The second characteristic affecting 'nutrient use efficiency' was the proportion of dry matter partitioned to stems as opposed to branches and needles. The N, P and K requirement for a unit increment in stem dry weight (including bark) was usually less than half of that for a unit increment of branches, and only about 5—15% of that for a unit increment of needle dry weight. Thus, relatively small differences in partitioning to stems increased the ratio of total dry matter producion per unit of nutrient taken up, all else being equal. Differences also existed in stem nutrient concentrations, perhaps reflecting differences in wood/bark ratios or the efficiency of nutrient withdrawal from woody tissues before they died. However, these differences had much less impact on clonal nutrient use efficiencies than the large differences in stem-crown partitioning.

The third characteristic related to 'nutrient use efficiencies' was needle longevity. In both species, clones that retained their needles longest tended to produce most dry matter per unit of nutrient taken up *(Table 4)*. We can think of two reasons why this might be so: (a) clones that retained their needles longest produced most photosynthate per unit of nutrient invested in needle construction and (b) clones with the largest needle biomass possessed the largest pool of the mobile nutrients which could be circulated within the trees, thereby lessening the demand on soil nutrients. However we could find no evidence that clones which retained their needles longest withdrew most nutrients from them before they were shed.

Conclusions

We conclude from this study that there are important opportunities to select conifer clones with relatively small demands on soil nutrients during their early years of growth. The 'ideotype' for high 'nutrient use efficiency' seems to be a tree with an inherently low nutrient concentration in its foliage, which partitions a high proportion of its new dry matter to stem, and which has good needle retention. Such trees may be well-suited to grow on nutrient-poor sites.

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Research on in vitro-techniques within the framework of poplar breeding – results and future trends

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Summary

In co-operation with the KWS Kleinwanzlebener Saatzucht AG at Einbeck we have been able to reproduce on a large scale by in vitro-cultures aspen clones bred at this institute and registered for trade. The extent to which the new propagation method contributes to the practical use of breeding success is explained.

In the field of resistance-breeding cheap and simple infection methods may be used by means of in vitro-culture techniques. The example of bacterial canker on black and

balsam poplars is given to show with what great efficacy such tests may be carried out today and in the near future.

Further research on plant regeneration by in vitro-cultures or from isolated protoplasts are presented in the form of an interdisciplinary research program which we have initiated. A consistent realization of this program will lead to new impulses for poplar cultivation.

Key words: high yield clones, large scale propagation, resistance breeding, genetic modification.

Zusammenfassung

In Zusammenarbeit mit der KWS Kleinwanzlebener Saatzucht AG in Einbeck ist es gelungen, am hiesigen Institut gezüchtete und zum Handel zugelassene Aspenklone über in-vitro-Kulturen in großem Umfang zu reproduzieren. Es wird dargelegt, in welchem Ausmaß die neue Vermehrungstechnik zur praktischen Nutzung von Züchtungserfolgen beiträgt.

Im Bereich der Resistenzzüchtung lassen sich auf dem Weg über in-vitro-Kulturtechniken einfache und kostengünstige Infektionsverfahren anwenden. Am Beispiel des Bakterienkrebses bei Schwarz- und Balsampappeln wird erläutert, mit welchem Wirkungsgrad solche Untersuchungen bereits heute und in naher Zukunft durchführbar sind.

Weitergehende Arbeiten zur Pflanzenregeneration durch in-vitro-Kulturen oder aus isolierten Protoplasten werden in Form eines von uns initiierten interdisziplinären Forschungsprogramms vorgestellt. Bei konsequenter Realisierung des Programms sind neue Impulse für den Pappelanbau zu erwarten.

Schlüsselwörter: Hochleistungssorten, Großvermehrung, Resistenzzüchtung, genetische Modifikation.

Introduction

The advantages of autovegetative propagation for poplars are known for a long time. By means of the conventional methods of rooting of cuttings large numbers of black and balsam poplars especially are reproduced each year in many countries and made available for practical cultivation.

However this method of reproduction cannot be used in the same way for all poplar species. Difficulties occur particularly when no or only few preformed or latent root primordia exist in the shoot. Thus it has not been possible despite many years' efforts to achieve with aspen a practicable large scale propagation by means of rooting of cuttings, or modified methods that would be financially acceptable. On the other hand these same species have been the subject of, in some cases, spectacular breeeding success (i.a. Einspahr, van Buijtenen and Peckham 1963; Melchior and Seitz 1966; Hattemer and Seitz 1967, Benson 1972; Zufa 1972; Fröhlich and Grosscurth 1973; Johnsson 1976; Weisgerber 1976, 1983; Mohrdiek 1977; Schmiedel 1982), so that practical forestry is greatly interested in the use of new high yield clones in sufficient plant numbers.

We have been trying therefore in the past years to develop new ways of solving the existing problems by the use of in vitro-techniques. Our research is based upon the recognition of the omnipotence of the plantcell as well as upon older fundamental publications (i.a. Haberlandt 1902; White 1932, 1934; cf. also Keller 1981). Besides these we have been stimulated by the success in auto-vegetative large scale propagation by means of tissue cultures that has been achieved since the seventies throughout the world with many plant species in agriculture, fruit-growing and horticulture (cf. i.a. Morel 1960; Fast 1980 with orchids). Bonga and Durzan (1982) gave comprehensive information on the present state of knowledge and the importance of this technique for forestry.

The measures which we are initiating for forest trees are carried out in close cooperation with the KWS Kleinwanzlebener Saatzucht AG at Einbeck, under the scientific direction of Dr. Barocka. Besides other species (i.a. *Abies* spec., *Acer pseudoplatanus, Fagus silvatica, Fraxinus excelsior, Prunus avium, Pseudotsuga menziesii, Quercus petraea* and *Qu. robur, Robinia pseudoacacia, Tilia cordata, Ulmus glabra)* research up to date on poplars has been concentrated principally upon the aspen *Populus tremula* and *P. tremuloides.*

In recent years authors have reported on similar reproduction methods based on tissue culture techniques (Winton 1971; Christie 1978; Chalupa 1981; Schmid 1981; Ahuja 1983). However it is not known to what extent the resulting plant material could be used for afforestation purposes. The ability to produce almost unlimited numbers of plants for practical purposes must meanwhile be regarded as a most important criterion for the suitability of a reproduction method.

Apart from the possibility of fast and relatively unproblematic plant production, in vitro-techniques offer considerable advantages which may be used for further breeding steps. Thus it seems to be relatively easy to initiate mutations for the purpose of polyploidy-breeding. In the field of resistance breeding many different tests are possible at very early stages of development and under standardized environmental conditions. As tissue cultures can be preserved for long periods of time by easy means and without being endangered by fungi, bacteria and virus there are good prerequisites for establishing and maintaining gene-banks. In the field of hybridization the somatic hybridization of partners whose combination by sexual means was not possible up to date (e.g. genus hybrids) would seem especially promising.

We report here upon the results of our research, carried out together with the KWS, on the mass propagation of aspen on the basis of the new propagation technique, and upon resistance tests on black and balsam poplars from in vitro-cultures. Further we present an interdisciplinary program on further methods of plant regeneration by means of organ cultures or from isolated protoplasts, together with some resulting perspectives for poplar breeding.

Autovegetative propagation of aspen by in vitro-cultures

The program set up with KWS at Einbeck was able to be carried out in two stages within a very few years: first with extensive preliminary research which led in 1980 to the development of a method of optimum employability. Since 1981, in a second phase, aspen clones bred at this institute and approved for trade are being propagated on a large scale and used for silvicultural purposes in Hesse and other Federal Lands.

The propagation method used has been described in detail by Fröhlich (1982) and Barocka *et al.* (1985). The most important steps are summarized here.

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1. Gathering and treatment of explants
   Meristematic tissue is removed in small pieces (2—4 mm) from the vegetation points of the shoot (apical and axillary buds). It is then sterilized in order to obtain plant material free of bacteria and fungi. Further treatment also takes place under sterile conditions.
2. Formation of callus and shoots
   The explants are transferred to an agar medium. To improve shoot development and oxygen supply shakable suspension cultures may be used. The media contains macro and micro nutrients, sacharoses, vitamins and hormones such as auxin, cytokinin and gibberellin. At a constant temperature of 25 to 29° C and under artificial light of 1000 to 2000 lux callus tissue forms on the wound-edges of the explants from which shoots then develop.
3. Shoot proliferation
   The further development towards differentiation of plantlets takes place in separate phases for shoot and root. First the shoot pieces are transferred to a growing medium designed especially to stimulate shoot proliferation.
4. Root proliferation
   Specific growth media are also available for rooting. In regulating the growth of shoot and root the auxin-cytokinin ratio is of prime significance since cytokinin influences the division of cells and auxin the elongation of cells.
5. Development in greenhouse and nursery
   When the plantlets show root and shoot differentiation they are transferred from the petri dishes into greenhouses where they are hardened off and finally transplanted to the nursery.

The timespan needed for the propagation of aspen by means of tissue culture by the method described is short: about 10 to 14 days are needed for the shoot to form and about 8 to 10 days for the root, so that plants grown in this way can be transplanted into the greenhouses already 3 or 4 weeks after the explant is taken.

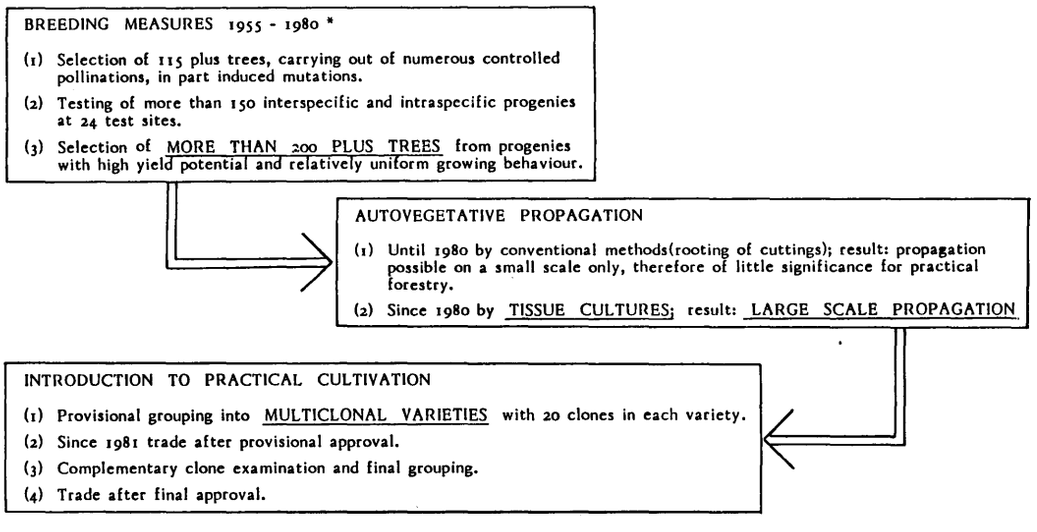
In 1981 it was possible for the first time at Einbeck to raise 50.000 plants from the triploid aspen clone 'Astria' by means of tissue culture. After one vegetation period they reached heights of 187 to 257 cm and a root-collar diameter of 13 to 23 mm with good root development.

In the following two years the propagation program was considerably extended; besides single clones we have, for the first time, included three aspen multiclonal varieties with a total of 49 clones. In 1983 we were able to grow approx. 150.000 plants from this material.

Figure 1 gives a survey of the measures we have carried through from the fundamental breeding work with aspen to the introduction of tested high yield clones into practical forestry. Here it can be clearly seen to what extent a fast mass propagation and development of plants by tissue culture, which is not dependent on the vegetation season, contributes to the practical usability of breeding results.

There have been many reports, the last by Fröhlich and Weisgerber (1985) on the breeding steps carried out in Hann. Münden with aspen and the results obtained up to the final establishment of multiclonal varieties as tested reproductive material conforming to official regulations. *Figure 1* shows that at the present time more than 200 plus trees are available for propagating and cultivating purposes which originate from progenies from controlled pollination of earlier series of tests; these have a high yield potential and relatively uniform growing behaviour. In the coming years therefore those aspen already being propagated can be complemented by a large number of other clones which have been tested as far as growth behaviour and state of health are concerned. We plan to collect this material into different groups as multiclonal varieties and to allow it to be used in practical forestry in this form only. By this means it is possible to prevent the establishment of large monoclonal stands which would be extremely exposed to biotic and abiotic dangers.

The aspen propagated up to now by the in vitro-method used at Einbeck were able to be used on a large scale for afforestation purposes, with the exception of those needed for special research projects. First results and experiences are thoroughly positive (Schulzke 1983). The healthy and fast-growing plants are becoming increasing popular in forest management as they may be used for many different



* Details refer to older test series. Different original material was used for more recent research which is not yet completed.

Fig. 1. — The use of breeding success with aspen *(Populus tremula, P. tremuloides)* for practical forestry by autovegetative large scale propagation by tissue cultures.

purposes, especially for the establishment of mixed stands with other deciduous tree species, but also with Norway spruce and Douglas fir.

Resistance tests with black and balsam poplars propagated by in vitro-cultures

Poplar cultivation in the Federal Republic of Germany and in the neighbouring west European countries has been suffering increasing harm in the last years from canker diseases caused by the bacterium *Xanthomonas populi* subsp. *populi* (Ridé) Ridé et Ridé.

Detailed observations on the biology of the pathogen and on clone-specific differences in susceptibility and resistance of the host plants have been compiled (i.a. Ridé 1963; Gremmen and Koster 1972; Gremmen and de Kam 1974; Ridé and Ridé 1978; FAO/CIP 1979).

At our institute in Hann. Münden Kechel (1982) succeeded in testing clone-specific resistance of different poplar species by the artificial infection of young plants. Inoculations are carried out generally on one-year-old shoots of poplars of one or more years of age by a method suited to an early test. The results of observations on the extent of the canker disease are available 2 to 3 years after infection and are of considerable importance when assessing a clone's suitability for cultivation. The method is easy to use, makes definite statements possible and is a sensible complement to field observations on many test sites with varying site conditions.

The success with in vitro-propagation of aspen motivated us to use this technique for the canker-resistance test also. We anticipated considerable rationalization from this method as the timespan, money and land needed for the observations could be reduced.

In the meantime the infection method was tried out and developed to practicability on plants raised from tissue cultures of black and balsam poplar clones with a known degree of susceptibility or resistance to canker respectively (Kechel and Böden 1984). After in vitro-propagation had been carried out at the KWS at Einbeck the plants were transplanted and infected in the greenhouse with bacterial suspensions in graduated concentration at different ages of between 1 1/2 weeks and 7 months. At the infection point at about 30 to 50% of the plant height a leaf and the bud situated between shoot and leaf stalk had been removed with a scalpel beforehand.

Clear canker symptoms became visible on the susceptible clones (e.g. *P.* × *euramericana* 'Brabantica') about 3 to 4 weeks after infection; after 2 months typical necroses developed. With the resistant clones on the other hand (*P.* × *euramericana* 'Forndorf', *P. trichocarpa* 'Columbia River') the wounds healed quickly.

Neither the age of the plants at infection nor the concentration of the bacterial suspension had a significant influence on the degree of canker susceptibility. The type and intensity of reactions to infection in all clones examined coincided well with disease symptoms known from field observations. After only a short testing period of the few weeks old plants therefore clear statements can already be made on the clone-specific differences in susceptibility to the pathogen of poplar canker.

A further rationalization of the method could probably be achieved if the resistance test could take place already during the in vitro-phase. Preliminary investigation on this subject is being carried out at present in cooperation with the KWS, and promises successful results.

In order to give a clear picture of the advance that has been achieved in the testing of poplar resistance to the pathogen of bacterial canker *Table 1* presents the most important criteria of the methods we have used hitherto and intend to use in the future. The knowledge of earlier years allowed only field observation to be made which could give only rough hints as to resistance behaviour specific to clone and species; on the other hand they occasioned considerable costs for the raising of plant material and for the establishment, management and repeated assessment of numerous test areas. A systematic evaluation of the material to be examined was made possible by the help of artificial infection. One may see from the table that the improvement described, which was achieved in steps, saves time and cuts costs. The advantages of resistance testing in conjunction with auto-vegetative propagation by in vitro-cultures are obvious. This is true for the unlimited availability of plant material independent of the season, which can be tested immediately, as well as for the great reduction in the duration of the tests.

In order to judge the different testing methods more easily we evaluated the criteria given in *Table 1.* The result is given in *Figure 2* where the efficacy shows to what extent each respective method leads quickly and cheaply to definite statements regarding the susceptibility to canker

Table 1. — Important criteria in testing the resistance of black and balsam poplars to the pathogen of bacterial canker, *Xanthomonas populi subsp. populi* (Ridé et Ridé).

Type of testing	carried out since	Propagation of the testing material	Place of raising	Type of infection	Time of infection	Place of testing	duration of testing
field test	1955	by cuttings in large numbers, therefore labour and time-consuming	nursery	natural	spring/ early summer	nursery and at least 2 to many test areas with different site conditions	at least 15 years
test in infection quarters	1975 (preparatory tests since 1964)	by cuttings taken in small numbers	nursery	artificial	spring/ early summer	nursery	3 years
test in the greenhouse	1982	by tissue cultures with no limit regarding time or numbers	tissue culture laboratory	artificial	independent of the season	greenhouse	3 months
test in the in-vitro-phase	1983 (as yet only preparatory)	by tissue cultures with no limit to time or numbers	tissue culture laboratory	artificial	independent of the season	tissue culture laboratory	a few weeks during the in-vitro-phase

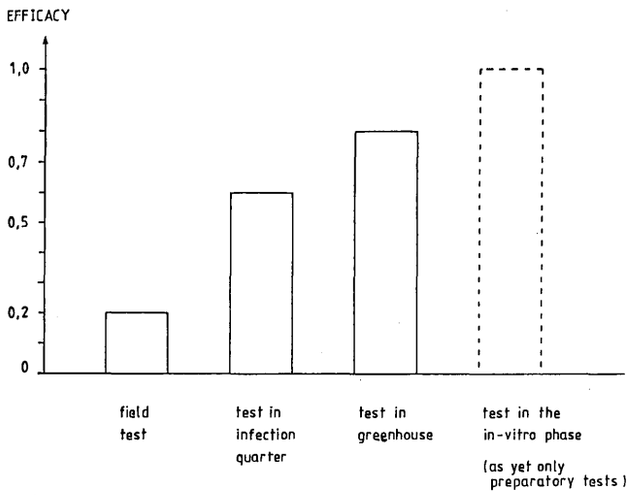


Fig. 2. — Resistance testing of poplars to the pathogen of bacterial canker. Estimated efficacy of different testing methods (comp. *Table 1*).

of the clones examined. The higher the efficacy, the better the assessment possibility would appear to be.

It may be seen that efficacy with the method of artificial infection is at its highest immediately after the in vitro-phase at the moment. An even better result may be obtained when the investigations on resistance during the in vitro-stage have been successfully completed.

The use of methods with high efficacy makes it possible even today to carry out in series big testing programs with black and balsam poplars, especially with new and promising populations created in hybrid breeding. The result of these measures, which can be carried out at short notice, will determine at our institute in future which individuals may be selected for further testing of their growth behaviour on test areas with different site-conditions.

The results shown here of our research into the canker resistance of black and balsam poplars in conjunction with in vitro-culture technique have a model quality. The extension of this investigation to other poplar species (section Leuce) and other pathogens is planned or already underway.

Future propagation and breeding program on the basis of in vitro-techniques

The success up to this point of the research described encourages us to embark upon a systematic extension of these measures in accordance with the breeding aims mentioned initially. It must be taken into consideration that silviculture has to rely to an increasing degree on the use of in vitro-culture techniques. The rapid spread of damage to forests caused by air pollution demands urgent breeding activity to preserve and propagate endangered tree species and populations, as well as to improve the stability and wood production of our forests. The availability of suitable plant material in sufficient amounts for reforestation in most severely damaged areas will in future often be possible only by in vitro-cultures.

Research in this field therefore assumes considerable silvicultural importance and urgency. For this reason we have decided to develop an inter-disciplinary research program on the basis of our investigations hitherto, in order to make the best possible use of relative knowledge on the subject and of experience in related fields of breeding. This should ensure fast action. We have already obtained the co-operation of institutes which have been working successfully for many years on in vitro-cultures with agricultural and horticultural plant species as also with fruit trees and vines, and which have achieved international recognition (cf. i.a. Reuther 1975, 1983; Li Liang-Cai and Kohlenbach 1982; Sonneborn *et al.* 1982).

The following measures are planned in connection with this joint research project:

1. Development of tissue culture techniques specific to different species to facilitate genetically identical mass propagation on the basis of already existent methods.
2. Carrying out of in vitro-test methods to test the resistance or tolerance of propagation material to fungi, bacteria and virus as well as to damage caused by abiotic factors.
3. Investigations into ploidy relations during and after the in-vitro phase and measures directed to breeding triploid plants.
4. Working out of suitable methods for isolating and cultivating protoplasts and for plant regeneration on a somatic basis.
5. Manipulations with genotypes by the introduction of foreign genetic material of special quality in protoplasts. This could take place with the help of plasmides and lead, for example, to an improvement in resistance characteristics.
6. Interspecific and intergeneric hybridization by fusion of protoplasts. This would make it possible to overcome barriers caused by incompatibilities which often impede the generative method, and to gain new genotypes with valuable resistance and growing characteristics.
7. Establishment of a living collection of valuable genotypes or those threatened by extinction.

The research program covers a wide spectrum of different tree species. For the first informative investigations in many areas poplars would seem to us to be the most suitable species as with this genus one can build on the positive results of many years' breeding work.

From a realization of this program we expect a favourable development in poplar cultivation within a few years, mainly for the following reasons.

1. The much too narrow clonal base used in most countries at present can be considerably enlarged as those clones hitherto difficult to propagate can in future be obtained commercially in large plant numbers.
2. New hybrids between different species and genus, also triploids and plant forms created by genetic manipulation will contribute to an increase in the supply of clones. It may be assumed also that these new clones will be capable of making especially good use of the site potential at hand and will be suited preferably for the cultivation of mixed stands with other tree species in many different ways.
3. Traditional monoclonal cultures have proved to be particularly susceptible to dangers (e.g. clone-specific calamities among black poplars caused by *Marssonina* spec., among balsam poplars caused by *Xanthomonas populi*, among grey poplars and aspen caused by *Pollaccia radiosa*). The resistance breeding measures planned will help to reduce cultivation risks considerably.
4. Furthermore by building up multiclonal varieties a noticeable increase in genetic variability can be achieved and epidemic diseases thus avoided to a large extent. Due to our breeding work with balsam poplars and aspen multiclonal varieties with 5 to 20 single clones

respectively are now already registered for trade in the Federal Republic of Germany.

The research on in vitro-techniques presented in this report is therefore of far-reaching practical significance. We are convinced that the measures already successfully begun or planned for the near future will open up new and hopeful perspectives for the breeding and cultivation of poplars in our country.

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Linkage Disequilibria Among Allozyme Loci in Natural Populations of Liriodendron tulipifera L.

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Summary

Linkage disequilibria between pairs of six isozyme loci were studied in natural populations of yellow poplar *(Liriodendron tulipifera* L.) from six locations in North Carolina. Genotypic data were used to estimate the Burrows composite measure of linkage disequilibrium. In three of the locations, linkage disequilibria were estimated for populations belonging to two different generations. Trees in the older generation were seed parents of seedlings studied in the younger generation. Extensive linkage disequilibrium was found in the seedling populations, whereas in the sexually mature populations only weak evidence for disequilibria was detected. In the seedling populations, disequilibria were found at five of the six locations studied. This result indicates that linkage disequilibria are widespread during the early seedling stage of development in this species.

Key words: Gametic Phase Disequilibrium, Burrows Measure, Yellow-Poplar, Correlation, Digenic Measure.

Zusammenfassung

Bei natürlichen Populationen von *Liriodendron tulipifera* auf 6 Standorten im nördlichen Carolina wurden Kopplungsgleichgewichte zwischen 6 Isoenzym-Loci-Paaren untersucht.

Um das Kopplungsgleichgewicht der zusammengesetzten Messung nach Burrows zu schätzen, wurden genotypische

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