Figure 7. — Adventitious shoot clusters of Norway spruce.

(e. g. winter buds or newly formed buds after flushing) were the factors mainly responsible for grafting success.

Changes of needle parameters (e. g. width) in grafted spruce buds confirmed similar results that were obtained with other conifers (EWALD et al., 1991). Further experiments are needed to test the influence of the juvenile rootstock on morphological and growth parameters of grafted organs.

Organogenesis has not been the only research interest during the last few years. In close cooperation with other scientific institutions, methods have been developed for the establishment of embryogenic lines of spruce (SÜSS et al., 1990) and larch, including early stages of plant regeneration. These first encouraging results elucidated to some extent the processes involved in plant regeneration via somatic embryogenesis in spruce and larch. The results demonstrated the developmental regulation is a delicately-controlled phenomenon in conifers. Additional research work must be done on the processes of both somatic embryogenesis and organogenesis in conifers to more fully understand it and to carry out an effective plant regeneration in vitro.

Literature

(see part II).

Aims and Results of Basic Research in the Institute of Forest Tree Breeding in Waldsieversdorf, Germany

II. The Use of Enzyme Gene Markers for Practical Breeding Tasks

By H. HERTEL*)

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Modern concepts for conservation of the genetic resources of forests and for breeding methods need support from genetic research. For more than 20 years, the use of enzyme gene markers in forestry has been a successful way of acquiring information on the genetic structure of provenances, populations and individual trees.

In the last few years, molecular genetic approaches, such as analysis of restriction fragment length polymorphisms (RFLPs) and DNA fingerprinting, have been applied to forest genetics, opening new possibilities for studies of the nuclear and organelle genomes. The older, well-tested method using isoenzyme markers, can still be applied to research on population genetics and breeding and is used in many institutes. A discussion of our work using isoenzyme methods follows.

Proteins were extracted from haploid (conifer endosperm) or diploid (buds, leaves, callus) tissues by homogenization in an extraction buffer. After the electrophoretic separation of proteins in a polyacrylamide or starch gel, the enzyme proteins were specifically stained. The isoenzyme

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patterns which become visible in this way, can be attributed to different gene loci and alleles.

For the identification of a polymorphic gene locus, it is necessary to prove the alternate occurrence of two different isoenzyme bands in the patterns of haploid endosperm of one tree in the relation of 1:1, the normal Mendelian relation after meiosis. The identification of a gene locus in angiosperms is not as easy as in conifers because they have no haploid tissues. In general, it is necessary to analyse the offspring of controlled pollinations or large numbers of progenies of single trees.

The first utilization of enzyme gene markers in the Department of Forest Tree Breeding in Waldsieversdorf was in 1987. We have focused our attention on practical breeding tasks with material from the following tree species: Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), beech (*Fagus sylvatica*), larch (*Larix decidua* and *L. kaempferi*) and Douglas-fir (*Pseudotsuga menziesii*) and we started the work with oak (*Quercus ssp.*) and black locust (*Robinia pseudoacacia*).

From analysis of 14 polymorphic gene loci in samples from several different seed stands of Scots pine, we found variation in the number of alleles and genotypes and in average heterozygosity. It was noteworthy that the stand with the best growth and morphological properties had the highest genetic variation in all parameters (HERTEL and EWALD, unpubl.). Another problem important for seed collection strategies was the nature of genetic differences among seed samples from distinct trees in one stand. Analysis using the two polymorphic loci AAT-B and AAT-C, with 5 and 4 alleles respectively, indicated that the genetic distances at these two gene loci between the paternal contribution of embryos in seed samples from four single trees are in the same range as the distances between the stands (HOFFMANN, 1991).

A high level of heterozygosity and genetic diversity as causes for survival capacity and adaptability are frequently been described for forest trees (MÜLLER-STARCK 1985; BERGMANN and SCHOLZ, 1989). In an experiment with mature beech trees from an air pollution-impacted stand near a cement mill, we compared the genetic structure of 26 pairs of injured and healthy trees by using 12 enzyme gene markers. The average heterozygosity in the group of healthy trees (Ho = 0.16) is significantly higher than in the group of injured trees (Ho = 0.11), although examination of single gene loci showed that differences in allele or genotype frequencies existed at only 2 of them (HERTEL and ZANDER, 1991).

Norway spruce was also investigated. Many spruce stands have died in part or completely in the Saxon Ore Mountains on a consequence of several decades of heavy SO_2 emissions. The conservation of spruce trees of various provenances surviving these conditions began 20 years ago. Clones were propagated by cuttings or grafting in order to establish clone collections, seed orchards, and mother tree plantations in areas with a lower level of air pollutants.

This material can now be used as a basis for the reforestation of Norway spruce stands after reduction of SO_2 pollution in the Saxon Ore Mountains. Since SO_2-related selection may have modified the genetic structure of our ex situ material in comparison with material from other spruce populations, genetic analysis in seed orchards should give recommendations for breeding or other measures to obtain seeds with a high level of genetic variation. The objective is to attempt to reconstruct progenies from native provenances with a maximum adaptive potential for stand establishment, i. e. with a high level of heterozygosity and genetic diversity.

Genotypes of 36 flowering clones of Norway spruce in the plantation Dahmsdorf near Waldsieversdorf were analysed by enzyme gene markers. In this material a mean heterozygosity of H_0 = 0.23 and a fixation index of F = 0.03, based on 18 polymorphic enzyme gene loci, appears adequate for the production of seeds with a high level of genetic variation for reforestation (HERTEL et al., 1991). Results indicate that the genetic structure is similar to that of spruce stands in the Tatra Mountains (MUONA et al., 1990).

There is no evidence of any substantial restriction of genetic variation. We found many rare alleles; of the 49 alleles, 15 have frequencies lower than 5%. It should,

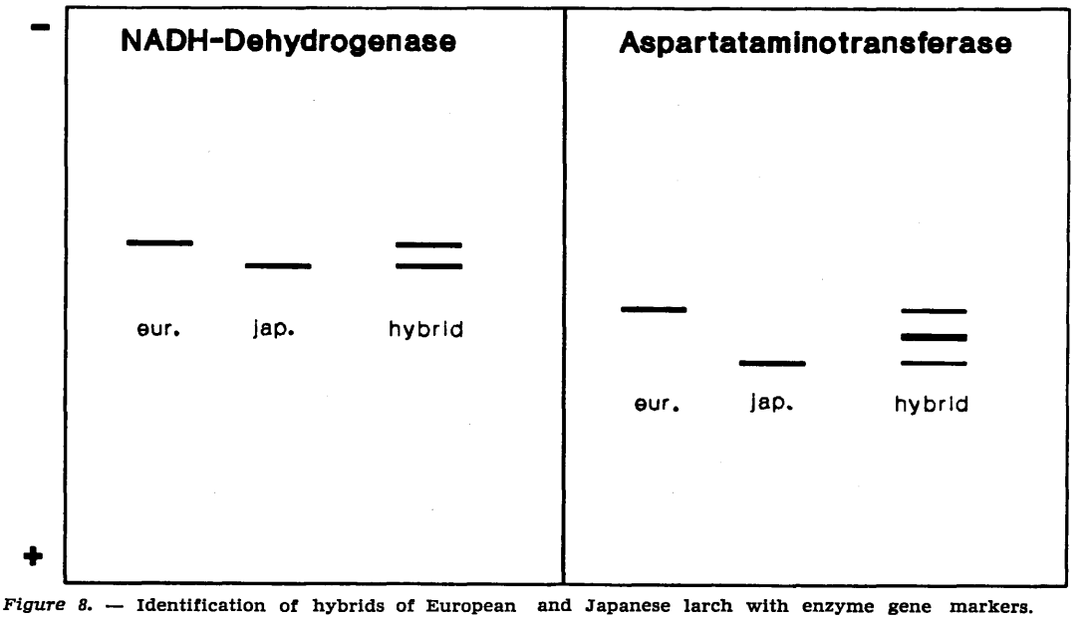

Figure 8. — Identification of hybrids of European and Japanese larch with enzyme gene markers.

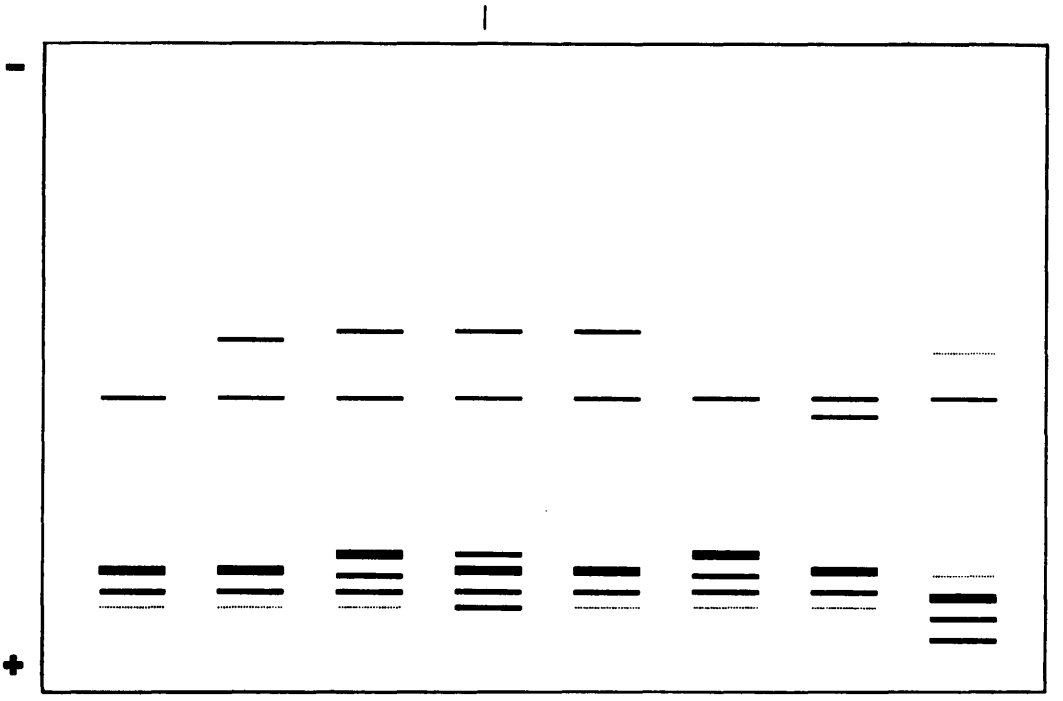

Figure 9. — Variation of leucine aminopeptidase patterns in Black locust leaves.

therefore, be possible to select clones for crosses with a higher level of expected heterozygosity in the offspring, due to a high number of rare alleles in parents. However we at all suggest considering the natural genetic structure with typical minor and major polymorphisms in the progenies.

Controlled pollination is useful for the combination of clones if they are unable to pollinate naturally because of different times of flowering or wide separation in the plantation.

One application of isoenzyme markers is the study of family relationships. In larch and Douglas-fir, the objective was the identification of hybrids of interspecific and intraspecific crosses.

In larch, we wished to determine the proportion of hybrids between European and Japanese larch in seeds from a hybrid seed orchard near Graupa in Saxony, using isoenzyme marker technique similar to that of BERGMANN and RUETZ (1987). The seed orchard was composed of one clone of Japanese larch and four clones of European larch. Two suitable gene loci were found useful for discrimination between the hybrid and European or Japanese larch. These were AAT-A and a NADH-dependent dehydrogenase. Both loci have two alleles, one with a faster migration in the Japanese clone and one with a slower migration in all of the four European clones in the homozygous state. Only hybrids of the two larch species exhibit the typical heterozygous enzyme pattern (*Fig. 8*). The proportion of hybrids from the five clones in the 1987 clonal seed samples ranged from 30% to 85% and probably depended on the location in the seed orchard and on time of flowering (BRAUN et al., 1990). In Douglas-fir, isoenzyme markers were useful to assure that only the hybrid progeny from crosses between the blue (*Pseudotsuga menziesii* var. *glauca*) and green form (*P. menziesii* var. *menziesii*) were propagated by biotechnological methods (SCHNECK, 1991).

The last example of the application of isoenzyme markers is the attempt to detect clonal differences within individuals of a population of black locust characterized by excellent stem form. Such individuals were selected in several stands, but it was necessary to consider the possibility that some of them might be genetically indentical because of the ability of black locust to regenerate from root suckers. When 9 enzyme systems were tested, variation was found only at 4 enzymes (EWALD et al., 1992). The highest number of different patterns occured in the enzyme leucine aminopeptidase (*Fig. 9*). It was possible to distinguish between 2 or 3 distinct groups of trees on the basis of isoenzyme patterns from the total of 4 to 7 trees tested in each stand. The groups that did not show any variation could consist of ramets of the same clone grown from root suckers. However, it cannot be excluded that some individuals of a group may originate from seed. Those individuals could not be discriminated by the isozymes used because of the limitation of the isozyme method. This specific case may illustrate the inadequacy of enzyme gene markers in comparison with RFLPs.

However, both methods have certain advantages and disadvantages, and both are likely to be applied to genetic research for breeding and for conservation of forest genetic resources in the future.

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Literature

AHUJA, M. R.: Somatic cell differentiation and rapid clonal propagation of aspen. Silvae Genetica **32** (3—4): 131—135 (1983). —

Bergmann, F. und Ruetz, W.: Identifizierung von Hybridlärchensaatgut aus Samenplantagen mit Hilfe eines Isoenzym-Markers. Silvae Genet. 36: 102—103 (1987). — Bergmann, F. and Scholz, F.: Selection effects of air pollution in Norway spruce (*Picea abies*) populations. Genetic effects of air pollutants in forest tree populations. Ed. Scholz, Gregorius and Rudin. Springer-Verlag (1989). — Braun, H., Hertel, H., Peschel, S. und Matschke, J.: Untersuchungen zum Anteil von Hybridlärchen aus einer Samenplantage. Beitr. Forstwirtschaft 24: 151—155 (1990). — Ewald, D., Naujoks, G., Hertel, H. und Eich, J.: Hat die Robinie in Brandenburg eine Zukunft? Allg. Forstztg., im Druck (1992). — Ewald, D., Naujoks, G. und Matschke, J.: Regeneration von Pappelpflanzen aus Antheren. Beiträge für die Forstwirtschaft 25: 16—18 (1991). — Ewald, D., Putenikhin, V. und Matschke, J.: Mikropfropfung adulter Koniferen — Möglichkeiten und Perspektiven. Allgem. Forstz. 17: 878—880 (1991). — Hertel, H., Ewald, C. and Matschke, J.: Genetic analysis in a *Picea abies* seed orchard — A contribution for reconstruction of SO_2 damaged populations in the Saxon Ore Mountains. Poster International Workshop Forest Genetics. Ufa, USSR. (1991). — Hertel, H. und Zander, M.: Genetische Unterschiede zwischen gesunden und geschädigten Buchen eines belastenden Bestandes. Berichte des Forschungszentrums Waldökosysteme, Reihe B, Bd. 22: 227—229 (1991). — Hoffmann, U.: Genetische Charakterisierung eines Kiefernsaatgut-Komplexes mit Enzym-Genmarkern. Diplomarbeit Martin-Luther-Universität Halle — Wittenberg (1991). — Hübl, S. und Zoglauer, K.: Entwicklung einer In-vitro-Vermehrungsmethode für züchterisch wertvolle Lärchen. Beiträge für die Forstwirtschaft 25: 18—20 (1991). — Matschke, J., Bolland, G., Ewald, D. und Schneck, H.: Möglichkeiten der beschleunigten Vermehrung von Braunmaserbirken. Beiträge für die Forstwirtschaft 21: 21—25 (1987). — Müller-Starck, G.: Genetic differences between "tolerant" and "sensitive" beeches (*Fagus sylvatica* L.) in an environmentally stressed adult forest stand. Silvae Genet. 34: 241—247 (1985). — Muona, O., Paule, L., Szmidt, A. E. and Kärkkäinen, K.: Mating system analysis in a central and northern European population of *Picea abies*. Scand. J. For. Res. 5, 97—102 (1990). — Naujoks, G., Ewald, D. und Matschke, J.: Ein Beitrag zur In-vitro-Kultivierung von Populus spec. Beiträge für die Forstwirtschaft 21: 102—106 (1987). — Schneck, V.: Identifizierung von Hybriden aus Kreuzungen zwischen Varietäten der Douglasie (*Pseudotsuga menziesii* [Mirb.] Franco) mittels Isoenzymanalyse. Beitr. Forstwirtschaft 25: 29—31 (1991). — Suess, R., Ewald, D. und Matschke, J.: Erzeugung somatischer Embryonen aus Saatgut der gemeinen Fichte (*Picea abies* [L.] Karst.). Beiträge für die Forstwirtschaft 24: 126—130 (1990). — Whitehead, H. and Giles, K.: Rapid propagation of poplars by tissue culture methods. N. Z. Journ. For. Science 7: 40—43 (1977).

Pressemitteilung

Veränderungen bei der BFH, Hamburg

Seit dem 1. 1. 1992 ist die Bundesforschungsanstalt für Forst- und Holzwirtschaft (BFH) mit neuen Einrichtungen in den neuen Bundesländern vertreten.

Die Umsetzung der Empfehlungen des Wissenschaftsrates zur Neugestaltung der agrar- und forstwirtschaftlichen Forschung der ehemaligen DDR hat zu einer personellen Verstärkung der BFH um insgesamt 65 Mitarbeiter/-innen geführt, darunter 20 Wissenschaftler/-innen. Entsprechend der Zielvorgabe stammen alle aus der ehemaligen Forschungsanstalt für Forst- und Holzwirtschaft, Eberswalde/Brandenburg.

In den neuen Bundesländern wurden 2 neue Institute gegründet sowie 2 Außenstellen zu schon vorhandenen Instituten in Hamburg. Im einzelnen handelt es sich am *Standort Eberswalde* um ein Institut für Forstökologie und Walderfassung, eine Außenstelle des Instituts für Ökonomie sowie eine Außenstelle des Instituts für Holzbiologie und Holzschutz, ferner am *Standort Waldsieversdorf* um ein Institut für Forstpflanzenzüchtung.

Im Zuge einer Neustrukturierung der Aufgabenverteilung wurden einige der bisherigen Arbeitsgebiete aus Hamburg in die neuen Institute verlagert mit der Folge, daß bei den bisherigen Instituten Umbenennungen erforderlich wurden. Das Institut für Weltforstwirtschaft und Forstökologie heißt jetzt — wieder wie in früheren Jahren — Institut für Weltforstwirtschaft. Aus dem Institut für Forstgenetik und Forstpflanzenzüchtung wurde das Institut für Forstgenetik.

In einer kleinen Feierstunde am 2. 1. 1992 in Eberswalde wurden die Arbeitsverträge ausgehändigt und die neuen Mitarbeiter/-innen in mehreren Reden, mal ernst, mal launig, begrüßt. Ministerialdirigent Wermann vom Bundesministerium für Ernährung, Landwirtschaft und Forsten (BML) betonte in seinem Grußwort vor den neuen Mitarbeiter/-innen und den aus Hamburg nach Eberswalde gekommenen Mitgliedern des Kollegiums und der Verwaltung der BFH, daß die Erwartungen seitens des BML hoch sind. Viele drängende Fragen des Ressorts warten auf wissenschaftlich fundierte Antworten.

Ein wesentliches Ziel für die nahe Zukunft ist es, daß die einzelnen Einrichtungen der neuen, größeren BFH, trotz der erheblichen räumlichen Entfernungen, möglichst schnell zu einer leistungsfähigen Einheit zusammenwachsen.

Der Bundesminister für Ernährung, Landwirtschaft und Forsten hat aufgrund der Wahl des Kollegiums der Bundesforschungsanstalt für Forst- und Holzwirtschaft (BFH) in Hamburg Herrn Professor Dr. C. Thoroe zum Leiter der BFH für die Amtszeit vom 1. 1. 1992 bis 31. 12. 1993 bestellt und Herrn Professor Dr. D. Noack zu dessen Vertreter.

Prof. Dr. C. Thoroe, der seit 1987 das Institut für Ökonomie der BFH leitet, löst Herrn Prof. Dr. H. H. Nimz ab, der das Amt des Leiters der BFH in den letzten 4 Jahren wahrgenommen hat.

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