

variables. Where the economic value is related to an environmental variable, nonlinear genotype-by-environment interactions are created. The environmental effect on value may be due to biotic variations or to a purely geographic effect such as closeness to a mill. Such a situation is described by OHBA (1984) for *Cryptomeria japonica*.

Implications

Since most genotype or provenance values are multiple trait composites for forestry, and since any departures from the simple assumptions of linearity and constancy can induce unexpected effects, the existence of rank-changing interactions may be very common in most tree breeding programs. Thus, in forest genetics research where multiple regression techniques are used with familiarity, there is obviously a need for multivariate analyses (NAMKOONG, 1967). As basis for provenance selection, multivariate analysis can be a substantial aid for any explicit value function (NAMKOONG, 1982).

Multiple-trait evaluations are needed to breed within populations and to choose individuals for single or multiple population breeding (NAMKOONG, 1976). Even in the absence of departures from linearity, the multiplicity of selection objectives may require such a diversity of performance types and value functions that multiple populations are required for economically efficient breeding. From an initial single provenance or population, several populations may be developed. In such cases, the set of individual genotypes in the separate breeding groups may abruptly switch among groups such that no individual is a member of more than one foundation population. Switching occurs if the objective functions are widely different, but it can also occur with small changes in the function, if the genotypic set is not convex in the trait space. That is, if all of the genotypes available for selection are good for some traits or

sites, and poor for others, and none are good for all, then even small changes in the value function can completely change the selected set of genotypes. Hence, selection must jointly consider the multiple nature of trait-by-environment responses, and the multiplicity of value functions.

In this paper, we have presented only linear models and value functions. The results are not qualitatively different for nonlinear functions, though they are more difficult to program.

It is significant that environmental effects on selection can commonly occur in the absence of traditionally defined genotype-by-environment interactions. These effects are uncovered only when multiple traits are evaluated. When nonlinear effects exist and value functions are not constant, there is no generally valid way to predict whether selection will be more or less complicated or will involve multiple or single populations. The effects of traits and values must be analyzed in detail for each environment.

Literature Cited

- ABOU-EL-FITTOUH, H. A., RAWLINGS, J. O., and MILLER, P. A.: Classification of environments to control genotype by environment interactions with an application to cotton. *Crop Science* 9: 135–140 (1969). — GIBSON, G. L.: Genotype-environment interaction in *Pinus caribaea*. Commonwealth Forestry Institute, Oxford. 112 pp. (1982). — NAMKOONG, G.: Multivariate methods for multiple regression provenance analysis. Proc. of 14th IUFRO Congress, Section 22, Munich. (1967). — NAMKOONG, G.: A multiple-index selection strategy. *Silvae Genetica* 25: 199–201 (1976). — NAMKOONG, G.: An application of biometrics in provenance analysis. *J. Tree Sci.* 1: 57–63 (1982). — NAMKOONG, G.: The influence of composite traits on genotype by environment relations. *Theoretical and Applied Genetics* 70: 315–317 (1985). — OHBA, K.: Genetics and breeding strategy of *Cryptomeria*. p 361–371. In: *Genetics: New Frontiers*, Proc. of XV International Congress of Genetics, Dec. 1983, New Delhi, India, Volume IV Applied Genetics. V. L. CHOPRA, B. C. JOSHI, R. P. SHARMA and H. C. BANSAL (eds.). Mohan Primalani, Oxford and IBH Publ. Co., New Delhi, India. 398 pp. (1984).

Are the EEC Directives on Forest Reproductive Material Genetically Adequate?¹⁾

By H. H. HATTEMER

Abteilung Forstgenetik und Forstpflanzenzüchtung,
Georg-August-Universität Göttingen,
Büsgenweg 2, 3400 Göttingen, Germany

(Received 30th September 1985)

Summary

Directives issued by the Council of the European Communities regulate the marketing of forest reproductive material in the member countries. In view of the long-term process of biological production in forestry, the genetic properties of forest seed are more important than those of short-lived crops. It is therefore desirable to have legislation which increases the purchaser's confidence in the phenotypic potential of forest reproductive material and the validity of the accompanying documents.

Because the Directives reflect the level of forest genetic knowledge of more than 20 years ago, their general conception as well as certain individual regulations conflict

with insights since gained in the field of population genetics. A discussion of these shortcomings illustrates the need for a revision of these Directives.

Key words: Forest reproductive material, certification, seed legislation, population genetics.

Zusammenfassung

Vom Rat der Europäischen Gemeinschaften erlassene Richtlinien regeln das Inverkehrbringen forstlichen Saat- und Pflanzguts in den Mitgliedsländern. Angesichts der langen Lebensdauer der Waldbäume sind in der Forstwirtschaft die genetischen Eigenschaften des Saat- und Pflanzgutes von größerer Bedeutung als bei der Begründung kürzerlebiger Pflanzenbestände. Es erscheint daher wünschenswert, eine Gesetzgebung zu haben, welche das Ver-

¹⁾ Dedicated to Prof. WOLFGANG LANGNER ON OCCASION OF HIS 80th birthday.

trauen des Käufers in das phänotypische Potential des Vermehrungsguts und in die Richtigkeit der Begleiddokumente festigt.

Da diese Richtlinien den forstgenetischen Wissensstand höchstens bis zur Mitte der 60er Jahre widerspiegeln, besteht eine Reihe von Konflikten zwischen sowohl der Grundkonzeption als auch einzelnen Vorschriften und der Populationsgenetik. Die Diskussion einiger ihrer Mängel belegt die Notwendigkeit einer Revision dieser Vorschriften.

Es ist aus diesem Grunde auch nur in Ausnahmefällen möglich, aus Freilandbeobachtungen ohne saubere Experimente wirklich zwingende Einsichten in physiologisch-ökologische oder gar genetische Gegebenheiten zu erhalten; sie werden erst auf der Basis experimentell unterbauter Befunde in vollem Umfang bedeutungsvoll. Darüber hinaus kann es auch nie gelingen, nur aus physiologisch-ökologischen Versuchen Rückschlüsse auf die Erbkonstitution zu ziehen, wenn nicht damit verbunden genetische Methoden angewendet werden.

MARQUARDT (1955)

I. Introduction

The potential of tree breeding for increasing forest yield has been repeatedly demonstrated. Numerous breeding projects comprising a variety of tree species and populations are presently being conducted in many countries.

We may define yield in the widest sense as a combination of such diverse components as volume and quality of wood, stem form, branch morphology, and health of trees. We may also define breeding in a wide sense to comprise both the testing of geographic races of forest trees and the artificial selection within these races.

Tree breeders are by no means the only people attempting to improve the phenotypes of trees. Those working in other fields of forestry, such as nutrition, protection, and silvicultural techniques, merely apply other methods. The breeder works towards changing the phenotypic mean of a tree population in a given set of environments by utilizing the genetic basis of an observed trait variation. By selecting superior phenotypes, he thus hopes to increase the frequencies of certain unknown genes. When reproducing a selected part of the population he expects not only to maintain the changed gene frequencies but also to attain a further frequency change in subsequent generations, instead of repeatedly starting anew at the original level of yield. This is one reason for the continuing attractiveness of the breeding approach, even though it is very time-consuming. Another reason is that artificial selection can sometimes improve the phenotype of several traits simultaneously.

In view of the importance of the seed, it is not at all surprising that legislation exists which aims not only at avoiding past failures in the choice of reproductive material but also at facilitating the transfer of breeding results into practical forestry. The IUFRO Working Party on Legislation on Forest Reproductive Material has assumed a commitment to foster the integration of genetic principles into the framework of pertinent legislation. In the present paper, an attempt is made to analyze genetic implications of existing legislation in the European Community. Reference is made to EEC Directives 404 of 1966 (ANONYMOUS 1966) and 445 of 1975 (ANONYMOUS 1975). Similar principles have been valid for the OECD Scheme of 1974 (ANONYMOUS 1974).

Like other member countries of both EEC and OECD, Germany has a Forest Seed Act (FSaatG; ANONYMOUS 1979). Some interesting details of the history of Directive 404 are presented by GORDON (1985). There is also discussion going

on about the general feasibility of the EEC Directives (FAULKNER 1986). A comparison of the laws of the member countries of the European Community was carried out by WEISGERBER (1981). Some of the practical consequences of the Directives were pointed out by HATTEMER (1985). All of this legislation applies only to marketed reproductive material. Only a few selected aspects or passages can be dealt with here. For the sake of brevity, the word 'seed' will refer to generative reproductive material, if not stated otherwise.

II. Undebated Advantages

The vital necessity for seed legislation is demonstrated by the following four considerations.

- 1) The legislation provides the buyer with documents from which he can readily find details of the location, altitude and type of underlying ecology of the site on which the present source was grown. This information is important if the source is an indigenous forest.
- 2) A requirement that all basic material must be approved enables the exclusion of such basic material from propagation that has previously been proven inappropriate. This use of prior information on characteristics such as poor resistance of a clone or poor growth of the progeny of a conifer stand may perhaps be of small economic scope, but it is nevertheless important in principle.
- 3) Rules for testing reproductive material set minimum standards for the procedure of selecting and testing. They may allow for objective evaluation of breeding results and their methodical exploitation for silvicultural use.
- 4) State control of the observance of the law serves to foster confidence in the documents accompanying a seed lot. Forest owners may thus accumulate data on their local experience with seed which definitely originated from a given source.

These points are important to breeders, producers, and individual forest owners, alike. The former can properly advertise their products. The latter can trust that they are drawing on the best possible source of seed for the initiation of the long-term process of forest production.

There may be additional positive aspects, but the above may well be those which come to a seed purchaser's mind when asked 'What if we had no legislation whatsoever?' They are important enough to advocate legislation as such.

III. Conflicts with Genetic Principles

1) *Genotype vs. Phenotype*

Both the 1966 Directive (in its preamble) and FSaatG (in its § 1) explicitly state that their motivation is to improve yield. Unfortunately, Article 1 of this Directive refers to 'genetic character' in an ambiguous way. Its preamble mentions the word 'genetic' as many as six times, but the context reveals that what is meant can only be the phenotype. The preamble furthermore contains an embarrassing confusion of the two concepts:

'Whereas genetic characters as understood at present in forestry work means the hereditary constitution, as opposed to the external features of reproductive material; whereas research has been undertaken on problems connected with the external features but has not yet been completed; whereas Community rules should therefore at present refer only to the genetic characters of reproductive material;'

The term 'genotype' of an individual may be used to indicate its nuclear genetic information in terms of nucleotide sequences or operationally in terms of identified Mendelian genes. It may also be used in terms of its reaction norm (JOHANNSEN 1911), but this definition makes sense only if substantial information on the reaction norm exists. While the text of the Directive claims to refer to the genotype, it can be interpreted to mean phenotypic trait expressions only. Trait expressions are in principle phenotypic, despite the fact that they may be partially or even completely conditioned by the genotype.

Both the preamble and Annex I of Directive 404 use the term 'genetic quality'; the preamble also uses 'genetic value', while the preambles of Directives 404 and 445 stress the necessity of using 'genetically superior' seed. This usage was retained in FSaatG and probably in the national legislation of other member countries. Hence, this legislation may raise unjustified high expectations on approved material, since only the genotype of an individual is invariant under varying environments. Although a phenotypic superiority may be genetically conditioned, 'genetic value' and 'genetically superior' are pretentious terms of speech. 'Genetic superiority' should be replaced by the more accurate term 'improved value for use' as defined in Directive 445. This latter term expresses both purpose and character of the present legislation more adequately; reference to this will be made below.

The criterion of 'improved value' indicates significant phenotypic superiority to a set of standards in an experimental series. However, as KRUSCHE (1983) and KRUSCHE and PADRO (1984) have shown, the superiority of a given progeny strongly depends on the experimental procedure used. Superiority of a given progeny with a given genotypic structure can hardly be called genetic if its *a priori* probability can be raised by testing it in a large number of locations or by including a large number of other experimental entries in the same test.

2) *Conserving Genetic Variation*

Recent considerations focus on the genetic variation both among and within populations of forest trees. Since genes for plasticity (i.e. ability of the individual to adjust itself to variable environments) appear to be very rare, only the presence of a large number of genes and hence genotypes in a population is likely to ensure its adaptability. Genetic variation in a population, which represents its adaptive potential, leads to the condition in which many gene loci are polymorphic. The environment of trees, as long-lived immobile organisms, will be more heterogeneous than that of other organisms; moreover, their environment can only be slightly manipulated by man. This postulate (GREGORIUS *et al.* 1979) has been supported by experimental findings in various organisms on the degree of heterozygosity (in the sense of LEWONTIN and HUBBY 1966), which measures the genetic multiplicity of individuals. Estimates of this parameter in nonendemic conifers by far exceed like estimates in any other living species hitherto sampled (NEVO *et al.* 1984). A high degree of flexibility in the continuous adaptation process in turn favorably influences the yield of stands established by purchased seed. It would also ensure sustained yield in future generations of forests for future generations of man.

A few additional words may be in order, since several European countries are severely affected by the present forest decline. Toxic chemical immissions do not only drastically reduce population size. For some time we have

known that the die-back must be selective (ROHMEDEK and v. SCHÖNBORN 1965) we have learned more recently that, depending on the nature of the environmental stress, certain genes and/or a large degree of heterozygosity favor the survival of a tree as long as the stress remains below a certain critical threshold; various pertinent references are given by GREGORIUS *et al.* (1985). Hence genetic variation is crucial (GREGORIUS *et al.* 1979, HATTEMER *et al.* 1982), and genetic multiplicity is a meaningful parameter (see review by ZIFHE 1982a).

This aspect received no consideration in the Directives. It was only later that new biochemical techniques paved the way for the period of worldwide, fruitful integration of biochemical and population genetics which allows the study of genetic variation. One important determinant of genetic variation is population size. However, the present legislation displays concern about population size in Annex I of Directive 404 solely in the context of inbreeding. A sufficient number of trees on a minimum area is recommended as a general precaution against inbreeding. It is doubtful whether this rule can provide an effective measure for avoiding inbreeding in subsequent generations as long as information on the degree of genetic relationship and of inbreeding in the basic material is not available (LANGNER 1959).

3) *Basic Material vs. Reproductive Material*

Fig. 1 illustrates modes of genetic change during the transition from basic material to termination of the rotation period of stands planted with the respective reproductive material.

In fact, all of our legislation on reproductive material applies exclusively to basic material. Whereas in the case of vegetative propagation of individuals this is in agreement with genetic principles, in the case of seed it is true only if the implicit assumption of genetic equilibrium holds true.

The equilibrium hypothesis cannot possibly refer to equilibrium at a gene locus controlling a metric trait which is maintained due to overdominance under artificial selection; breeding progress would then have to be expected to come to a halt soon. But breeding progress is never questioned anywhere in the legislation. Therefore, Table 1 lists the single assumptions inherent in the compound hypothesis of panmictic equilibrium. Some of these are formulated in a way which permits their experimental validation. For instance, assumptions 3 to 6 replace the conventional assumptions of random mating and equal fecundity, which would have read:

- A. The mating system is entirely random with respect to the controlling gene locus; this implies that:
 - (a) For carriers of any one genotype, the potential set of mates is identical to the set of all fertile and sexually compatible individuals.
 - (b) There are no mating preferences in this set (any such preferences could be based either on genotype or genealogy).
- B. The number of offspring arising from any mating is independent of the genotypes of the respective mates.

This formulation has been in use in animal genetics. However, in non-dioecious plants it is not necessarily meaningful to consider the absence of mating preferences if the variation of the sexual function is continuous. Moreover, it is impossible to test any of these assumptions separately.

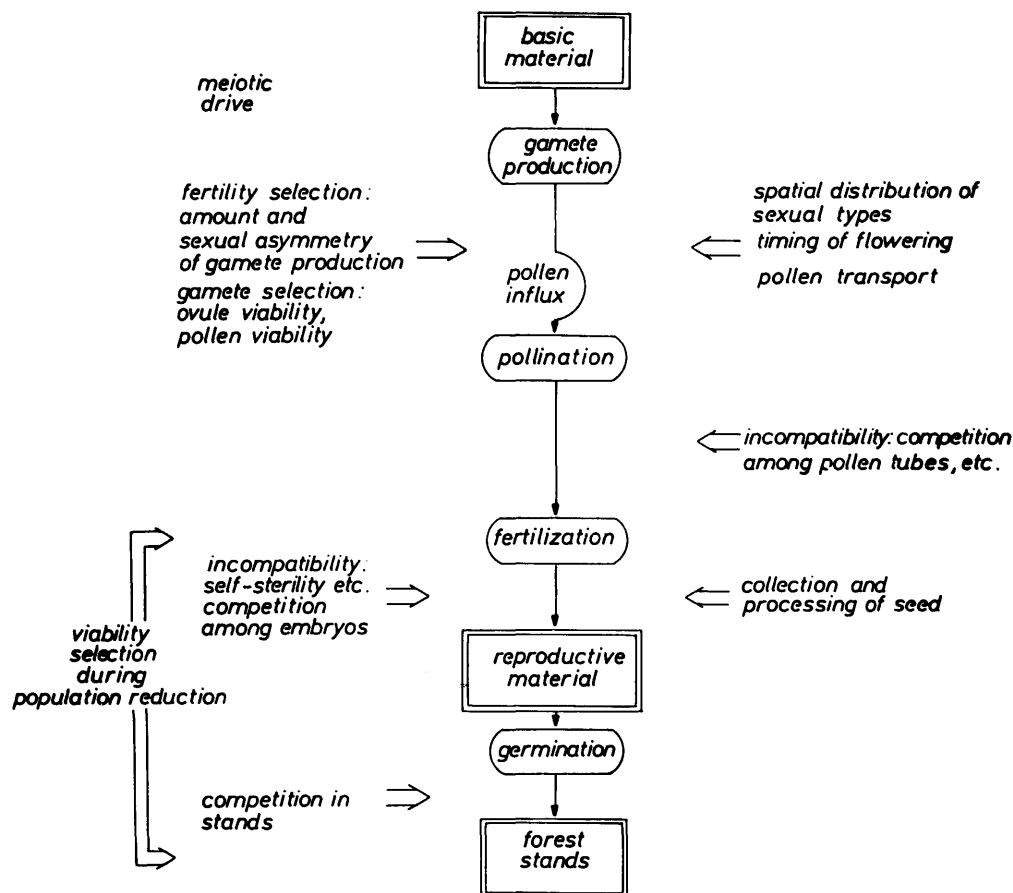


Fig. 1. — Genetics of the production of generative reproductive material and the development of stands planted with it. Only those elements are shown which reportedly exert an influence on the genetic structure (after ZIEHE 1982).

Theoretical work has been indispensable in identifying these assumptions and in drawing conclusions on the specific effects of departures from these ideal conditions. For instance, in a theoretical study, ZIEHE (1982b, 1983) showed that the interplay between sexual asymmetry and partial selfing may lead to a HARDY-WEINBERG structure. MÜLLER-STARCK (1982b) and MÜLLER-STARCK *et al.* (1983) presented experimental evidence that these two effects can compensate each other to produce something close to a HARDY-WEINBERG structure in orchard-produced seed. This result also stands as a warning against making inferences on genetic equilibrium solely on the grounds of an observed HARDY-WEINBERG structure (SPIESS 1977, l. c. p. 429 ff., VALENZUELA 1985, ZIEHE and HATTEMER 1985).

Only assumption 5 has been found to be compatible with the reproductive system realized in coniferous seed orchards.

Estimates of the percentage of foreign pollen effective in fertilization (assumption 9) are necessarily conservative, with the true percentage possibly being much greater. This is particularly true if pollen flow is monitored at a single gene locus and the foreign pollen source carries an allele serving as a marker in the heterozygous state. Much pollen contamination can also be expected from inside sources, such as from grafts representing admixtures to any of the orchard clones (LINARES and HATTEMER, in press).

The experimental evidence was mainly derived from enzyme gene loci used as markers. Hardly representing an

atypical group, they may be involved in controlling economic traits. Hence there exists convincing and relevant experimental evidence against the general validity of the equilibrium hypothesis.

It follows from Table 1 that, contrary to general opinion, the efficiency of breeding and the rationale of the respective legislation rest on numerous assumptions. Some of these deviate drastically from actual conditions prevalent in tree populations. In contrast, intensive population-genetic research teaches us to explicitly and carefully consider biological facts rather than to rely on mere assumptions when predicting population structure.

A breeder who *a priori* mistrusts all models and assumptions is by no means able to make himself independent of them. Even if inclined to stick to an empirical approach, he would still rely on the inherent but cogently refuted assumption that his artificial selection represents the only source of genetic change in a tree population.

Whether a given lot of reproductive material is to be put on the market as 'selected' material is determined solely by the properties of its basic material. However, as the purchaser of seed does not buy the basic material, he is interested in the phenotypic potential of the genetic information carried by the seed itself. It is clear that in the absence of foreign pollen contamination, seed collected from a given stand exclusively carries genes contained in the parent stand. However, the seed would even then never represent that stand genetically. Segregation at meiosis,

Table 1. — Prerequisites of genetic equilibrium in the context of the legislation. Assumptions which have been found to be invalid by experiment are marked with an asterisk*; research results given in the references qualify or refute the general validity of the respective assumption. Assumptions marked with two asterisks** are hardly ever testable in forest trees.

<p>I. Single-locus theory.</p> <ol style="list-style-type: none"> 1. Any allele at the locus possesses equal frequency among carriers of all sexual types. (This, of course, does not apply in the absence of variation in type.) *2. Meiosis is regular, so that segregation is random, and gamete selection is absent (CHELIJAK et al. 1984). *3. Fertility selection is absent, i. e. carriers of all genotypes produce the same number of gametes (LINHART et al. 1979, MÜLLER-STARCK et al. 1983). *4. Sexual symmetry is present, i. e. the ratio of the number of female gametes to the number of male gametes is constant for all genotypes (MÜLLER-STARCK 1982b, 1985, MÜLLER-STARCK and ZIEHE 1984). 5. Fusion of gametes is random. If an angiospermous species possesses a system of prezygotic genetic incompatibility, this assumption is <i>a priori</i> false unless linkage equilibrium exists with the gene loci controlling the incompatibility. *6. The frequencies of the alleles in the zygotes of the subsequent generation are the same as those among the gametes of the preceding generation (MÜLLER-STARCK et al. 1982, 1983; counter-example by CHUNG 1981). 7. The population reproduces in separated generations. 8. The size of the population is effectively infinite. *9. No gene flow into the population exists (SQUILLACE 1977, SQUILLACE and LONG 1981, MÜLLER-STARCK 1982a, FRIEDMAN and ADAMS 1982, 1985, ADAMS 1983, NAGASAKA and SZMIDT 1985). *10. Viability selection among the zygotes is absent so that the genotypic structure is invariant over ontogenetic phases (LUNDKVIST 1980, KIM 1985). <p style="text-align: center;">II. Multi-locus theory.</p> <ol style="list-style-type: none"> **11. The above conditions hold for arbitrary gene loci. **12. Linkage equilibrium holds among the controlling loci.
--

and recombination at both meiosis and fertilization also play a role in determining the genotypic structure of the seed. Last but not least, factors related to the mating and/or the selection system are responsible for the fact that the genes of the parent stand are found in varied associations and/or varied frequencies in the stands established with its seed. An example was presented by ROBERDS and CONKLE (1984). Needless to say, the environment of parent and progeny stands are never the same. Silvicultural treatment of the basic material prior to approval may further contribute to the phenotypic difference between basic material and progeny stands.

The Directives cover also vegetative propagules. Some items of Table 1 do not apply to this material.

4) Delimiting Regions of Provenance

The testing of geographic provenances has shown that offspring of a stand may more closely resemble offspring of an adjacent stand than offspring of a more distant stand. This observation was apparently taken into account in the present legislation. In Article 3 of Directive 445, the delimitation of regions of provenance is based on 'areas subject to practically uniform ecological conditions on which are found stands showing similar phenotypic or genetic characters'. It must be stated that, in general, indigenous stands growing under like ecological conditions need not possess similar genetic characteristics. Nor need genetically similar indigenous stands growing side by side owe their similarity

in appearance to similar ecological conditions. We neither know which ecological conditions exert an influence on genotypic structures nor what their effects are. We have only limited information on population differentiation (cf. BERGMANN 1975, 1984 for examples in Norway spruce). It is therefore doubtful whether delimiting regions means delimiting populations. This matter becomes all the more obscure if delimitation is applied to planted, possibly non-indigenous stands or even to their phenotypic characteristics, as long as the degree of rigidity of their genetical control at the level of stands is unknown.

Although delimitation may simplify legal control (STERN 1969), it does not necessarily possess genetic relevance. It has to be asked whether the statement made in the preamble of Directive 404, namely that 'approval of basic material and, consequently, delimitation of regions of provenance are fundamental to selection' is even applicable in member countries where seed is largely produced in planted stands under regular management. Though delimitation within a single member country may be less meaningful, it does possess importance within the entire region of the Community, as GORDON (1985) points out.

5) Keeping Lots Separate

Article 8 of Directive 404 prescribes that individual lots of reproductive material be kept separate and that they be labelled according to as many as six criteria below the

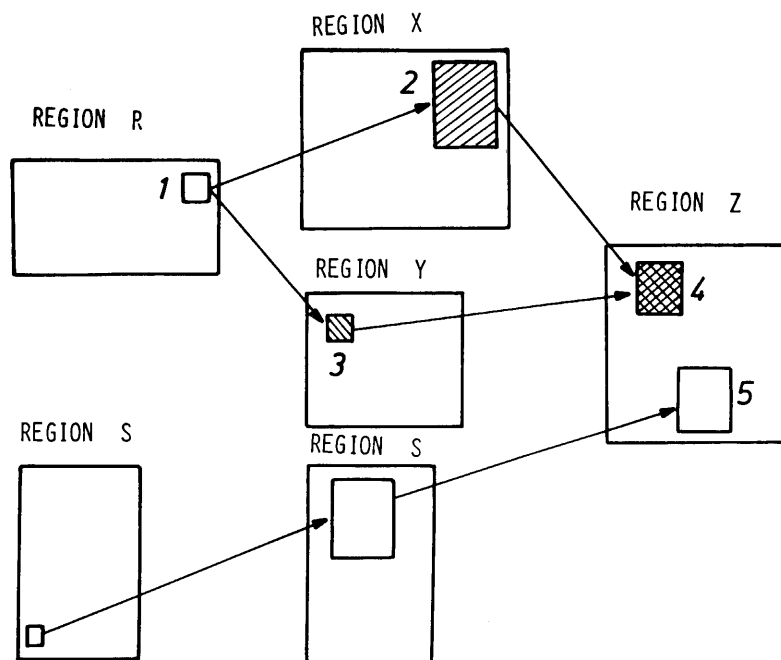


Fig. 2. — According to the legislation, regions of provenance are delineated within pre-existing areas of planted forests. The schematic illustration shows some consequences of keeping lots of 'selected' reproductive material separate. For an explanation see text.

species level. Among these are the region of provenance and the year of ripening of the seed.

It was pointed out that the genetic significance of the region of provenance is doubtful in non-indigenous forests. As demonstrated in Fig. 2, this Article even yields contradictory implications. Thus the reasoning behind this example may be equally difficult to explain to a geneticist and to a person for whom this legislation is binding: Seed from stands 1 to 4 may not be mixed, because the stands are located in different regions. This disregards the eventuality that stands 2 to 4 have a common parent stand. However, seed from stands 4 and 5 may be mixed, even though their remote parent stands were subsequently assigned to different regions of provenance.

Comments on the criterion of year of ripening are of a more genetic character. Since it was demonstrated that reproduction is not panmictic, we must assume the existence of genetic differentiation among seed lots collected from one and the same basic material in different years. Some data from a pine orchard presented in Tab. 2 indicate marked genetic distances between the genetic structures of seed and their invariant panmictic expectation as derived from the genotypic constitution of the orchard clones at a marker locus. These distances are smaller for the bulked seed, so that after bulking, the reproductive material represented the genetic constitution of the basic material more closely.

There may be good reasons for keeping lots separate, which would justify their being assigned a higher priority than that given to the possible genetic relevance. It would therefore be premature to advise against Article 8 on these grounds. But we must consider the genetic implications of applying these rules. One of these is that by the separate marketing of seed produced in different years, annual fertility changes within one basic material are projected into structures of stands established from that seed.

The genetic differentiation of seed produced by a basic material in different years was inferred by LANGNER (1967), who also drew conclusions for seed legislation.

The legislation prescribes that lots be kept separate only prior to the planting of forests. On the other hand, what is the likelihood that the individual forest owner will buy several lots at a time and mix them in order to approximate the basic material more closely or to attain a certain level of genetic variation in his forest?

6) Direct vs. Indirect Control

It is typical of legislation predominantly concerned with basic material that its controls focus on observing the path taken by reproductive material from the site of collection up to the person or body finally marketing the reproductive material. The genetic characters of the reproductive material could have been so entirely disregarded only in legislation conceived prior to and during the mid-sixties. GREGORIUS *et al.* (1984) demonstrated how genetic methods of reconstructing descent can support or partly replace the use of conventional methods and, in addition, which further information can be gained from studying the actual genetic characters of the reproductive material.

Annex II of Directive 445 makes the following statement on tested material:

'1.2 Every care shall be taken that the reproductive material, including the standards, is representative of the basic material being studied.

1.3 If during tests it is proved that the reproductive material does not possess at least the characters:

- which identify its basic material, then such reproductive material must be immediately eliminated;

This Directive thus has to be interpreted as explicitly requiring the study of descent by analyzing genotypes.

7) Selected Reproductive Material

Approval of basic material for the production of selected reproductive material has been criticized for being

'phenotypic selection'. However, this formulation is just as correct as with any other artificial selection process, since the breeder selects an individual either by its own phenotype or by the phenotypes of its relatives, i.e. its progeny. The salient point with Directive 404 is the lack of a provision for progeny testing, since it defines selected reproductive material as

'... material whose qualities are such as to make it suitable for reproductive purposes and which has no characters undesirable for the production of wood...'

It is difficult to perceive what is meant by these qualities. If this Directive presupposes constancy of the phenotypic structure over generations, then this fundamentally requires constancy of both the genotypic structure and the environment over generations. Under random assignment of the genotypes to ecological conditions in subsequent generations, it is impossible in forestry that an identical phenotypic structure can ever rearise.

This passage of the legislation still implicitly assumes genetic efficiency of stand selection in that the mean of the phenotypic structure of progeny stands is expected to be no worse than the mean of that of the basic material. Various examples of problems associated with 'phenotypic selection' are also reported by GORDON (1985).

Justified expectations for an improved phenotype of stands established with selected seed could at best be based on an experimental result verifying, for example, a close relationship between the average expression of traits in basic material and in stands established with their seed. This is not meant to question the breeding efficiency of all selection. Nevertheless, only something like a correlation observed between random stands and their offspring, when planted with an appropriate design over several environments, would once and for all allow an objective empirical

estimate of the efficiency of selection among stands without progeny testing. To the present author's knowledge, such a result for the traits listed in Annex I of Directive 404 has never been published. We only have to envisage that the selection of stands in the field must be much less efficient than the selection among progenies in a planned experiment. It is therefore open to discussion whether much breeding progress is to be expected from the use of selected material. Forest history might consider this period as being inglorious for forest genetics and forest tree breeding during which state authorities advocated these rules for 'scientific reasons'. This period will hopefully not be of grave consequence to the forest itself.

IV. Outlook

It is clear that the future legislation should first abstain from proliferating misunderstandings about genetics. It should therefore either (a) strictly avoid the use of genetic terminology, or it should (b) be revised in order to meet genetic requirements.

(a) The former alternative would imply the exclusion of any genetic and breeding terminology. This would in turn remove the essential motivation for the rules: According to their preambles, the sole justification of the Directives is genetic, and rules without a genetic background would deteriorate to mere red tape. Such rules will be difficult to 'sell' to anyone for whom the legislation is binding. In any case, the important genetic implications of the rules will continue to exist.

(b) For this reason, the latter alternative is preferable. It entails the integration of the results of genetic research into revised Directives. It is self-evident that legislation on forest reproductive material regulates a subject of an intrinsically population genetic nature.

Table 2. — Changes in genetic structure between basic material and seed in a seed orchard (*Pinus sylvestris* L.) as monitored by genetic distances at an LAP gene locus.¹⁾

I. Comparisons among the seed produced in three consecutive years (sample size was 640 seeds each)

comparison	genotypic distance ²⁾	genic distance
1976 vs. 1977	.13	.07
1976 vs. 1978	.13	.07
1977 vs. 1978	.02 ³⁾	.01 ³⁾

II. Comparisons between the panmictic expectation and the seed produced during the three years.

year of ripening of seed	genotypic distance ²⁾	genic distance
1976	.07	.04
1977	.10	.04
1978	.10	.05
seed of these three years mixed in equal proportions	.06	.02

¹⁾ The data were taken from MÜLLER-STARCK *et al.* (1972). The genetic distances were computed according to GREGORIUS (1974).

²⁾ After pooling frequencies of reciprocal embryo genotypes.

³⁾ Statistical hypotheses involved independence in contingency tables (part I) and goodness of fit (part II). All χ^2 -test quantities were significant except these two cases.

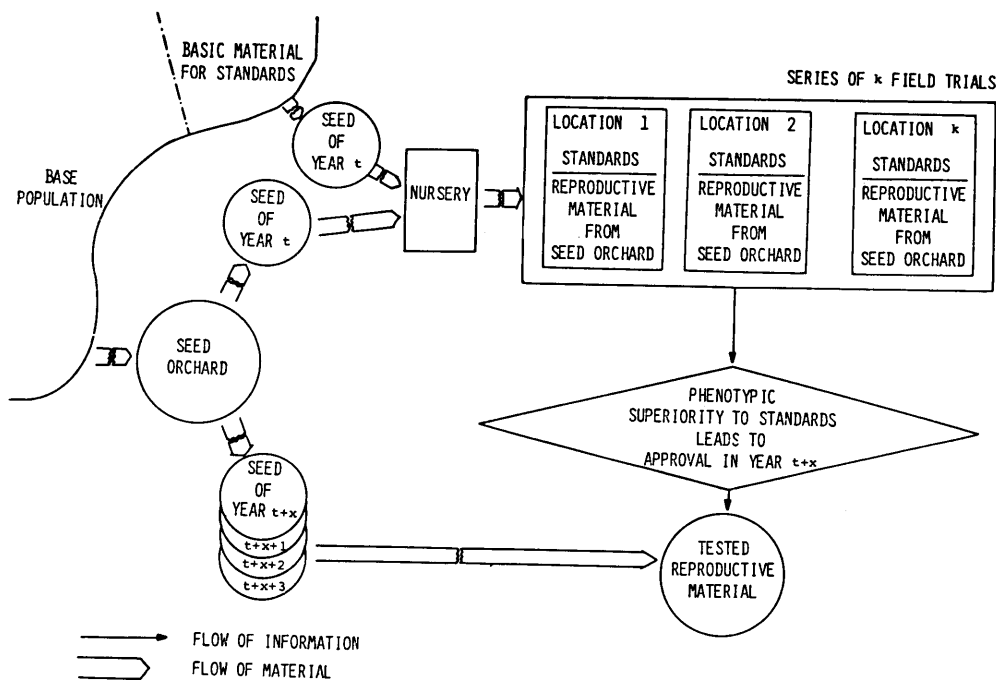


Fig. 3. — Procedure for approval of a seed orchard for the production of tested reproductive material. Broken arrows indicate changes in genetic structure. x denotes the length of the test period. Some institutions might mix seed produced in several years for establishing the field tests; this practice would not be reflected in this figure. The arrow leading to tested reproductive material is also broken, because the seed of years $t+x, t+x+1, \dots$ is hardly homogeneous and the germination is possibly selective.

Although the approval of tested reproductive material should be encouraged, three aspects remain to be observed: 1) Since the maintenance of genetic variation is not ensured by merely improving the phenotype of a finite number of strains, lines, clones, varieties, etc., future legislation should be primarily oriented towards the genotype rather than the the phenotype of trees. One should also consider that the protection of genetic variation in reproductive material provides the principal source of genetic variation in production populations.

2) Although progeny tests may reveal instances of locally improved value for use, there is still a need for integrating the genetic structure of the reproductive material itself into the legislation. As indicated in Fig. 3, there exists evidence of changed genetic structures at all points indicated (cf. Tab. 1). Consequently, fluctuations in genetic structure among individual seed lots collected from one and the same basic material have to be envisaged. We do not yet know how important these fluctuations may be for the level of forest yield nor for the adaptive potential of forests. In any case, the reproductive material never possesses the genotypic structure of the basic material.

3) As long as we live in a world of fraud and imposture, no one is safe from deception. Control measures on samples of reproductive material should therefore become routine. This should apply both to private companies and state institutions marketing reproductive material. After all, the preamble of Directive 445 calls for 'rules which are as strict as possible'.

V. Acknowledgements

The author is heavily indebted to M. ZIEBE for help in compiling Table 1, and to E. G. GREGORIUS for linguistic advice. H. J. MUHS and two anonymous reviewers provided helpful suggestions for revision of an earlier version of the manuscript.

VI. Literature Cited

- ADAMS, W. T.: Application of isozymes in tree breeding. P. 381—400 in: TANKSLEY, S. D. and TH. J. ORTON (eds.) *Isozymes in Plant Genetics Breeding. Part A.* Amsterdam, Oxford, New York, 1983.
- ANONYMOUS: Council Directive of 14 June 1966 on the marketing of forest reproductive material (66/404/EEC). *Off. J. Europ. Comm.* 2326/66, p. 161—167, 1966.
- ANONYMOUS: OECD Scheme for control of forest reproductive material moving in international trade. OECD Directorate for Agriculture and Food. Paris 1974.
- ANONYMOUS: Council Directive of 26 June 1975 amending Directive No 66/404/EEC on the marketing of forest reproductive material (75/445/EEC). *Off. J. Europ. Comm.* No L 196, 14—23, 1975.
- ANONYMOUS: Gesetz über forstliches Saat- und Pflanzgut. *Bundesgesetzblatt Teil I, Nr. 47, 1242—1262, 1979.*
- BERGMANN, F.: Herkunft-Identifizierung von Forstsaatgut auf der Basis von Isoenzym-Genhäufigkeiten. *Allgem. Forst- u. Jagd-Ztg.* 146, 191—195, 1975.
- BERGMANN, F.: Ein besonderer Fall geographischer Variation an zwei Enzym-Genloci der Fichte (*Picea abies*). P. 8—14 in: *Verh. 3. Arbeitstagung Forum Genetik-Wald-Forstwirtschaft 1983, Göttingen, 1984.*
- CHELLAM, W. M., K. MORGAN, B. P. DANCIC, C. STROBECK and F. C. H. YEH: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* LAMB. *Theor. Appl. Genet.* 69, 145—151, 1984.
- CHUNG, M. S.: Biochemical methods for determining population structure in *Pinus sylvestris* L. *Acta Forestalia Fennica* 173, 28 p., 1981.
- FAULKNER, R.: Are our tree seed and plant regulations working well? *Forestry and Timber*, September issue, p. 15—18, 1986.
- FRIEDMAN, S. T. and W. T. ADAMS: Genetic efficiency in pine seed orchards. P. 213—224 in: *Proc. 16th Southern Forest Tree Improvement Conf.*, Blacksburg, 1982.
- FRIEDMANN, S. T. and W. T. ADAMS: Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). *Theor. Appl. Genet.* 69, 609—615, 1985.
- GORDON, A. G.: Are EEC seed regulations really in Britain's interest? *Forestry and British Timber*, November issue, p. 22—25, and December issue, p. 22—23, 1985.
- GREGORIUS, H.-R.: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. *Silvae Genetica* 23, 22—27, 1974.
- GREGORIUS, H.-R., F. BERGMANN, G. MÜLLER-STARCK und H. H. HATTEMER: Genetische Implikationen waldbaulicher und züchterischer Maßnahmen. *Allgem. Forst- u. Jagdztg.* 150, 30—41, 1979.
- GREGORIUS, H. R., H. H. HATTEMER und F. BERGMANN: Über Erreichtes und kaum Erreichbares bei der „Identifikation“ forstlichen Vermehrungsguts.

Allgem. Forst- u. Jagdztg. 155, 201–214, 1984. — GREGORIUS, H. R., H. H. HATTEMER, F. BERGMANN und G. MÜLLER-STARCK: Umweltbelastung und Anpassungsfähigkeit von Baumpopulationen. *Silvae Genetica* 34, 230–241, 1985. — HATTEMER, H. H.: Genetische Aspekte des EG-Rechts über forstliches Saat- und Pflanzgut. Forum Genetik-Wald-Forstwirtschaft, Verh. 4. Arb. Tagung, Göttingen, 124–143, 1985. — HATTEMER, H. H., H.-R. GREGORIUS, M. ZIEHE und G. MÜLLER-STARCK: Klonanzahl forstlicher Samenplantagen und genetische Vielfalt. Allgem. Forst- u. Jagdztg. 153, 183–191, 1982. — JOHANNSEN, W.: The genotype conception of heredity. *Amer. Nat.* 45, 129–159, 1911. — KIM Z. S.: Viability selection at an allozyme locus during development in European beech (*Fagus sylvatica* L.). *Silvae Genetica* 34, 181–186, 1985. — KRUSCHE, D.: Optimale Versuchsplanung und Nutzen-Kosten-Analyse von Vergleichsprüfungen zur Zulassung von Ausgangsmaterial nach dem Gesetz über forstliches Saat- und Pflanzgut. P. 39–45 in: Verh. 3. Arbeitstagung, Forum Genetik-Wald-Forstwirtschaft, Freiburg, 1983. — KRUSCHE, D. and A. PADRO: Planning the size of variety field trials with forest trees. *An. Inst. Nac. Invest. Agr., Ser. For., Num. 8*, 33–43, 1984. — LANGNER, W.: Inzuchtgefahren bei der Saatgutgewinnung in Beständen und Samenplantagen. Allgem. Forstzeitschr. 14, 325–326, 1959. — LANGNER, W.: Gedanken und Vorschläge zur Novellierung des Forstlichen Saat- und Pflanzgesetzes. Allgem. Forstzeitschr. 22, 571–573, 1967. — LEWONTIN, R. C. and J. L. HUBBY: A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* 54, 595–609, 1966. — LINARES BENSIMÓN, C. und H. H. HATTEMER: Abstammungsrekonstruktion in der Forstpflanzenzüchtung. (in press). — LINHART, Y. B., J. B. MITTON, D. M. BOWMAN, K. B. STURGEON and J. L. HAMRICK: Genetic aspects of fertility differentials in ponderosa pine. *Genet. Res.* 33, 237–242, 1979. — LUNDKVIST, K.: Kan den biokemiska genetiken göra den framtida skogsträdsförädlingen effektivare? *Sver. Skogsvårdsförb. Tidskr.* 78, 148–152, 1980. — MARQUARDT, H.: Über die wissenschaftlichen Grundlagen eines forstlichen Artgesetzes. Allgem. Forstzeitschr. 10, 37–41, 1955. — MÜLLER-STARCK, G.: Tracing external pollen contribution to the offspring of a Scots pine seed orchard. P. 176 in: SPETHMANN, W. (ed). *Proc. IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies Including Multiclonal Varieties*. Escherode 1982a. — MÜLLER-STARCK, G.: Sexually asymmetric fertility selection and partial self-fertilization. 2. Clonal gametic contributions to the offspring of a Scots pine seed orchard. *Silva Fennica* 16 (2), 99–106, 1982b. — MÜLLER-STARCK, G.: Reproductive success of genotypes in *Pinus sylvestris* L. in different environments. P. 118–133 in: GREGORIUS, H.-R. (ed.). *Population Genetics in Forestry. Lecture Notes in Biomathematics* 60, 1985. — MÜLLER-STARCK, G., M. ZIEHE, F. BERGMANN, H. R. GREGORIUS und H. H. HATTEMER: Die Samenplantage als Instrument der Ver-

mehrung von Waldbäumen. Allgem. Forst- u. Jagdztg. 153, 220–229, 1982. — MÜLLER-STARCK, G., M. ZIEHE and H. H. HATTEMER: Reproductive systems in conifer seed orchards. 2. Reproductive selection monitored at an LAP gene locus in *Pinus sylvestris* L. *Theor. Appl. Genet.* 65, 309–316, 1983. — MÜLLER-STARCK, G. and M. ZIEHE: Reproductive systems in conifer seed orchards. 3. Female and male fitnesses of individual clones realized in seeds of *Pinus sylvestris* L. *Theor. Appl. Genet.* 69, 173–177, 1984. — NAGASAKA, K. and A. E. SZMIDT: Multilocus analysis of external pollen contamination of a Scots pine (*Pinus sylvestris* L.) seed orchard. P. 134–138 in: GREGORIUS, H.-R. (ed.). *Population Genetics in Forestry. Lecture Notes in Biomathematics* 60, 1985. — NEVO, E., A. BEILES and R. BEN-SHLOMO: The evolutionary significance of genetic diversity: Ecological, demographic and life history correlates. P. 13–213 in: MANI, G. S. (ed.). *Evolutionary Dynamics of Genetic Diversity. Lecture Notes in Biomathematics* 53. Berlin, Heidelberg, New York, Toronto 1984. — ROBERTS, J. H. and M. TH. CONKLE: Genetic structure in loblolly pine stands: Allozyme variation in parents and progeny. *For Sci.* 30, 319–329, 1984. — ROHMEDE, E. and A. V. SCHÖNBORN: Der Einfluß von Umwelt und Erbgut auf die Widerstandsfähigkeit der Waldbäume gegenüber Luftverunreinigung durch Industrieabgase. Ein Beitrag zur Züchtung einer relativ rauchresistenten Fichte. *Forstwiss. Cbl.* 84, 1–13, 1965. — SPIESS, E. B.: *Genes in Populations*. Wiley and Sons, New York, 1977. — SQUILLACE, A. E.: Use of monoterpene composition in forest genetics research with slash pine. P. 227–238 in: *Proc. 14th Southern Forest Tree Improvement Conference*. Gainesville 1977. — SQUILLACE, A. E. and E. M. LONG: Proportion of pollen from non orchard sources. P. 15–19 in: FRANKLIN, E. C. (ed.). *Pollen Management Handbook*. Washington D.C., 1981. — STERN, K.: Zukünftige Methoden bei der Auswahl anerkannter Bestände. *Der Klenger und Forstbaumschuler, Folge* 2, 1–5, 1969. — VALENZUELA, C. Y.: Algebraic and epistemological restrictions in studies on HARDY-WEINBERG equilibrium. *Amer. Nat.* 125, 744–746, 1985. — WEISGERBER, H.: Das neue Gesetz über forstliches Saat- und Pflanzgut. Fortschritt und Probleme aus der Sicht der Forstpflanzenzüchtung. *Der Forst- u. Holzw.* 36, 349–352, 1981. — ZIEHE, M.: Quantifizierung genetischer Variation. Pages 41–49 in: Verh. 2. Arbeitstagung, Forum Genetik-Wald-Forstwirtschaft, Göttingen, 1982a. — ZIEHE, M.: Sexually asymmetric fertility selection and partial self-fertilization. 1. Population genetic impacts on the zygotic genotypic structure. *Silva Fennica* 16 (2), 99–106, 1982b. — ZIEHE, M.: Genotypic frequencies of the offspring generation under selection on female or male gamete production in partially self-fertilizing plant populations. *Göttingen Res. Notes in Forest Genetics*, No. 5, 16 pages, 1983. — ZIEHE, M. and H. H. HATTEMER: Neuere Erkenntnisse über Asymmetrie-Effekte in der sexuellen Reproduktion von Waldbäumen. *Allg. Forst- u. Jagdztg.* 156, 225–231, 1985.

Short Note: Identifizierung von Hybridlärchensaatgut aus Samenplantagen mit Hilfe eines Isoenzym-Markers*)

VON F. BERGMANN and W. RUETZ

Institut für Forstgenetik und Forstpflanzenzüchtung,
Forstliche Biometrie und Informatik
der Universität Göttingen, Büsgenweg 2, D-3400 Göttingen

Bayerische Landesanstalt
für Forstliche Saat- und Pflanzenzucht,
Forstamtsplatz, D-8221 Teisendorf
(Eingegangen 12. Dezember 1986)

Zusammenfassung

Mit Hilfe von Isoenzymmustern eines Enzymsystems (Shikimat-Dehydrogenase) gelang es, Herkunftsproben der beiden Lärchenarten *Larix decidua* und *L. kaempferi* zu unterscheiden. Dadurch wurde die Möglichkeit eröffnet, Hybridlärchensaatgut zu identifizieren, so daß der Anteil an Hybriden im Saatgut von Hybridlärchen-Samenplantagen eingeschätzt werden kann. Die Verwendungsmöglichkeit

dieses Verfahrens wurde bei Saatgutproben einer zweiklonigen Hybridlärchen-Samenplantage demonstriert.

Abstract

Applying the enzyme system of shikimate dehydrogenase it was possible to distinguish the two larch species *Larix decidua* and *L. kaempferi* on the basis of different isozyme patterns. This result was used to identify interspecific hybrid seeds (embryos), so that the proportion of such hybrids in seed lots from seed orchards consisting of clones of both

*) Herrn Prof. Dr. W. LANGNER zum 80. Geburtstag gewidmet.