Poplar Commission of the Federal Republic of Germany

Report
on Activities Related to
Poplar, Aspen and Willow
Cultivation and Utilisation
in the Federal Republic of Germany

Period: 1996-1999

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C O N T E N T S

Annexes: 1. List of persons and institutions involved
2. References

I POLICIES AND LEGISLATION

1 Policies

a) Studies about the cultivation of poplars and willows in short rotation plantations

Experimental cultivation of fast-growing tree species, especially of poplars and willows, has been taking place in Germany since 1976. Since 1982, the Federal Government has been promoting research activities at various locations in Germany, launching a new pilot project in 1997 designed to clarify issues related to the production of poplar industrial wood in short rotation periods on a 100 ha area in Hesse and Saxony. This research work serves to settle questions relating to the practical cultivation of fast-growing tree species and their use. The studies conducted in the past few years have contributed to answering basic questions regarding the cultivation of fast-growing tree species as already set out in the last report. Economic studies have shown that the relatively high costs of investment possibly yield a return if promotional funds within the framework of set-aside can be used.

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

It has been possible since September 1993 to cultivate perennial crops on agricultural areas as renewable resources within the framework of set-aside; 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 retrospectively as of January 1995, preserves the arable status of set-aside areas planted with fast-growing tree species. However, only few farmers opted for this alternative cultivation so that only 100 – 500 ha are subject to this type of cultivation.

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

Due to the cultivation of hybrid poplars and the destruction of riparian forests, there has been a sharp decline in the occurrence of pure European black poplar (*Populus nigra* L.). Efforts are being made to conserve the remaining poplars through *in situ* and *ex situ* measures. Germany also takes part in the international network within the framework of the EUFORGEN programme for the genetic conservation of European black poplar and the white poplar (*Populus alba* L.).

2 Legal measures

No changes as against 1996

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II. STATISTICAL AND ECONOMIC DATA

1 Statistical data

No significant changes as against 1996.

2 Production

The significance of poplar and willow cultivation as a basis for raw materials is to be rated low in the Federal Republic of Germany. There are no exact data on the current levels of felling of poplar raw wood, since cuts and sales of poplar raw wood are recorded and entered together with the wood species group beech. Annual poplar felling is estimated at 150,000 to 300,000 m^3.

There are no recent surveys on the utilisation of poplar raw wood.

3 Imports and exports

The foreign trade of the Federal Republic of Germany in poplar wood is insignificant all in all. Exports clearly exceed imports.

4 Market trends

A distinct poplar wood market has not developed in the Federal Republic of Germany. Accordingly, the wood-working and processing industry is not geared to this type of wood. All in all, poplar wood sales are unsatisfactory because of scattered availability and resulting low supplies.

5 Management and administration of plantations

No significant changes as against 1996.

III. TECHNICAL DATA

1 Identification, registration and varietal control

a) Identification

The previous methods for poplar clone identification were solely based on morphological and phenological characters. The methods are no longer sufficient for clonal mixtures, in particular, to guarantee a clear distinction of all clones. Biochemical methods (isoenzyme analyses and molecular genetic methods) are, therefore, increasingly being used for identification.

These methods of analysis are also used for genetic conservation. Because of the easy vegetative propagation by cuttings, black poplars hybrids have been spread in Europe for around 200 years. This applies, in particular, to crossings between the eastern cottonwood (*P. deltoides* Bartr.) and the European black poplar, whose progeny is termed *Populus x euramericana.* As the European black poplar has been crossed with the eastern cottonwood as well as with hybrid clones, a genetic contamination of the native black poplars cannot be ruled out. Due to the similarity between the two tree species, these species and their species hybrids cannot be distinguished with absolute certainty by their morphological characters. Clear-cut distinctions between the representatives of the pure European black poplars and the black poplar hybrids are only possible with isoenzymes or with molecular genetic analyses.

b) Approval

The approval of basic material to obtain tested reproductive material takes place on the basis of multiannual experimental plantations under the national Act on Forest Reproductive Material and the relevant legal and administrative regulations issued thereunder in the category "Tested Reproductive Material". Since 1997 the Saxon Agency for Forestry (Sächsische Landesanstalt für Forsten) has applied for the approval of four aspen progenies *(Populus tremula L.)* as basic material to obtain Tested Reproductive Material. The approval agency is the forestry agency of Bautzen. The progenies concerned 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

The progenies have the following features:

1. Graupa I: good adaptability
2. Graupa II as well as Bärenstein I and II: above-average growth production (height, diameter)

Recommendations for the use of the progenies:

- establishing pioneer crops,
- mixture by single trees in deciduous tree stands,
- temporary mixture in deciduous and coniferous tree stands,
- underplanting in coniferous pure stands with fail patches
- stabilising unstable coniferous pure stands,
- replanting of lacunose crops or natural regeneration.

c) Registration

Approved clones and clonal mixtures of poplars are entered in an arboricultural register. The

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keeps the central arboricultural register for poplars.

d) Varietal control

Due to weak demand on the market, varietal control is of no importance.

2 Cultivation

No significant changes as against 1996.

3 Breeding and selection

a) Normal rotation

No changes as against 1996.

b) Short rotation

The breeding and testing programmes with poplars, aspen and willows supported by the Federal Government at the locations of Abbachhof, Canstein and Oldenburg were completed in 1996. The last 1996 report by the National Poplar Commission already informed about the key results of cultivation and about the obtained biomass yields of poplars, aspen and willows as well as about ecological aspects. Merely the results of the economic studies were not included in this report and are added here.

The subject being studied is a plantation established with cuttings on an arable area with poplar hybrid clones. The cultivation of willows or aspen was not studied. The investment costs to establish a plantation are high and vary according to the number of plants which in turn depend on the utilisation of the produced woody biomass. Given a use for energy purposes with high plant numbers and short rotation periods, costs totalling DM 3,500/ha were calculated. If the biomass is used for industrial purposes with lower plant numbers and longer rotation periods, the planting costs amount to DM 1,500/ha without fences respectively.

Apart from the investment costs, the harvest and transportation constitute significant cost factors, which heavily depend on the production target. Given the use of specially designed harvesting machines, harvesting wood chips for energy uses at max. 4-year rotations, harvest costs of DM 20/t were ascertained. Longer rotation periods result in larger dimensioned material, which necessitates the use of conventional harvest techniques.

To assess the economic situation of short-rotation areas planted with poplars, given an energy use of woody biomass and an average volume production (10 t/ha) and lifespan (7 rotations) of the plantation, production costs between DM 55 to 85 /t absolutely dry matter of woody biomass can be expected. Leaving the operating risk out of account and expecting proceeds of DM 100/t absolutely dry matter of produced biomass, an annuity (at a 3.5% interest rate) of between DM 250 and 500 /ha can be expected. At proceeds of DM 120/t the situation looks better. Yet, at proceeds under DM 80 /t and under 8 t/ha a positive result cannot be expected.

Under the prevailing framework conditions, short-rotation cultures do not represent a serious competition to conventional agricultural food production. Matters are quite different regarding the set-aside areas. Here, the chances of realising woody biomass production for energy uses are quite good. Yet, in the event of productivity losses or cuts in volume production by only 20%, losses can already occur. Due to the fixed cost burden, the operating life of a plantation also exerts a key influence on costs. Biotic damage can be regarded as another element of uncertainty. With increasing expansion of areas, hitherto unimportant harmful organisms can cause significant economic damage due to better possibilities of development. The central question arising for farmers is the price at which the produced material can be utilised. The lack of purchasers and the low demand can be seen as reasons for the few short-rotation plantations so far being established.

4 Protective measures

No special damaging events occurred in the case of poplars and willows.

5 Exploitation and utilization

a) Exploitation

Normal rotation

The exploitation of poplars and willows under normal rotation takes place according to normal forestry practices. Poplar raw wood is graded according to the legal provisions on statutory grades for raw wood.

Short rotation

The woody biomass derived from experimental cultivation is used for specific experiments or for energy uses in the form of wood chips.

b) Utilization

A newly developed method to produce mechanical pulp (alkaline peroxide thermo mechanical pulp-APTMP) can open up market outlets for poplar wood. The benefits of this process should lie, *inter alia*, in the great strength, whiteness and drainage capacity of the mechanical pulp as well as in the low energy costs. A paper manufacturer plans to establish larger areas to produce poplar wood in short rotation (10-12 years) in the catchment area of the Heidenau industrial site near Dresden.

To produce poplar industrial wood for these purchasers, the Federal Government has been promoting a pilot project since 1997 to plant poplars with a 10-year production period on set-aside agricultural areas. Various research institutions are involved in this endeavour. All in all, 50 ha of model areas were established at various locations in Hesse and 50 ha in Saxony. Results are not available as yet.

6 Other Studies and Activities

a) Gene Conservation

In Strasbourg in 1990 the Ministerial Conference on the Protection of Forests in Europe adopted Resolution S2 "Conservation of Forest Genetic Resources". Subsequently, EUFORGEN was created, a European programme to conserve forest genetic resources. Within the framework of five networks it focuses in particular on specific tree species and/or groups of tree species. One aims at combining, increasing and disseminating international expertise in distribution, biology, genetics and threats to these species and at working out recommendations for effective conservation measures and sustainable utilisation measures. In this context international cooperation takes account of the transboundary occurrence of many tree species. Germany joined the EUFORGEN programme in 1998 and participates, *inter alia*, in the *Populus nigra* and *Populus alba* network.

Even before Germany's accession to the EUFORGEN programme various *Länder* had made great efforts to identify and map the rare occurrences of pure European black poplars. All in all some 3,500 individuals of European black poplar have been identified. Most of these trees are remnants of old stands of riparian forests which have been destroyed or of residual stands of former plantations. First the purity of the tree species is being determined with the aid of morphological characteristics. In cases of doubt *Populus nigra* can be clearly distinguished from *Populus x euroamericana* with the help of biochemical methods (isoenzyme and molecular genetic analyses).

Within the framework of the EUFORGEN network a core collection of black poplar clones has been set up in Casale Monferrato, Italy. Germany contributed 2 clones to this collection. The network is also developing a data base relating to the euro-wide occurrence of European black poplars. Data on the occurrence of European black poplars in Germany are being gathered to enter them into this data base.

As intact riparian forests are rare, *in-situ* conservation of remaining species is paramount. The possibility of cooperating with the World Wide Fund for Nature (WWF) is currently being examined. Following propagation by cuttings and *in vitro* propagation, *ex situ* conservation takes place in conservation plantations and clonal collections. The network is beginning to gear its work also to the white poplar (*Populus alba*), which is also endangered in parts of Europe.

Together with several other institutions from seven countries, two German institutions also participate in the research project "EUROPOP", financed by the European Union since 1998. These institutions are the Hessian *Land* Institute for Forest Management, Forest Research and Ecology in Hann. Münden as well as the Institute for Forest Genetics, Tree Breeding, Forest Biometrics and Informatics of the University of Göttingen. The project studies the genetic diversity of the European black poplar, evaluates the biological diversity in riparian forests ecosystems and develops conservation strategies.

b) Gene transfers in aspen

The studies of genetically modified aspen clones have been continued. In spring of 1996 a release test was started on the premises of the Institute for Forest Genetics and Forest Tree Breeding of the Federal Research Centre for Forestry and Forest Products. Eight transgenic lines with a total of 256 plants were planted in a randomised block with four replications and 96 additional control plants. The hybrid clone Esch5 (*Populus tremula x P. tremuloides*) and the clones Brauna 11 and W52 (*Populus tremula*) were used as parent clones.

Parent clone	Transgenic lines
Esch5 (hybrid aspen, female)	Esch5:35S-*rolC*#1, -#3, -#5, -#16
Brauna11 (aspen, female)	Brauna11:35S-*rolC*#2
W52 (aspen, male)	W52:35S-*rolC*#2
Esch5 (hybrid aspen, female)	Esch5:rbcS-*rolC*#4, -#18

The construct *rolC*, used for transformation, has been derived from the *Agrobacterium rhizogenes* and was used either under the control of the constitutive cauliflower mosaic virus 35S promoter (35S-*rolC*) or the light-inducible promoter of the small sub-unit of ribolose biphosphate carboxylase (rbcs-*rolC*).

The transformation method and the expression of the morphological and physiological characteristics in transgenic aspens have already been described in the last report (1996). The experiments focus on:

- expression and stability of the construct
- interactions between mycorrhizal fungi and transgenic aspen clones
- hormone and carbohydrate metabolisms in *rolC* transgenic aspen clones and their possible effects on phytopathological features

These studies have not been concluded yet, preliminary results can be summarised as follows:

i) Expression and stability

To characterise the phenotype of the transgenic plants under outdoor conditions, characteristics like foliation, plant height, stem diameter, branching characteristics and leaf size have been measured. The 35S-*rolC* transgenic plants sprouted approximately one week earlier and the rbcs-*rolC* transgenic plants only a few days before the control plants. Hormone analysis in buds demonstrated that transgenic plants contained lower concentrations of abscisic acid, a phytohormone responsible for bud and seed dormancy. Measurements of plant height and stem diameter indicated that the 35S-*rolC* transgenic plants showed much less growth, whilst the rbcs-*rolC* plants developed like the non-transformed plants. The determination of the branches clearly demonstrated a difference between the various clones: while in the 35S-*rolC* transgenic plants of the parent clone Esch5 there was a decrease in the number of branches, the parent clones Brauna11 and W52 showed an increase. It was possible to observe morphologically and phenotypically visible reversions (visible deviations from the expected 35S-*rolC* phenotype) in two transgenic lines. While only six plants expressed the 35S-*rolC* phenotype in the 35S-*rolC* transgenic lines of the parent clone Brauna11, the leaves of 26 plants were larger. These leaves, however, did not completely correspond to the leaf size of the control clone Brauna11. In one 35S-*rolC* transgenic line of the parent clone Esch5 two plants (out of a total of 32 plants) were found with visible reversions. However, in contrast with the 35S-*rolC* transgenic plants of the parent clone Brauna11 they included only parts of the plants (twigs and leaves). All these observations were made in 1997, and could be observed again on the same plants in 1998. In addition, in the 35S-*rolC* line of the parent clone Esch5 four other plants showed reversions in 1998. In all other transgenic lines no more reversions were observed until the end of 1998.

In the 35S-*rolC* transgenic plants of the parent clone Brauna11, molecular studies demonstrated that it was possible to find the expected fragments after PCR analyses using specific primers. Currently one is searching for a *rolC*-specific transcript in large and small leaves within the framework of Northern experiments. Neither PCR analyses nor Southern and Northern experiments succeeded in finding *rolC*-specific signals in reverted leaves, occurring occasionally, of 35S-*rolC* transgenic plants of the parent clone Esch5, although small leaves of the same plants, which corresponded to the 35S-*rolC* phenotype, expressed the *rolC*-specific signal. This indicates the "disappearance" of the *rolC* gene, the causes of which are to be further analysed. In order to do so, the flanking regions of the transposon are to be cloned and sequenced at the respective integration site in the genome of the transgenic plants. The expected findings will shed some light on the structure of the integration site and possibly indicate the reasons for unstable expressions and/or sequence-related characteristics in lines with stable expressions.

ii) Interactions between mycorrhizal fungi and transgenic aspen clones

Genetic modification of forest plants can only be advantageous to the utilisation of the plants if neither the formation nor the functionality of mycorrhiza are adversely affected. This is why the following questions should be explored before using transgenic trees to a great extent in forestry: Is the transgenic trees' ability to form mycorrhiza qualitatively or quantitatively restricted? Might genes be horizontally transferred from transgenic plants to mycorrhiza fungi as a result of the close contact between the symbiosis partners? Which are possible consequences of such a horizontal gene transfer?

At present the mycorrhization of transgenic aspens is being studied within the framework of the first release test with genetically modified forest plants in Germany. Currently one is quantifying the colonisation of the roots by ectotrophic and arbuscular mycorrhiza to record the mycorrhiza status. In addition, the diversity of mycorrhiza populations is determined with the aid of anatomical and molecular characteristics. Neither in the level of mycorrhization nor in the diversity of the mycorrhiza populations has it been possible to find clear differences between transgenic aspen lines and control plants. By contrast, there were many significant differences between the various aspen lines as regards one of the four most frequent ectomycorrhiza morphotypes in respect of frequency and stages of development. The fungus partner of this type of mycorrhiza has been isolated, and it is easy to cultivate it. Currently a model system is being set up to study interaction between this fungus and the different aspen lines under *in vitro* conditions.

iii) Hormone and carbohydrate metabolisms and effects on physiological features

For *rolC* transgenic aspens the project focuses on the question how the *rolC* gene influences the formation of low-molecular carbohydrates and how this causes changes in the spectrum and infestation with fungus pathogens. A field experiment is available to these studies, located on the premises of the Institute for Forest Genetics and Forest Tree Breeding of the Federal Research Centre for Forestry and Forest Products. The Institute cooperates in this project with the Max-Planck-Institute for Breeding Research in Cologne.

At the beginning the crowns of the outdoor aspens were classified. Through the iterative classification procedure the estimates of their infestation with *Pollaccia radiosa* and *Melampsora sp.* on the leaves lead to percentages ranging between 5 and 60%. It is possible to identify a classification relating to the different transformants. Resistance reactions were not found. This is also true for previous infestation results in *rolC* transgenic potatoes. The infestation level as far as *Melampsora sp.* is concerned was slightly higher than the *P. radiosa* level. The infestation pattern of both fungi shows a high degree of correspondence, the correlation coefficients were always $r > 0.9$. These studies will be continued next year and compared with the laboratory tests. A first laboratory infestation-test with *P. radiosa* on non-transformed aspen clones showed infestation differences between an early flushing (high degree of infestation) and a late flushing clone (low degree of infestation). These results correspond to the expectation value. The infection was carried out with 4,000 conidia per 20µl of solution. At 20°C, infestation symptoms developed after 10-12 days.

IV. GENERAL INFORMATION

1. National Poplar Commission

Chairman of the national Poplar Commission is the head of the Directorate-General 5 of the Federal Ministry of Food, Agriculture and Forestry.
The Secretariat of the national Poplar Commission is maintained by the Federal Ministry of Food, Agriculture and Forestry.

2. References

See Annex 2.

3. Relations with other countries

a) Exchange of cuttings, plants and pollen

Cuttings, planting stock and pollen were exchanged with many scientific institutions from Europe, Asia, North and South America:

- Institute for Forest Sciences, Budapest II., Hungary,
- C.E.M.A.G.R.E.F., Ministère de l'Agriculture, 45290 Nogent- Sur-Vernission, France,
- The Agricultural Institute, Oak Park Research Centre, Carlow, Ireland,
- Station d'Amélioration des Arbres Forestiers, Ardon, 45160 Olivet, France,
- Research Institute for Forestry and Landscape Planning, "De Dorshkamp", 6700 AA Wageningen, Netherlands,
- Universität Freiburg, Schweiz, Institut für Botanische Biologie und Phytochemie, CH-1700 Freiburg, Switzerland,
- Eidgenössische Technische Hochschule, CH-8092 Zürich, Switzerland,
- Rijkostation voor Populierenteelt, B-9500 Geraardsbergen, Belgium,
- Forstliche Bundesversuchsanstalt - Institut für Forstpflanzenzüchtung -, A-1131 Wien, Austria,
- Instituto di Sperimentazione per la Pioppicoltura, 15033 Casale Monferrato, Italy,
- Poplar Research Station, Gazi/Ankara, Turkey,
- Ontario Tree Improvement and Forest Biomass Institute, Maple, Ontario, Canada,
- Direcion de Bosques y Porques, Fontana y Ameghiono, 9200-Esquel (Chubut), Argentina,
- The Chinese Academy of Forestry, Beijing, China,
- Kibbutz Gonen, D.N. Galilelion, 12130, Israel
- Beijing College of Forestry, Beijing, China,
- Research Institute of Forests and Rangeland, P.O. Box 13-116, Teheran, Iran,
- Institute of Forest Genetics, Suweon, Kyunggido, Korea,
- VULHM, Station Uherske Hradiste, CR,
- Finnische Forstliche Forschungsanstalt Maisala, Finland,
- University of Minnesota, North Central Experiment Station, 1861 Highway 169 East, Grand Rapids MN 55744, USA,
- Swedish University of Agricultural Sciences, Division of Energy Forestry, Box 7072, 75007 Uppsala, Sweden.

Annex 1

This report is primarily based on the technical contributions of the following persons and institutions:

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Vorsitzender des Kontrollausschusses
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Annex 2

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