</sup>
Endangerment	Total number	Red list	Total	Total	Total number
-	Total number	species	number	number	Total number
Tree/shrub genera					
Prunus	6	6	6	8	6
Salix	6	5	9	3	5
Populus	5	5	3	3	2
Frangula	5	3	0	2	2
Cornus	3	2	1	2	1
Lonicera	3	2	3	3	2
Rubus	3	2	1	7	1
Ulmus	3	2	3	3	3
Betula	2	2	1	2	2
Crataegus	2	2	1	4	1
Pyrus	2	2	0	0	1
Quercus	2	2	2	2	2
Rhamnus	2	1	0	3	3
Ribes	2	1	0	2	1
Humulus	2	0	1	1	1
Fraxinus	1	1	1	1	0
Malus	1	1	0	0	1
Rosa	1	1	0	0	1
Sorbus	1	1	0	0	1
Ulex	1	1	0	2	0
Corylus	1	0	0	1	1
Hedera	1	0	1	0	0
Ligustrum	1	0	0	0	0
Euonymus	0	0	1	0	0
Hippophae	0	0	1	0	0
Sambucus	0	0	0	1	0

Source of information and groups included:

1 Ebert and Rennewald (1991); Rhopalocera and Grypocera.

2 Weidemann (1986, 1988); Rhopalocera and Grypocera

3 Blab and Kudrna (1982); Rhopalocera, Grypocera and Zygaenidae

4 SBN (1991); Rhopalocera

On the basis of the data from Baden-Württemberg (based on actual, verified field observations, while the other sources are based on literature records only), the genus *Populus* is an important food source for three groups of Lepidoptera. It is ranked third after *Prunus* and *Salix*. Moreover, all recorded species depending on *Populus* are listed as threatened species. According to the other less reliable sources of information, *Populus* has an average importance as a food source for these groups of Lepidoptera.

According to Koch (1984, cited in Brockmann 1991), 117 or 10% of the 1200 species of butterflies (Rhopalocera) are associated with the genus *Populus*. The number of butterfly species whose larvae feed on the different poplar species is listed in Table 6. From this table it can be seen that only a very small number of butterfly species associated with the genus *Populus* restrict themselves to indigenous black poplar alone. Most of them are able to feed equally on all poplar species and hybrids; they are thus oligophagous on the genus *Populus*. A rather large number of them even feed on *Populus* and *Salix*. Within the genus *Populus*, *P. tremula* seems to be by far the most important host for the monophagous Lepidoptera.

All poplar species	78
Populus alba	2
Populus tremula	46
Populus nigra	11
Populus nigra subsp. italica	2
Populus × euramericana	5
Populus deltoides	1
Populus and Salix species	74

**Table 6.** Associations of butterfly species (Rhopalocera) with poplars (and willows) according to Koch (1984). The numbers indicate how many species feed on each host

Food preferences of moth species belonging to the Bombycoidea and Sphingoidea were analysed by Peterson (1984). The study included 277 moth species of Central Europe, of which 255 were found in Germany with 104 of them being threatened. The larvae of most of these species are phytophagous and only occasionally feed on roots or wood. Table 7 lists the number of moth species associated with the most important genera of trees and shrubs.

The genus *Populus* is ranked second after *Salix*. More than two thirds of the associated 32 species feed exclusively or primarily on poplars. Together with *Quercus*, the genus *Populus* is a food source for the highest number of threatened moth species in Germany. *Populus* even hosts the highest number of threatened species which primarily depend on a genus.

Another group of important phytophagous species are the beetles (Coleoptera), especially long-horned beetles (Cerambycidae), bark beetles (Scolytidae), weevils (Curculionidae), fungus weevils (Anthribidae) and leaf beetles (Chrysomelidae).

A total of 175 species of long-horned beetles exist in Germany; 101 of them are listed as threatened or extinct. Only a small proportion of the Cerambicidae is phytophagous; most of the species live on decaying wood of dead trees and shrubs, which explains the high proportion of threatened species. Table 8 lists trees and shrubs which serve as food sources for the larval stage of phytophagous long-horned beetles in Central Europe, based on data from Koch (1992).

With 40 species of phytophagous long-horned beetles, the genus *Populus* is ranked in sixth position. Poplars seem to be a rather important host species for this group of herbivores, since half of these species are classified as threatened in Germany.

A total of 946 species of weevils (Rhynchophora) exist in Germany, with 318 species classified as threatened. While the fungus weevils (Anthribidae) and the bark beetles (Scolytidae) mostly feed on wood, the true weevils (Curculionidae) are mostly phytophagous.

Bark beetles are primarily associated with conifers (Table 9). As regards broadleaf species, *Populus* is ranked in fifth position having only three associated species fewer than *Quercus*.

The group of true weevils (Curculionidae) contains mostly species of phytophagous beetles which generally feed in a polyphagous manner on certain plants (Blab *et al.* 1984). An overview of species–host associations is presented in Table 10. Most species are polyphagous on broadleaves. For the mono- or oligophagous species, however, the genus *Populus* seems rather important, being the third most important host after *Salix* and *Quercus*. Of the species that feed primarily on *Populus*, however, only a small number are classified as endangered in Germany.

**Table 7.** Host genera for the larvae of moth species (Bombycoidea and Sphingoidea). The numbers indicate how many species feed on each host, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Peterson (1984)

Tree/shrub genera	Nu	mber of m	oth specie	S
	1	2	3	4
Salix	35	17	13	6
Populus	32	22	14	9
Quercus	30	17	14	8
Betula	25	9	12	5
Polyphagous on trees and shrubs	23	0	7	0
Polyphagous on broadleaves	22	0	4	0
Fagus	17	5	4	0
Alnus	13	4	5	3
Prunus	10	1	4	0
Tilia	9	1	5	1
Rubus	9	3	3	0
Picea	7	1	2	0
Pinus	6	2	2	1
Abies	6	0	2	0
Sorbus	5	0	2	0
Corylus	5	0	1	0
Ulmus	4	1	2	1
Crataegus	4	1	1	0
Carpinus	4	1	1	1
Acer	3	2	2	1
Polyphagous on conifers	3	0	1	0
Larix	3	1	0	0
Lonicera	2	0	1	0
Fraxinus	2	0	1	0
Malus	2	1	0	0
Rosa	2	0	0	0
Ligustrum	1	0	0	0
Viburnum	1	0	0	0
Ribes	1	0	0	0
Euonymus	1	0	0	0
Juniperus	1	0	0	0

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

**Table 8.** Species of long-horned beetles (Cerambycidae) in Central Europe whose larvae feed on trees and shrubs. The numbers indicate how many species feed on each host, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Koch (1992)

Tree/shrub genera	Number of Cerambycidae species						
	1	2	3	4			
Quercus	91	32	34	15			
Fagus	56	7	24	2			
Pinus	53	22	24	10			
Picea	49	15	21	7			
Salix	42	7	15	2			
Populus	40	8	20	6			
Ulmus	36	0	14	0			
Castanea	35	2	14	0			
Prunus	35	2	8	0			
Alnus	34	2	15	1			
Betula	33	1	7	0			
Tilia	31	6	14	3			
Acer	24	2	11	1			
Abies	24	1	11	0			
Corylus	24	2	8	0			
Pyrus	24	0	2	0			
Carpinus	20	0	9	0			
Juglans	19	0	11	0			
Larix	19	3	6	1			
Malus	19	1	2	0			
Aesculus	15	2	9	1			
Fraxinus	15	0	6	0			
Polyphagous on broadleaves	15	0	0	0			
Polyphagous on conifers	9	0	1	0			
Robinia	8	0	5	0			
Crataegus	8	0	2	1			
Rhamnus	5	1	2	1			
Euomymus	5	0	0	0			
Sorbus	3	0	2	0			
Juniperus	3	0	1	0			
Rosa	3	0	0	0			
Lonicera	2	2	1	1			
Rubus	2	0	1	0			
Viburnum	2	0	0	0			

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

**Table 9.** Species of bark beetles (Scolytidae) occurring in Central Europe whose larvae feed on trees and shrubs. The numbers indicate how many species feed on each host, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Koch (1992)

Tree/shrub genera	Number of Scolytidae species						
	1	2	3	4			
Pinus	63	42	13	8			
Picea	50	21	9	4			
Abies	31	7	5	3			
Larix	31	2	4	0			
Quercus	17	8	2	0			
Fagus	17	5	1	0			
Ulmus	15	8	4	3			
Populus	14	6	2	1			
Pseudotsuga	14	0	2	0			
Carpinus	12	0	1	0			
Acer	11	0	3	0			
Fraxinus	10	4	1	0			
Alnus	7	3	3	1			
Corylus	7	2	1	1			
Castanea	7	2	0	0			
Tilia	6	2	1	0			
Betula	6	1	0	0			
Juglans	6	0	0	0			
Juniperus	6	0	0	0			
Salix	4	2	2	0			
Pyrus	4	0	1	0			
Polyphagous on broadleaves	4	0	0	0			
Rhamnus	3	1	1	1			
Sorbus	3	0	1	0			
Crataegus	2	0	0	0			
Malus	2	0	0	0			
Robinia	2	0	0	0			

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

A total of 463 different species of leaf beetles (Chysomelidae) occur in Germany. Of these, 183 are either extinct or classified as endangered. The species of this ecologically rather homogeneous group of beetles are mostly oligophagous herbivores feeding on different parts of the plants. The genus *Populus*, ranked in fourth position after *Salix*, *Corylus* and *Quercus*, provides important hosts for this group of herbivores (Table 11). A high proportion of these leaf beetles primarily live on the genus *Populus*, although only a few of them are listed as threatened.

For the fungus weevils (Anthribidae), which are a group with a rather restricted number of species, *Populus* is not an important host species (Table 12).

**Table 10.** Species of weevils (Curculionidae) occurring in Central Europe whose larvae feed on trees and shrubs. The numbers indicate how many species feed on each host, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Koch (1992)

Tree/shrub genera	Number of Curculionidae species						
	1	2	3	4			
Polyphagous on broadleaves	108	0	11	0			
Salix	66	41	8	5			
Quercus	63	41	17	11			
Populus	41	27	4	4			
Polyphagous on conifers	37	0	1	0			
Alnus	34	6	5	1			
Pinus	32	23	6	4			
Betula	28	10	2	1			
Picea	27	8	6	4			
Fagus	24	6	8	1			
Crataegus	22	4	10	5			
Corylus	18	3	3	0			
Rosaceae	18	9	2	1			
Abies	12	4	4	1			
Prunus	12	5	3	2			
Ulmus	11	7	2	0			
Acer	10	7	6	4			
Rubus	9	4	2	1			
Carpinus	9	0	1	0			
Fraxinus	8	6	1	1			
Castanea	6	0	2	0			
Pyrus	6	2	1	0			
Larix	6	1	0	0			
Sorbus	5	1	3	1			
Ligustrum	4	1	0	0			
Rosa	4	0	0	0			
Tilia	3	0	0	0			
Euonymus	1	1	1	1			
Juglans	1	0	1	0			
Cornus	1	1	0	0			
Lonicera	1	1	0	0			
Frangula	1	0	0	0			
Malus	1	0	0	0			

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

In summary, the genus *Populus* hosts a rich complex of phytophagous herbivores. For many species of butterflies, moths and beetles, *Populus* is an important food source. Poplars are exclusive or primary hosts to a high proportion of threatened insect species, especially species of butterflies, moth and long-horned beetles. Poplars thus play an important role in the conservation of a large number of threatened species of herbivores and other species which are associated with them or depend on them.

The current base of knowledge does not permit the ranking in order of importance of the different poplar species as host plants. It seems, however, that monophagous herbivores are the exception rather than the rule. Long-horned beetles, butterflies, true weevils, fungus weevils and leaf beetles seem to utilize the whole genus. Many of the specialized species

even extend their potential hosts to the genus *Salix*. It is possible that the content of salic acid in the Salicaceae is responsible for the attractiveness of the two genera as a food source for many herbivores. It is known from a number of insects that they transform salic acid to a carbol-like substance which serves as a defence mechanism against predators. It is in fact striking that a number of genera in most of the mentioned groups of herbivores have become specialized to live on *Salix* and *Populus* (for example *Saperda*, *Trypophloeus*, *Zeugophora*, *Chalcoides* or *Melasoma*).

**Table 11.** Number of species of leafbeetles (Chrysomelidae) in Central Europe whose larvae feed on trees and shrubs. Given are the number of species feeding on the listed hosts, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Koch (1992)

Tree/shrub genera	Number of Chrysomelidae species					
	1	2	3	4		
Salix	89	42	31	11		
Corylus	39	2	16	2		
Quercus	35	14	15	8		
Populus	35	20	7	2		
Betula	34	1	16	1		
Alnus	20	3	8	1		
Crataegus	20	5	3	1		
Prunus	11	1	2	0		
Sorbus	7	0	3	0		
Pinus	6	5	3	2		
Ulmus	5	3	3	2		
Rosa	4	0	3	0		
Abies	4	1	2	1		
Rubus	4	1	1	0		
Polyphagous on broadleaves	4	0	0	0		
Fraxinus	3	0	2	0		
Juglans	2	0	2	0		
Carpinus	2	0	2	0		
Tilia	2	0	1	0		
Rhamnus	2	0	0	0		
Fagus	2	0	0	0		
Pyrus	1	0	1	0		
Mespilus	1	0	1	0		
Ostrya	1	0	1	0		
Picea	1	0	1	0		
Larix	1	0	0	0		
Juniperus	1	0	0	0		
Viburnum	1	0	0	0		
Cornus	1	0	0	0		
Acer	1	0	0	0		

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

**Table 12.** Number of species of fungus weevils (Anthribidae) in Central Europe whose larvae feed on trees and shrubs. Given are the number of species feeding on the listed hosts, differentiated according to their dependence and status of threat in Germany. After Hondong (1994), based on data from Koch (1992)

Tree/shrub genera	Number of Chrysomelidae species						
	1	2	3	4			
Quercus	14	6	3	1			
Fagus	12	3	3	0			
Alnus	10	0	4	0			
Salix	10	0	2	0			
Crataegus	8	0	4	0			
Corylus	7	0	1	0			
Betula	6	1	4	1			
Prunus	6	0	4	0			
Carpinus	5	1	2	0			
Tilia	5	0	2	0			
Malus	5	0	2	0			
Pinus	4	1	2	0			
Ulmus	4	0	1	0			
Populus	4	0	1	0			
Picea	3	0	2	0			
Polyphagous on broadleaves	3	0	1	0			
Frangula	2	0	1	0			
Sorbus	2	0	1	0			
Fraxinus	1	0	1	0			
Abies	1	0	0	0			
Acer	1	0	0	0			
Rhamnus	1	0	0	0			
Polyphagous on conifers	1	0	0	0			

1 = number of species feeding on the genus.

2 = number of species which primarily feed on the genus.

3 = number of species which are threatened in Germany.

4 = number of threatened species which primarily feed on the genus.

# Poplar plantations and biodiversity

Poplar plantations are often criticized as being unnatural and highly artificial compared to natural forests. In Central Europe, especially in Germany and Switzerland, the cultivation of poplars has decreased drastically in the face of opposition from nature conservation organizations (e.g. Naturschutzbund Deutschland, Späth 1992; Allard and Dufour 1997). The main arguments against poplar plantations can be summarized as follows:

- Hybrid poplars are introduced species. Since they are not part of the natural ecosystems, they may have negative effects on the native fauna and flora.
- The monospecific, single age stand structures, compared to natural forests, have negative impacts on fauna, flora and the landscape.
- Poplars are often cultivated on alluvial sites, replacing highly diverse and highly structured floodplain forest with stands composed of one species, one or few clones and little or no vertical structure.

# Hybrid poplars and their influence on biodiversity

The issue of negative effects of hybrid poplars on the native fauna was discussed by Blab and Kudrna (1982).They claimed that two species of butterflies, the lesser purple emperor

(*Apatura ilia*) and the poplar admiral (*Limenitis populi*) have become endangered as a direct result of the cultivation of poplar hybrids. They argued that the young larvae of both species starved because they were unable to feed on the thicker, tougher leaves of the introduced 'Canadian poplars'. It has subsequently been shown that no such negative effect exists and that Blab and Kudrna (1982) wrongly interpreted and generalized an earlier observation made by Friedrich (1966). Friedrich observed that the larvae of *Apatura ilia* did not consume the offered leaves of *P. balsimifera* for 2 days, grew less and developed faster into the next larval stage but performed normally when feeding on other poplar species (*P. pyramidalis, P. × canadensis, P. tremula* and *P. nigra*). At the same time he observed dead larvae on *P. balsimifera* for oviposition, obviously attracted by its more intense scent. He speculated that *A. ilia* larvae may be 'trapped' by *P. balsimifera* since the females preferred it for oviposition but the larvae were not able to consume the leaves. *L. populi* females were also especially attracted by *P. balsimifera*, although eggs were also frequently found on *P. tremula, P. nigra* and *P. pyramidalis*.

In conclusion, Friedrich writes that, even if P. balsimifera seemed to be more attractive for both species for oviposition, both the size of the trees and their position in the stand as well as the microhabitat were much more important for the attractiveness to the butterflies than the species itself. Although Friedrich's observation has never been scientifically investigated, Blab and Kudrna (1982) reported Friedrich's observation of a possible 'trap effect' of P. balsimifera as if it were a scientifically proven fact. Moreover, they incorrectly extended his observation to both butterfly species and to 'Canadian poplars' without any proof or additional data. Blab and Kudrna were obviously unable to distinguish between balsam poplars (which in fact have thicker and somewhat tougher leaves) and 'Canadian poplars', a name which was commonly used for all P. × *euramericana* hybrids (i.e. crosses between P. nigra and either P. deltoides or P. angualata). Since then, hybrid poplars have had the reputation of having negative effects on the native fauna. It is interesting to note here that Friedrich (1966) came to exactly the opposite conclusion. He believed that the decreasing populations of both butterfly species were primarily a result of the diminishing surface of poplar plantations as an important food source. This example has been described in some detail because it illustrates that facts and assumptions regarding negative effects of cultivated hybrids are often not clearly separated, and that the arguments often lack a scientific basis and are merely misinterpretations or ill-founded generalizations.

There is in fact no scientific evidence for the belief that hybrid poplars or introduced species have negative effects on the native fauna because they are not part of the natural system. Instead, available data tends to suggest that specialized herbivore-host associations operate at the level of the genus rather than the species. Hybrid poplars seem to be utilized by herbivores as a food source in the same way as the native species. In some cases, the introduced species are even preferred over the native species, as the two examples of Apatura ilia and Limenitis populi mentioned above demonstrate. Moth species, for example, form the most significant group of invertebrates in poplar plantations that are presently known (Prater 1993). A wide range of macro-moths are also found on native poplars (Table 13). Since most of the planted hybrids have P. nigra as one parent, it can probably be assumed that most of these moth species also feed on plantation poplars. In fact, studies on leaf grazing by Dagley (1987, cited in Prater 1993) showed that a substantial number of the moth species listed in Table 13 were also present in poplar plantations. The caterpillars occurred on all cultivars examined although it was significantly less on 'Serotina' then on other cultivars. This is an indication that cultivars of hybrid poplars may differ in their value for biodiversity (see later).

Xanthia ocellaris	Pale-lemon sallow	
Acronicta megacephala	Poplar grey	
Furcula bifida	Poplar kitten	
Eligmodonta ziczac	Pebble prominent	
Pheosia tremula	Swallow prominent	
Pterostoma palpina	Pale prominent	
Clostera curtula	Chocolate tip	
Cerura vinula	Puss moth	
Smerinthus ocellata	Eyed hawk	
Laothoe populi	Poplar hawk	

**Table 13.** Typical macro-moths found on poplars, according to Prater (1993)

In contrast, there is evidence that host resistance to insect pests can affect associated species such as arthropods, fungi and birds. According to Dickson and Whitham (1996) or Campbell and Eikenbary (1990), plant resistance traits may affect aphid distribution and performance, for example. Aphids in turn may affect other species in different ways. Dickson and Whitham (1996) described such an interaction chain in natural hybrid cottonwood stands in northern Utah. Plant resistance traits affected the distribution of a common leafgalling aphid (Pemphigus betae), which in turn influenced other community members. A richer arthropod community was observed on trees with high aphid densities relative to those with low aphid densities. Exclusion of the gall aphids on susceptible trees resulted in a 24% decrease in species richness and a 28% decrease in relative abundance of the arthropod community. In addition, exclusion of aphids also caused a two- to threefold decrease in foraging and/or presence of three taxa of aphid enemies, i.e. birds, fungi and insects. These results suggest that resistance traits may have a direct or indirect influence on associated species from different trophic levels. Removing certain genotypes from the populations, for example by breeding activities, or reducing the natural variability of resistance traits in the planting material may thus have serious indirect effects on the diversity of associated species.

While such negative effects may be a consequence of planting any improved material, they may be of special significance for poplars for the following reasons. Poplars are bred very intensively in Europe. According to Kleinschmit (2000), poplars come in fourth place after pine, spruce and oak regarding breeding activities. The reason for this is the high economic value of poplar plantations in Europe. France, for example, has 250 000 ha of poplar plantations which allow for a annual harvest of 3.4 million m<sup>3</sup> of round wood. Regarding annual cut, poplar is the most important broadleaf in France followed by oak with 3.1 million m<sup>3</sup> and beech with 2.3 million m<sup>3</sup> (Villar 1998). Poplars are more susceptible to pests (insects, fungi, bacteria, virus and microplasms) which cause important economic losses than other commercial tree species (Villar 1998). In his compilation of insects associated with poplars, Delplanque (1998) lists more than 650 insects, most of which develop at the expense of the poplars. Consequently, the planting material used for plantations has been strongly selected for resistance traits against major poplar pests. Due to strong selections in the breeding programs, genetic variability is comparatively low in the employed material. Commonly, only a few clones are propagated and used for the plantations. For example in France, currently only 23 cultivars are nationally registered, only 10 of them are propagated and used for the plantations while only 5 make up 80% of the planted area (Villar 1998). Since resistance traits against parasites and fungi are important selection criteria in poplar breeding, and because genetic variation in the used planting material is very low, indirect effects on associated species are likely and probably more severe in poplars compared to other species.

According to Barkman (1958) and Hoffmann (1993), hybrid poplars support a rather high species richness of epiphytes. *P.* × *canadensis* (*P.* × *euramericana*) appears to be relatively rich in epiphyte species compared to other hosts, especially in polluted areas. In the moderately to slightly polluted areas in West Flanders, hybrid poplars were just as rich in epiphyte species as old *Quercus* and *Fraxinus* trees. Poplar cultivars with a rough, rather soft but grooved bark such as '*Robusta*' supported a high species richness while very old, large and strongly grooved specimens with a hard bark and cultivars with a relatively soft and smooth bark were very poor in species (Hoffmann 1993).

In summary, poplar cultivars seem to have negative effects of on biodiversity of associated species. However, these negative impacts are rather a result of the genetic makeup of the planting material used (strongly selected material with very low genetic variability—few clones only) than a consequence of utilizing hybrids or foreign species to which the native fauna is not adapted.

#### Structural features of poplar plantations and biodiversity

There is some scientific evidence that both structure and composition of poplar plantations may have a negative effect on diversity of animals and plants. Poplar plantations are characterized by a much lower structural diversity than natural floodplain forests. In most cases, artificial plantations are monospecific, single-aged stands with little or no vertical structure. These structural features have a direct effect on associated species. It is a commonly observed pattern that mixed-aged stands have a higher level of biodiversity than even-aged stands. Waltz and Whitham (1997), for example, have demonstrated that plant development affects arthropod communities which may then, in a chain of interaction, have a cascade of effects on other species. Their investigation showed that mature zones of cottonwoods (i.e. crowns, flowering branches) supported 23% higher species richness and 108% higher relative abundance of arthropods than juvenile ramets of the same genotypes. In addition, of 17 common arthropod taxa, 8 showed a significantly higher abundance on one developmental zone over the others; 4 were more abundant on mature zones; while 4 were more frequent on juvenile ramets. These results suggest that habitat variability resulting from different developmental stages of host plants with varying levels of nutrition, chemical defence, leaf toughness and other factors affects species richness and abundance and contributes to increased biodiversity in uneven-aged stands. It is highly probable that other species of the food chain, such as predators, will also be affected. For example, Dickson and Whitham (1996) showed that within individual cottonwood trees, avian predators disproportionately foraged on branches where gall aphids (P. betae) were most abundant. After experimental reduction of the gall aphids, avian predation declined threefold relative to control branches. Plant development in time also alters branch architecture which, for example, can affect nest site selection by birds (McArthur and McArthur 1961; Martinsen and Whitham 1994; Waltz and Whitham 1997). Artificial poplar plantations have very little or no developmental variability, and consequently are expected to support less biodiversity than natural floodplain forests with their high structural diversity.

In fact, natural floodplain forests support a very high avian diversity and abundance (Table 14). They are very important for species conservation (Gepp et al 1985) and impacts of forestry, such as transformation into poplar plantations, have been well studied. Compared to natural floodplain forests, avian diversity and abundance is much lower in poplar plantations, as the following examples illustrate.

Floodplain and location	Area of investigation (km <sup>2</sup> )	Number of bird species
Enns/Trautfels	1	50
Donau/Petronell	4.1	64
Rhein delta	4	>70
Donau/Stopfenreuth	8.4	74
March–Thaya floodplains	40	117

**Table 14.** Observed numbers of bird species in floodplain forests of Austria (Gepp *et al.* 1985)

A drastic reduction in the number of species and abundance of birds in poplar plantations has been described in floodplain forests of the Rhine in Baden, Germany by Späth (1981) (Table 15).

**Table 15.** Number of bird species and breeding pairs in floodplain forests and poplar plantations, based on data from Späth (1981)

Type of stand	Age	Number of	Number of	Number of	Size of
		species	breeding	samples	samples
			pairs/10 ha		(ha)
Querco-Ulmetum	100	36–40	155	2	11–12
Querco-Ulmetum	40	32	144	1	12
<i>Querco-Ulmetum</i> with	80	25	142	1	9
hybrid poplars					
Querco-Carpinetum	93	31–35	111	3	9–10
Salicetum albae	50	35–36	109	2	10–15
Hybrid poplars	60	20	82	1	13
Hybrid poplars with	93	26–29	76	2	10–15
understory					
Hybrid poplar with	40	15	32	1	4.4
understory					

Similar results were also reported by Handke and Handke (1982) for floodplain forests of the upper Rhine in Germany (Table 16).

Table 16.	Number	of	bird	species	and	breeding	pairs	in	floodplain	forests	and	poplar
plantations	, after Ha	ndk	ke an	d Handke	e (198	32)						

Type of stand	Number of	Number of	Number of	Area
	species	breeding	samples	surveyed
		pairs/10 ha		(ha)
Querco-Ulmetum	48	238–296	4	20.5
Salicetum albae, rarely flooded	43	190–288	2	7.7
Salicetum albae, regularly flooded	35	75–130	2	3.9
Hybrid poplar plantations	33	46–87	2	23.0

Finally, Späth and Gerken (1985) reported results for floodplain forests in Baden, Germany (Table 17).

According to Bogliani (1988), 10 species of birds nested in poplar plantations even if the ground layers were completely absent (mechanically removed); they were primarily canopy or secondary cavity nesters. Six species nested in the lower strata but only if a shrub layer was present. Other species which commonly occur in natural floodplain forests were very scarce or absent in the plantations. The very low density of some forest passerines was obviously related to the low structural diversity of the poplar plantations.

Type of stand	Number of species	Number of breeding pairs/ 10 ha	Number of samples	Area surveyed (ha)
Querco–Ulmetum	32–40	103–144	3	38.7
Salicetum albae	35–36	103–118	2	25
Ulmo–Carpinetum	31–35	114–117	2	19
Hybrid poplar plantations	5–29	6–27	3	23

**Table 17.** Number of bird species and breeding pairs in floodplain forests and poplar plantations, after Späth and Gerken (1985)

Both age and structure of the stands thus have an influence on species diversity and abundance. According to Karthaus (1990) or Dorsch and Dorsch (1991), the density of the understory is of special significance for bird density. Likewise, Anderson and Ohmart (1983) showed that vegetation density and diversity were both important predictors of avian community measures at the habitat level. Avian density was more closely related to variation in the vegetation than was avian diversity. Poplar plantations generally have no understory or only a poorly developed one, which has direct negative effects on the bird community. In addition, the arthropod community may be negatively affected (see above) which indirectly contributes to a decrease in avian diversity and abundance.

Similarly, Twedt *et al.* (1999) described higher species richness, diversity and territory density of birds in mature (> 30 years) bottomland hardwood stands than in young (6–9 years old) cottonwood (*P. deltoides*) plantations in the Mississippi alluvial valley. Tree species diversity, angular canopy cover, and midstory density were positively associated with bird species assemblage in the mature bottom hardwood stands, whereas vegetation density at ground level was positively associated with bird communities in cottonwood plantations. The authors conclude that mature hardwood forests are twice as valuable for bird conservation as cottonwood plantations, primarily because of the higher variability in composition and structure of the stands.

In contrast, a higher avian diversity in poplar plantations than in alluvial forests has been reported by Godreau *et al.* (1999). Their results, however, show a change in species composition in poplar plantations with semi-open landscape and urban park or garden species and less forest species.

Positive effects of poplar plantations on a number of bird species were also reported by Prater (1993) for the United Kingdom. In particular, the threatened golden oriole (*Oriolus oriolus*) and the barn owl (*Tyto alba*) were found to be associated with poplar plantations in fenland. While the barn owl did not nest within the woodlots but used the young plantations for hunting, the golden oriole is considered a key species for poplar plantations in this region. Its breeding population size has increased as a result of the increasing number of poplar plantations during the 1980s. It is interesting to note here that golden orioles prefer cultivars with big leaves and an early bud break like '*Robusta*' as nesting habitats and clearly select against cultivars with the opposite characteristics such as '*Serotina*'. According to Prater (1993), the positive effects of poplar plantations on bird species in fenland are partly a result of the small amount of suitable woodlands other than poplar plantations as breeding habitats. This example clearly indicates that the biological value of poplar plantations may differ according to the overall situation and always needs to be evaluated in a broader context.

Negative effects of the artificial poplar plantations on natural vegetation have been reported by Schuldes and Kübler (1991) and Hügin (1981). Due to the light crown cover of poplar plantations, neophytes such as *Solidago canadensis*, *Solidago gigantea*, *Reynoutria* 

*japonica, Reynoutria sachalinense, Impatiens glandulifera, Helianthus tuberosus* or *Hertacleum mategazzianum* may develop optimally and become invasive in the stands, reducing the species richness of the original ground vegetation. Likewise, Schnitzler and Muller (1998) describe the invasion of *Fallopia japonica*, a close relative of *F. sachalinensis*, in plantations of cultivated poplars. It may be assumed that the invasion of these plants may not only change floral diversity but also faunal diversity.

According to Barnaud *et al.* (1996), poplar plantations lead to a 'simplification of the original ecosystem' (vegetation as well as animals, especially birds). The change in vegetation cover is the result of either soil treatment (tilled sites for planting), herbicide treatment, mechanical removal of vegetation competition (weeds and bushes) or the changes in light conditions due to the wide spacing of poplars compared to natural forest, or a combination of several of these factors. According to Daudon (1994), the proportions of nitrophilous species such as nettles (*Urtica dioeca*) and big-leafed species are much higher in artificial poplar plantations than in natural forests. In the Garonne valley in France, Karinski (1997) showed that 75 out of 182 plant species found in poplar plantations are typical followers of such artificial plantations.

In summary, there is some scientific evidence that biodiversity in poplar plantations is less than that of natural floodplain forests on the same sites. Negative effects are primarily related to two causes: firstly, the genetically rather uniform, highly selected planting material of low genetic variability used for plantations may negatively affect the arthropod community and all dependent species, such as birds and other predators, resulting in a reduced faunal diversity. Secondly, habitat variability, resulting from both different developmental stages (with varying nutrition, chemical defences, leaf toughness and other factors affecting species richness and abundance) and vertical structures, which contributes to a high biodiversity of arthropods and associated species, is much smaller in poplar plantations than in natural floodplain forest, resulting in a reduced biodiversity. Moreover, changes in arthropod diversity or light conditions result in changes of overall species composition in both animals and plants.

#### References

- Allard, M.-O. and Y. Dufour. 1997. Les impacts floristiques et paysagers du développement de la populiculture en zone prairiale. Héterogénités spatiales et temporelles à différentes échelles. Le cas de la vallée de l'Ouanne [Floristic and environmental impacts of the revelopment of poplar cultivation in floodplains. The case of the Ouanne valley] Rapport de stage, CEMAGREF, Division Ressources génétiques et plants forestiers, Nogent-sur-Vernisson, France.
- Anderson, B.W. and R.D. Ohmart. 1983. Avian and vegetation community structure and their seasonal relationships in the lower Colorado River Valley. Condor 85:392–405.

Barkman, J.J. 1958. Phytosociology and Ecology of Cryptogamic Epiphytes. Van Gorcum, Assen.

- Barnaud, G., C. Fauque, E. de Feraudy and L. Mermet. 1996. Observatoire National des Zones Humides: études préparatoire [National Humid Zone Observatory: preparatory studies]. Rapport intermédiaire, MNHN, IEGB.
- Blab, J. and O. Kudrna, 1982. Hilfsprogramm für Schmetterlinge. Ökologie und Schutz von Tagfaltern und Widderchen [A programme to conserve butterflies. Ecology and conservation of butterflies and Zygaenid moths]. Naturschutz aktuell No. 6, Greven, Germany.
- Blab, J., E. Nowak, W. Trautmann and H. Sukopp. 1984. Rote Liste der gefährdeten Tiere und Pflanzen in der Bundesrepublik Deutschland [Red List of endangered animals and plants in the Federal Republic of Germany]. Naturschutz aktuell 1.
- Bogliani, G. 1988. Densità e scelta dell'habitat degli ucelli nidificanti in pioppeti coltivati [Density and choice of habitat of nesting birds in cutivated poplar]. Riv. ital.Orn. Milano 58 (3–4):129–141.

Brockmann, E. 1991. Die Kulturpappel ('Hybridpappel'), eine Gefahr für die Natur? [Poplar hybrids, are

they a danger for nature?] Vogel und Umwelt 6 (3-4):231-236.

- Campbell, R.K. and R.D. Eikenbary. 1990. Aphid–plant Genotype Interactions. Elsevier, Amsterdam, The Netherlands.
- Carter, C., R.Cobham and R. Lloyd. 1979. Towards a new landscape. Pp. 101–130 *in* After the Elm (B. Clouston and K. Stansfield, eds.). Heinemann, London, UK.
- Daudon, M. 1994. Flore et végétation des Basses Vallées Angevines (Maine-et-Loire): effets de divers modes d'utilisation du milieu [Flora and vegetation of the lower Angevin valleys (Maine-et-Loire): effects of various uses of the environment. Ligues pour la protection des oiseaux, Communauté Economique Européenne, Ministère de l'Environnement, Brussels, Belgium.
- Delplanque, A. (ed.) 1998: Les insectes associés aux peupliers [The insects associated with poplars]. Editions Memor, Brussels, Belgium.
- Dickson, L.L. and T.G. Whitham. 1996. Genetically based plant resistance traits affect arthropods, fungi, and birds. Oecologia 106:400–406.
- Dorsch, H. and I. Dorsch. 1991. Abhängigkeit der Vogelbesiedlung von der Vegetationsstruktur einer Pappelpflanzung [Dependence of avian diversity on vegetation characteristics in a poplar plantation]. Acta Ornithoecol. Jena 2(3):231–259.
- Ebert, G. and E. Rennewald (eds.). 1991. Die Schmetterlinge Baden-Württembergs [Butterflies of Baden-Württemberg (Germany)]. Band 1: Tagfalter I, Band II: Tagfalter II. Ulmer Verlag, Stuttgart, Germany.
- FAO. 1979. Poplars and Willows in Wood Production and Land Use. Forestry Series 10, FAO of the United Nations, Rome, Italy.
- Friedrich, E. 1966. Die Schillerfalter [Purple emperor butterflies]. Neue Brehm Bücherei, Band 505, Wittenberg-Lutherstadt, Germany.
- Georgiev, G. and S. Beshkov. 2000. New and little-known lepidopteran phytophages on poplars (*Populus* ssp.) in Bulgaria. Anzeiger für Schädlingskunde/J. Pest Science 73(1):1–4.
- Gepp, J., N. Baumann, E.P. Kauch and W. Lazowski. 1985. Auengewässer als Ökozellen [The importance of floodplains as ecological niches]. Grüne Reihe des Bundesministeriums für Gesundheit und Umwetschutz No. 4, Vienna, Austria.
- Gerken, B. 1980. Über Tiergemeinschaften der Rheinaue—zur Bedeutung des Wasserhaushaltes und des Zustandes der Waldvegetation [Faunal communities in the floodplains of the river Rhine—importance of the water regime and forest vegetation]. Pp. 352–372 *in* Berichte Internationales Symposium Internationale Vereinigung Vegetationskunde, Rinteln, Germany, 1979.
- Gerken, B. 1988. Auen, verborgene Lebensadern der Natur [Floodplains, the hidden veins of nature]. Rombach, Freiburg, Germany.
- Grechkin, V. and A.I. Vorontsov. 1962. Insect Pests and Diseases of Poplars and their Control. Goslesbumizdat, Moscow, Russia (in Russian).
- Godreau, V., B. Faivre, P. Guichard and B. Frochot. 1999. Breeding bird community in hybrid poplar plantations and alluvial forests in floodplain of Saone (France). International Poplar Symposium, Orleans, 13–17 September. Programme with abstract: 39.
- Handke, K. and U.Handke. 1982. Ergebnisse sechsjähriger Brutvogel-Bestandesaufnahmen im NSG 'Lampertheimer Altrhein', Kr. Bergstrasse (1974–1979) [Results of a six year census of breeding birds in the 'Lampertheimer Altrein' nature reserve, Kr. Bergstrassse (1974–1979)]. Z. Vogelkunde und Naturschutz in Hessen, Vogel und Umwelt 2:75–124.
- Heydemann, B. 1982. Der Einfluss der Waldwirtschaft auf die Wald-Ökosysteme aus zoologischer Sicht [The influence of forestry on forest ecosystems from a zoological perspective]. Schriftenreihe Deutscher Rat für Landespflege 40:926–943.
- Hoffman, M. 1993. Verspreiding, fytosociologie en ecologie van epifyten en epifytengemeenschappen in Oost en West-Vlaanderen [Distribution, phytosociology and ecology of epiphytes and epiphyte communities in east and west Flanders]. PhD thesis, University of Gent, Belgium.
- Hondong, H. 1994. Populus. Übersicht über die Arten und Sektionen, Standort und Gesellschaftsanschluss,

Gefährdung, Fauna, Epiphyten [Populus. Overview of species and sections, sites and associated vegetation, risks, fauna, epiphytes.]. Institut für Landespflege der Universität Freiburg, Germany.

- Hügin, G. 1981. Die Auenwälder des südlichen Oberrheintales—Ihre Veränderung und Gefährdung durch den Rheinausbau [Floodplains of the southern upper Rhine valley—their transformation and endangerment caused by the expansion of navigability]. Landschaft und Stadt 13(2):78–91.
- Karinski, C. 1997. La diversité floristique sous peupleraie: une approche en vallée de Garonne (rapport der BTS) [Diversity of flora in cultivated poplar: an approach in the Garonne valley (BTS report)]. Cemagref, Nogent-sur-Vernisson/INP ENSAT, Toulouse, France.
- Karthaus, G. 1990. Zur ornithoökologischen Bedeutung von Bachufergehölzen in der Kulturlandschaft [The ecological importance for birds of river bank groves in a rural setting]. Natur und Landschaft 65(2):51–57.
- Kleinschmit, J. 2000. Tree breeding and plantations in Europe: A regional situation report. IUFRO WP 2.02.00 and 2.08.00 meeting in Kuala Lumpur, Malaysia, 7 August 2000 (unpublished).
- Koch, K. 1992. Die Käfer Mitteleuropas. Ökologie, Band 3 [Beetles of Central Europe. Volume 3: Ecology]. Goecke und Evers Verlag, Krefeld, Germany.
- Koch, M. 1984. Wir bestimmen Schmetterlinge [The identification of butterflies]. Neumann Verlag, Leipzig, Germany.
- LFU 1996. Pappeln an Fliessgewässern [Poplars on river systems]. Handbuch Wasser 2, Landesanstalt für Umweltschutz Baden Württemberg No. 30, Karlsruhe, Germany.
- MacArthur, R.H. and J.W. MacArthur. 1961. On bird species diversity. Ecology 42:594–598.
- Martinsen, G.D. and T.G. Whitham. 1994. More bird nests in hybrid cottonwood trees. Wilson Bulletin 106:474–481.
- Peterson, M. 1984. Grundlagen eines Hilfsprogrammes für Schmetterlinge (Bombyces und Sphinges) [A basic programme to save butterflies (Bombyces and Sphinges)]. Diplomarbeit am Institut für Landespflege der Universität Hannover, Germany.
- Prater, A. J. 1993. Poplars and wildlife. Quarterly Journal of Forestry 84(4):314–319.
- SBN 1991. Tagfalter und ihre Lebensräume. Schweiz und angrenzende Gebiete. Arten, Gefährdung, Schutz [Butterflies and their habitats. Switzerland and neighbouring regions. Species, risks and protection]. Hollinger Verlag, Zürich, Switzerland.
- Schnitzler, A. and S. Muller. 1998. Ecology and biogeography of highly invasive plants in Europe: giant knotweeds from Japan (*Fallopia japonica* and *F. sachalinensis*). Rev. Ecol. Terre Vie 53(1):3–38.
- Schuldes, H. and R. Kübler. 1991. Neophyten als Problempflanzen im Naturschutz [Neophytes, problematic plants in nature conservation]. Umwelt Kommunal Umweltarchiv 133–34.
- Southwood, T.R.E. 1961. The number of species of insect associated with various trees. J. Anim. Ecol. 30:1– 8.
- Späth, V. 1981. Die Bedeutung der letzten naturnahen Rheinauenwälder für die Vogelwelt [The importance of the last natural floodplain forests of the Rhine for birds.]. Bericht deutsche Sektion internat. Rat Vogelschutz 21:91–96.
- Späth, V. 1992. Naturschutz im Wald [Nature conservation in the forest]. Naturschutzbund Deutschland, e.V. Landesverband Baden-Württemberg, Kornwestheim, Germany.
- Späth, V. and B. Gerken. 1985. Vogelwelt und Waldstruktur. Die Vogelgemeinschaften badischer Rheinauenwälder und ihre Beeinflussung durch die Forstwirtschaft [Avifauna and forest structure. Bird communities in the floodplains of the river Rhine in Baden and how they are influenced by forestry]. Ornith. Jahrb. Baden-Württemberg 1:7–56.
- Tittizer, T. and F. Krebs (eds.) 1996. Ökosystemforschung: Der Rhein und seine Auen [Ecosystem research: the river Rhine and its floodplains]. Springer, Berlin, Germany.
- Twedt, D.J., R.R. Wilson, J.L. Henne-Kerr and R.B. Hamilton. 1999. Impact of forest type and management strategy on avian densities in the Mississippi Alluvial Valley, USA. Forest Ecol. Manage. 123:261–274.
- Villar, M., 1998. Caractéristiques des peupliers et de la populiculture [Characteristics of poplars and their

cultivation]. Pp. 5–9 *in* Les insectes associés aux peupliers. (ed. A. Delplanque). Editions Memor, Brussels, Belgium.

- Walter, Th., M. Umbricht and K. Schneider. 1998. Datenbank zur Fauna in Auen [Floodplain fauna database]. Professur für Natur- und Landschaftsschutz, ETH Zürich (*www.nls.umnw.ethz.ch/8aua/Titel.html*)
- Waltz, A.M. and T.H. Whitham. 1997. Plant development affects arthropod communities: opposing impacts of species removal. Ecology 78(7):2133–2144.
- Weidemann, J.J. 1986. Tagfalter. Band 1: Entwicklung, Lebensweise [Butterflies. Volume 1: Development and living]. Neudam Verlag, Melsungen, Germany.
- Weidemann, J.J. 1988. Tagfalter. Band 2: Biologie—Biotopschutz [Butterflies. Volume 2: Biology—ecology—habitat protection]. Neumann-Neudamm Verlag, Melsungen, Germany.

# Introgressive hybridization in *Populus*: consequences for conservation

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The aim of this paper is to initiate a framework for evaluating the consequences of introgressive hybridization in natural poplar populations, based on current research and published findings. The topics addressed include: (1) concepts and terminology, (2) the reproductive biology of poplar, (3) barriers to hybridization and (4) examples of natural hybridization in poplar.

A major issue for the conservation of native populations is the extent to which genes from exotic species will infiltrate into the native species and cause impacts in the natural population. This is of particular concern for forest trees because they are virtually undomesticated (Bradshaw and Strauss 2001, in Difazio *et al.* 2004), they have the potential for spatially extensive gene flow and they can have large effects on ecosystem processes and biological diversity when they are the dominant life form (Peterken 2001). If a common, sexually compatible species is sympatric with a smaller population of a rare species, then the rare species is vulnerable to extinction via hybridization (genetic assimilation) (Ellstrand 1992).

The genus *Populus* is tremendously varied, with 22 to about 85 species (depending on the interpretation of a 'species') distributed throughout the Northern Hemisphere, in both the temperate and subtropical zones (Farmer 1996; Eckenwalder 1996) (Table 1). The opportunities to generate novel genotypes through hybridization are enormous. Intercrossability among species is one of the foundations of breeding work in the genus Populus (e.g. Zsuffa 1974; Eckenwalder 1996; Stettler et al. 1996). Crossing programmes have revealed both the wide extent of potential intercrossability and its very real limitations (Zsuffa 1974). The description of the earliest cultivars dates back to 1775 (P. serotina Hart) and 1814 (P. regenerata Henry), and numerous others followed (Zsuffa 1975). The impressive silvicultural qualities of the cultivated hybrids (fast growth, good form and easiness of vegetative propagation) have led to widespread production of cultivated poplar plantations in Europe and North America. The use of hybrid poplars expanded from use in windbreaks to producing wood, fibre and fuel, and to remediation of contaminated sites. The presence of many hybrid poplar plantations is frequently considered to pose a severe potential threat for the natural populations of European black polar (Populus nigra L.) in Europe (e.g. Cagelli and Lefèvre 1995; Arens et al. 1998; Lefèvre et al. 2001). A complete review of gene flow in Populus is beyond the scope of this paper.

#### Natural hybridization: concepts and terminology

Various definitions of the terms 'natural hybridization' or 'introgressive hybidization' and 'hybrids' have been used in scientific literature. The term 'hybrid' can be restricted to organisms formed by cross-fertilization between individuals of different species (Rieseberg and Carney 1998). Alternatively, hybrids can be defined more broadly as the offspring between individuals from populations 'which are distinguishable on the basis of one or more heritable characters' (Harrison 1990 in Rieseberg and Carney 1998). Similarly, introgression or introgressive hybridization can be defined narrowly as the movement of genes between species mediated by backcrossing or more broadly defined as the transfer of genes between genetically distinguishable populations.

**Table 1.** Species of each section with their generalised distribution along Eckenwalder (1996). Species designated as s. l. (*sensu lato*) include others that are often recognized as distinct in the literature and which might be retained as subspecies. Some other species also contain additional subspecies or varieties.

	Abaso	Turanga	Leucoides	Aigeiros	Tacamahaca	Populus
W. Eurasia and N. Africa	-	euphratica	_	nigra	laurifolia	<sup>alba</sup> tremula
E. Eurasia	– eup	hratica pruinosa	lasiocarpa glauca,s.l.	nigra, s.l.	ciliata szechuanica yunnanensis suaveolens, s.l. simonii, s.l. laurifolia	alba adenopoda gamblei sieboldii tremula, s.l.
E. Africa N. America	– Ilicifoli mexicana –		– heterophylla	– deltoides fremontii	– balsamifera trichocarpa angustifolia	– simaro- aguzmanantlensis monticola grandidentata tremuloides

A focus on interspecific hybrids requires consideration of species concepts. Unfortunately, the term species has a wide variety of definitions ranging from concepts based on the ability to interbreed to those based on common descent (Rieseberg and Carney 1998). Perhaps the most widely accepted definition is Mayr's (1963 in Rieseberg and Carney 1998) biological species concept: 'species are groups of interbreeding natural populations which are reproductive isolated from all other such groups'. This concept is useful for studies of hybridization and speciation, but it would deny species status of hybridizing taxa if applied stringently (Rieseberg and Carney 1998). Thus, here we will refer to biological species as groups of interbreeding populations that are 'genetically isolated' rather than 'reproductively isolated' from other such groups.

Introgression is achieved in three phases: (1) initial formation of F1 hybrids, (2) their backcrossing to one or another of the parental species, and (3) natural selection of certain favourable recombinant types. This process is simply gene flow between species. Gene flow involves many factors affecting dispersal, establishment, competition, and survival (Difazio *et al.* 2004).

It is important to differentiate between natural hybridization among native species in the absence of human activities, and 'exotic' hybridization events brought about by human disturbances and introductions. For example, major human disturbances have enabled the European white poplar (*P. alba* L.), native to southern Europe, to initiate naturally occurring hybrids on three continents: the grey poplar (*P. × canescens* = *P. alba* × *P. tremula*) in Europe, Rouleau' s poplar (*P. × roulwauiana* = *P. alba* × *P. grandidentata*) in eastern North America, and Chinese white poplar (*P. × tomentosa* = *P. alba* × *P. adenopoda*) in China (OECD 2000). In each area, European white poplar hybridized with a native aspen. In North America, North Europe and China, European white poplar itself was not native, but was introduced as an ornamental tree.

Natural hybridization or introgression can have various consequences (Arnold 1997). At one extreme, natural hybridization may cause a merging of hybridizing species, groups or populations. At the other extreme, introgression may lead to selection for conspecific matings and increased reproductive isolation (Howard 1986 in Martinsen *et al.* 2001) or speciation (=formation of barriers to reproduction) (Martinsen *et al.* 2001).

#### Reproductive biology of Populus and seedling establishment

Populus species are predominantly dioecious and obligatory outcrossers; individual trees are

either male or female. In both sexes, the flowers are clustered in pendulous catkins. Males commonly initiate flowering before females, ensuring that pollen is in the air when the first females are receptive (Farmer and Pitcher 1981). Both sexes flower in early spring (March-April) approximately 1–2 weeks before leaf initiation during the flood peak period along rivers (Braatne et al. 1996). Once chilling requirements have been fulfilled, increasing spring temperatures regulate the seasonal timing of flower emergence (Pauley 1950). Variation in flowering date is due to differences among trees; in *P. deltoides*, this is highly heritable (Farmer 1976 in OECD 2000). The variation in flowering date extends the pollination period from 2 to 3 weeks. Wind-dispersed pollen germinates within the first few hours after pollination. Fertilization takes place several days later and is normally complete within 2 weeks (Farmer and Pitcher 1981). The seed maturation process lasts 4-6 weeks (Larsson 1976 in Barsoum 2001). During this period, the female catkins elongate and swelling green capsules appear along their length (Barsoum 2001). When ripe, the fruit capsules will eventually split in warm dry weather, releasing seeds embedded in significant quantities of pappus (i.e. long white, silky hairs attached to the seed). The pappus of the seeds promotes wind dispersal over great distances; in addition to wind-pollination and obligatory outcrossing, this results in high rates of migration, high gene flow and genetic diversity (Legionnet and Lefèvre 1996).

The timing and duration of flowering and the length of the seed maturation process are related to both the photoperiod and ambient temperatures and therefore vary from one locality to the next, with implications for the timing of seed release (Pauley 1950; Mahoney and Rood 1998 in Barsoum 2001). Poplars are prolific seed producers. A typical 12-m tall P. deltoides specimen was estimated to produce almost 28 million seeds in one season, and estimates for P. tremula have ranged up to 54 million seeds (Schreiner 1974 in OECD 2000). Seed dispersal typically coincides with declining river flows following springtime snowmelt and storm flows, thereby increasing the probability of seeds landing in favourable microsites along the river channel (Braatne et al. 1996). Seedlings colonize moist, recently exposed soil along gravel bars, sandbars and riverbanks within the riparian corridor (Braatne 1999). Poplar seeds are small (2 mm) with little or no endosperm (energy storage), and seed viability is very short, generally lasting only 1-2 weeks under natural conditions (Braatne et al. 1996). Light and continual moisture is required for germination. If river levels decline too rapidly, seedlings succumb to drought stress. Seedlings that establish on moist soils at lower river levels are subject to later removal or damage by the scouring of ice and floodwaters (Braatne et al. 1996). Collectively, these environmental constraints contribute to the infrequent establishment of seedlings, on the order of every 10-20 years depending on climatic conditions and channel morphology (Braatne et al. 1996).

The age of reproductive maturity varies among native species, from 5 to 10 years, yet in some natural populations flowering may not occur until the trees are 15–20 years old (Braatne *et al.* 1996; Stanton and Villar 1996; Braatne 1999). In contrast, hybrid poplars grown in well-maintained plantations commonly attain reproductive maturity in 4 years (Braatne 1999). Poplar species are also capable of asexual, or vegetative, reproduction as an alternative to regeneration from seed. Asexual reproduction is promoted only by flood disturbances when, through extended periods of submergence and/or mechanical damage to parent plants, dormant primordia in roots and shoots are stimulated to produce new shoots and roots (Barsoum 1998 in Barsoum 2001).

#### Isolating mechanisms limiting gene flow

Several authors report on mechanisms that limit gene flow between the hybrid and the parental species. Reproductive isolating mechanisms are generally divided into two categories, based on whether they act before or after fertilization. Mechanisms that act to prevent mating or fertilization are referred to as **prezygotic**, whereas those that act to reduce the viability or fertility of the hybrid zygote or later-generation hybrid offspring are referred to as **postzygotic** (Rieseberg and Carney 1998).

#### **Prezygotic barriers**

Prezygotic isolating mechanisms in plants include **habitat and temporal barriers**, barriers to gene flow due to **pollinator preference** (i.e. ethological isolation, not for wind-pollinated forms) as well as **gametic competition** or **incompatibility**. In *Populus* species, which are wind pollinated and dioecious with one exception, the opportunity for natural hybridization can be limited if flowering period is non-overlapping.

In the context of poplar breeding programmes, interspecific incompatibility due to gametic incompatibility is intensively studied. Interspecific mixtures of viable incompatible and killed compatible (mentor/recognition) pollen have been used to achieve incompatible mating, with variable success (e.g. Stettler 1968; Knox *et al.* 1972; Stettler *et al.* 1980).

Interspecific pollen competition has also been observed in *Populus* in experimental studies. Rajora (1989) observed competition among pollen of P. deltoides, P. nigra and P. maximowiczii in fertilizing P. deltoides ovules. The low frequencies of interspecific matings relative to conspecific matings in pollen-mix controlled crosses suggest conspecific pollen advantage (Rajora 1989). As a result, the relative fertilization success of a pollen species depends upon the species constitution of the pollen mix. Similar results were obtained by Benetka et al. (2002) and Vanden Broeck et al. (2003) in cross experiments with P. nigra females. When  $P. \times$  canadensis pollen was used in mixtures with P. nigra pollen to controlpollinate P. nigra females, most of the seedlings were fathered by P. nigra. The mean percentage of introgressive hybridization obtained in the controlled cross experiments by Benetka et al. (2002) and Vanden Broeck et al. (2003) was 7.14 and 4.6, respectively. When twice as much pollen of *P*. × *canadensis* than pollen of *P*. *nigra* was used in the pollen mix crosses, the frequency of hybrid seedlings was higher then in the case of equal pollen proportions, although the difference was not statistically significant (Vanden Broeck et al. 2003). Pollen competition was also studied in detail for Louisiana irises. These studies have focused on conspecific pollen advantage, including measurements of pollen tube lengths in conspecific and heterospecific styles and examination of hybrid seed production following different types of controlled pollinations (for a review, see Rieseberg and Carney 1998). Although several studies suggest that faster growth of conspecific pollen tubes might be acting as a barrier to hybridization, its importance relative to postzygotic factors, like for example hybrid inviability resulting in embryo abortion, could not be determined.

#### **Postzygotic barriers**

Common postmating reproductive barriers include **hybrid sterility**, **hybrid weakness or inviability**, **and hybrid breakdown** in which first-generation (F1) hybrids are vigorous (hybrid superiority due to heterosis), robust and fertile, but later-generation hybrids are weak or non-viable (Rieseberg and Carney 1998). Poplar hybrids are characterized by reduced fertility relative to parental species; pollen and seed viability is significantly lower in F1 hybrids (Stettler *et al.* 1996; Braatne 1999). Interspecific crosses in *Populus* can result in F1 genotypes that are more susceptible to insect and pathogen attack than parental species (Eckenwalder 1984a; Whitham *et al.* 1999). This reduction in defence mechanism has been observed in poplar hybrids growing in commercial plantations and natural zones of hybridization (Braatne 1999). However, recent analyses have found that poplar hybrids are not uniformly unfit, but rather are genotypic classes that possess lower, equivalent or higher levels of fitness relative to their parental taxa. Schweitzer *et al.* (2002) found that F-
generations of *P. fremontii* × *P. angustifolia* and backcross generations can be just as fit as the parent taxa. F-generations produced as many viable seed as *P. angustifolia* and backcross genotypes produced as many viable seeds as both parent taxa. Moreover, hybrids produced nearly two and four times as many ramets from root sprouts as *P. angustifolia* and *P. fremontii* respectively. Extensive variability in viability and fertility is also observed within and between hybrid generations from the same interspecific cross. Therefore, extremely low fertility or viability of early-generation hybrids (e.g. F1, F2, B1) does not necessarily prevent extensive gene flow and the establishment of new evolutionary lineages (Arnold *et al.* 2001).

Over the past few decades, considerable progress has been made towards understanding the genetic basis of hybrid inviability, sterility and breakdown (Rieseberg and Carney 1998). In crosses between chromosomally divergent species, sterility is typically attributed to the effects of chromosomal rearrangements on meiotic pairing. However, this assumption has recently been questioned and several authors have suggested that genic factors may explain much of the loss of fertility typically attributed to chromosomal rearrangements (for a review see Rieseberg and Carney 1998). In *Populus*, incompatibility in the temporal patterns of capsule and embryo maturation is a critical factor regulating fertility. If the capsules and embryos of hybrid females lack synchronous patterns of development, capsules will mature before embryos, resulting in the production of non-viable seed (Stettler *et al.* 1996).

#### Hybrids as filters

Detailed studies of the genetic basis of hybrid breakdown between subspecies in rice suggest that hybrid weakness appears to result from the break-up of coadapted gene complexes that affect fitness traits. If many genes contribute to fitness traits, then much of the genome may be resistant to introgression because of linkage. These results also suggest that species genomes are often differentially permeable to introgression, where certain portions of the genome are open to the incorporation of alien alleles, but introgression is restricted in other parts of the genome. Different selection pressures for different genomic regions were also observed in *Populus* (Martinsen *et al.* 2001).

The results of Martinsen et al. (2001) indicate that the hybrid zone between natural populations of P. fremontii and P. angustifolia (P.  $\times$  hinckleyana = natural hybrid between P. *fremontii* × *P. angustifolia*) located near the river Weber (Utah, USA), acts as an evolutionary filter, preventing the introgression of most genes but allowing others to introgress. Different introgression rates (localized introgression, slow-dispersed and fast-dispersed introgression) among genetic markers suggest that there are different selection pressures for different genomic regions. A few well-dispersed, negative-acting genes could prevent most of the genome from introgressing. Because large DNA segments are more likely to carry such negative genes, backcrosses carrying large DNA segments would be selected against. Consistent with this hypothesis, selective filtering of the genome appears to be most intense in the very earliest backcross generations (e.g. at the hybrid zone boundary) where the average introgressing segment is likely to be 25 cM or larger. The arrangement of genes and their action will have a major impact on the genomic pattern and rates of introgression. If hybrid populations act as evolutionary filters, there are several important implications. First, species barriers are maintained in the face of hybridization. Second, a strong filter should prevent the introgression of deleterious genes while allowing introgression of beneficial ones. Finally, a filter helps explain the existence and long-term persistence of hybrid zones (Martinsen *et al.* 2001).

#### Natural hybridization among native poplar species

Despite the numerous barriers to hybridization, natural hybridization among native species is common in *Populus*. Natural poplar hybrids are regularly found wherever different species

come into contact with one another (Eckenwalder 1984b,c). Hybridization between species of different sections is comparatively restricted. Only section Aigeiros (cottonwoods) and section Tacamahaca (balsam poplars) are freely intercrossable (Eckenwalder 1984b,c). These two sections, like others in the genus, differ somewhat in habitat as well as in range. Balsam poplars are trees of boreal and montane habitats, whereas cottonwoods occupy lower elevations of middle latitudes. They are largely allopatric in Eurasia but are broadly sympatric in North America, with overlapping ecological preferences. They contact each other and hybridize primarily where topographic diversity brings their distinctive habitats into proximity. Such localities, although widespread, are restricted (about 10-15 km) and populations containing hybrids represent a small fraction of all populations of each parent species. Most hybrids in the field appear to be first-generation crosses (Eckenwalder 1984c) although advanced-generation hybrids were also observed (Braatne 1999; Martinsen et al. 2001). The hybrids show little tendency to invade the characteristic habitats of either of their parents but tend to remain in the hybridized habitat (Eckenwalder 1984b,c). There are exceptions to this ecological barrier to hybridization, and in local areas the habitats of cottonwood and balsam poplar parents may be virtually indistinguishable and thus not limiting to the hybrids. This is probably the case with *P. balsamifera*, *P. deltoides*, and *P. × jackii* in part of their broad range of sympatry (Ronald et al. 1973 in Eckenwalder 1984c).

In North America, near the Weber River in Utah, USA, *P. fremontii* hybridize with *P. angustifolia* where their distinctive habitats come into proximity. This hybrid zone has been the object of a long series of studies. Pure populations of *P. fremontii* occur at the lower elevations (below 1300 m above sea level), *P. angustifolia* is found at higher elevations (above 1470 m) (Keim *et al.* 1989). In the 13-km hybrid zone, introgression is unidirectional; F1 hybrids only backcross with pure *P. angustifolia* (absence of hybrid × hybrid and hybrid × *P. fremontii* crosses) (Keim *et al.* 1989). This restricted hybridization process means that the hybrid zone is not self-perpetuating and will presumably become extinct in the absence of a *P. angustifolia* population (Keim *et al.* 1989).

In North America, natural hybridization between species of cottonwoods and balsam poplars has occurred over at least the last 12 million years (Eckenwalder 1984c). The long-term consequences of natural hybridization appear to have involved exploitation of increased variability and novel gene combinations (Eckenwalder 1996). Whitham *et al.* (1999) illustrate that plant hybrid zones are dynamic centres of ecological and evolutionary processes for plants and their associated communities. The intermediate genetic differences between the parental species will result in the greatest genetic variation in the hybrid zone, which in turn will have a positive effect on biodiversity (Whitham *et al.* 1999).

#### Natural hybridization between native and domesticated poplar species

Numerous exotic poplar species have been introduced to Europe as well as into North America for the establishment of shelterbelts, windbreaks and wood production in urban, suburban and agricultural landscapes. The most common non-native poplars in Europe include P. × *canadensis*, P. × *generosa* (cultivated hybrids between P. *trichocarpa* and P. *deltoides*), P. *trichocarpa*, P. *alba* (non-native in northern Europe) and P. *nigra* cv. *italica* (Lombardy poplar).

#### **North America**

Exotic poplars introduced to the temperate regions of North America include Lombardy poplar and *P. alba*. *P. alba* was introduced to North America at least 200 years ago, but it has not yet become naturalized probably because planted individuals have been propagated from only a few clones, virtually all of which are female (Little *et al.* 1957 in Braatne 1999). Lombardy poplar is widely planted in North America but reports on local hybridization

between this species and either of the native cottonwoods *P. deltoides* or *P. fremontii* with which it frequently occurs are sparse (Eckenwalder 1982). This paucity of hybrids may be attributed to the sex of the introduced *P. nigra*. All black poplars planted in North America are from a single staminate clone, Lombardy poplar, and its pollen may not be able to compete with pollen of *P. deltoides* or *P. fremontii* in fertilizing ovules of these species in natural populations (Baker 1951 in Braatne 1999). Occasionally, in North America non-native poplars colonize adjacent riparian corridors via asexual propagation, yet there are no reports of these species displacing native cottonwood populations (Braatne 1999).

#### Europe

In Europe in the eighteenth century, spontaneous hybridization occurred between the introduced *P. deltoides* and the native *P. nigra*, giving rise to the widely cultivated *P.* × *canadensis* (Eckenwalder 1982). While the activities on poplar breeding and cultivation developed in Europe, human activities in floodplain areas including agriculture, urbanization and hydraulic engineering resulted in the destruction and modification of the habitat of native black poplar. Moreover, in many countries of the temperate regions clones of *P.* × *canadensis* were massively planted for wood production to replace the autochthonous black poplar resources on alluvial floodplains. This resulted in a severe reduction in population size and habitat fragmentation of the European black poplar all over Europe. A secondary effect of the human activities and the introduction of cultivated hybrid poplars is the potential for natural hybridization between the introduced and the native poplars. Habitat modification followed by introgressive hybridization can lead to the extinction of a rare plant species (Ellstrand 1992).

The situation in the United Kingdom and in Belgium illustrates the risk of extinction of this species in the margins of its range area. In these countries, black poplar is considered to be one of the rarest native trees (Cottrell et al. 2002; Vanden Broeck et al. 2002). Several authors (e.g. Cagelli and Lefèvre 1995; Frison et al. 1995; Heinze 1997) mention the potential threat of introgression of genes of P. × canadensis into the native P. nigra. Cultivated poplar plantations contain very few clones and contribute to a large extent to the pollen and seed pools (Cagelli and Lefèvre 1995). Not only exotic hybrids are involved, but also pure *P. nigra* varieties like the male tree 'italica' distributed all over the continent (Cagelli and Lefèvre 1995), or the female variety 'thevestina' that was planted as an ornamental tree in Eastern Europe (Bordács et al. 2002). However, only recently evidence for introgressive hybridization of P. × canadensis in the offspring of black poplar females was found (Vanden Broeck et al. 2004). In Belgium, genes of P. deltoides were detected in the open-pollinated offspring of a black poplar female, surrounded by  $P. \times$  canadensis hybrids and in the absence of conspecific males (Vanden Broeck et al. 2004). This is in contrast with other studies, were no introgression of P. deltoides genes in the offspring of P. nigra females was found, even when flowering male trees of *P.* × *canadensis* were present in the vicinity (Rajora 1986; Heinze 1997; Janssen 1998; Benetka et al. 1999; Tabbener and Cottrell 2003). In the latter studies, the female black poplars investigated were surrounded by conspecific males.

These results are in conformity with the results from the controlled cross experiments with interspecific pollen mixes, suggesting conspecific pollen advantage. In a mixed pollen cloud, pollen of *P. nigra* may be more successful than that from *P.* × *canadensis* in pollinating female black poplar (Vanden Broeck *et al.* 2003; Tabbener and Cottrell 2003). However, if no pollen of their own species is present, *P. nigra* females are pollinated successfully by pollen of *P.* × *canadensis*. Therefore, low levels of introgression from *P.* × *canadensis* are expected in natural populations of *P. nigra* where there are several male black poplars close to the female trees. This is confirmed by the results of the European research project EUROPOP where the genetic diversity of black poplar populations along the borders of six river systems (Danube,

Drôme, Ebro, Rhine, Ticino and Usk) was studied (Vanden Broeck *et al.* 2003, 2004a). Although genes of *P. deltoides* and/or *P. trichocarpa* were detected in young poplar seedlings colonizing the banks of the river Meuse in Belgium (Vanden Broeck *et al.* 2003, 2004), the Waal and Meuse rivers in the Netherlands (Beringen 1998), the river Ebro in Spain (Agúndez *et al.* 2001), the river Danube in Austria (Heinze 1998; Krystufek 2003) and in the Czech Republic (Benetka *et al.* 1999). It is most likely that these introgressed seedlings originate from hybrid × hybrid crosses or from open-pollinated *P. × canadensis* females (Beringen 1998; Benetka *et al.* 2002; Vanden Broeck *et al.* 2003). These findings indicate that cultivated poplars are reproductive along several river systems in Europe and that they may compete with the native species in colonizing new habitats. Some of the introgressed seedlings seemed to be well adapted as they survived the river dynamics over several years.

#### Conclusion

The numerous barriers to hybridization are paradoxical in light of the widespread occurrence of natural hybrids (Arnold 1997). A solution to this paradox rests with the same explanation that is given for evolution in general. The successful establishment of hybrid populations is determined by events that, in many organisms, occur only rarely. Furthermore, this establishment may initially be non-adaptive or even maladaptive for the hybridizing pairs, but may lead to adaptive evolution through the production of hybrid genotypes that are more fit than their parents in the parental or novel habitats. Significantly, the barriers to initial hybridization may subsequently aid in the isolation of hybrid lineages, thus promoting divergent evolution (Arnold 1997). Therefore, natural hybrids can have important consequences for evolution and conservation biology. They serve as filters, preventing the introgression of most genes but allowing others to introgress throughout the range of the recipient species (Martinsen *et al.* 2001). Furthermore, natural hybrid zones can be centres of diversity and may provide essential habitat for rare species (Whitham *et al.* 1999). These natural zones of hybridization are unique and worthy of special efforts to promote their conservation and protection (e.g. Whitham *et al.* 1999; Martinsen *et al.* 2001).

On the other hand, the presence of gene flow between different species may not always be a blessing (Ellstrand 1992). Gene flow between a common (introduced) species and a rare species can create outbreeding depression (i.e. fitness reduction following hybridization) resulting in a lower realized reproductive potential. A second potential hazard from introgressive hybridization is the genetic assimilation of the rare form by the more numerous taxon (Ellstrand 1992; Arnold 1997). Genetic assimilation involved the loss of the rare form through asymmetric gene flow from the more numerous taxon. Ellstrand (1992) stated that interspecific gene flow is perhaps the greatest gene flow hazard in plant conservation.

#### References

- Agúndez D., Fluch S., Maestro C., Alba N. 2001. Introgression genetica procedente de plantaciones de hibridos en rodales naturales de *Populus nigra* [Genetic introgression from plantations of hybrids into natural stands of *Populus nigra*]. Pp. 546–552 *in* Actas del III Congreso Forestal Español Vol. II.
- Arens P., H. Coops, J. Jansen and B. Vosman. 1998. Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. Mol. Ecol. 7:11–18.
- Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, Oxford, UK.
- Arnold, M.L., E.K. Kentner, J.A. Johnston, S. Cornman and A.C. Bouck. 2001. Natural hybridisation and fitness. Taxon 50(1):93–104.
- Barsoum, N. 2001. Regeneration—requirements and promotion measures. Pp. 16–24 *in* EUFORGEN Technical Bulletin: *In situ* conservation of *Populus nigra*. (F. Lefèvre, N. Barsoum, B. Heinze, D. Kajba, P. Rotach, S.M.G. de Vries and J. Turok, eds.). IPGRI, Rome, Italy.

Benetka V, K. Vacková, I. Bartáková, M. Popísková and M. Rasl. 2002. Introgression in black poplar

(Populus nigra L. ssp. nigra) and its transmission. J. Forest Sci. 48(3):115–120.

- Benetka, V., J. Mottl, M. Vacková and M. Dubský. 1999. Estimation of the introgression method in *Populus nigra* L. populations by means of isozyme gene markers. Silvae Genet. 48:218–223.
- Beringen, R. 1998. Natuurlijke verjonging en hybridisatie bij populieren (*Populus nigra* en *P.* × *canadensis*) [Natural regeneration and hybridization in *Populus*]. Gorteria 24:139–147.
- Bordács S., A. Borovics and I. Bach. 2002. Genetic diversity of natural populations and gene bank of black poplar in Hungary. Pp 93–106 *In* Genetic Diversity in River Populations of European Black Poplar— Implications for riparian eco-system management (B.C. Van Dam and Bordács S., eds.). Csiszár Nyomda, Budapest, Hungary.
- Braatne J.H., S.B. Hood and P.E. Heilman. 1996. Life history, ecology, and conservation of riparian cottonwoods in North America. Pp. 57–85 *in* Biology of *Populus* and its Implications for Management and Conservation (R.F. Stettler, H.D. Bradshaw, JR., P.E. Heilman and T.M. Hinckley, eds.). NRC Research Press, National Research Council of Canada, Ottawa, Canada.
- Braatne, J.H. 1999. Biological aspects of hybrid poplar cultivation on floodplains in western North America—A Review. Document No. 910-R-99-002, U.S. Environmental Protection Agency, Washington, DC, USA (http://www.epa.gov/r10earth/offices/ecocomm/poplars.pdf).
- Cagelli, L. and F. Lefèvre. 1995. The conservation of *Populus nigra* L. and gene flow with cultivated poplars in Europe. Forest Genet. 2:135–144.
- Cottrell, J.E., H.E. Tabbener and G.I. Forrest. 2002. Distribution of variation in British Black Poplar: the role of human management. Pp. 73–84 *in* Genetic Diversity in River Populations of European Black Poplar—Implications for riparian eco-system management. (B.C. Van Dam and Bordács S., eds.). Csiszár Nyomda, Budapest, Hungary.
- DiFazio, S.P., G.T. Slavov, J. Burczyk, S. Leonardi and S.H. Strauss. 2004. Gene flow from tree plantations and implications for transgenic risk assessment. Pp. 405–422 *in* Plantation Forest Biotechnology for the 21st Century. (C. Walter and M. Carson, eds.) Research Signpost, Kerala, India.
- Eckenwalder, J.E. 1982. *Populus × inopina* hybr. Nov. (*Salicaceae*), a natural hybrid between the native North American *P. fremontii* S. Watson and the introduced Eurasian *P. nigra* L. Madroño 29:67–78.
- Eckenwalder, J.E. 1984a. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in section Aigerios and Tacamahaca. I. Population studies of *P. × parryi*. Can. J. Bot. 62:317–324.
- Eckenwalder, J.E. 1984b. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in section Aigerios and Tacamahaca. II. Taxonomy. Can. J. Bot. 62:325–335.
- Eckenwalder, J.E. 1984c. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections Agerios and Tacamahaca. III. Paleobotany and evolution. Can. J. Bot. 62:336–342.
- Eckenwalder, J.E. 1996. Systematics and evolution of *Populus*. Pp 7–32 *in* Biology of *Populus* and its Implications for Management and Conservation. Part I, Chapter 1 (R.F. Stettler, H.D. Bradshaw, JR., P.E. Heilman and T.M. Hinckley, eds.). NRC Research Press, National Research Council of Canada, Ottawa, Canada.
- Ellstrand, N.C. 1992. Gene flow by pollen: implications for plant conservation genetics. Oikos 63:77-86.
- Farmer, R.E. and J.A. Pitcher 1981. Pollen handling for Southern hardwoods. Pp. 77–83 in Pollen Management Handbook (E.C. Franklin, ed.) Agriculture Handbook 587. USDA Forest Service, Washington, DC, USA.
- Farmer, R.E. Jr. 1996. The genecology of *Populus*. Pp. 33–35 *in* Biology of *Populus* and its Implications for Management and Conservation. (R.F. Stettler, H.D. Bradshaw, JR., P.E. Heilman and T.M. Hinckley, eds.). NRC Research Press, National Research Council of Canada, Ottawa, Canada.
- Frison, E., F. Lefèvre, S. De Vries and J. Turok (compilers). 1995. Populus nigra network. Report of the first meeting, Izmit, Turkey, 3–5 October 1994. IPGRI, Rome, Italy.
- Heinze, B. 1998. Molekulargenetische Unterscheidung und Identifizierung von Schwarzpappeln und Hybridpappelklonen [Molecular genetic differentiation and identification of black poplars and hybrid

poplar clones]. FBVA-Berichte 105. Schriftenreihe Der Forstlichen Bundesversuchanstalt Wien, Vienna, Austria.

- Heinze, B. 1997. A PCR marker for a *Populus deltoides* allele and its use in studying introgression with native European *Populus nigra*. Belg. J. Bot. 129:123–130.
- Janssen, A. 1998. Artbestimmung von Schwarzpappeln (*Populus nigra* L.) mit Hilfe von Isoenzymmustern und Überprüfung der Methode an Altbäumen, Absaaten von kontrollierten Kreuzungen und freien Abblüten sowie Naturverjüngungen. [Species determination of black poplar (*Populus nigra* L.) based on isozymes and isozyme analysis of black poplar trees, half-sib offsprings and full sib progenies originating from controlled crosses]. Pp. 32–42 *in* Die Schwarzpappel-Hessischen Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie (H. Weisgerber and A. Janssen, eds.), Vol. 24. Hann. Münden, Germany.
- Keim, P., K.N. Paige, T.G. Whitham and K.G. Lark. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*—occurrence of unidirectional introgression. Genetics 123(3):557–565.
- Knox, R.B, R.R. Willing and A.E. Ashford. 1972. Pollen-wall proteins: role as recognition substances in interspecific incompatibility in poplars. Nature 237:381–383.
- Krystufek, V. 2003. Population genetic analysis of *Populus nigra* in Austria using nuclear and chloroplast DNA markers. PhD thesis, University of Vienna, Austria.
- Lefèvre, F., N. Barsoum, B. Heinze, D. Kajba, P. Rotach, S.M.G. de Vries and J. Turok. 2001. EUFROGEN Technical Bulletin. *In situ* conservation of *Populus nigra*. IPGRI, Rome, Italy.
- Legionnet, A., and F. Lefèvre. 1996. Genetic variation of the riparian pioneer tree species *Populus* nigra L.1. Study of population structure based on isozymes. Heredity 77(6):629–637.
- Martinsen, G.D., T.G. Whitham, R.J. Turek and P. Keim. 2001. Hybrid populations selectively filter gene introgression between species. Evolution 55(7):1325–1355.
- OECD. 2000. Consensus document on the biology of *Populus* L. (poplars). Series on Harmonization of Regulatory Oversight in Biotechnology No. 16, Organisation for Economic Co-operation and Development, Paris. ENV/JM/MONO(2000)10.
- Pauley, S.S. 1950. Flowering habits in Populus. Genetics 35:684.
- Peterken, G.F. 2001. Ecological effects of introduced tree species in Britain. Forest Ecol. Manage.141:31-42.
- Rajora, O. P. (1986). Mating systems and fertilizing pollen gene pools of *Populus deltoides* Marsh. and *P. nigra* L. clones located in different compatible species neighbourhoods as inferred from isozyme analysis. Pp. 134–141 *in* Proceedings of the 20th Canadian Tree Improvement Association, Ottawa.
- Rajora, O.P. 1989. Pollen competition among *Populus deltoides* Marsh., *P. nigra* L. and *P. maximowiczii* Henry in fertilizing *P. deltoides* ovules and siring its seed crop. Sexual Plant Reproduction 2:90–296.
- Rieseberg, L.H. and S.E. Carney 1998. Tansley Review No. 102. Plant Hybridization. New Phytologist 140:599–624.
- Schweitzer, J.A., G.D. Martinsen and T.G. Whitham. 2002. Cottonwood hybrids gain fitness traits of both parents: A mechanism of their long-term persistence? Am. J.Bot. 89(6): 981–990.
- Stanton, B.J. and M. Villar. 1996. Controlled reproduction of *Populus*. Pp. 113–138 in Biology of *Populus* and its Implications for Management and Conservation. (R.F. Stettler, H.D. Bradshaw, JR., P.E. Heilman and T.M. Hinckley, eds.). NRC Research Press, National Research Council of Canada, Ottawa, Canada.
- Stettler, R.F., L. Zsuffa and R. Wu. 1996. The role of hybridization in the genetic manipulation of *Populus*. Pp. 87–112 *in* Biology of *Populus* and its Implications for Management and Conservation. (R.F. Stettler, H.D. Bradshaw, JR., P.E. Heilman and T.M. Hinckley, eds.). NRC Research Press, National Research Council of Canada, Ottawa, Canada.
- Stettler, R.F., R. Koster and V. Steencackers 1980. Interspecific crossability studies in poplars. Theor. Appl. Genet. 58:273–282.
- Tabbener, H.E. and J.E. Cottrell. 2003. The use of PCR based DNA markers to study the paternity of poplar seedlings. Forest Ecol. Manage. 179:363–376.
- Vanden Broeck, A. 2004. Potential gene flow between native European black poplar (Populus nigra L.) and

cultivated poplars in Belgium. Ph.D. thesis, University Ghent, Ghent, Belgium.

- Vanden Broeck, A.H., V. Storme, K. Cox *et al.* 2002. Mating system of *P. nigra* in a mixed poplar stand composed of *P. nigra*, *P. deltoides* and *P. × euramericana*.). Pp 53–61 *in* Genetic Diversity in River Populations of European Black Poplar—Implications for riparian eco-system management B.C. (Van Dam and Bordács S., eds.). Csiszár Nyomda, Budapest, Hungary.
- Vanden Broeck, A., P. Quataert, I. Roldán-Ruiz, E. Van Bockstaele and J. Van Slycken. 2003. Pollen competition in *P. nigra* females revealed by microsatellite markers. Forest Genet. 10(3):219–227.
- Vanden Broeck, A., V. Sotrme, J.E. Cottrell *et al.* 2004. Gene flow between cultivated poplars and native black poplar (*Populus nigra* L.): A case study along the river Meuse on the Dutch–Belgian border. Forest Ecol. Manage. 197(1–3):307–310.
- Whitham, T.G., G.D. Martinsen, K.D. Floate, H.S. Dungey, B.M. Potts and P. Keim. 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. Ecology 80(2):416–428.

Zsuffa, L. 1974. The genetics of Populus nigra L. Ann. Forest. (Zagreb) 6/2:29:53.

### Common action plan for Populus nigra

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In this paper, I want to discuss whether the Network could benefit from a common action plan for the conservation of (mainly) *Populus nigra*. I propose to coordinate and integrate Network and national activities to a level of jointly executing conservation and restoration projects across national borders, with outside funding, according to a constantly revised plan set up by the Network itself.

EUFORGEN has a mission to ensure the effective conservation and the sustainable utilization of forest genetic resources in Europe. Present achievements, in general, include the identification of priority species, setting up networks of specialists, compiling information on individual national conservation efforts, standardizing the language and some technical methodologies, and issuing broadly applicable guidelines.

So, what is left to be done? Have we achieved what we wanted? In 'ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe', goals for 'effective conservation' have to be set. This could be summarized, for instance, as 'to ensure the largely unrestricted evolution of tree species in Europe now and in the foreseeable future, by letting the full forces of evolution act upon a species'. The goal for 'sustainable use' could be set as 'to avoid complete inhibition of natural evolution by management practices' Species loss, fragmentation, depletion of the gene pool, and its deliberate and irreversible change should be avoided. Are our activities sufficient to achieve this? They may be, or they may not. In any case, it would help to have a plan of how to achieve the goals – a common action plan.

#### The Populus nigra example

This Network has seen the establishment of national *ex situ* measures (clone collections), the writing of guidelines, conservation efforts for use in breeding, the establishment of national *in situ* measures (protected areas), and so on. I think that especially the coordination of national practical conservation measures *in situ* cand and should be improved: there are different levels of activities in different countries, 'white spots' on the map, and different regulations for conservation measures in different countries. While the Network has been successful in disseminating this information effectively to relevant people in different countries, we have been less successful in encouraging the establishment of such *in situ* measures in regions where they are most needed. What is rare in one country may be common across the border, and what is difficult to achieve in one country on a stand-alone basis may be much easier with a little outside help of good friends. We could benefit from a common framework for all conservation efforts – a plan. This plan could comprise:

- setting the priorities for the different parts of the natural distribution range for *P. nigra*
- recommending and coordinating practical measures in different regions or countries through logistic, legislative, *ex situ* and *in situ*, and socio-economic measures
- monitoring the progress of these activities, to provide feedback into periodical updates of the plan
- securing the long-term (continuous) nature of the efforts.

I mean not just 'paper', but an invitation for 'action'. Possible steps on the way to setting up such a plan could include the preparation, from the Network reports, of a 'density map' overlaid onto the distribution map, which gives the census of *P. nigra* left in the different countries or regions, and to compare it with a map of suitable habitats and potential

restoration sites. Examples of general distribution maps for *P. nigra* exist (e.g. Cagelli and Lefèvre 1995; Heinze and de Vries 1998; Weisgerber 1999) but I think that they give a far too optimistic impression of the future of the species. A distribution map does not give information on the actual status of a species. As a local example from the Danube in Austria, a comparison of potential and actual sites of *P. nigra* showed a great discrepancy (Heinze 1998, after Ruhm 1990). On a larger geographical scale, a map produced by the WWF Danube-Carpathian programme (see *http://www.carpathians.org/danube.htm, http://www.wwf.org.uk/researcher/places/000000060.asp*) shows the restoration potential for floodplains in the Danube Basin, and indicates problematic areas.

The next step would be to discuss the 'density map' in conjunction with the amount of measures taken in the different regions or countries, and with the quality of these measures (e.g. size of reserves, genetic diversity in the *ex situ* collections, etc.) in close collaboration with relevant scientists. Network meetings offer an excellent forum for this activity. From these discussions, a priority list for action can be derived. For example, the Network could discuss and decide how urgent it is to set up *in situ* reserves in the margin of the distribution range, in clearly addressed regions, and so on. However, one of the most important steps is to discuss how to execute the required action cooperatively, where necessary. This may especially be true for country border regions, smaller countries, or for all the different players concerned in a larger country. A plan remains just a plan on paper if we do not support the implementation of the necessary action.

#### Action

The Network could prepare the necessary elements of such a plan from the cumulated information that is largely already available in the Network Reports: general distribution, status of the species in the different countries/regions, measures already taken, quality of the measures (whether they conform with issued guidelines, e.g. with the Technical Bulletin for *in situ* conservation, Lefèvre *et al.* 2001). All this could be compiled in a 'species status report'. Use of a geographical information system (GIS) would be very important for all this information. Countries could indicate positions of *in situ* and *ex situ* sites in such a system. On the basis of the compiled information, the botanical definition of subspecies, varieties etc. will be much easier and can be standardized across the distribution range. It can also form the data basis for provenance trials in the future—it would be desirable to have a network of such trials across the distribution range, in order to verify the distinct nature of regional genetic resources. From these data, priority areas for action can be chosen, and measures recommended.

The next step is crucial. I think that Network members are not always able to initiate the putting into practice of the plan in all countries. We need to help each other more than we have done in the past, and we need outside help. I think that invitations to scientists, non-governmental organizations (NGOs such as forestry organizations, WWF, etc.), and other key interest groups to participate in an appropriate form, on a case-by-case basis, may be helpful. The socio-economic situation of the individual countries must be considered; in most cases it will be absolutely necessary to directly involve local people, and to convince them of the benefits this programme can offer for them. We should also deepen our contacts in the remaining species range outside Europe, to a level suitable for executing common projects. Supporting the implementation of action could for instance involve, as the **future 'routine' work of the Network**:

- formulating the necessary tasks, splitting them into short-term projects, and organizing their realization (project planning)
- organizing funding for the specific projects (EC, the World Bank, UNESCO, FAO, the

Rockefeller Foundation)

• **monitoring the success** of all the actions, e.g. by excursions, local training sessions, symposiums and conferences *in situ*. This could also serve to raise public awareness locally and globally, and to increase technical skills and knowledge where it is most needed.

Although this is a big task, it can be done in small steps, with achievable goals.

#### New approach

What is different about this approach? It contains a commitment for cross-national common measures, as suggested and planned by a Network of experts; collaboration with all players involved; and alternative ways of financing. I think it should not be legally binding—an attractive programme will gather its own momentum, and we will hardly convince lawmakers and governments that they should support 'forced interference' from other countries. I think that copying some of the 'lobbying' and 'public awareness' measures of successful NGOs would be more successful. The EUFORGEN *P. nigra* Network would act as the long-term facilitator of this plan, thereby also ensuring that the Network itself has a long-term future. At the end of this process we may see a network of *in situ* reserves which is constantly updated and adjusted to new scientific findings and new technical possibilities.

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#### References

- Cagelli, L. and F. Lefèvre. 1995. The conservation of *Populus nigra* L. and gene flow with cultivated poplars in Europe. Forest Genet. 2:135–44.
- Lefèvre, F., N. Barsoum, B. Heinze *et al.* 2001. EUFORGEN Technical Bulletin: *In situ* conservation of *Populus nigra*. IPGRI, Rome, Italy.
- Heinze, B. and S.M.G. de Vries. 1998. Integration nationaler Generhaltungsprogramme: das EUFORGEN-Netzwerk Schwarzpappel in Österreich und den Niederlanden [Integrating national and international programmes for gene conservation: the EUFORGEN network black poplar in Austria and the Netherlands]. Pp. 178–188 *in* Erhaltung genetischer Resourcen im Wald—Normen, Programme, Maßnahmen (T. Geburek and B. Heinze, eds). Ecomed-Verlagsgesellschaft, Landsberg, Germany.
- Ruhm, W. 1990. Jagd—Fischerei—Naturschutz im Stauraum Altenwörth [Hunting—fishing—conservation in the retention area of the Altenwörth hydroelectric power station]. Diploma thesis, Universität für Bodenkultur, Vienna, Austria.
- Weisgerber, H. 1999. *Populus nigra* Linné, 1753 (*Populus nigra* III-2). Chapter 16th supplement 6/99. Pp 1–18 in Enzyklopädie der Holzgewächse—Handbuch und Atlas der Dendrologie (P. Schütt, H.J. Schuck, U.M. Lang and A. Roloff, eds.). Ecomed-Verlagsgesellschaft, Landsberg, Germany.

# *In situ* regeneration of black poplar (*Populus nigra* L.) along the river Eder

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The floodplains of the river Eder near Fritzlar were declared as a nature reserve in 1981. The strategy developed by the nature conservation authorities in Kassel included, among other measures, the regeneration of the *Populus nigra* L. population. Various attempts, which will be described in this report, were conducted with varying success.

#### Description of the nature conservation area

The nature reserve is located 4 km east of Fritzlar, a town 25 km south of Kassel right in the middle of Germany. The area, including riparian floodplain forests, gravel pits and floodplains, is approximately 70 ha, stretching 3 km along the river Eder.

- Site conditions are characterized as follows:
- elevation: 160 m above sea level
- soil texture: riverside soil with top 15–70 cm loam above 3–4 m of gravel
- total annual precipitation: 550–600 mm
- minimum monthly precipitation occurs in March
- average annual temperature: 8.5 °C

The nature reserve is home for 36 different plant associations including 383 species, 13 of which are highly endangered. The importance of the area is also indicated by the occurrence of 49 species of songbirds breeding and 9 amphibian species (Stiegmeier and Freitag 1988).

As far as Hesse is concerned, the existence of black poplar stands and *Salix purpurea* associations are of special importance.

#### Purpose of nature conservation in the area

According to the declaration of 7 December 1981, the area should contribute to the conservation of rare animals and plants. The purpose of the nature reserve is the sustainable preservation of this biotope as a habitat for endangered birds, amphibians, reptiles, insects and other rare animals, and as a location for plants with very specific site requirements. Activities that might destroy or even disturb the area are banned and measures are taken to maintain or develop the area for conservation purposes. In this context, the regeneration of black poplar plays an important role.

#### Black poplar population along the river Eder

The assessment and inventory of *P. nigra* in Hesse found about 800 individuals still existing, mostly as single trees or small groups. Only three stands were identified, one of them on the river Eder (Janssen and Walther 1997). The population consists of about 100 mature trees. Their physiological condition is rather poor as there are no young trees or natural regeneration. The black poplars were identified first by phenotypic characteristics, and later verified by isozymes (Janssen 1997).

According to documents dating from 1859, the area had almost no forest cover at that time. The present forests originate from trees along the banks of the meandering river. Particularly in the 1950s, enrichment plantings were done using both black poplars and hybrid poplars. Between 1959 and 1981, large areas of the floodplain forests disappeared as a result of intensified gravel quarrying. The declaration of the nature reserve prevented further destruction.

It is unclear which parts of the forests originate from natural regeneration. In 1913, however, the construction of a dam 25 km upstream reduced the natural river dynamics, and it is likely that natural regeneration was poor after that because erosion and new sedimentation only occurred on a small scale. Therefore, there was a lack of open fresh substrates necessary for natural regeneration of black poplar. This situation was similar to that in other European countries (Weisgerber 1999; Barsoum 2000; Rotach 2000). Some trees with an estimated age of 50–60 years most likely originate from natural regeneration, as there was large-scale flooding when the dam was destroyed during World War II.

#### Measures proposed and implemented

As the regeneration of the old poplars is one of the main aspects of the strategy for maintaining the biotope, several measures were planned and conducted in close cooperation between forest and nature conservation authorities.

To assist natural regeneration, bare soil was created artificially and three plots were fenced to protect seedlings of poplars and willows from browsing. Five times a year, the area was treated with a rotary cultivator to destroy competing vegetation but this method was later discontinued as it did not show any success. The most interesting action planned from the ecological point of view was a controlled flooding of the area. It was intended to reintroduce the natural dynamic by creating a flood using water from the reservoir in the upper reaches of the river. However, because of concerns raised by farmers, fishermen and owners of gravel pits this action has not yet been realized.

Some areas, especially floodplains and branches of the river, were cut off from being flooded by normal seasonal high waters by small dams constructed in earlier years. Destruction of these dams should re-establish a direct connection with the main stream, improving the water supply of the soils. Also, high water levels were thought to create some bare soil. These measures were started in 1998, but, as a consequence of the disturbed natural river dynamics, the results were not very promising. Only exceptional rainfall led to a noticeable increase of the water level. Recent assessments by the nature conservation authorities showed that some natural regeneration has taken place and it will be very interesting to monitor whether the seedlings will survive (E. Funk, pers. comm.).

For artificial regeneration, planting stock was raised from cuttings. In 1988, cuttings were taken from the already identified old black poplars with the aim of establishing both a clone collection and a stool bed. Plants with a 1-year-old shoot on 2-year-old roots were produced at the nursery of the Hessian Forest Research Station for planting on suitable sites. In spring and autumn 1992, forest workers of the District Forest Office in Fritzlar planted 840 poplars manually using hole-planting techniques. The plants were protected against browsing. With very few exceptions, all the poplars died during the first year due to insufficient water supply. Lethal damage caused by mammals, mice in particular, or insects, was not observed.

In 1999, based on experiments, a new planting technique was chosen that might overcome the problems resulting from lack of precipitation and the unpredictable river dynamic. The soil conditions also had to be considered, as the natural water regime was disturbed. The ground water level will go down during summer as result of the regulation of the river. Low precipitation would not allow high water availability in the soil, and the coarse stratum of gravel interrupts the capillarity.

The traditional method of planting sets seemed appropriate. The production of sets took place in the nursery, where mother plants in the clone collection were cut back regularly. They built the suitable 1- or 2-year-old shoots, which had to be 3–4 m in length. Then 90 sets were planted immediately into holes prepared by drilling machines. The sets were sunk up to two thirds of their length to enable contact with the ground water. First results showed

that 90% of the plants have survived. In 2003, some of the poplars reached heights of 4–5 m, although some showed only a little growth because of damage caused by game animals and insects (Janssen *et al.* 2000).

#### Conclusions

It is questionable whether native black poplar can be maintained in the nature reserve through natural regeneration. Nevertheless, black poplars are important for the existence of many other species in different stages of succession and this makes *in situ* conservation extremely valuable. Thus, artificial measures should be undertaken to ensure the ecological function black poplars have in this specific environment. The planting of sets raised from mature trees at least helped to overcome the problems related to the complete loss of certain stages of successional development.

The future prospects of establishing new riparian forests seem rather limited. Although the annual report on the situation of German forests published by the Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL 2003) highlights the importance of forests with respect to protection against flood disasters, only very little activity has taken place. In Hesse, farmers raised concerns about the loss of highly productive land, despite financial subsidies that will be granted for planting of riparian forests.

A bit of optimism may be admissible because of some administrative activities. The Regional Council of Kassel has initiated a conference involving various stakeholders adjacent to the river Eder and the Eder reservoir, including water management, tourism, agriculture, nature conservation and industry. The aim of the conference is to exchange views and begin to develop a common strategy. The national importance of the river Eder nature reserve will help to stress the demands of nature conservation.

For the time being, the forestry division is trying to focus interest on the establishment of riparian forest as compensation for the loss of forest converted to settlements or other types of infrastructure. The compensation of forest conservation by afforestation is a legal obligation, and re-establishment of riparian forests could fulfil nature and landscape aspects too.

#### References

- Barsoum, N. 2000. The balance of black poplar (*Populus nigra*) regeneration strategies as a function of hydrology on floodplains. Pp. 46–50 *in Populus nigra* Network: Report of the sixth meeting, 6–8 February 2000, Isle sur La Sorgue, France (S. Borelli, S. de Vries, F. Lefèvre and J. Turok, compilers). IPGRI, Rome, Italy.
- BMVEL. 2003. Bericht über den Zustand des Waldes 2002 [Report on the situation of the forests in Germany in 2002]. Bundesminister für Verbraucherschutz, Ernährung und Landwirtschaft, Bonn, Germany.
- Janssen, A. 1997. Unterscheidung der beiden Schwarzpappelarten *Populus nigra* L. und *P. deltoides* Marsh. sowie der Arthybride *P. × euramericana* (Dode) Guinier mit Hilfe von Isoenzymmustern [Differentiation of two black poplar species (*Populus nigra* L. and *Populus deltoides* Marsh.) and the hybrid species *P.x euramericana* by means of isozyme patterns]. Die Holzzucht 51:17–23.
- Janssen, A. and P. Walther. 1997. Die Schwarzpappel in Hessen. Kartierung, Identifizierung, Erhaltung und Vermehrung [Black poplar in Hesse. Mapping, identification, conservation and regeneration]. Allgemeine Forstzeitschrift/Der Wald 52:968–969.
- Janssen, A., P. Walther, and R. Schulzke. 2000. Sicherung von Schwarzpappelvorkommen im Auenbereich der hessischen Eder am Beispiel einer standortangepaßten Pflanzmethode [Conservation of a black poplar population in the riparian forests of the River Eder in Hesse by means of a site-adapted cultivation method]. Angewandte Landschaftsökologie 37:291–293.

- Rotach, P. 2000. The situation of floodplain forests, the natural habitats of black poplar. Pp. 11–14 *in Populus nigra* Network: Report of the sixth meeting, 6–8 February 2000, Isle sur La Sorgue, France (S. Borelli, S. de Vries, F. Lefèvre and J. Turok, compilers). IPGRI, Rome, Italy.
- Stiegmeier, I. and H. Freitag 1988. Pflegeplan für das Naturschutzgebiet Ederauen [Concept for the maintenance and development of the natural conservation area of Ederaue]. Unpublished report) University of Kassel, Germany.

Weisgerber, H. 1999. Populus nigra L. In Enzyklopädie der Holzgewächse, Landsberg, Germany.

# Evaluation of genetic resources of black poplar (*Populus nigra* L.) in North-Rhine Westfalia, Germany

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As a large-scale measure to improve the ecological value of certain floodplain areas along river Rhine and some of its tributaries in North-Rhine Westfalia, it is planned to establish new and extended stands of pure, autochthonous black poplar (*Populus nigra* L.) to restore the natural ecosystem in these areas. Centres of activity will be areas in the Wahner Heide (a former military training area) and the nearby Agger valley. In order to select genetically unambiguous black poplar clones (saplings) and diaspores (seed) to meet this ambitious goal, all black poplar trees (464 altogether) have been re-assessed by the isoenzyme method (Rajora 1989a,b,c; Janssen 1997). The trees had been previously identified and mapped as 'true' *P. nigra* individuals either by morphological criteria, by cpDNA sequences or by isozyme electrophoretic pattern. Moreover, clone separation, also employing isoenzyme patterns, was included in this investigation.

In case of black poplar, clone assessment has not been undertaken before; only one comment dealing with this subject has come to our knowledge (Natzke 1988). This additional information allows for maximum clone (and thus genetic) heterogeneity at the planting sites chosen for re-establishment of *P. nigra*. A theoretical and practical introduction to sample preparation and starch gel electrophoresis techniques was kindly provided by Dr A. Janssen (Hessische Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie; Hann. Münden).

#### Methods

The investigation presented here includes nearly all trees previously classified by different methods as pure-bred *P. nigra*. It was our objective to reinvestigate these data by employing a uniform classification technique. Samples were taken from all mapped and marked trees from all natural stands in North-Rhine Westfalia and, in addition, from the *P. nigra* clonal archive at Welver. Buds were collected between October and December 2000.

For a detailed description of enzyme extraction, isoenzyme separation and pattern identification we refer to Hellenbrand (2001) and van Schyndel (2001). For discrimination between pure-bred *P. nigra* L. individuals and *P. × euramericana* hybrids (= species classification) the following enzymes were employed: phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), glutamatoxaloacetate transaminase (GOT) and leucine aminopeptidase (LAP). Clone discrimination took place on basis of the zymogram patterns for malate dehydrogenase (MDH), isocitrate-dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGDH) and shikamate dehydrogenase (SKDH). The discriminative value of the former two enzymes by far exceeded that of the latter two.

#### Results

#### Methodological aspects

In general, reliability and reproducibility are essential for any method employed for species classification. Since all of the trees in this investigation had already been pre-classified by different methods, the approximate level of conformity between them can be estimated from

our results.

Nine out of 246 trees had been identified previously as *P. nigra* solely by morphological criteria, but 2 of them (22%) now turned out to be hybrids. A total of 222 trees had been classified as pure-bred black poplars by cpDNA analysis, but discrepancies were observed for 4 of these (1.8%), which turned out to be hybrids. A total of 253 trees had already been investigated by the isoenzyme method and 6 of these (2.4%) yielded deviating results. In this case, there are indications that these trees were originally confused during sampling and/or analysis.

It can be concluded that the cpDNA and isoenzyme methods yield highly consistent results. However, not enough data were available to allow for a representative comparison of the morphological and isoenzyme methods.

#### Vegetative versus generative propagation

From the data on species identification and clone discrimination, in combination with the distribution pattern of black poplar throughout North-Rhine Westfalia, distinct ecological conclusions can be drawn for the future work on black poplar. These conclusions, though admittedly speculative in part, may also have consequences for the reforestation programmes of *P. nigra* which are presently being developed.

The *status quo* of black poplar in North-Rhine Westfalia can best be characterized by some basic quantitative facts. In total, 130 out of 232 pure-bred *P. nigra* individuals in North-Rhine Westfalia and in the Münsterland region can be assigned to 40 clones, yielding an average clone size of about three individuals. Most certainly, these 'micro-clones' are the vegetative offspring of just one tree in each case.

Only seven stands in these two regions are made up of more than 10 trees. From these, only 30–50% are genetically independent and the rest result from vegetative propagation. As a rare exception, genetic heterogeneity proved to be very large in case of two stands near Wesel, which are part of the largest black poplar population in the North-Rhine area (102 trees altogether). In these two stands, 32 out of 36 trees (89%) are genetically independent. Many young seedlings were found on that site. It may be safe to assume that under these conditions sexual propagation is dominant. A second 'hot spot', which shows natural propagation (generative and vegetative), is the sites near Soest. Ecological conditions may be more favourable in these locations than elsewhere and thus they should be investigated systematically with the aim of best-possible protection and perhaps extension.

In Westfalia, only six black poplar stands consist of nine or more individuals. They contain a total of 134 trees, of which 110 can be assigned to one of the 16 genetically different clones; only 24 trees are individuals in a genetic sense. Adding these 16 clones to the 24 individuals, just 40 different genotypes of *P. nigra* could be traced in Westfalia (27% of all trees, counting one clone as one mother tree). Each of the small isolated stands in this area has less than nine *P. nigra* trees and these represent, with a few exceptions, one single clone.

In summary, altogether 464 *P. nigra* trees could be identified in North-Rhine Westfalia. Twelve trees which appeared in the earlier records could no longer be traced. In addition, 5 trees exist only as vegetative offsprings in the clone archives in Welver. Of these 464 trees, only 253 trees (54%) are genetically independent. This number comprises all clones plus all individual trees (solitaires). The low numbers illuminate impressively how endangered *P. nigra* is in North-Rhine Westfalia.

#### Conclusions and recommendations

What are the conclusions from these findings? We tend to favour the interpretation that the small, isolated black poplar stands, most of which represent only one clone (evidently

originating from a single tree), are relict sites which developed by regression of larger populations within recent decades (Schulze and Vornam 1997; Franke *et al.* 1997). Some of these sites, especially those in urban areas, may instead originate from human activity.

Many of the trees forming small natural stands are over-aged, consequently showing reduced vitality which in turn may hamper both flowering (which would be a futile effort anyway, because of lack of sexual partners) and the production of vital vegetative offspring of any kind (Holzberg 1998) Occurrence of the latter has been questioned (Joachim 2000) but it has also been defended (Natzke 1998). In any case, vegetative propagation—if there is still propagation at all (it has not been observed everywhere)—must be considered the prevailing form of propagation of *P. nigra* in North-Rhine Westfalia. Generative propagation, on the other hand, is a rather rare event.

From our results, we are forced to assume that trees maintained in the clonal archives of North-Rhine Westfalia (e.g. Welver) represent a rather limited number of clones, because clone analysis was not performed accurately. According to our results, 'surplus' clone material should be replaced by clones which have not yet been included in the archives. Since autochthonous trees are available only to a very limited extent in North-Rhine Westfalia, it seems indispensable to rely primarily on vegetative clone material (*ex situ* conservation). In addition, the maintenance of an *ex situ P. nigra* gene resource (genebank) may help to guarantee the future of endangered black poplar. In the light of our results, greater effort must be undertaken to keep 'genetic twins'—i.e. the offspring of identical clones—far apart, when material from the *P. nigra* clone archives is being selected for reforestation purposes. Instead, as many different clones as possible should be brought together, relying on the results of the biochemical clone analysis of this project.

Nevertheless, generative propagation should be promoted by ecological measures in the field wherever possible. There are several reasons for this. One argument in favour of this propagation model is that vegetative material has an unknown, but possibly very high, biological age. It may be a second- or third-generation tree with a purely vegetative reproduction history, which eventually was included in the *P. nigra* clone archives. If so, genetic degeneration, i.e. an accumulation of unfavourable somatic mutations over time, cannot be excluded. This in turn might well reduce the vitality and perhaps also the pathogen resistance of offspring derived from such a tree. With this in mind, every individual seeding black poplar tree in North-Rhine Westfalia deserves the highest level of protection to maintain diversity in the region's *P. nigra* gene pool.

#### Acknowledgements

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#### References

- Franke, A., H.G. Jaeschke and C. Seyd, 1997. Erfassung letzter Schwarzpappelvorkommen (*Populus nigra* L.) im baden-württembergischen Teil der Oberrheinischen Tiefebene [Documentation of the last occurrences of black poplar (*Populus nigra* L.) in the Baden-Württenberg part of the upper Rhine valley]. Die Holzzucht 51:5–14.
- Hellenbrand, B. 2001. Vergleichende gelelektrophoretische Analysen der Isoenzymmuster mutmaßlich autochthoner Schwarzpappelbestände in Westfalen zwecks Untersuchung der Reinrassigkeit und Klonabgrenzung bei *Populus nigra* L. [Comparative gel electrophoretic analyses of isoenzyme patterns of presumbly autochthonous stands of black poplar in Westfalia in order to achieve characterization of genetically pure *Populus nigra* L. lines including clone separation.] Staatsexamensarbeit, Köln, Germany.

Holzberg, H. 1998. Vermehrungsstrategien von Populus nigra L. unter naturnahen Bedingungen und die

künstliche Nachzucht im Kampbetrieb [Propagation stategies of *Populus nigra* L. under approximately natural conditions and artificial propagation in a nursery]. *In* Die Schwarzpappel; Symposium, 13–14 Mai 1998 in Ha. Münden (H. Weisgerber and A. Janssen, eds.). Mitteilungen der Hessischen Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie 24:91–98.

- Janssen, A. 1997. Unterscheidung der beiden Schwarzpappelarten *Populus nigra* L. und *Populus deltoides* Marsh. sowie ihrer Arthybride *P. × euramericana* (Dode) Guinier mit Hilfe von Isoenzymmustern [Discrimination between the two *Poplar* species *Populus nigra* L. and *Populus deltoides* Marsh, including their hybrid *P. × euramericana* (Dode) Guinier on the basis of isoenzyme patterns.]. Die Holzzucht 51:17– 23.
- Joachim, H.F. 2000. Die Schwarzpappel (*Populus nigra* L.) in Brandenburg [The black poplar (*Populus nigra* L.) in Brandenburg]. Landesforstanstalt Eberswalde. Ministerium für Landwirtschaft, Umweltschutz und Raumordnung Brandenburg, Germany.
- Natzke, E. 1998. Erfassung, Identifizierung, Vermehrung und Wiederansiedelung der Schwarzpappel (*Populus nigra* L.) in Sachsen-Anhalt [Documentation, identification, propagation and reforestation of the black poplar (*Populus nigra* L.) in Sachsen-Anhalt]. *In* Die Schwarzpappel; Symposium, 13–14 Mai 1998 in Hann. Münden (H. Weisgerber and A. Janssen, eds.). Mitteilungen der Hessischen Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie 24:91–98.
- Rajora, O.P. 1989a. Genetic structure and and identification of *Populus deltoides* based on allozymes. Genome 32:440–448.
- Rajora, O.P. 1989b. Identification of some *Populus deltoides* Marsh. × *P. nigra* L. clones, developed in North America, with the aid of allozymes. Euphytica 43:207–213.
- Rajora, O.P. 1989c. Characterization of 43 *Populus nigra* clones representing selections, cultivars and botanical varieties based on their multilocus allozyme genotypes. Euphytica 43:196–206.
- Schulze, L. and B. Vornam. 1997. Generhaltungsarbeit für die reinrassige Schwarzpappel [Measures directed towards the conservation of the gene pool of the pure-bred black poplar.] Allgemeine Forstzeitschrift/Der Wald 52:966–967
- van Schyndel, P. 2001. Untersuchungen zur Rainrassigkeit und Klonabgrenzung bei der Schwarzpappel. Vergleichende gelelektrophoretische Analysen der Isoenzymmuster mutmaßlich autochthoner *Populus nigra* L. Bestände im Rheinland und im Münsterland [Investigations on genetic purity and clone separation of the black poplar. Comparative gel electrophoretic analyses of isoenzyme patterns of presumedly autochthonous *Populus nigra* L. stands in the Rhein and Münster lands.]. Staatsexamensarbeit, Köln, Germany.

# Micropropagation of *Populus nigra* L.: a potential contribution to gene conservation and tree improvement

#### Gisela Naujoks and Georg von Wühlisch

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Because of their rapid growth, capability of reproduction by cuttings and good mating potential, black poplars have been cultivated in Europe for the past 200 years and intensive tree improvement by hybridization with other poplar species has been carried out 80 years. At present, the existence of pure European black poplar (*Populus nigra* L.) is severely threatened because the species is not able to regenerate itself naturally due to the lack of suitable habitats. Therefore measures are necessary to secure the genetic resources by vegetative propagation of the identified remnant individuals of pure black poplar. One example of these attempts was the elaboration of micropropagation methods with material from old trees growing on the floodplains of the Oder and Elbe rivers.

#### Material and methods

Plant material from adult trees (100–130 years old) was harvested in spring or early summer. After surface desinfection, the explants were transferred to nutrient media containing different growth regulators for induction of tissue culture (Table 1). Adventitious shoot formation was achieved during several 4-week propagation cycles.

Elongated shoots were rooted *in vitro* and transplanted to soil under plastic foil cover in the greenhouse to ensure high air humidity conditions. After stepwise acclimatization, the black poplar plantlets were grown in the nursery. Two or three-year-old plants were then transferred to field plantations.

Mother trees			Nutrient medium		
Location	Number	Explant type	Culture initiation	Propagation	Rooting
	of trees				
Mittlere Elbe	44	Buds	WPM (Lloyd and McCown 1980)		WPM
biosphere reserve			+ BAP 0.25–1.0 mg/l		+ IBA 0.5 mg/l
(Sachsen-Anhalt)			+ NAA 0.02 mg/l		+ NAA 0.1 mg/l
Oder floodplain	10	Shoot tips,	LS (Linsmaier and	LS	B (Boulay 1979)
(Brandenburg)		nodal	Skoog 1965)	+ BAP 1.0 mg/l	+ IBA 0.1 mg/l
		segments	+ BAP 0.2 mg/l	+ NAA 0.1–0.2 mg/l	+ NAA 0.05 mg/l
		-	+ NAA 1.0 mg/l	-	-

Table 1. Material and methods used for micropropagation of Populus nigra L.

B, medium according to Boulay (1979); BAP, 6-benzyl-aminopurine; IBA, indole-3-butyric acid; LS, medium according to Linsmaier and Skoog (1965); NAA, α-naphtalene acetic acid; WPM, Woody Plant Medium (Lloyd and McCown 1980).

#### Results and conclusions

Three-year-old micropropagated black poplars from the mother trees of the biosphere reserve Mittlere Elbe were used in 1997 for a gene conservation plantation on the original floodplain sites (altogether 534 plants from 33 clones). These plantlets showed good growth in the field and the root quality was better than in plants derived from cuttings of the adult trees.



Figure 1. Micropropagated black poplar plantlets: A, 4 weeks after root induction; B, 8 weeks after transfer to the soil.



Figure 2. Root quality of 3-year-old black poplars: A, field-grown cutting; B, pot-grown cutting; C, micropropagated plant.

The micropropagated trees derived from mother trees of the river Oder floodplain are growing in a clonal archive and can be used as donor trees for propagation of cuttings. Since tissue culture has a rejuvenating influence, rooting success is expected to be better than with rooting of cuttings from adult mother trees. The experiments confirmed that micropropagation methods are suitable to be included in black poplar gene conservation strategies.

Well-established tissue cultures are a precondition for virus elimination in plants. Approaches to produce virus-free black poplars might be carried out in the future using thermotherapy combined with meristem culture.

#### References

- Boulay, M. 1979. Propagation *'in vitro'* du Douglas par micropropagation de germination aseptique et culture de bourgeons dormants (*Pseudotsuga menziesii* (Mirb.) Franco) [In vitro propagation of Douglas fir by micropropagation of aseptic germination and culture of dormant shoots]. *In* Micropropagation des arbres forestiers, AFOCEL 12:67–75.
- Linsmaier, E.M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18, 100–127.
- Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Int. Plant Prop. Soc. Proc. 30:421.

### Programmes Seventh EUFORGEN *Populus nigra* Network Meeting Osijek, Croatia, 25–27 October 2001

Wednesday 24 October-arrival of participants

#### Thursday 25 October

#### **Opening of the meeting**

- 09:00 Welcome (Host country and Chair of the *Populus nigra* Network)
- 09:15 Introduction (EUFORGEN Secretariat)
- 09:30 Adoption of the agenda and nomination of rapporteurs

#### **Country updates**

09:45 Highlights of progress made in countries (round-the-table discussion)

10:30 Coffee break

- 10:45 Highlights of progress (continued)
- 11:30 Report on the International Poplar Commission (D. Kajba)
- 12:00 Technical Bulletin (dissemination and implementation) (S.M.G. de Vries)
- 13:00 Lunch

#### In situ conservation

14:30 Results and recommendations of the EUROPOP project and their practical application (F. Lefèvre)

16:00 Coffee break

#### Ex situ conservation

16:30 Update on EUFORGEN core collection of *P. nigra* clones (L. Vietto)

17:00 Establishment of core collection of *P. alba* clones (I. Bach)

#### Friday 26 October

#### Sharing responsibilities for the conservation of genetic resources in Europe

09:00 Concept, results of the EUFORGEN Inter-Network meeting, the case of *Populus nigra* (S.M.G de Vries, B. Heinze, EUFORGEN Secretariat)

10:15 Links with non-member or non-European countries

10:30 Coffee break

#### **Biodiversity**

11:00 Biodiversity and genetic resources in *Populus* stands (P. Rotach) **Research** 

12:00 Discussion on further research needs and opportunities (all)

13:00 Lunch

#### Documentation

- 14:30 Update on European *P. nigra* clone database (L. Vietto)
- 15:00 Update on *P. alba* clone database (C. Maestro)

- 15:30 EUFORGEN Database/ Information platform (EUFORGEN Secretariat)
- 15:45 Bibliography (EUFORGEN Secretariat)
- 16:00 Coffee break

#### **Public awareness**

- 16:30 CD-ROM (S.M.G. de Vries)
- 17:00 Poster (S.M.G. de Vries)
- 17:30 Listserver (EUFORGEN Secretariat)
- 17:45 Other public awareness initiatives

#### Saturday 27 October

Field trip to Drava and Danube rivers and Nature Park 'Kopacki rit'

- 15:00 Adoption of the report
- 16:30 Coffee break
- 17:00 Date and place of next meeting
- 17:30 Conclusions and closure

Sunday 28 October-departure of participants

# Eighth EUFORGEN *Populus nigra* Network Meeting Treppeln, Germany, 22–24 May 2003

Wednesday 21 May - arrival of participants

#### Thursday 22 May

#### **Opening of the meeting**

08:30 Welcome (Host country and Chair of the *Populus nigra* Network) Adoption of the agenda and nomination of rapporteurs

08:45 Network outputs and EUFORGEN update (J. Koskela)

#### Country introductory reports and updates

09:15 Country introductory reports: Bosnia and Herzegovina, and Ireland Country updates

- 10:30 Coffee break
- 11:00 Country updates (continued)
- 13:00 Lunch

#### 14:00 Country updates (continued)

- Technical guidelines and common action plan
- 14:30 Technical Guidelines and leaflet (J. Koskela)
- 15:00 Common action plan for *Populus nigra* (B. Heinze)

#### Public awareness and communication

15:30 Slide collection and CD-ROM (S. de Vries) EUFORGEN Web site (J. Koskela) Poster presented at the DYGEN conference ID sheet for *P. alba* (I. Bach)

#### Friday 23 May

#### Ex situ conservation

- 08:30 Core collections (I. Bach, L. Vietto) Population collection (I. Bach)
- 10:30 Coffee break

#### **Research facilitation**

11:00 Proposals

Introgression and gene flow in *Populus* (A. Vanden Broeck/J. Van Slycken)

#### Documentation

- 12:00 Clonal database (L. Vietto) *Populus nigra* bibliography (J. Koskela)
- 13:00 Lunch

#### In situ conservation of black poplar in Germany

14:00 River Eder (R. Schulzke) River Elbe (H. Koss) River Rhine (M. Weidner) 17:00 Visit to the nature reserve Ziltendorfer Niederung
Saturday 24 May
Field trip to Kietz Island, Kuestrin
15:00 Wrap-up session

Any other business
Date and place of next meeting
Adoption of the minutes and summary of the meeting

Sunday 25 May—Departure of participants

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