the dominant ones. Furthermore, all recessive genes may not have complete penetrance (semi-lethals) and, what is more, endosperm lethal recessives may also be involved.

Key words: Empty grains, recessive embryolethal genes, recessive endospermlethal genes, general- and specific combining ability, individual breeding values, reciprocal differences.

Zusammenfassung

Die Frequenzen der Hohlkörner in Kiefernsamen *(Pinus silvestris)* sind nach freiem Abblühen und nach Individualkreuzungen untersucht worden. Die folgenden Kreuzungspläne wurden verwendet: 29 Mütter mit 4 durchgehenden Vätern, 40 Eltern mit Kreuzungen jeder Eltern in einem partiellen diallelen Plan und einem vollständigen Diallel mit 6 Eltern.

In den Prozentsätzen der Hohlkörner liegen große Individualunterschiede vor.

Es hat sich eindeutig gezeigt, daß die Entstehung der Hohlkörner im wesentlichen von der Homozygotisierung rezessiver Gene abhängig ist, die den Embryo zum Absterben bringt. Die Additivität ist hoch (hohe allgemeine Kombinationseignung) bei großer Streuung der additiven Werte.

Spezifische Kombinationseignung hat sich nicht gezeigt. Dies kann dadurch erklärt werden, daß eine große Anzahl loci gegeben ist, und deshalb spezifische Genkombinationen selten werden.

Es können erhebliche Verschiedenheiten im Hohlkornprozent reziproker Kreuzungen auftreten. Daß ein Individuum einen höheren Hohlkornanteil hervorruft, wenn es als Mutter verwendet wird, beruht möglicherweise darauf, daß ein solches Individuum nebst embryolethalen auch endospermlethale Gene besitzt. Ist dagegen der Hohlkornanteil höher, wenn das Individuum als Vater fungiert, kann dies andeuten, daß das Individuum über eine hohe Anzahl Archegonien pro Ovulum verfügt.

Dieser Lethalgenmechanismus, der in seiner Auswirkung Inzucht verhindert und folglich den s-Allel Systemen der Angiospermen entspricht, ist zweifellos von einer großen Anzahl Allelpaare aufgebaut.

Der Mechanismus ist nicht nur durch das Vorkommen von mehreren Archegonien pro Ovulum kompliziert, sondern auch dadurch, daß die rezessiven Allele wahrscheinlich eine niedrigere Frequenz in den Populationen haben als die dominanten Allele. Es ist auch anzunehmen, daß nicht alle rezessiven Gene eine vollständige Penetranz besitzen (Semilethale), und daß es nicht nur embryolethale, sondern auch endospermlethale rezessive Gene gibt.

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A simple method of estirnating rates of self-fertilization by analysing isozymes in tree seeds

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If it is possible to identify an individual pollen of a certain tree by detecting its genes in the diploid embryo tissue of seeds, one should be able to estirnate rates of self-fertilization, as well as fertilization probabilities in general. The knowledge of the parameters is the well-known basis for investigations concerning the mating system of trees, especially the estimation of coefficients of inbreeding and kinship. The applicability of isoenzyme techniques for identifying genes in needle and endosperm tissues of trees is proved by numerous investigations (review see FERET and BERGMANN, 1976). In addition, experiments with the purpose of estirnating rates of self-fertilization were performed by RUDIN (1976) by means of needle analysis of ***Pinus silvestris.***

Because self-fertilization affects the whole genome equally, it can be sufficiently demonstrated by investigating one polymorphic gene locus.

Several conditions required

Using diploid material for isozyme analysis, monameric enzymes should be preferred, as they do not cause hybrid band-configurations in heterozygous individuals so that the genotype can be identified directly by the isozyme phenotypes in the zymogram. Therefore, our investigations began with the analysis of the monomeric system of leucine aminopeptidase (LAP) in Norway spruce *(Picea abies* (L.)

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KARST.), the genetic control of which is known. It was demonstrated by gamete segregation (BERGMANN, 1973) as well as by crossing experiments (LUNDKVIST, 1974) that the LAP system in Norway spruce is controlled by two distinct gene loci (LAP-A, LAP-B) with different codominant alleles, which are phenotypically represented by distinct isozyme bands.

In addition, it must be ascertained whether the analysed isozyme is active in endosperm as well as in embryo tissue and whether isozyme patterns controlled by the same alleles are identical in endosperm and embryo. This can be assumed, since in more than 98% of all analysed seeds of one individual tree, the detected endosperm allele was found to be identical to one allele in the corresponding embryo tissue (see results). Additional analyses of embryo tissues proved the embryo isozyme phenotype to be uniform.

Generally an estimation of self-fertilization or other fertilization probabilities by isozyme analysis can be performed only if rare alleles are detectable in the investigated tree population.

Materials and Methods

In January 1975 cones from 86 trees of Norway spruce were collected separately from a continuous part of a 91-year-old stand that was cut down recently. The position of each tree was marked in a map. Because flowering in 1974 was extraordinarily intensive, there were only a few trees without cones.

LAP analysis was performed by homogenizing endosperm of dormant seeds and separating the isozymes by means of starch gel zone-electrophoresis in a modified discontinuous buffer system after POULIK (1957). For a detailed description see BERGMANN (1973). The embryos were treated separately by the same method.

The material was analysed in two steps: firstly, the LAP-phenotypes of all spruce trees were identified to find trees with suitable rare alleles. This was done by pure endosperm analysis using a sample of six seeds per tree.

Secondly, a sample of about 1000 seeds per selected "marker tree" was investigated by analysing the embryo and the corresponding endosperm of each individual seed grain. Comparing both patterns it is easy to detect the allele originating from the pollen genome in the patterns of the embryo, because the allele from the marker tree genome is known by its endosperm pattern analysed in the first step.

If the investigated gene locus is homozygous for two rare, but identical alleles, all seeds originated from self-fertilization can easily be detected by this method, since their endosperm and embryo patterns have to be identical. It is assumed that the fertilization probability for pollen of other trees bearing the same allele is equal to zero because of their great distance to each other.

If the marker tree is heterozygous at the investigated gene locus, only two out of the four possible genotypes originating from self-fertilization can be detected definitely: seeds with only the rare allele in endosperm and embryo, as well as those with a frequent allele in the endosperm and the rare one added in the embryo. Others can originate from both self-fertilization and cross-pollination.

Results of endosperm and embryo analysis

In our investigation only the genotypes at the LAP-B locus were regarded, since the isozyme phenotypes of those at the LAP-A locus are often difficult to discriminate. Analysing endosperms of a total of 86 trees, four alleles were identified at the LAP-B locus in accordance with the results presented by BERGMANN(1973), namely the single isozyme bands B_1, B_2, B_3, and the double isozyme band B_4. For better illustration the isozyme phenotypes of all genotypes at the LAP-B locus are surveyed schematically in figure 1. Each of the five observed LAP-B genotypes B_1B_1, B_1B_2, B_2B_2, B_2B_3, and B_2B_4) corresponds with the two LAP isozyme patterns found alternatively in the haploid tissue of the endosperm. Out of 86 trees two carried the quite rare allele B_4 and one the most rare allele B_3 (see BERGMANN, 1974).

The B_2B_3 tree was selected as the marker tree because of its simple LAP-pattern and the fact that the neighboring trees up to a distance of at least 40 meters carried neither allele B_3 nor B_4.

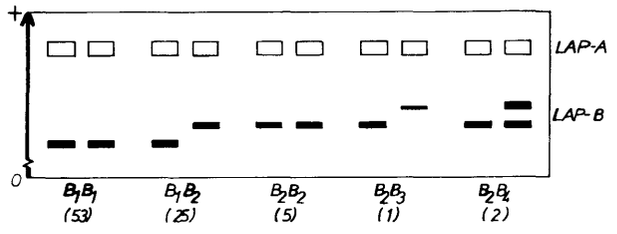

Figure 1. — LAP phenotypes of endosperm of 86 spruce trees. Distinctions in LAP-A region are not marked. The LAP-B genotypes and their frequencies are written below.

The results of endosperm and embryo analysis of seeds of the individual marker tree are presented in *table 1*. Only 98% of all anaysed seeds are included. The zymograms of the rest either could not be identiifed precisely, or they contained different patterns in endosperm and embryo and were considered for the present as experimental error.

Table 1. — Alleles at LAP-B locus in endosperm and corresponding embryo of 987 seeds of one marker tree (nomenclature see *fig. 1*). Number of seeds in parentheses.

Allele in endosperm	Alleles in corresponding embryo		
B_3♀ (459)	B_3♀B_3♂ [13]	B_3♀B_2♂ (81)	B_3♀B_1♂ (365)
B_2♀ (528)	B_2♀B_3♂ [44]	B_2♀B_2♂ (325)	B_2♀B_1♂ (159)

Subsequently genotypes are considered as ordered pairs of alleles, the first of which denotes female and the second male contribution.

Assuming that the rare allele B_3 is contributed only by the marker tree, the genotypes B_3B_3 and B_2B_3 definitely originate from self-fertilization and represent two out of a total of four selfed genotypes.

The genotypes B_3B_2 and B_2B_2 can originate from self-fertilization as well as from cross-fertilization, because B_2 is a common allele in the population.

The genotypes B_3B_1 and B_2B_1 definitely originate from cross-fertilization.

Expected rate of self-fertilization within viable seeds

The estimation of the rate of self-fertilization must be based on the frequency of the genotypes B_3B_3 and B_2B_3. If both had the same frequency, the expected rate of self-fertilization could be derived easily by doubling their portion from all seeds, assuming that the percentages of the non-detectable genotypes B_3B_2 and B_2B_2 are equal to those of the detected ones B_3B_3 and B_2B_3.

The presented results indicate a remarkable deviation in frequencies between B_3B_3 and B_2B_3 (13 : 44 that is 1 : 3.4) which cannot yet be explained satisfactory. Doubling their portions means evaluating a lower limit of the rate of self-fertilization if it can be assumed that both heterozygotes are equally frequent and that the non-detectable homozygote B_2B_2 is at least as frequent as B_3B_3.

In this case the rate of self-fertilization can be expected to be at least 11.6% (2 X (13 + 44) = 114 selfed seeds out of 987). Having in mind that the deviation in frequencies may be caused by the rare allele B_3, all genotypes which are not homozygous with respect to B, can be assumed to be at least equally viable. In this case the given value can be calculated more precise so that the rate of self-fertilization increases to 14.7% (13 + (3 X 44) = 145 selfed seeds out of 987).

Further aspects

The application of the described method will be extended to estimate rates of self-fertilization and other fertilization probabilities of and between individual trees in stands as well as in seed orchards.

If more investigations are performed, possible uncertainties have to be dealt with such as occurrance of distinct isozyme patterns of the same allele in endosperm and embryo or deviations in the frequencies of the two detectable LAP phenotypes. In addition the estimations of rates of self-fertilization have to be improved, if cross-fertilization by gametes of other marker trees has to be taken in account.

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Summary

Analysing isozymes in endosperm and embryo tissue it is possible to estimate rates of self-fertilization of individual trees. Comparing for example leucin-aminopeptidase isozyme patterns of embryo and endosperm tissue of individual seeds of a certain "marker tree", two out of totally four genotypes originating from self-fertilization can be identified definitely. Preliminary results are given within a stand of Norway spruce: the rate of self-fertilization of one spruce tree was estimated to be 14.7%.

Key words: Self-fertilization, Leucin-aminopeptidase (LAP), seed analysis, Norway spruce.

Zusammenfassung

Anhand der Analyse von Isoenzymen in Endosperm- und Embryogewebe ist es möglich, eine Abschätzung der Selbstbefruchtungsraten von Einzelbäumen vorzunehmen. Vergleicht man z. B. Leucinaminopeptidase-Isoenzymmuster aus dem Embryo- und Endospermgewebe einzelner Samen eines bestimmten Genotyps, so lassen sich zwei von insgesamt vier aus Selbstbefruchtung stammende Typen präzise identifizieren. Ein vorläufiges Ergebnis wird aus einem Fichtenbestand mitgeteilt: Die Selbstbefruchtungsrate einer Fichte wird auf 14,7% geschätzt.

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Variance components and gains in volume growth of Virginia Pine (Pinus virginiana Mill.)

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Introduction

Since most estimates of additive genetic variance components in forest tree breeding are based on experiments in only one environment, these estimates may be inflated by the presence of a genotype-environment interaction (NAMKOONG, SNYDER and STONECYPHER, 1966; STONECYPHER, 1966; EVANS and THOR, 1971). These interactions usually are low (KING, 1965, WELLS and WAKELY, 1966; WRIGHT, 1973). However, if it is assumed that most forest trees are heterozygous for most characteristics, seed collected from random trees in a provenance will probably most closely resemble a "hybrid" blend with considerable genetic diversity. Crop breeders generally recognize that genetic diversity contributes to phenotypically consistent performance (ALLARD, 1961; REICH and ATKINS, 1970).

One objective of this paper is to evaluate and compare the genotype-environment interaction at the provenance and open-pollinated progeny levels in Virginia pine *(Pinus virginiana* MILL.). Another objective is to obtain estimates of heritability and gain in volume growth of this species.

Materials and Methods

Plantations were established in 1967 from open-pollinated seed collected from 13 stands, four in Kentucky and

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