Poplar Commission of the Federal Republic of Germany

Poplars, Aspens and Willows in Germany: Exploitation, Conservation, Research

Period: 2000 - 2003

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I Policies and Legislation

1 Policies

a) Studies on short-rotation cultivation of poplars and willows

Studies on the cultivation of poplars and willows in short rotation periods take place primarily in Hesse, Bavaria and Saxony. Roughly 400 ha of such experimental plantations have been planted with the goal of clarifying all questions on practical cultivation, economic viability and the selection of suitable species and varieties. The high costs of establishing plantations and the long-term capital tie-up, however, so far do not correspond to the relatively low revenues after deduction of harvesting costs. Yet, seen under the aspects of CO_2 fixation, the search for alternative energy sources and the exploitation of set-aside agricultural areas, this type of cultivation may have enormous potential. By creating suitable framework conditions and promotional measures, a large area potential could be used to sustainably produce a renewable raw material for industrial and energy exploitation.

b) Cultivation of poplars and willows on set-aside agricultural areas

Since September 1993, it has been possible to cultivate perennial crops on agricultural areas as renewable resources on set-aside land; these crops also include fast-growing tree species with a rotation period of a maximum of 10 years. The Act on Equal Status for Set-Aside and Agriculturally Used Areas (*Gesetz zur Gleichstellung stillgelegter und landwirtschaftlich genutzter Flächen*) (Federal Law Gazette I p. 910), which came into force with retroactive effect in January 1995, preserves the arable status of set-aside areas planted with fast-growing tree species. However, only few farmers decided in favour of this alternative cultivation so that only 400 ha are used for this type of cultivation, most of them operated as experimental or pilot plantations.

c) Genetic conservation of the European black poplar and white poplar

The population of pure black poplar has been greatly reduced by efforts to make larger rivers navigable and canalized and the resulting destruction of riparian forests, beginning with the Rhine correction planned by Colonel Tulla that was begun in 1817. Efforts have been made to preserve the remaining population through *in situ* and *ex situ* measures. The gene banks of several Federal Länder (NW, RP, HE, SN, BB, ST) contain black poplars of certified origin for renaturation measures in the respective region. In the Land of Brandenburg two black poplar stands have been approved for harvesting generative reproductive material for genetic conservation purposes in accordance with the Act on Forest Reproductive Material (*Forstvermehrungsgutgesetz - FoVG*). Germany is part of the international network of the EUFORGEN Programme for genetic conservation of the indigenous black poplar.

2 Legal measures

The following legal provisions are of relevance for poplars and willows:

a) Act on Forest Reproductive Material (*Forstvermehrungsgutgesetz - FoVG*) of 29 May 2002 (Federal Law Gazette I p. 1658)

This act implements Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material (Official Journal L 011, 15/01/2000 p. 0017-0040) in national law. All poplar species and artificial poplar hybrids with the exception of willows are subject to the act.

Vegetative reproductive material (clones) of poplars may only be sold under the category "Tested Reproductive Material".

b) Regulation implementing the Act on Forest Reproductive Material (*Forstvermehrungsgut-Durchführungsverordnung – FoVDV)* of 20.12.2002 (Federal Law Gazette I p. 4711, 2003 I p. 61)

Some of the ordinances that were once in force were summarized in this ordinance and therefore repealed (Forestry Seed Control Book Ordinance (*Forstsaat-Kontrollbuchverordnung*) of 1983 and the Forestry Seed Notification Ordinance (*Forstsaat-Meldeverordnung*) of 1997). According to this ordinance, master certificates must be issued for reproductive material in general and clones or clonal mixtures in particular according to a specific template. The marketable reproductive material must be extensively labelled, e.g. the variety or, in the case of clonal mixtures, all clones of the mixture. Genetically modified material can only be marketed under the category "tested". It must be labelled, whether it is genetically modified.

The ordinance also regulates what information must be contained in the delivery papers, what books and records must be kept by the forestry seed or plant enterprises to record the entire lifecycle of the reproductive material as well as the notification obligation for exports and imports to or from another member state of the European Union.

c) First Ordinance Amending the Forestry Seed Origin Ordinance (*Erste Verordnung zur Änderung der Forstsaat-Herkunftsgebietsverordnung – FoVHgV*) of 15 January 2003 (Federal Law Gazette I p. 238,)

This ordinance determines the regions of origin for “Selected Reproductive Material” of the tree species covered by the act, including the poplar (§§2 and 5 of the act) and regulates the form and content of the master certificates required for the transport of generative propagation material from approved stands, seed orchards, clones and clonal mixtures. Reproductive material for the poplar genus is almost without exception sold as Tested Reproductive Material. In poplar cultivation the choice of a site-adapted species or variety is of chief importance. Therefore, for the genus poplar only one region of origin was determined, which encompasses the entire national territory.

d) Forest Reproductive Material Approval Ordinance (*Forstvermehrungsgut-Zulassungsverordnung – FoVZV*) of 20 December 2002 (Federal Law Gazette I p. 4721, 2003 I p. 50)

This ordinance regulates the approval requirements for basic material.

For generative reproductive material of the poplar, approval of basic material to acquire Selected Reproductive Material requires the following: the basic material must be superior compared with other stands in particular with regard to volume yield, form and health and be at least average with regard to the remaining evaluation characteristics.

A minimum area of 0.25 ha and a minimum age of 20 years are prerequisites for approval. In addition, the stand must include at least 20 fruit-producing trees, of which at least 10 must be harvested at every harvest.

The ordinance allows approval for two clone seed orchards for generative reproductive material of the poplar as starting material for Tested Reproductive Material. This means that not only clones from vegetative propagation can be provided to users as forestry reproductive material for poplars, but also complete families originating from controlled hybridization, for example for aspens, as long as they have proven their suitability for cultivation as Tested Reproductive Material under given environmental conditions. The advantages of this are the simple and inexpensive production of such generative reproductive material using seed as well as the larger genetic diversity of the reproductive material.

Vegetative reproductive material must always fulfil the strict demands of Tested Reproductive Material. Clonal mixtures should be favoured for approval over single clones.

Information on the approved stand must be entered in a Crop Authorization Register for generative poplar starting material, as for other tree species. An Arboriculture Register is kept for vegetative poplar starting material.

e) Plant Inspection Ordinance amended by the Tenth Ordinance on the Amendment of the Plant Inspection Ordinance (*Pflanzenbeschauverordnung*) of 5 June 2003 (Federal Law Gazette I no. 24 p. 799) last amended by: "Fourth Ordinance on the Amendment of Legal Plant Protection Provisions" (*Vierte Verordnung zur Änderung pflanzenschutzrechtlicher Vorschriften*) of 26 November 2003 (Federal Law Gazette I no. 57 p. 2438).

This ordinance regulates conditions and restrictions in the export and import of plants and plant products in the interests of plant protection. There are also conditions for poplars and willows and their products. Appendix 1 of this ordinance cites important harmful organisms, the import of which is banned: poplar rust (*Melampsora medusae*) and poplar *Septoria* canker (*Mycosphaerella populorum*). The bacterial canker (*Xanthomonas populi*) listed in the previous ordinance is no longer cited.

II Statistical and Economic Data

1 Statistical data

Active planting of poplars and willows in Germany's forests is increasingly rare, primarily due to the low sales potential for poplar wood. Aspens occur only in mixed pioneer crops on large calamity areas. It is therefore difficult to record area percentage figures; however we must presume that actual distribution is underestimated. Poplars and aspens take up an area of at least 100,000 ha, or roughly 1% of forested area. Area distribution of willows in forests is, by contrast, much lower.
The area of poplar plantations on formerly agriculturally-used areas outside the forests is about 400 ha.

2 Production

The significance of poplar and willow cultivation as a basis for raw materials is minor in the Federal Republic of Germany. We have no precise data on current levels of poplar raw wood felling since poplar felling and sales are recorded and entered together with the timber species group beech. Annual poplar felling is estimated at 150,000 to 300,000 m^3.
There are no separate records on the use of poplar raw wood.

3 Imports and exports

Exports of poplar timber from the Federal Republic of Germany are generally low, but distinctly higher than imports.

4 Trends

The market for poplar timber in the Federal Republic of Germany has not developed to any major significance. Accordingly, the wood-working and processing industry is not equipped for this type of wood. Poplar wood sales are generally unsatisfactory because of scattered availability and the resulting low supply. Current prices for standard poplar log varieties are cited at 26 €/solid metre by the Land Forest Administration of Lower Saxony. This price is roughly 50% lower than the price of the cheapest other tree species, e.g. pine.

III Technical Data

1 Identification, registration and variety control

a) Identification

The methods once used for poplar clone identification were based solely on morphological and phenological properties. The methods were no longer sufficient to guarantee a clear distinction of all clones, in particular for clonal mixtures. Hence, for years now biochemical methods (isozyme analyses and molecular-genetic methods) have been used increasingly for identification.

These analysis methods are used for genetic conservation as well. Because of the ease of propagation using wood cuttings, black poplar hybrids have become widespread in Europe over the past 200 years. This applies in particular for hybrids of the Eastern Cottonwood (*P. deltoides* Bartr.) and the European black poplar (*P. nigra* L.), whose progeny is termed *Populus x euramericana*. Since the European poplar crosses with the Eastern Cottonwoodas well as with hybrid clones, widespread genetic contamination of the native black poplars cannot be excluded. Due to the similarity between the two tree species, these species and their species hybrids cannot be distinguished by morphological characteristics with 100% certainty. However, both isozyme and molecular-genetic analyses can be used to differentiate with certainty between the pure European black poplars and black poplar hybrids.

b) Approval

New clones are approved on the basis of experimental plantations lasting many years under the provisions of the Act on Forest Reproductive Material (*Gesetz über forstliches Vermehrungsgut - FoVG*) and the accompanying ordinances under the category "Tested Reproductive Material". Since 1997 the Saxon Regional Institute for Forests has submitted four aspen progenies (*Populus tremula* L.) for approval of starting material for attaining Tested Reproductive Material. The approval office is the Forest Administration Bautzen. The progenies are:

Bärenstein I:	clone 3105/IA	x	clone 3110/IIIA
Bärenstein II:	3105/IA	x	60
Graupa I:	69	x	97
Graupa II:	5085	x	236

These progenies have the following special properties

Graupa I: Good adaptability

Graupa II and Bärenstein I and II: Above-average growth production (height, DBH)

The recommendations for the use of the progenies are:

- Establishing pioneer crops
- Single-tree mixture in deciduous stands
- Temporary mixture in deciduous and coniferous stands
- Underplanting in sparse pure coniferous stands
- Stabilization of unstable pure coniferous stands
- Improvement of sparse cultures or natural rejuvenation

Two stands of *Populus nigra* for attaining generative reproductive material have been approved for the purpose of genetic conservation or renaturation of riparian forests in Brandenburg, approval office Eberswalde:

1.) Oder island Küstrin AfF Müllrose dept. 2334 a 2 row 1, approx. 2.21 ha
2.) Ziltendorfer lowland AfF Müllrose dept. 5456, approx. 1.00 ha

c) Registration

Approved poplar clones and clonal mixtures are entered in an Arboriculture Register. The central agency for the Arboriculture Register for poplars is

Regierungspräsidium Kassel
Steinweg 6
D-34117 Kassel

d) Variety control

Due to the low demand for reproductive material from the poplar, variety protection is not employed.

2 Cultivation

Poplars, aspens and willows are no longer cultivated to any notable extent. Germany also no longer has any nurseries that cultivate and offer these special products. The consequence is that display orchards of old poplar varieties are neglected and that aspen seed orchards are not maintained.

3 Breeding and selection

a) Conventional rotation

No breeding or selection took place for exploitation in conventional cultivation during the period under report.

b) Short rotation

The government supported cultivation and testing programmes for poplars, aspens and willows of prior years at the sites of Abbachhof (Bavaria), Canstein Haferfeld, Georgenhof (Hesse), Oldenburg (Lower Saxony) and more recently the sites Methau and Thammenhain (Saxony), Bornim, Spremberg (Brandenburg) as well as Wöllershof, Neuhof, Schwarzenau and Coburg (Bavaria) are still in the research phase or have already been completed. A number of further, smaller cultivation experiments have been initiated in different parts of Germany. In general, yields of 10 tonnes of dry mass per hectare can be calculated annually, which can be achieved with the available aspen and balsam poplar varieties in ten-year rotation periods.

The investment costs for establishing a plantation are high and vary depending on the number of plants, which in turn depends on the utilization purpose for the woody biomass produced. Costs of € 1,800 per hectare are calculated for energy exploitation with a large number of plants and short rotation periods, establishment costs for industrial utilization of the biomass with lower numbers of plants and longer rotation periods are € 800/ha, in both cases not including fencing.

. In addition to investment costs, harvesting and transport are major cost factors, which are very dependent on the production objective. With the use of specially constructed harvesting machinery that harvest wood chips for energy exploitation at a maximum 4-year growth, harvesting costs of € 45-82/tonnes dry mass have been registered. Longer rotation periods result in thicker harvest material, which results in higher harvesting costs.

To evaluate the economic situation of poplar short-rotation plantation areas for energy use of woody biomass and an average plantation volume yield (10 t/ha) and lifetime (7 rotations), production costs between 30 and 45 €/t absolutely dry weight woody biomass are calculated. If the operating risks are not taken into account and revenues of € 50/t absolutely dry weight are calculated for the produced biomass, annuities (at 3.5% interest) of between 125 and 250 €/ha can be anticipated.

Under the present circumstances, short rotation areas are no competition to agricultural food production. The case is different for set-aside areas due to government funding, where the chances of realizing woody biomass production for energy exploitation are better. However, production lapses or volume yield reductions of only 20% can lead to losses. In addition, due to its burden of fixed costs, the lifetime of a plantation is a significant cost-influence factor. Biotic damages are another uncertainty. Increased spread of areas could cause significant economic damages due to better development opportunities of previously insignificant harmful organisms. The primary risk for farmers is, however, the price at which the produced material can be utilized. If oil prices remain at their present high for a long time or above it temporarily, woody biomass could be competitive as an energy source. Yet, thus far the oil price always drops again, making the demand for energy wood still too low. For this reason hardly any short rotation plantations have been established.

4 Protective measures

Exceptional damage events are not known for poplars, aspens and willows, making protective measures only necessary in individual cases.

5 Exploitation and utilization

a) Exploitation

Poplars and willows grown under conventional rotation periods are exploited according to normal forestry practices. Poplar raw wood is graded according to the statutory provisions on raw wood grades.

The woody biomass produced by the experimental plantations is used for special experiments or as wood chips, e.g. for wood-based panels, cellulose, but for energy purposes in particular.

b) Utilization

Organic fuel

A newly developed process for the production of automotive fuel will be used in Freiberg, Saxony by the company Choren Fuel. From 2005, it will use wood chips to produce 15,000 t of this fuel (sunfuel) annually as a renewable energy source in a pilot plant. The funding made possible by the Renewable Energy Act (*Erneuerbare Energien Gesetz - EEG*), which came into force in 2000, will be used to make the process economically profitable. The process is designed to use wood chips from different tree species; however it is quite possible that wood chips produced in short rotation periods on poplar plantations may be used in particular.

6 Other studies and activities

a) Genetic conservation

In 1990 in Strasbourg, the Ministerial Conference on the “Protection of Forests in Europe” passed Resolution S2 on the conservation of forest genetic resources. This led to the establishment of the European EUFORGEN programme for conservation of forest genetic resources. Five networks were set up for specific tree species or groups of tree species, with the aim of summarizing, multiplying and disseminating the international status of knowledge on the distribution, biology, genetics and endangerment of the selected species and to draw up recommendations for effective conservation and sustainable exploitation measures. An international alliance takes into account the cross-border distribution of many tree species. Germany joined the EUFORGEN programme in 1998 and participates in the *Populus nigra* network as well as the other networks. The network also focuses on the white poplar (*Populus alba*), which is endangered throughout Europe as well.

Even before joining the EUFORGEN programme, intensive efforts were made in Germany in the scope of the Federal-Länder working group “Conservation of Forest Genetic Resources”, to determine and map the rare pure black poplar population. It ascertained a total of approximately 5,000 black poplar individuals. Most of these are old trees, remnants of earlier, destroyed riparian forests, or from residual stands of earlier cultivation. The species purity of the trees is first ascertained based on morphological characteristics. In cases of doubt, *Populus nigra* can be distinctly differentiated from *Populus x euramericana* using biochemical methods (isozyme and molecular genetic analyses).

The genetic analyses of North-Rhine Westphalian black poplars revealed that statistically every second poplar is a vegetative descendant of another poplar. The 466 identified purebred, tested individuals actually only represented 253 genetically-independent black poplars. The reason for this is the planting of clones or shoot sprouting from the stump after exploitation or root suckering. The total number of black poplars in Germany will presumably possess similar relations.

In the scope of the EUFORGEN network a core collection of black poplar clones was established in Casale Monferrato, Italy, two of which were contributed by Germany. The collection serves as a display orchard for supplying experimental material, e.g. for use in projects such as “EUROPOP” mentioned below.

Due to the rarity of intact riparian forests, *in-situ* conservation of the remaining population in riparian forests is of priority. In this context, the network is striving to make alliances with organizations such as the World-Wide-Fund for Nature (WWF). The network is setting up a database on the Europe-wide population of black poplars in which data on the black poplar population in Germany will also be compiled.
Ex-situ conservation takes place in conservation plantations and display orchards using propagation with cuttings and *in-vitro* propagation.

"EUROPOP", a research project funded by the European Union and concluded in 2001, involved institutions from seven countries including two institutions from Germany: the Regional Institute Hesse-Forst, Hann.-Münden and the Institute for Forest Genetics and Forest Tree Breeding, Forest Biometry and Informatics of the University of Göttingen. The project researched the genetic diversity of the European black poplar, evaluated the biodiversity in riparian forest ecosystems and developed conservation strategies.

The project did genetic, phenotype and phenological (in particular flowering phenology) analyses of over 1,500 black poplar individuals from 23 populations on eight larger rivers in nine non-city-states of western Europe. It showed that the remaining populations in the relict stands have genetically differentiated and adapted to the local conditions. Therefore, renaturation measures should make use of local starting material from an original population, if possible.

b) Gene transfers in aspens

The studies of genetically modified (transgenic) aspen clones were continued in the period under report.

i) Establishment of two field release experiments

In the spring of 1996 a field release experiment was begun with 35S-*rolC* and rbcS-*rolC* transgenic aspens on the grounds of the Institute for Forest Genetics and Forest Plant Breeding of the Federal Research Institute for Forestry and Forest Products. The objective of the first field release experiment was to examine the expression and stability of the foreign genes over a period of five years. Other studies were carried out on the interactions between mycorrhiza fungi and the root systems of transgenic aspen clones as well as on the hormonal and carbohydrate metabolisms in *rolC* transgenic aspen clones and their potential effects on phytopathological properties. The experiment was concluded in October 2001 and the results were described in the last Poplar Report (BML, 2000).
In 2000 a second field release experiment was begun with genetically modified aspens, also on the grounds of the Institute for Forest Genetics and Forest Plant Breeding in cooperation with the University of Tübingen. The aim of this field release experiment was to

quantify a possible horizontal gene transfer by the transgenic trees to the mycorrhiza fungi living in symbiosis with the root system. In a three-year study, after examining roughly 100,000 different mycorrhiza, no evidence of horizontal gene transfer was found.

ii) Vegetative and generative propagation capacity of transgenic poplars

During the above described first field release experiment with transgenic aspens and hybrid aspens begun in 1996, in 1998 one three-year old tree formed two female flower buds, two years later the number increased to twelve flower bud-bearing trees. Interestingly, all twelve trees carried the 35S-*rolC* gene construct. This result indicates that the observed early blossom formation is specific for the transferred construct and can therefore not be transferred to transgenic trees with other constructs.

From 1999 to 2001 small plants were found on the field which were evident progeny from root suckers. Examination of the transgenic status revealed that roughly half of them were genetically modified.

Vegetative propagation via root suckers is, as we know, common in poplars. Hence, when estimating the risks of transgenic poplars and other species that have tendencies to vegetative propagation, in addition to the generative propagation capacity, vegetative propagation should be considered as well.

iii) Properties of timber from genetically modified trees

Material for the first introduction experiment that ended in October 2001 was used to investigate the properties of timber from 35S-*rolC* and rbcS-*rolC* genetically modified trees. The wood samples were analyzed for (a) the wood's ultrastructure, (b) chemical contents (lignin, cellulose), and (c) physical-technical properties (e.g. strength). The results of these studies were published in the *Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft* (2004), Hamburg, volume 214. The most important results are cited below:
Microscopic studies of the cambium and xylem revealed that 35S-*rolC* transgenic lines manifest very thin year rings, compared to non-transgenic Esch5 controls and rbcS-*rolC* transgenic lines. RbcS-*rolC* transgenic poplars as well as the controls had late wood fibres with much thickened cell walls in the xylem near the cambium, while the 35S-*rolC* transgenic lines often showed late wood fibres with less thickened walls. In chemical studies lignin pyrolysis products and scaffold polysaccharides were analysed. Combined with a multivariate primary component analysis it was possible to differentiate the wood tissue of both the control samples and the rbcS-*rolC* transgenic lines for lignin pyrolysis products from the 35S-*rolC* transgenic aspens. Moreover, in spite of comparatively high fluctuations within the collective of the unmodified Esch5 controls as well as the rbcS-*rolC* and 35S-*rolC* transgenic aspens, the results suggested that the control trees possess a higher amount of scaffold polysaccharides than the transgenic trees. These differences were especially distinct in the cellulose content. Interestingly, the transgenic 35S-*rolC* transgenic aspens contained roughly 4% more Klason lignin. This finding is explained by the results

of the physical examination of the wood, which show that 35S-*rolC* transgenic aspens have increased raw densities compared with rbcS-*rolC* transgenic and control plants.

iv) Expression stability of foreign genes under stress

In a project funded by the Federal Office for Environment and the Ministry for Environment, Nature and Forests of the Land Schleswig-Holstein, the Institute for Forest Genetics and Forest Plant Breeding of the Federal Research Institute for Forestry and Forest Products investigated the expression and stability of foreign genes in genetically modified aspens in controlled climate chamber experiments with simultaneous application of stress (temperature and UV light). Two different gene constructs with the *uidA* gene (GUS expression) were selected: 35S-*uidA* and rbcS-*uidA*. A total of 20 climate chamber experiments were carried out with different transgenic lines.

The overall result ascertained that there was no evidence of principle instabilities in GUS expression under stress, although morphological growth repression was found at high temperatures. In summary, the conclusion is that the expression of foreign genes in transgenic poplars after application of stress varies between the different lines carrying one T-DNA copy. The reasons for the line-dependent GUS expression patterns observed may be the different promoters (rbcS *versus* 35S) as well as the respective different genomic integration sites of the T-DNA. Transgene repeats (two T-DNA copies) at the site of insertion do not necessarily lead to deactivation of the transgene.

v) Specific environmental effects of transgenic woody plants

As part of the Federal Ministry of Education and Research funding plan “Safety Research and Monitoring” in the Federal Government’s “Biotechnology 2000” programme, a joint project called “Specific Environmental Effects of Transgenic Woody Plants” is being funded from 2002 until 2007. This joint project is coordinated by the Institute for Forest Genetics and Forest Plant Breeding of the Federal Research Institute for Forestry and Forest Products. Six working groups are involved in this project which deals with questions of the biological safety of genetically modified woody plants such as poplar. This takes into account the necessity to recognize possible ecological and/or environmentally relevant consequences involved with the introduction of genetically modified poplars in due time and, if necessary, prevent them. The establishment of this joint project – in cooperation with working groups working in other joint projects on comparable research approaches with annual and biannual, herbaceous plants – aims to make the risks of exploitation of transgenic poplars calculable and examine conditions that ensure ecologically tolerable field release of transgenic poplars.

vi) Establishment of male and female sterility in transgenic poplars

Due to the lack of barriers in domestic forest ecosystems, genetically modified and non-genetically modified trees can hybridise freely. Therefore, introgression of the genes transferred by genetic engineering (transgenes) in the gene pool of the respective species is anticipated ("vertical gene flow"). One possible strategy to prevent vertical gene transfer is the induction of male and/or female sterility, without preventing the formation of flower organs. Furthermore, it is very important to ensure lifelong stability of the genetically-induced sterility.

However, studies on the safety of transgenic forest plants suffer from the problem that trees have long generation periods. For example, poplars require 8 to 20 years before entering the generative phase. Therefore, it is important in these studies to first promote induction of flowering in transgenic poplars in order to be able to carry out studies on sterility. Four different gene constructs were used in this project to promote flowering ability. Early formation of blossoms was observed in two- to five-month-old transgenic plants containing the 35S-*Leafy* gene construct.

All existing sterility constructs of male and female "normal blossoming" poplar clones have already been transformed. These include BpMADS1::*Barnase*, PTA29::*Barnase*, PTA29::*Vst1*, C/GPDH-C::*Barnase* and C/GPDH-C::*Vst1*. Moreover, a number of lines have already been produced, which possess both a sterility and an early blossom gene. Hence, it will be possible for the first time to reach findings on the fertility/sterility of the blossoms formed by the early-blooming poplar lines transformed with sterility constructs in a short time span of two to three years.

vii) Phytoremediation of areas polluted with heavy metals

Trees can be used for phytoremediation of soils polluted by heavy metals. In a project by the University of Freiburg, Chair for Tree Physiology, transgenic poplars were produced with an increased glutathione concentration. In greenhouse studies, these transgenic poplars revealed great potential for absorbing and detoxifying heavy metals and pesticides. They absorb increased quantities of the heavy metals and store them in their leaves.

Glutathione plays a central role in protecting the plant from various stress and environmental factors. It can "detoxify" toxic compounds using chemical reactions. The result is compounds (conjugates) which are stored in the vacuoles of the plant cells: membrane enclosed cell segments.

However, before these plants can be used in practice, they must be tested for possible harmful environmental effects. An field release experiment was established to clarify whether the increased glutathione content has an influence on the soil microflora in the root area. In addition, the study will examine whether horizontal gene transfer to associated mycorrhiza fungi is possible.

viii) Evidence of foreign genes in timber

For precautionary consumer protection it is extremely important that methods be drawn up that reveal genetic modifications in decorticated timber, so that genetically modified timber put on the market can be identified. Therefore, the Institute for Forest Genetics and Forest Plant Breeding of the Federal Research Institute for Forestry and Forest Products took wood samples of transgenic trees and non-transgenic control plants of field released transgenic poplars. Genomic DNA was extracted from various fractions (cambium bark, early and late wood) of freshly harvested samples as well as from wood samples stored for five and twelve months. The highest DNA contents were found in the cambium bark, although the quality of the DNA was low. PCR and Southern Blot studies could find both aspen-specific and genetically transferred genes in early and late wood. This was the first time that foreign genes could be found in timber from genetically modified trees stored for up to twelve months.

c) Genome research in poplars

i) Marking genes with transposons

Trees are characterized by a large number of specific properties, such as long life span and intensive timber formation, which undoubtedly are under strict genetic controls. We can assume that a number of genes are involved in these processes. The objective of the project carried out by the Institute for Forest Genetics and Forest Plant Breeding of the Federal Research Institute for Forestry and Forest Products is to isolate and characterize regulatory and other gene sequences specific for trees. Representatives of the *Populus* (poplar) genus are available for these studies. Poplars can be considered as a model system for trees since these plants possess a relatively small genome and are at the same time easily accessible for genetic modifications via the *Agrobacterium tumefaciens* system. Furthermore, the poplar is only the third species in the plant kingdom after *Arabidopsis* and rice in which the total genomic sequence is known.

The approach is derived from the results of earlier studies. These revealed for the first time that it is possible to genetically transfer a transposon (*Ac* from maize) into the genome of poplars and that *Ac* leave their original position in the construct and can re-integrate somewhere in the genome (transposition). Moreover, it was ascertained that a transposition of *Ac* still occurs in six-year-old transgenic plants.

The results can be summarized as follows:

(a) A large percentage of the new positions of *Ac* in the genome of the aspen are in or near coding regions. This result illustrates that in haploid poplars *Ac* can be used for knock-out experiments (in which genes are “knocked out” to discover their function through changes in the phenotype) or in diploid poplars for activation tagging experiments (increasing the activity of genes to find out what their function is through changes in the phenotype).

(b) A comparison of the integration in coded regions in *Ac* and T-DNA showed that *Ac* is better suited for a gene tagging approach than T-DNA.

ii) Gender differentiation in poplars

The majority of higher plants on earth are hermaphrodite or bisexual. Less than 4% of plant species are, however, dioecious. The two Salicaceae genii, *Salix* and *Populus*, are among the dioecious, lignifying angiosperms. The aim of a research project funded by the German Research Society (*Deutsche Forschungsgemeinschaft*) is to develop molecular markers for male or female aspens (*Populus tremula* L.). The available material originated in hybridization experiments between the European (*Populus tremula* L.) and the American (*Populus tremuloides* Michx.) aspens. The F1 plants have by now reached the generative phase so that the gender of the plants could be ascertained.

First, the poplar genome was examined using the AFLP technique. The parent and progeny were first tested in a "bulk segregant analysis", then in single individual analyses with more than 200 different primer-enzyme combinations (PECs) and surveyed for polymorphisms. So far, nine DNA fragments have been identified that reveal gender-relevant separation. Three of these fragments were cut out of the AFLP gel, cloned and sequenced. Database searches showed that one of these fragments reveals high similarity to a known sequence from cDNA libraries of poplar blossoms. Presently a gene map of both parents is being made on which both the AFLP and the microsatellite markers will be mapped. In addition, a genomic BAC library of *Populus tremuloides* was created.

d) Studies on the gene flow in aspens

In the arboretum of the Institute for Forest Genetics and Forest Plant Breeding, Grosshansdorf, of the Federal Research Institute for Forestry and Forest Products (BFH) in 2002, five female trees were identified that blossomed and formed seeds in the same year. The progeny of two trees were then examined. A total of 47 different, putative paternal genotypes were identified and mapped in the above arboretum. High-resolution microsatellite gene sites were used to investigate what influence neighbouring trees have on gene flow of wind pollinators or whether a significant amount of the pollen originated from trees located farther away.

Microsatellite gene sites have proven to be the most suitable markers for paternity analysis. In both the old poplars and their progeny frequent and rare alleles were found for each microsatellite gene site. After a paternity analysis, the results were evaluated as follows:

(a) The large majority (60-70%) of male parent trees identified in the fertilization of both female parent trees are located in the direct proximity of the female parent trees (in a radius of only roughly 30 to 60 metres).

(b) On the other hand, this means that 30 to 40% of the reproductively effective pollen originates from poplars growing outside the arboretum. The result indicates that reproductively-effective poplar pollen can travel over distances of more than 100 metres.

e) Production of haploid poplars

Haploid plants are very prevalent starting material both in plant breeding and genome studies. However, all previous efforts to preserve haploid trees for the long term were unsuccessful, since spontaneous adaptation to diploid status was ascertained early. Therefore, a few experiments were initiated to produce haploid poplar lines. The selected approach was the isolation and cultivation of microspores, and the subsequent regeneration of haploid plants. These experiments resulted in the availability of at least six different haploid lines. One of the lines grows well in spite of its haploid status. Another line has maintained its haploid status for over two years.

IV General Information

1 National Poplar Commission

Chairman of the national Poplar Commission is the head of Department 5 of the Federal Ministry of Consumer Protection, Food and Agriculture (Rural Regions, Social Order, Plant Production, Forestry and Forest Products).
The Secretariat of the national Poplar Commission is maintained by the Federal Ministry of Consumer Protection, Food and Agriculture.

2 Literature

See appendix.

Appendix 1

This report is primarily based on the specialized contributions of the following individuals and institutes:

Bundesforschungsanstalt für
Forst- und Holzwirtschaft
Leuschnerstrasse 91
21027 Hamburg
www.bfafh.de

Fachagentur für Nachwachsende Rohstoffe e.V. (FNR)
Dr. Ing. A. Schütte
Dorfplatz 1
18276 Gülzow
www.fnr.de

Bund-Länder-Arbeitsgruppe
Forstliche Genressourcen und Forstsaatgutrecht
www.genres.de/fgr/blag/

Hessen-Forst
Forsteinrichtung, Information, Versuchswesen
Prof.-Oelkers-Strasse 6
34346 Hann.-Münden
www.hessen-forst.de

Regierungspräsidium Kassel
Baumzuchtregister
Steinweg 6
34117 Kassel

Sächsische Landesanstalt für Forsten
Graupa
Bonnewitzer Str. 34
01827 Graupa
www.forsten.sachsen.de/laf

Forstassessor M. Hofmann
Forschungsinstitut für schnellwachsende Baumarten
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Appendix 2

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