COMMISSION OF THE
EUROPEAN COMMUNITIES

COORDINATION OF
AGRICULTURAL RESEARCH

EWG-SYMPOSIUM DER BIOCHEMIE FÜR WALDBÄUME

EEC SYMPOSIUM ON FOREST TREE BIOCHEMISTRY

SYMPOSIUM CEE SUR LA BIOCHIMIE DE L'ARBRE FORESTIER

1977

EUR 5885 DE-EN-FR

KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN
COMMISSION OF THE EUROPEAN COMMUNITIES
COMMISSION DES COMMUNAUTÉS EUROPÉENNES

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EEC SYMPOSIUM ON FOREST TREE BIOCHEMISTRY
SYMPOSIUM CEE SUR LA BIOCHIMIE DE L'ARBRE FORESTIER

organized by the
Directorate-General for Agriculture
in Brussels from 25-27 January 1977

Chairman: D.T. Seals

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1977

Eur 5885 DE-EN-FR

Published by the

COMMISSION OF THE EUROPEAN COMMUNITIES

Directorate-General
'Scientific and Technical Information and Information Management'

Bâtiment Jean Monnet

LUXEMBOURG

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A bibliographical slip can be found at the end of this volume

Printed in Belgium

Catalogue number: CD-NK-77-011-3A-C

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FOREWORD

The symposium of forest biochemists which is the subject of this publication was convened by the Divisions for Forestry and for the Co-ordination of Agricultural Research of the Commission of the European Communities at the suggestion of the Directors of Forest Research in the nine Member States. They considered that the development and wider application of biochemical methods in forestry could be of considerable benefit to both forestry research and practice in the EEC, especially in connection with tree breeding and the identification of provenances. A symposium was considered the best way of promoting these objectives.

Mr. D.T. Seal, the Chief Research Officer (North) of the Forestry Commission in Great Britain, planned the detailed programme of the symposium, acted as chairman and prepared the report. He was helped by Dr. G.I. Forrest and Dr. J.J. Philipson also of the Forestry Commission and by Mrs. Helen Parkes of the Forestry Division of the Commission of the European Communities.

This publication is introduced by the Chairman's report; in it he gives a brief review of the subjects covered by the symposium and makes recommendations in the light of the discussions. These recommendations will be considered by the Commission of the European Communities in consultation with the Directors of Forest Research in the EEC. The main body of the publication is devoted to the papers presented by the participants.

The Commission takes this opportunity to express its most cordial thanks to the Chairman Mr. Seal, his helpers and all participants for their respective contributions to the symposium and to this publication. These constitute a sound foundation for further progress in the development and application of biochemical methods in forestry.

F.C. HUMMEL

Head of Forestry Division
Commission of the European Communities

ACKNOWLEDGEMENTS

The symposium generated informative papers and discussions, useful suggestions and valuable personal contacts. This successful outcome of a partly experimental proposal was due to the support and facilities provided by the Forestry Division of the Commission of the European Communities and to the willing contributions, on paper and in discussion, by participants from Member States and from Finland, Sweden and the United States of America. As a 'non-biochemical' chairman I was very grateful for such willing help from all these quarters.

D. T. SEAL
Symposium Chairman

EEC SYMPOSIUM ON FOREST TREE BIOCHEMISTRY

CHAIRMAN'S REPORT

INTRODUCTION

The symposium was convened by the Divisions for Forestry and for the Co-ordination of Agricultural Research of the Commission of the European Communities and held in Brussels on 25th to 27th January 1977. Nineteen participants from EEC countries were nominated by their respective Directors of Research and included forest and agricultural research scientists using biochemical methods or having a direct interest in their application. Three participants, from Finland, Sweden and the USA, were experts in the applications of biochemical methods in forest genetics and also represented the views of the International Union of Forest Research Organisations (I.U.F.R.O.). In addition to exchanging current information, an objective of the symposium was to examine possible ways of improving the application of biochemical methods to forest research in the EEC. The results of that examination are summarised in this Report for consideration by the Directors of Forest Research of the member states.

Biochemical methods currently used in forest research were examined in four groups according to the type of substance used for analysis and the symposium papers are being presented in the same groups, namely methods based on Terpenes, Iso-enzymes, Polyphenols, and Other Substances. Comparable methods applied in research into the genetics of agricultural crop plants were explained by the agricultural scientists. The symposium finally discussed the future application of biochemical methods in forest research in the EEC. The different methods and their applications are very well described in the symposium papers and it is necessary only to summarise their main features here.

METHODS OF ANALYSIS AND THEIR APPLICATIONS

Terpenes

The resin of conifers consists of a mixture of terpenes which vary from the simplest monoterpenes to more complex sesquiterpenes and diterpenes such as resin acids. There are two methods of analysis. Analysis of

monoterpenes is by direct gas-liquid chromatography and is relatively rapid. The other method for a more complete analysis involves prior steam distillation and is slower.

Terpene analysis is limited to conifers, and differences between resins from different sources within the same tree confine comparisons to resin of a single type (cortical resin is commonly used). The method is not effective with seeds or with plants less than about two years old but the discovery by Bernard-Dagan and Baradat in France of precursors to resins in very young seedlings, and current research in the USA on seed resins, may ultimately remove these limitations in some species.

The terpene composition or 'profile' of conifer resins shows features which are strongly inherited, stable under different environmental conditions and often linked to geographical origin, sometimes revealing a clinal distribution. Terpene composition can therefore be useful as a measure of variation within a species, to distinguish sub-specific populations, to characterise populations based on other criteria such as morphology and as an aid to the verification or identification of origin. The value of terpene analysis for these purposes depends on the pattern of distribution of the features of terpene composition in the species and the amount of data available for reference. Where the distribution pattern is very well known, for example in Pinus elliotii (Paper No 1), the approximate origin can be deduced. Similarly, where the range of material involved is narrow and its terpene characteristics are well known, eg a group of provenances, genotypes or clones, then terpene analysis is a reliable guide to origin, and may be of value to trade in certain circumstances.

Some features of terpene composition are under single gene control and these are valuable in selecting material for breeding and in tracing inheritance through the breeding process, particularly in seed orchards. Terpene composition has been used for these purposes in France (Paper No 4) as well as in the USA.

Within the EEC, terpene analysis following steam distillation is in regular use in France (Paper No 4) and monoterpene analysis is in continuous use in the UK (Papers No 2 and 3).

Iso-enzymes

Many enzymes in plant material occur naturally in multiple forms, known as iso-enzymes because of the minor variations in their molecular structure. The iso-enzyme composition is a direct reflection of the gneotype of the plant. Iso-enzymes are usually extracted from leaves or seed and separated by electrophoresis.

This method is applicable to broadleaved as well as conifer species although applications to broadleaved species in Europe are at an exploratory stage and mainly concerned with Fagus and Quercus in Germany. The seeds of broadleaved species present some special difficulties but these will probably be surmounted by current research.

Many characteristics of iso-enzyme occurrence and composition are strongly inherited and stable, and as with terpenes, these characteristics are an aid in characterising species, sub populations and provenances.

Precise identification of unknown origin is not yet feasible by iso-enzyme analysis but some features show clear clinal distributions and approximate origin can then be deduced. However, the method is not at present suitable for large scale verification of origin.

Iso-enzyme analysis is an aid in characterising genotypes for selection and breeding. The occurrence of some rare alleles, identifiable by iso-enzyme analysis, also allows the method to be used in inheritance studies in stands and in seed-orchards.

The method is under active development in Sweden (Paper No 5). Within the EEC the method is in continuous development in Germany (Papers No 6 and 7) and has been used in France (Paper No 8).

Polyphenols

The polyphenols are a diverse group of compounds occurring in all higher plants. Thet vary from relatively simple components which may be colourless or coloured (eg coloured flower and fruit pigments), to polymers of high molecular weight which include the tannins and bark and heartwood pigments. Polyphenols are synthesised in the plant from sugars and their formation is influenced by various factors including the availability of carbohydrates and nitrogen. This extensive group of substances has been less used than terpenes and iso-enzymes to characterise commercial forest trees.

The use of polyphenols is at an early stage in forest genetics but these substances are extensive and important in forest trees and justify continuing investigations. The variability of some phenolics, while it may reduce their value in genetic studies, may allow of different applications in forest research in future.

Within the EEC the use of polyphenols is being developed in Denmark (Paper No 9) and some studies have been carried out in the UK.

Other Substances

Serological methods, based on antibody - antigen reactions have been used in general plant taxonomy but are not yet sufficiently widely applied in forest research for their potential to be clear. The most advanced applications appear in Finland (Paper No 10) and Japan. In Finland serological methods have been used to study the relationship between pine species. No continuing application of this method is known in forest research within the EEC.

The characterisation of tree material by the extraction and quantification of nucleic acids does not appear to be a method sufficiently developed for extensive application and is not known as a continuing application in forest research in the EEC.

A number of other biochemical methods with specialised applications were not discussed. Amino acid analysis for forest entomological research and foliar analysis of mineral nutrients for example, were regarded as beyond the scope of the symposium.

POTENTIAL APPLICATIONS

The symposium clearly revealed the present role of biochemical methods as a means of defining plant material at every level, from species to genotype, to assist in tree improvement by selection and breeding. In this role biochemical analyses provide a valuable additional dimension to morphological and biometric methods. A requirement for this kind of application are biochemical characteristics which are highly inherited and stable. An alternative use of biochemical analysis, namely using features which are sensitive to environmental change to diagnose and measure such changes, is not currently so prominent but may have important future applications.

Biochemical methods are likely to be increasingly used in other areas of forest research, especially in tree physiology, forest pathology, and wood utilisation. Strong and general interest was expressed at the symposium in the possibility of joint EEC studies by forest pathologists and biochemists of Fomes annosus, a disease where resistance in the host tree appears to be linked to biochemical features.

Interest was also expressed in biochemical characterisation of Ulmus as an aid to breeding for resistance to Ceratocystis ulmi. Less urgent but potentially important applications foreseen at the symposium were in the diagnosis of tolerance to environmental factors such as drought, waterlogging, low nutrition and atmospheric pollution. Biochemical methods are, of course, already applied in such studies but their use is likely to increase in future.

Biochemical methods may also be applied to conservation, for example

in the preservation of a range of genotypes in natural woodland.

FUTURE DEVELOPMENT IN THE EEC

A reasonable range of biochemical methods is being actively developed by individual member countries and there is no need for control by the Community as a whole to secure future development.

There are obvious possibilities for profitable collaboration between individual member countries, particularly those with a common interest in the improvement of a single species and especially between countries with different levels of expertise and facilities in biochemical analysis and interpretation. The Commission of the European Communities could usefully facilitate such collaboration by the means recommended below. Where all or most countries have a distinct common interest an initiative could usefully be taken by the Directors of Forest Research to organise specific projects on a Community level and one such project is recommended at the end of this part of the Report.

Biochemical methods are generally being improved and extended and the main need within the Community is to ensure that users and potential users of such methods are kept well informed of developments, especially in their potential applications. This can most effectively be done by-

(a) EEC Biochemical Seminars

These should include scientists using biochemical methods and potential users of such methods. They could normally be held at intervals of two to three years but should be timed to supplement and not duplicate similar meetings arranged by I.U.F.R.O., of which the 1974 Biochemical Workshop held at Göttingen was an excellent example.

(b) Exchanges of Scientists

The value of even short exchange visits by scientists working

on similar projects involving biochemical application can hardly be over-rated as a means of acquiring relevant, complete and detailed information. There is of course nothing to prevent individual member countries arranging such visits now, but they do not appear to be used as much as they should and Directors of Research could usefully instigate more exchanges of this kind.

(c) Training

The scale of biochemical applications in EEC forest research does not justify formal, centralised training courses at present. If the appropriate authorities would agree however, training in analytical methods could be provided on request and for individuals, or small numbers. Training in methods based on terpenes could be provided in France, on mono-terpene analysis in the UK, on iso-enzymes in Germany, and on polyphenols in Denmark. Such training would be 'on the job' rather than formal but would be quite adequate for the purpose.

Training in an analytical technique alone could be complete in about 2 weeks.

(d) Biochemical data banks

The value of any biochemical method of characterising plant material rests largely on the extent of data available for reference. Central data banks of biochemical analysis results were discussed by the symposium but there is difficulty and expense in formalising the central collection and banking of biochemical data. On the other hand it is considered feasible and important for individual research stations to store their own analysis results and make these available on request. The seminars and exchanges of scientists recommended above would help to ensure that everyone was aware of the range of data available. The need to standardise

biochemical methods within the EEC was considered but, as most procedures are either already standard or produce directly comparable results there is no strong case for this. In any case a universal standardisation would be preferable and more usefully achieved by I.U.F.R.O.

One special project involving the biochemical analysis of European material of Douglas fir (Pseudotsuga menziesii) is recommended for joint action within EEC for two reasons:-

(1) The direct interest in improvement of this species in most member countries.

(2) The need, agreed by forest biochemists at the symposium, to establish whether or not different biochemical analyses, applied to identical trees, together provide a more precise characterisation than is possible by methods applied in isolation.

In outline, the proposal is that samples be taken from selected provenances of Pseudotsuga menziesii in the European I.U.F.R.O. and other provenance trials and subjected to analysis of terpenes in France, iso-enzymes in Germany, polyphenols in Denmark, and monoterpenes in the UK. The number of provenances and trials used should be restricted, at least in the first instance, so that analysis time in the laboratories concerned is kept to acceptable limits. The project would be completed by the exchange, interpretation and publication of results by the research stations concerned.

The combination of results from this project and data from the U.S.A. could form the basis of a chemo-taxonomic map for Douglas fir which would be a valuable aid to selection and breeding of this species by Member States. Dr. Squillace warned that if Douglas fir is to be

mapped in this way, however, it should be done soon because of the rapid depletion of the remaining natural stands.

Time did not allow of proposals in more detail at the symposium and a small working group would be required to plan the project. However, the resources required are small, and the results likely to be of direct benefit to Member States and of interest to forest geneticists in I.U.F.R.O.

D T SEAL
Symposium Chairman
Edinburgh
March 1977

APPENDIX

The following references were kindly provided by Dr A E Squillace and are of interest in connection with the proposed Douglas fir project.

Rudlogg, E. von. 1972. Chemosystematic studies in the genus Pseudotsuga I. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas fir. Can. Jour. Bot. 50: 1025 - 1040.

Rudloff, E. von. 1973. Geographic variation in the terpene composition of the leaf oil of Douglas fir. Pure and Applied Chemistry 34: 401 - 410.

Rudloff, E. von. 1973. Chemosystematic studies in the genus Pseudotsuga (3). Population differences in British Columbia as determined by volatile leaf oil analysis. Can. Journ. Forest Res. 3: 443 - 452.

Zavarin, E. and K. Snajberk, 1973. Geographic variability of monoterpenes from cortex of Pseudotsuga menziesii. Pure and Applied Chemistry 34: 411 - 433.

Zavarin, E. and K. Snajberk, 1975. Pseudotsuga menziesii chemical races of California and Oregon. Biochemical Systematics and Ecol. 2: 121 - 129.

SYMPOSIUM CEE SUR LA BIOCHIMIE DE L'ARBRE FORESTIER

RAPPORT DU PRESIDENT

INTRODUCTION

Le symposium a été organisé par les divisions Forêt et Coordination de la Recherche Agricole de la Commission des Communautés Européennes et s'est tenu à Bruxelles du 25 au 27 janvier 1977. Dix-neuf participants, venant des divers pays de la CEE, avaient été désignés par leurs directeurs de recherche respectifs et comprenaient des scientifiques spécialisés dans la recherche forestière et agricole, qui utilisent des méthodes biochimiques ou qui sont directement intéressés à l'application de celles-ci. Trois autres participants, venant de Finlande, de Suède et des Etats-Unis, étaient experts dans les applications de méthodes biochimiques dans le domaine de la génétique forestière et défendaient également les vues de l'Union internationale des instituts de recherche forestière (I.U.F.R.O.). En plus de l'échange d'informations courantes, le symposium avait pour objectif l'étude des moyens qui permettraient d'améliorer l'application de méthodes biochimiques à la recherche forestière au sein de la CEE. Les résultats de cet examen sont résumés dans le présent rapport qui sera soumis à l'approbation des directeurs de la recherche forestière dans les Etats Membres.

Les méthodes biochimiques couramment utilisées pour la recherche forestière furent examinées et classées en quatre groupes distincts suivant le type de substance utilisée pour l'analyse et les mémoires du symposium sont présentés ici d'après cette même classification, c'est-à-dire : méthodes basées sur les terpènes, les iso-enzymes, les polyphénols et autres substances. Des méthodes comparables utilisées pour la recherche dans le domaine de la génétique des plantes de culture agricole furent exposées par des spécialistes en agronomie. Enfin, les discussions portèrent sur l'application future des méthodes biochimiques dans le domaine de la recherche forestière au sein de la CEE. Les différentes méthodes, ainsi que leurs applications, sont très bien décrites dans les mémoires du symposium et il ne reste plus qu'à résumer ici leurs caractéristiques principales.

LES METHODES D'ANALYSE ET LEURS APPLICATIONS

Terpènes

La résine des conifères est constituée d'un mélange de terpènes allant des monoterpènes les plus simples jusqu'aux sesquiterpènes et diterpènes plus complexes tels que les résines acides. Dans ce cas, il y a deux méthodes d'analyse. L'analyse des monoterpènes s'effectue par simple chromatographie de partage de phase (liquide - gazeuse) et est relativement rapide. L'autre méthode qui permet une analyse plus complète implique au préalable un entraînement à la vapeur et est plus lente.

L'analyse des terpènes n'est applicable qu'aux conifères et les différences entres les résines provenant de différents endroits dans le même arbre limitent les comparaisons à un seul type de résine (c'est la résine corticale qui est généralement utilisée). La méthode n'est pas valable pour des graines ou des plantes ayant moins de deux ans d'âge. Cependant, la découverte faite en France par Bernard-Dagan et Baradat concernant l'existence de précurseurs de résine dans les très jeunes plants et la recherche en cours aux Etats-Unis sur les résines de graines permettront en fin de compte de lever les présentes limitations dans le cas de certaines espèces.

La composition terpénique ou "profil" des résines de conifères présente des caractéristiques qui sont fortement héréditaires, stables dans des conditions d'environnement différentes et souvent liées à l'origine géographique, révélant parfois une distribution clinale. La composition terpénique peut dès lors être utile en tant que mesure de la variation à l'intérieur d'une espèce, pour différencier des populations sous-spécifiques, pour caractériser des populations basées sur d'autres critères tels que la morphologie et, enfin, elle peut aider à la vérification et à l'identification de l'origine. A cet effet, la valeur de l'analyse terpénique dépend du schéma de distribution des caractéristiques de la composition terpénique dans l'espèce et de la quantité de données de référence disponibles. Lorsque le schéma de distribution est bien connu, comme dans le cas du Pinus elliotti (document N° 1), on peut déduire l'origine rapprochée. De même, lorsque la gamme du matériel végétal impliqué est restreinte et que ses caractéristiques terpéniques sont bien connues, par exemple un groupe de provenances, génotypes ou clones, l'analyse terpénique est alors un guide sûr pour déterminer

l'origine et peut, dans certaines circonstances, être précieuse pour l'exploitation.

Certains traits de la composition terpénique sont sous le contrôle d'un gène unique et sont précieux pour sélectionner le matériel végétal destiné à la reproduction et pour suivre l'hérédité à travers le processus de reproduction, notamment dans les vergers à graines. A cet effet, on a utilisé la composition terpénique en France (document N° 4) ainsi qu'aux Etats-Unis.

Au sein de la CEE, l'analyse des terpènes qui implique un entraînement à la vapeur est communément pratiquée en France (document N° 4) et l'analyse du monoterpène continue à être utilisée au Royaume-Uni (documents Nos 2 et 3).

Iso-enzymes

Dans le tissu végétal, la plupart des enzymes se trouvent à l'état naturel sous de multiples formes et sont connus sous le nom d'iso-enzymes, à cause des faibles variations de leurs structures moléculaires. La composition de l'iso-enzyme est une image directe de génotype de la plante. On extrait habituellement les iso-enzymes des feuilles ou des graines et on les sépare par électrophorèse.

La présente méthode est applicable aux espèces feuillus ainsi qu'aux conifères, bien qu'en Europe les applications aux feuillus en soient encore au stade exploratoire et concernent principalement le Fagus (hêtre) et le Quercus (chêne) en Allemagne. Les graines des espèces feuillus présentent certaines difficultés particulières, mais celles-ci seront probablement surmontées grâce aux recherches en cours.

La plupart des caractéristiques relatives à l'occurence et à la composition de l'iso-enzyme sont fortement héréditaires et stables. Comme dans le cas des terpènes, elles permettent de caractériser les espèces, les sous-populations et les provenances.

L'analyse de l'iso-enzyme ne permet pas encore l'identification précise d'une origine inconnue, mais certains traits présentent de nettes distributions clinales et on peut, dès lors, déduire l'origine rapprochée. Cependant, la

méthode n'est pas pour l'instant adaptable à une large échelle de vérifications d'origine.

L'analyse de l'iso-enzyme aide à caractériser le génotype pour la sélection et la reproduction. L'occurence de certaines allèles rares, identifiables par l'analyse de l'iso-enzyme, permet également d'utiliser la méthode lors d'études d'hérédité en peuplements forestiers homogènes ou en vergers à graines.

La méthode est dans une phase active de développement en Suède (document N° 5). Au sein de la CEE, la méthode continue à se développer en Allemange (documents Nos 6 et 7) et a été utilisée en France (document N° 8).

Polyphénols

Les polyphénols constituent un autre groupe de substances présentes dans toutes les plantes supérieures. Ils vont de composés relativement simples qui peuvent être incolores ou colorés (par exemple : les pigments des fleurs et fruits colorés) jusqu'à des polymères de haut poids moléculaire, qui comprennent les tannins et les pigments de l'écorce et du bois de coeur. Les polyphénols sont synthétisés dans la plante à partir de sucres et leur formation est influencée par divers facteurs et notamment par la disponibilité en hydrates de carbonne et en azote. Ce large groupe de substances a été moins utilisé que les terpènes et les iso-enzymes pour caractériser les arbres forestiers propres à l'exploitation.

En génétique forestière, l'utilisation des polyphénols est encore au stade de l'expérimentation, mais le nombre et l'importance de ces substances dans les arbres forestiers justifient la poursuite des recherches dans ce domaine. Bien que la variabilité de certains produits phénoliques puisse diminuer leur valeur au point de vue des études génétiques, elle est, par contre, susceptible de permettre à l'avenir diverses applications en recherche forestière.

Au sein de la CEE, l'utilisation des polyphénols est en développement au Danemark (document N° 9) et certaines études ont été effectuées au Royaume-Uni.

Autres substances

Des méthodes sérologiques, basées sur des réactions anticorps-antigène, ont été utilisées en taxonomie végétale, mais elles n'ont pas encore été suffisamment appliquées en recherche forestière pour définir leur valeur potentielle dans ce domaine particulier. Les applications les plus avancées sont réalisées en Finlande (document N° 10) et au Japon. En Finlande, des méthodes sérologiques ont été utilisées pour étudier les liens de parenté entre les diverses espèces de pins. Au sein de la CEE, il n'y a, à notre connaissance, aucune application suivie de la présente méthode dans le domaine de la recherche forestière.

La caractéristique d'un tissu végétal provenant d'un arbre par extraction et quantification des acides nucléiques, n'apparaît pas comme une méthode suffisamment développée pour permettre une large application et, à notre connaissance, elle n'a pas d'application suivie en recherche forestière au sein de la CEE.

Certaines autres méthodes biochimiques ayant des applications spécialisées n'ont pas été discutées. On a considéré que, par exemple, l'analyse des acides aminés pour la recherche forestière au point de vue entomologique et l'analyse foliaire des composants nutritifs minéraux sortaient du cadre du présent symposium.

APPLICATIONS POTENTIELLES

Le symposium a révélé clairement le rôle actuel des méthodes biochimiques : elles constituent un moyen de définir un tissu végétal à n'importe quel niveau, de l'espèce jusqu'au génotype, afin d'aider à l'amélioration de l'arbre par sélection et reproduction. Dans ce rôle, les analyses biochimiques fournissent une précieuse dimension supplémentaire aux méthodes morphologiques et biométriques. Pareille application exige des caractéristiques biochimiques stables et fortement héréditaires. Un autre emploi de l'analyse biochimique, c'est-à-dire la détermination de particularités qui sont sensibles aux changements d'environnement dans le but de diagnostiquer et de mesurer de tels changements, est peu répandu pour l'instant mais il est susceptible d'offrir à l'avenir d'importantes applications.

Il est vraisemblable que les méthodes biochimiques seront de plus en plus utilisées dans d'autres domaines de la recherche forestière et plus spécialement en physiologie de l'arbre, en pathologie forestière et enfin dans le domaine de l'exploitation du bois. Lors du symposium, on a noté un vif intérêt général en ce qui concerne la possibilité d'entreprendre avec la participation de biochimistes et de pathologistes forestiers des études CEE sur le Fomes annosus (maladie du rond des pins), une maladie où la resistance de l'arbre hôte apparaît liée à des caractéristiques biochimiques.

On a noté aussi un certain intérêt pour la caractérisation biochimique de l'Ulmus (orme) en tant qu'aide au chef de la reproduction pour la résistance à Ceratocystis ulmi (maladie de l'orme). Le symposium a permis de prévoir des applications moins urgentes mais potentiellement importantes en ce qui concerne le diagnostic de tolérance aux facteurs d'environnement tels que la sécheresse, l'engorgement, la malnutrition et la pollution atmosphérique. Pareilles études ont évidemment déjà recours aux méthodes biochimiques, mais il est vraisemblable que l'usage de ces dernières ne cessera d'augmenter.

Des méthodes biochimiques peuvent aussi être appliquées aux problèmes de la conservation, par exemple : la préservation d'une gamme de génotypes dans les pays naturellement boisés.

DEVELOPPEMENT FUTUR AU SEIN DE LA CEE

A l'heure actuelle, une gamme déjà appréciable de méthodes biochimiques est activement développée par différents pays membres et il ne s'avère pas nécessaire que la Communauté dans son ensemble exerce un contrôle pour assurer la poursuite du développement.

Il existe des possibilités évidentes de collaboration fructueuse entre différents Etats membres, particulièrement entre ceux qui ont un intérêt commun dans l'amélioration d'une espèce donnée et plus spécialement encore entre pays possédant des niveaux différents d'expertise et d'équipements dans l'analyse biochimique et dans son interprétation. La Commission des Communautés Européennes pourrait utilement faciliter une telle collaboration grâce aux moyens recommandés ci-dessous. Dans le cas où tous les pays ou la plupart d'entre eux ont un intérêt commun bien marqué, les directeurs de la recherche forestière pourraient utilement prendre l'initiative d'organiser des projets spécifiques à l'échelon communautaire et dans cette optique un projet est recommandé à la fin de cette partie du rapport.

En règle générale, les méthodes biochimiques sont continuellement perfectionnées et leur domaine d'application élargi. Au sein de la Communauté, il est primordial de s'assurer que les utilisateurs et utilisateurs potentiels de telles méthodes soient bien tenus au courant des développements particulièrement dans le chef de leurs applications potentielles. Pour ce faire, les moyens les plus efficaces sont :

(a) Séminaires biochimiques CEE

Ces séminaires doivent réunir des scientifiques qui utilisent les méthodes biochimiques, ainsi que des utilisateurs potentiels de telles méthodes. Ils pourraient être normalement organisés tous les deux ou trois ans et devraient être programmés de façon à compléter et non à reproduire des réunions similaires organisées par l'I.U.F.R.O., dont le séminaire biochimique qui se tient à Göttingen en 1974 fut un excellent exemple.

(b) Echanges de scientifiques

La valeur des visites d'échange - même de courte durée - effectuées par des scientifiques travaillant sur des projets simi-

laires qui comportent une application biochimique est de la plus haute importance en tant que moyen d'acquérir des informations utiles, complètes et détaillées. Rien n'empêche bien entendu les différents Etats membres d'organiser, dès maintenant, de telles visites. Toutefois, ce moyen ne semble pas être utilisé autant qu'il le faudrait et c'est pourquoi les directeurs de recherche pourraient valablement susciter plus d'échanges de ce genre.

(c) Foramation

A ce jour, l'étendue des applications biochimiques dans la recherche forestière de la CEE ne justifie pas l'organisation formelle et centralisée de cours de formation. Cependant, si les autorités en place y consentent, l'apprentissage des méthodes analytiques peut être fourni sur demande, soit à titre individuel, soit par petits groupes. Une formation dans les méthodes basées sur les terpènes peut être obtenue en France, sur l'analyse des monoterpènes au Royaume-Uni, sur les isoenzymes en Allemagne et sur les phénols au Danemark. Pareille formation serait plutôt du type "sur le tas" que du type "formel", mais elle serait tout-à-fait adéquate à remplir son objectif. L'apprentissage limité à une seule technique analytique pourrait être effectué en deux semaines environ.

(d) Banques de données biochimiques

La valeur de toute méthode biochimique destinée à caractériser un tissu végétal se base largement sur l'étendue des données de référence disponibles. Les banques centrales de données de résultats d'analyse biochimique furent discutées lors du symposium, mais il s'avère difficile et coûteux de donner une forme conventionnelle à un système centralisé de collecte et de mise en banque des données biochimiques. D'autre part, on a considéré comme possible et important pour les diverses stations de recherche que ces dernières rassemblent et emmagasinent leurs propres résultats d'analyse et les communiquent à qui en fait la demande. Les séminaires et les échanges de scientifiques, déjà recommandés ci-dessus, aideraient à garan-

tir que tout le monde ait connaissance de la gamme des données disponibles. On a considéré la nécessité d'unifier les méthodes biochimiques au sein de la CEE, mais, comme la plupart des méthodes sont déjà unifiées ou donnent directement des résultats comparables, il n'y a pas de gros problèmes dans ce domaine. De toute façon, une unification universelle serait préférable et elle serait utilement réalisée par l'I.U.F.R.O.

Dans le cas d'une projet spécial concernant l'analyse biochimique des tissus végétaux des types européens du Douglas vert (Pseudotsuga menziesii), on a recommandé une action collective au sein de la CEE et ce, pour deux raisons:

(1) La plupart des pays membres trouvent un intérêt direct dans l'amélioration de la présente espèce.

(2) La nécessité, reconnue par les biochimistes forestiers lors du symposium, d'établir si oui ou non des analyses biochimiques différentes, appliquées à des arbres identiques, fournissent de concert une caractérisation plus précise que celle qui peut être obtenue par des méthodes appliquées séparément.

En grandes lignes, la proposition est la suivante : des échantillons seront prélevés sur des provenances sélectionnées de Pseudotsuga menziesii dans la branche européenne de l'I.U.F.R.O. et sur d'autres essais de provenance et soumis à l'analyse des terpènes en France, des iso-enzymes en Allemagne, des polyphénols au Danemark et des monoterpènes au Royaume-Uni. Le nombre de provenances et d'essais devrait être limité, du moins au début, afin que le temps d'analyse dans les laboratoires concernés se maintienne dans des limites acceptables. Pareil projet serait compliqué par l'échange, l'interprétation et la publication des résultats par les stations de recherche concernées.

La combinaison des résultats du présent projet et les données fournies par les Etats Unis pourrait former la base d'une carte chimiotaxonomique pour le Douglas vert, qui aiderait grandement les Etats membres lors de la sélection et de la reproduction de cette espèce. Le Docteur Squillace a signalé que si l'on veut dresser une pareille carte, il faut le faire au plus vite étant donné l'épuisement rapide des derniers peuplements naturels des Douglas verts.

Lors du symposium, le temps n'a pas permis d'établir des propositions plus détaillées et de petits groupes de travail devraient maintenant être créés pour arrêter le plan du projet. Toutefois les ressources nécessaires sont faibles et il est vraisemblable que les résultats seront au bénéfice direct des Etats membres et intéresseront les spécialistes en génétique forestière au sein de l'I.U.F.R.O.

D T SEAL
Président du Symposium
Edinbourg
Mars 1977

APPENDIX

Dr. A.E. Squillace a fourni les références suivantes en ce qui concerne le projet proposé du sapin de Douglas.

Rudlogg, E. von. 1972. Chemosystematic studies in the genus Pseudotsuga I. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas fir. Can. Jour. Bot. 50: 1025 - 1040.

Rudloff, E. von. 1973. Geographic variation in the terpene composition of the leaf oil of Douglas fir. Pure and Applied Chemistry 34: 401 - 410.

Rudloff, E. von. 1973. Chemosystematic studies in the genus Pseudotsuga (3). Population differences in British Columbia as determined by volatile leaf oil analysis. Can. Journ. Forest Res. 3: 443 - 452.

Zavarin, E. and K. Snajberk, 1973. Geographic variability of monoterpenes from cortex of Pseudotsuga menziesii. Pure and Applied Chemistry 34: 411 - 433.

Zavarin, E. and K. Snajberk, 1975. Pseudotsuga menziesii chemical races of California and Oregon. Biochemical Systematics and Ecol. 2: 121 - 129.

EWG - SYMPOSIUM DER BIOCHEMIE FÜR WALDBÄUME

BERICHT DES VORSITZENDEN

EINLEITUNG

Das Symposium wurde von den Abteilungen für Forstwirtschaft und die Koordination landwirtschaftlicher Forschung der Kommission der Europäischen Gemeinschaften einberufen und fand am 23. bis 27. Januar 1977 in Brüssel statt. 19 Teilnehmer aus Mitgliedsstaaten wurden von den jeweiligen Leitern der Forschungsabteilungen ernannt, und zu ihnen zählten land- und forstwirtschaftliche Wissenschaftler, die mit biochemischen Methoden arbeiten oder unmittelbar an deren Anwendung interessiert sind. Drei Teilnehmer aus Finnland, Schweden und den Vereinigten Staaten waren Experten für die Anwendung biochemischer Methoden in der Forstgenetik und vertraten gleichzeitig den Standpunkt der I.U.F.R.O. (Internationale Union der forstwirtschaftlichen Forschungsorganisationen). Neben dem Austausch der neuesten Informationen wurde im Laufe des Symposiums versucht, Wege zu einer Verbesserung der Anwendungsmöglichkeiten biochemischer Methoden in der forstlichen Forschung zu prüfen. Die Ergebnisse dieser Untersuchung werden in diesem Bericht zusammengefasst, welcher den Leitern der forstlichen Forschungsabteilung zur Begutachtung vorgelegt werden. Die derzeit in der forstlichen Forschung verwendeten biochemischen Methoden wurden je nach der für die Analyse verwendeten Substanzen in 4 Gruppen eingeteilt, und die hier vorliegenden Kongressunterlagen halten sich an dieselbe Einteilung, nähmlich Terpen-, Isoenzym-, Polyphenolmethoden und Methoden mit anderen Substanzen. Ähnliche Forschungsmethoden, die auf der Genetik von landwirtschaftlichen Anbauprodukten angewendet werden, wurden von Landwirtschaftexperten erläutert. Schliesslich wurden anlässlich des Symposiums zukünftige Anwendungsmöglichkeiten biochemischer Methoden in der forstwirtschaftlichen Forschung innerhalb der Gemeinschaft besprochen. Die verschiedenen Methoden und ihre Anwendung sind in den Kongressunterlagen ausführlich beschrieben und es gilt hier lediglich, die wichtigsten Merkmale zusammenzufassen.

ANALYSEVERFAHREN UND DEREN ANWENDUNG

Terpene

Das Harz von Nadelhölzern besteht aus einer Terpenmischung, die von den einfachsten Monoterpenen bis zu komplexer zusammengesetzten Sequiterpenen und Diterpenen wie z.B. Harzsäuren reichen können. Es gibt 2 Analyseverfahren. Die Analyse der Monoterpene erfolgt direkt durch Flüssiggaschromatographie und ist ein relativ rasches Verfahren. Die andere und etwas vollständigere Methode erfordert eine vorherige Wasserdampfdestillation und dauert etwas länger.

Die Terpenanalyse kann nur bei Nadelhölzern angewendet werden, und Unterschiede der Harze von verschiedenen Stellen eines einzigen Baumes beschränken den Vergleich auf eine einzige Harzart (im allgemeinen wird Rindenharz verwendet). Diese Methode kann nur auf mehr als 2 Jahre alte Pflanzen angewendet werden, doch seit der Entdeckung von Vorläufern von Harzen in ganz jungen Pflanzen durch die französischen Forscher Bernard-Dagan und Baradat in Frankreich, sowie durch laufende Forschungsarbeiten über Pflanzenharze in den Vereinigten Staaten gilt diese Beschränkung für gewisse Baumarten nicht mehr.

Die Terpenzusammensetzung oder das "Profil" von Nadelholzharzen weist stark vererbte Merkmale auf, die unter verschiedenen Umweltbedingungen unverändert bleiben und oft mit der geographischen Herkunft in Zusammenhang stehen und vereinzelt auf eine klinale Verteilung hinweisen.

Die Terpenzusammensetzung kann sich daher für die Massung von Schwankungen innerhalb einer Baumart als nützlich erweisen, um subspezifische Populationen zu unterscheiden oder Populationen zu beschreiben, die auf anderen wie z.B. morphologischen Kriterien basieren, sowie als Hilfe bei der Ermittlung oder Feststellung der Herkunft. Die Effizienz der Terpenalalyse zu diesem Zwecke hängt von der Verteilung der Markmale der Terpenzusammensetzung bei der Baumart ab, sowie von der Menge der vorhandenen Bezugsdaten. In Fällen, in denen die Verteilung ausreichend bekannt ist, wie z.B. bei der Pinus elliotii (Dokument Nr. 1), kann die Abstammung einigermassen abgeleitet werden. Ähnlich erweist sich in Fällen, in denen wenig Material vorliegt und die Terpenmerkmale ausreichend bekannt sind, wie z.B. eine Gruppe von Provenienzen, Erbtypen oder Klonen , ist die Terpenanalyse als verlässlich für die Feststellung der Herkunft und kann unter gewissen Unständen im Handel von Nutzen sein.

Einige Merkmale der Terpenzusammensetzung sind durch die einfache Genkontrolle gesteuert und sind besonders bei der Auswahl von Material für Züchtungen sowie der Feststellung der Vererbung während der Züchtung, besonders bei Pfropfanlagen von Nutzen. Die Terpenzusammensetzung wurde für diese Zwecke in Frankreich (Dokument Nr. 4) und in den Vereinigten Staaten verwendet.

Innerhalb der Gemeinschaft wird die Terpenanalyse nach Wasserdampfdestillation regelmässig in Frankreich (Dokument Nr. 4) und die Monoterpenanalyse ständig im Vereinigten Königreich verwendet (Dokument Nr. 2 und 3).

Isoenzyme

Viele Enzyme kommen als Isoenzyme in verschiedener Form natürlich in Pflanzen vor, da ihre Molekularstruktur nur geringe Schwankungen aufweist. Die Zusammensetzung der Isoenzyme gibt direkten Aufschluss über den Erbtyp der Pflanze. Im allgemeinen werden die Isoenzyme aus Blättern oder Samen gewonnen und durch Elektrophorese getrennt.

Diese Methode ist sowohl auf Laubhölzer als auch auf Nadelhölzer anwendbar, doch befindet sich die Anwendung auf Laubhölzer in Europa noch in der Versuchsphase und befasst sich vorwiegend mit Fagus und Quercus in Deutschland. Bei Samen von Laubholzarten ergeben sich besondere Schwierigkeiten, die jedoch vermutlich mit Hilfe der derzeitigen Forschungsarbeiten überwunden werden können.

Viele Merkmale des Vorkommens und der Zusammensetzung von Isoenzymen sind streng vererbt und unveränderlich und ebenso wie bei den Terpenen sind diese Merkmale bei der Ermittlung von Baumarten, Subpopulationen und Provenienzen von grossem Nutzen.

Die genaue Feststellung einer unbekannten Herkunft ist mit Hilfe der Isoenzymanalyse derzeit nicht möglich, doch zeigen einige Merkmale deutlich klinale Verteilungen, wodurch der Ursprung annähernd abgeleitet werden kann. Allerdings kann diese Methode für eine weitgehende Feststellung der Herkunft noch nicht verwendet werden.

Die Isoenzymanalyse ist für die Feststellung des Genotyps bei der Auswahl und Züchtung von Nutzen. Durch das Vorhandensein einiger seltener Allele, die mit Hilfe der Isoenzymanalyse festgestellt werden können, kann diese Methode auch auf Erbuntersuchungen bei Beständen und Pfropfanlagen verwendet werden.

An der Entwicklung dieser Methode wird in Schweden aktiv gearbeitet (Dokument Nr. 5). Innerhalb der Gemeinschaft wird diese Methode laufend in Deutschland weiterentwickelt (Dokument Nr. 6 und 7) und sie wird in Frankreich verwendet (Dokument Nr. 8).

Polyphenole

Polyphenole sind verschiedene Verbindungen, die in allen höheren Pflanzen zu finden sind. Sie reichen von relativ einfachen farbigen oder farblosen Bestandteilen (z.B. farbige Blumen- und Fruchtpigmente) bis zu Polymeren mit hohem Molekulargewicht, wie z.B. Gerbsäuren, Rinden- und Harzholzpigmente. Polyphenole werden aus Zucker in Pflanzen synthetisiert, und ihre Bildung hängt von verschiedenen Faktoren ab, unter anderem dem Vorhandensein von Kohlehydraten und Stickstoff. Diese grosse Gruppe von Substanzen wurde seltener als die Terpene und Isoenzyme für die Kennzeichnung von Erwerbsbäumen verwendet.

Die Verwendung von Polyphenolen ist in der Forstgenetik noch selten, doch sind weitere Forschungsarbeiten auf Grund der Bedeutung und des ausgedehnten Vorhandenseins dieser Substanzen in Waldbäumen gerechtfertigt. Die Veränderlichkeit einiger Phenole macht diese zwar für gewisse genetische Untersuchungen weniger brauchbar, doch könnte sich in Zukunft in der forstlichen Forschung andere Anwendungsbereiche finden.

Innerhalb der Gemeinschaft wurde der Einsatz von Polyphenolen in Dänemark entwickelt (Dokument Nr. 9), und einige Untersuchungen wurden im Vereinigten Königreich durchgeführt.

Andere Substanzen

Serologische Methoden, die auf Antikörper-Antigenreaktionen beruhen, wurden in der allgemeinen Pflanzentaxonomie verwendet, doch werden sie in der Forstforschung noch zu wenig eingesetzt, um über ihre Möglichkeiten Aussagen machen zu können. Die Anwendung ist in Finnland (Dokument Nr. 10) und Japan am weitesten entwickelt. In Finnland wurden serologische Methoden für die Untersuchung der Beziehungen zwischen Kiefernarten verwendet. Innerhalb der Gemeinschaft ist nichts über eine ständige Anwendung dieser Methode in der Forstforschung bekannt.

Die Kennzeichnung von Baummaterial durch Extraktion und quantitative Bestimmung von Nukleinsäuren scheint für eine ausgedehnte Anwendung noch nicht ausreichend entwickelt zu sein und wird in der forstlichen Forschung innerhalb der Gemeinschaft noch nicht laufend eingesetzt. Eine Reihe anderer biochemischer Methoden für spezielle Untersuchungen wurden nicht angeführt. Die Aminosäurenanalyse für entomologische forstliche Forschungszwecke und die Blattanalyse von Mineralnährstoffen fielen z.B. unserer Ansicht nach nicht in den Bereich des Symposiums.

ANWENDUNGSMÖGLICHKEITEN

Während des Symposiums wurde deutlich die Rolle beleuchtet, welche biochemische Methoden bei der Definition von Pflanzenmaterial von der Art bis zum Genotyp für eine Verbesserung des Baummaterials durch Auswahl und Züchtung spielen. Dabei stellen die biochemischen Analysen eine wertvolle Ergänzung der morphologischen und biometrischen Methoden dar. Eine Voraussetzung für diese Art der Anwendung sind streng vererbte und unveränderliche biochemische Merkmale. Ein anderer Einsatz eines biochemischen Analyseverfahrens, das sich bei der Diagnose und Messung der Veränderung jener Merkmale bedient, die auf Umwelteinflüsse anfällig sind, ist derzeit noch nicht sehr weit entwickelt, doch könnten sich in der Zukunft wichtige Anwendungsbereiche ergeben.

Biochemische Methoden werden vermutlich immer häufiger in anderen Bereichen der forstlichen Forschung verwendet werden, insbesondere in der Baumphysiologie, der Waldpathologie und der Holzverwendung. Allgemeines und starkes Interesse fand anlässlich des Symposiums die Möglichkeit gemeinsamer gemeinschaftlicher Forschungsarbeiten von Forstpathologen und Biochemikern über Fomes annosus, eine Krankheit bei der die Resistenz des Wirtsbaumes mit biochemischen Merkmalen in Zusammenhang zu stehen scheint.

Interesse fand auch die biochemische Kennzeichnung von Ulmus als Hilfe bei der Züchtung zur Resistenz gegen Ceratocystis ulmi und weniger dringende, doch möglicherweise wichtige Anwendungen, die anlässlich des Symposiums besprochen wurden, liegen in der Feststellung der Verträglichkeit von Umweltfaktoren, wie Dürre, Feuchtigkeit, schlechte Versorgung mit Nährstoffen und Umweltverschmutzung.

Selbstverständlich werden biochemische Methoden bereits bei solchen Untersuchungen verwendet, doch werden sie in Zukunft vermutlich noch häufiger eingesetzt werden.

Biochemische Methoden können ebenso für die Erhaltung z.B. einer Reihe von Erbtypen im Naturwald angewendet werden.

KÜNFTIGE ENTWICKLUNG IN DER GEMEINSCHAFT

Eine ganz Reihe biochemischer Methoden wird derzeit von den einzelnen Mitgliedsstaaten aktiv entwickelt und es bedarf keiner Kontrolle von seiten der Gemeinschaft, um die Entwicklung in Zukunft sicherzustellen.

Es gibt eindeutig Möglichkeiten für eine fruchtbare Zusammenarbeit zwischen einzelnen Mitgliedsstaaten, insbesondere jenen, die gemeinsam an der Verbesserung einer einzelnen Art interessiert sind und besonders zwischen Ländern mit verschiedenen Erfahrungen und unterschiedlichen Möglichkeiten, biochemische Analysen durchzuführen und zu interpretieren.

Die Kommission der Europäischen Gemeinschaften könnte eine solche Zusammenarbeit mit Hilfe der nachstehend angeführten Mittel erleichtern. In Fällen, in denen alle oder die meisten Länder ein bestimmtes gemeinsames Interesse zeigen, könnten die Leiter der forstlichen Forschungsabteilungen die Initiative ergreifen und Sonderprojekte auf gemeinschaftlicher Ebene organisieren, wobei ein solches Projekt am Ende dieses Berichtes empfohlen wird.

Die biochemischen Methoden werden allgemein verbessert und ausgebaut. Das wichtigste Ziel innerhalb der Gemeinschaft besteht darin, die Benützer und eventuellen Benützer solcher Methoden ausreichend über die Entwicklungen zu informieren, insbesondere über mögliche Anwendungsbereiche.
Dies kann am besten erfolgen durch:

a) Gemeinschaftliche Seminare für Biochemie

   Bei solchen Seminaren sollten Wissenschaftler vertreten sein, die sich biochemischer Methoden bedienen, sowie potentielle Benützer solcher Methoden. Sie sollten gewöhnlich in Abständen von 2 bis 3 Jahren stattfinden. Allerdings sollten sie so angesetzt werden, dass sie ähnliche von der IUFRO organisierte Veranstaltungen, wie z.B. das biochemische Seminar in Göttingen im Jahre 1974, ergänzen, jedoch nicht wiederholen.

b) Austausch von Wissenschaftlern

Die Bedeutung von selbst nur kurzen Austausch besuchen von Wissenschaftlern, die an ähnlichen Projekten über biochemische Methoden arbeiten, kann durchaus als Instrument bezeichnet werden, mit dessen Hilfe einschlägige, vollständige und genaue Informationen erhalten werden können.

Selbstverständlich kann nichts die Mitgliedsstaaten daran hindern, solche Austauschbesuche derzeit zu veranlassen, doch könnten diese durchaus häufiger stattfinden, und die Leiter der Forschungsabteilungen könnten zweifellos zu einem stärkeren Austausch dieser Art anregen.

c) Ausbildung

Der Anwendungsbereich biochemischer Methoden in der forstlichen Forschung innerhalb der Gemeinschaft ist zu klein, um derzeit formelle, zentrale Ausbildungslehrgänge zu rechtfertigen. Wären allerdings die zuständigen Behörden bereit, so könnte eine Ausbildung für analytische Methoden auf Anfrage für Einzelpersonen oder kleine Personengruppen vorgesehen werden. Eine Ausbildung für Terpenanalyseverfahren könnte in Frankreich, für Monoterpenanalyseverfahren im Vereinigten Königreich, für Isoenzymanalysen in Deutschland und Polyphenolanalysen in Dänemark organisiert werden. Es würde sich dabei eher um eine praktische Ausbildung am Arbeitsplatz als um eine theoretische Ausbildung handeln, die jedoch für diesen Zweck durchaus ausreichen würde. Eine Ausbildung in Analysetechniken konnte innerhalb von za. 2 Wochen erfolgen.

d) Biochemische Datenbanken

Der Vorteil jeglicher biochemischer Methode zur Bestimmung von Pflanzenmaterial hängt weitgehend vom vorhandenen Bezugsmaterial ab. Die Möglichkeiten, zentrale Datenbanken für Ergebnisse von biochemischen Analysen zu schaffen, wurden anlässlich des Symposiums besprochen, doch ergeben sich Schwierigkeiten und Unkosten bei einer zentralen Erhebung und Speicherung biochemischer Daten. Andererseits ist es möglich und auch wichtig, dass einzelne Forschungsstellen ihre eigenen Analyseergebnisse speichern und sie auf Anfrage zugänglich machen. Die oben empfohlenen Seminare und der Austausch von Wissenschaftlern würde dazu beitragen, dass man sich allgemein über das Ausmass der verfügbaren Daten bewusst wird. Die Notwendigkeit einer Normung von biochemischen Methoden innerhalb der Gemeinschaft wurde in Betracht gezogen, doch da die meisten Verfahren entweder bereits genormt sind oder zu vergleichbaren Ergeb-

nissen führen, ist dies nicht unbedingt ein dringliches Anliegen. Auf jeden Fall wäre eine allgemeine Normung vorzuziehen, die am besten von der IUFRO geschaffen werden könnte.

Ein Sonderprojekt einer biochemischen Analyse für europäische Douglasien (Pseudotsuga menziesii) wird aus 2 Gründen für ein gemeinsames Projekt der Gemeinschaft empfohlen:

1) Wegen des direkten Interesses an einer Verbesserung dieser Baumart in den meisten Mitgliedstaaten;

2) Wegen der von den beim Symposium anwesenden Biochemikern anerkannten Notwendigkeit, gleiche oder verschiedene biochemische Analyseverfahren für identische Baumarten zu finden und gemeinsam eine genauere Kennzeichnung zu schaffen, was durch isoliert angewendete Methoden nicht möglich ist.

Es wird vorgeschlagen Proben zu sammeln von ausgewählten IUFRO und sonstigen Europäischen Provenienzversuchen; diese Proben sollten dann in Frankreich einer Terpenanalyse, in Deutschland einer Isoenzymanalyse, in Dänemark einer Polyphenolanalyse und im Vereinigten Königreich einer Monoterpenanalyse unterzogen werden. Die Zahl der Provenienzen und Proben sollte zumindest anfangs begrenzt sein, um die für die Analyse aufgewendete Zeit in den betreffenden Labors entsprechend in Grenzen zu halten. Das Projekt würde durch den Austausch, die Interpretation und die Veröffentlichung der Ergebnisse durch die betreffenden Forschungsstellen ergänzt werden.

Auf Grund der gemeinsamen Ergebnisse dieses Projekts und Daten aus den Vereinigten Staaten könnte eine chemisch-taxonomische Karte für Douglasien erstellt werden, welche bei einer Selektion und Zucht dieser Baumart in den Mitgliedstaaten von grossem Nutzen sein könnte. Dr. Squillace gab zu bedenken, dass falls eine solche Karte für Douglasien erstellt werde, dies bald erfolgen sollte, da die noch vorhandenen natürlichen Bestände rasch abnehmen.

Aus Zeitgründen war es anlässlich des Symposiums nicht möglich, die Vorschlage eingehender zu erörtern und für die Planung des Projekts wäre die Bildung einer kleinen Arbeitsgruppe erforderlich. Die notwendigen Mittel

wären gering und die Ergebnisse wären wahrscheinlich für die Mitgliedsstaaten von direktem Nutzen und für die Forstgenetiker bei der IUFRO von direktem Interesse.

D.T. SEAL,
Vorsitzender des Symposiums
Edinburgh

März 1977

ANHANG

Die folgenden Hinweise zur Literatur wurden freundlicherweise von Dr. A.E. Squillace im Zusammenhang mit dem Vorschlag für ein Douglasienprojekt erwähnt.

Rudlogg, E. von. 1972. Chemosystematic studies in the genus Pseudotsuga I. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas fir. Can. Jour. Bot. 50: 1025 - 1040.

Rudloff, E. von. 1973. Geographic variation in the terpene composition of the leaf oil of Douglas fir. Pure and Applied Chemistry 34: 401 - 410.

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SESSION		PAPER	
SEANCE	I	DOCUMENT	1
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MONOTERPENE COMPOSITION OF CORTICAL OLEORESINS IN Pinus elliottii AND ITS UTILITY IN GENETICS RESEARCH.

COMPOSITION DES OLEORESINES DES TISSUS CORTICAUX CHEZ Pinus elliottii SON UTILISATION DANS LES RECHERCHES DE GENETIQUE.

MONOTERPENZUSAMMENSETZUNG DES HARZES KORTIKALEN GEWEBES BEI Pinus elliottii UND DIE ANWENDBARKEIT IN DER GENETISCHEN FORSCHUNG.

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SUMMARY

The concentrations of 4 of the 5 major monoterpenes in cortical oleoresin of Pinus elliottii are controlled by single genes, with high being dominant or partially dominant over low amounts. Environmental effects on monoterpene composition are small. Large differences occur between trees, and examples of 15 of the 16 possible phenotypes have been found. Distinctive patterns of geographic variation occur for each of the 4 monoterpenes shown to be simply inherited, with clinal trends being a dominant feature over much of the species range. Such detailed knowledge of variation and inheritance permit use of monoterpene compositions as gene markers for studying genetic problems. Uses include identifying relatives and seed origins and determining the degree of selfing and of wild pollen contamination in seed orchards.

RESUME

Les concentrations de 4 des 5 monoterpènes principaux présents dans l'oléorésine des tissus corticaux de Pinus elliottii sont contrôlées par un seul gène, le caractère "forte concentration étant dominant ou partiellement dominant sur le caractere "faible concentration". Les effets du milieu sur la composition monoterpénique sont faibles. On observe de grandes différences entre arbres et on a rencontré 15 des 16 phénotypes possibles. Il a été montré que les lois de variabilité géographique sont différentes pour les 4 monoterpènes à hérédité simple ; la tendance à une variation clinale prédomine sur une grande partie de l'aire de l'espèce. Une telle connaissance détaillée de la variabilité et du mode d'hérédité rend possible l'utilisation des compositions monoterpéniques comme gènes marqueurs pour étudier les problèmes de génétique. Parmi les utilisations possibles, on peut noter l'identification des apparentés et de l'origine des graines ainsi que la détermination du taux d'autofécondation ou du taux de contamination par du pollen sauvage en vergers à graines.

ZUSAMMENFASSUNG

Die Konzentrationen von 4 der 5 Hauptmonoterpenkomponenten im Harz des kortikalen Gewebes bei Pinus elliottii werden von einzelnen Genen kontrolliert, hierbei ist hoher Gehalt dominant oder teilweise dominant über geringen Gehalt. Umweltbedingte Effekte auf die Zusammensetzung der Monoterpene sind gering. Zwischen Bäumen kommen grosse Unterschiede vor und Beispiele von 15 der 16 möglichen Phänotypen sind gefunden worden. Für jede der 4 einfach vererbten Monoterpenen kommen unterscheidbare Muster der geographischen Variation vor, wobei über einen grossen Teil des Verbreitungsgebiets der Art der klonale Trend vorherrschend ist. Solch detaillierte Kenntnisse über Variation und Vererbungsmodus erlauben es, die Monoterpenzusammensetzung als Genmarker für die Analyse genetischer Fragen zu verwenden. Die Anwendung umfasst die Identifizierung von Verwandten und den Ursprung von Saatgutproben sowie die Ermittlung des Selbstungs- bzw. Fremdungsgrades in Samenplantagen.

MONOTERPENE COMPOSITION OF CORTICAL OLEORESIN IN *Pinus elliottii* AND ITS UTILITY IN GENETICS RESEARCH

Interest in monoterpene composition at our laboratory originated from our research to develop strains of *Pinus elliottii* Engelm. that would yield large amounts of oleoresin for gum naval stores. The monoterpenes occurring in this species vary greatly in value, and the original objective of this monoterpene research, beginning in 1961, was to increase yield of the most valuable component, β-pinene (Squillace and Fisher 1966). This work was fruitful, but we also soon learned that monoterpene composition could help solve other tree breeding problems. Hence, monoterpene composition became a major part of our research program. In this paper I briefly summarize findings on the variation and inheritance of monoterpene composition and give some examples of how we use this information in our genetics research.

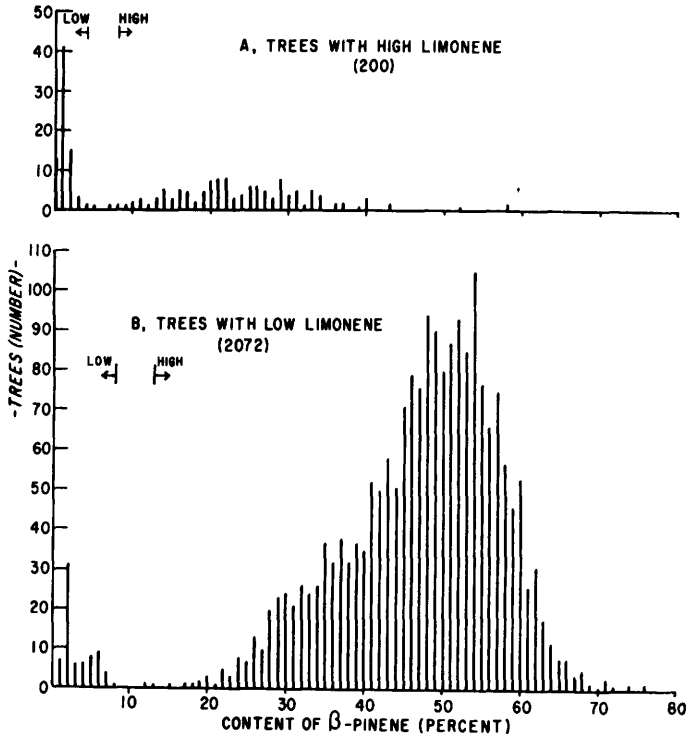
INDIVIDUAL TREE VARIATION AND INHERITANCE

The oleoresin of *P. elliottii* consists of about 20 percent monoterpenes, the remainder being mainly resin acids. The monoterpene fraction of oleoresin from xylem tissue consists mostly of α-pinene, β-pinene and β-phellandrene. Frequently camphene, myrcene, α-phellandrene, and limonene occur as minor constituents, while traces of Δ-3-carene, and γ-terpinene occur occasionally. In oleoresin from cortical tissue of branch tips the same constituents occur but the amounts of myrcene and/or limonene can be very high in some trees.

The oleoresin of stem xylem is of greatest commercial importance, but the oleoresin from cortical tissue has proved to be the most useful for genetics studies. The composition of monoterpenes in stem xylem oleoresin often varies with height in the tree and depends partly upon distance from the live crown (Roberts 1970 and Franklin 1976). This effect tends to complicate sampling procedures for stem xylem oleoresin. The oloeresin in cortical tissue, on the other hand, is relatively constant within the crown. This desirable feature, plus the fact that cortical oleoresin has 5 major constituents compared to 3 in xylem oleoresin, has led us to favor cortical oleoresin for genetics studies. Monoterpene composition in oleoresin of needles tends to be similar to that of branch cortical tissue. However, we usually use the latter, because of its relative ease of collection - most trees readily exude a droplet of oleoresin sufficient for analysis when branch tips are excised. Occasionally we have to extract oleoresin from cortical tissue with a solvent; this procedure is also satisfactory.

The relative amounts of most of the monoterpenes are usually either high or low. That is, frequency distributions for oleoresin from a large number of trees are usually bimodal, (Figures 1A and B). One complication is that the location of modes can be affected by the presence or absence of other major constituents. For example, the mode for high β-pinene is lower in trees containing high limonene (Fig. 1A) than in trees containing low limonene (Fig. 1B). After study of over 2000 trees, we developed the classification scheme given in Table 1 for 4 of the major constituents in *P. elliottii*. Since clear evidence of bimodality for α-pinene is lacking, this constituent is excluded from Table 1.

Figure 1.—Frequency distributions for β-pinene.



Bimodality in the frequency distribution for a monoterpene suggests that the relative amount of it is controlled by a single gene with dominant gene action. Studies of parents and their self- and cross-pollinated offspring show that this is indeed the case for all constituents showing clear bimodality (Table 2) (Squillace 1971, 1976a). Curiously, high was found to be dominant over low in all cases.

As the work progressed we were able to identify enough genotypes to study the degree of dominance expressed by monoterpenes. Preliminary indications are that dominance is partial rather than complete in most cases (Table 3). In cases of incomplete dominance we may eventually be able to distinguish heterozygotes from homozygotes, enhancing the utility of monoterpenes as gene markets.

Environmental effects on monoterpene composition of cortical oleoresin of P. elliottii are small (Squillace and Fisher (1966) and Gansel and Squillace (In press)).

Since 4 of the major monoterpenes show bimodality, we can classify trees into 16 phenotypes as indicated in Table 4. Note that many of the trees fall into a few of the phenotypic classes, but appreciable numbers occur in other classes. Only one class lacks representatives.

Table 1.—Criteria used for classifying trees as having high or low amounts of each monoterpene. (From Gansel and Squillace, In press)

Monoterpene	Low	High	Constraint condition
	—Percent—		
β-pinene	0–4	8+	high limonene
	0–8	12+	low limonene
myrcene	0–6	9+	high limonene
	0–4	7+	low limonene
limonene	0–8	16+	none
β-phellandrene	0–2	4+	none

Note especially that two trees occur which, lacking genes for high amounts of the monoterpenes known to be simply inherited, are almost entirely α-pinene. At the other extreme, some trees contain the high allele for all 4 monoterpenes.

GEOGRAPHIC VARIATION

In one of our studies, we sampled trees originating from all portions of the species range. Trees were classified as having either high or low amounts of each of the 4 monoterpenes shown to be simply inherited. Distinctive patterns occurred. For example no trees having high β-pinene were found in extreme south Florida (Fig. 2). From this point the percentage of such trees increased rapidly to the north. A plateau was reached where all trees had high β-pinene. Clinal patterns, with plateaus in some cases were also found for other monoterpenes (Figs. 3 to 5).

UTILITY OF MONOTERPENE COMPOSITION

Identification of Seed Origin

The geographic patterns of variation in monoterpene composition offer great potential for identifying the approximate geographic origin of seed used in plantations of unknown origin. We recently had an opportunity to test the procedure on 3 plantations that were planted in west Florida in about 1936. On the basis of uncertain records, the seed were believed to have come from several counties in northeast Florida. We sampled 30 trees in each plantation and concluded that the seed used for each were of roughl the same geographic origin (Table 5). Hence, we used the averages to estimate their geographic origin. Taking each of the 4 chemicals successively, we eliminated areas where the seed could not likely have originated (Fig.6). All but two areas were eliminated, a small one in south-east Mississippi and a larger one in north-east Florida and south-east Georgia. Hence, the analysis suggested that the purported origin given in the plantation records, north-east Florida, was correct.

Identification of Seed Orchard Seed

Because of differences in monoterpene composition among both individuals and seed sources, I believe that it would be possible to identify seed

Table 2.—Segregation data for inheritance of four monoterpenes in branch cortical oleoresin of slash pine.

Type of mating	Families	Individuals			
		Observed		Expected	
		High	Low	High	Low
		Number			
		β-pinene			
BB x —	78	980	0	980.0	0.0
Bb x Bb	8	86	25	83.2	27.8
Bb x bb	2	9	10	9.5	9.5
bb x bb	0	—	—	—	—
		Myrcene			
MM x —	2	32	0	32.0	0.0
Mm x Mm	17	151	44	146.2	48.8
Mm x mm	45	278	330	304.0	304.0
mm x mm	26	1	278	.0	279.0
		Limonene			
LL x ll	1	31	0	31.0	0.0
Ll x Ll	0	—	—	—	—
Ll x ll	2	14	19	16.5	16.5
L- x ll	1	2	0	1.0	1.0
Ll x wind	1	7	4	5.5	5.5
ll x ll	90	0	1052	.0	1052.0
		β-phellandrene			
PP x —	63	769	14	783.0	0.0
Pp x Pp	8	138	48	139.5	46.5
Pp x pp	17	43	49	46.0	46.0
pp x pp	2	1	50	.0	51.0

Table 3.—Degree of dominance for simply-inherited monoterpenes in *P. elliottii*.

Phenotypic group 1)	Homozygous dominants		Heterozygotes		Homozygous recessives		Degree of dominance 2)
	Basis, trees	Average content	Basis, trees	Average content	Basis, trees	Average content	
	No.	%	No.	%	No.	%	
			β-pinene				
BM1P	156	47.0	12	44.3	60	3.1	0.88
BmLP	1	29.0	25	22.8	28	1.1	.56
Bm1P	257	51.0	8	39.2	9	5.7	.48
Weighted average 3)							.63
			Myrcene				
BM1P	2	34.5	228	21.7	1076	1.0	.24
			Limonene				
BMLP	1	37.0	11	38.4	548	.5	1.08
bmLP	1	75.0	5	70.0	9	.3	.87
bmLp	1	89.0	1	89.0	2	.0	1.00
Weighted average 3)							1.01
			β-phellandrene				
BM1P	168	18.6	61	11.5	83	.4	.22
Bm1P	183	16.4	24	11.3	269	.5	.36
BmLP	1	33.0	1	14.0	3	.0	-.15
Weighted average 3)							.28

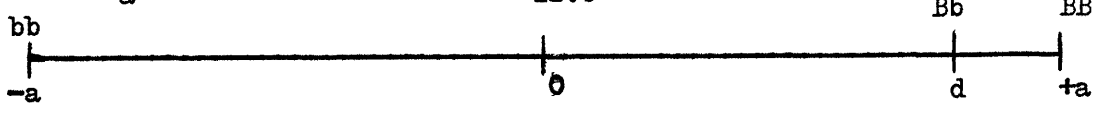
1) B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

2) Computed by methods outlined in Kempthorne (1957, p. 373). For example, the degree of dominance for β-pinene group BM was computed as follows:

$$a = \frac{47.0 - 3.1}{2} = 22.0$$

$$d = 22.0 - (47.0 - 44.3) = 19.3$$

$$\frac{d}{a} = \text{degree of dominance} = \frac{19.3}{22.0} = .88$$



3) Weighted by number of homozygous dominants and heterozygotes involved.

Figure 2.—Percent of trees having high β-pinene (From Gansel and Squillace, In Press).

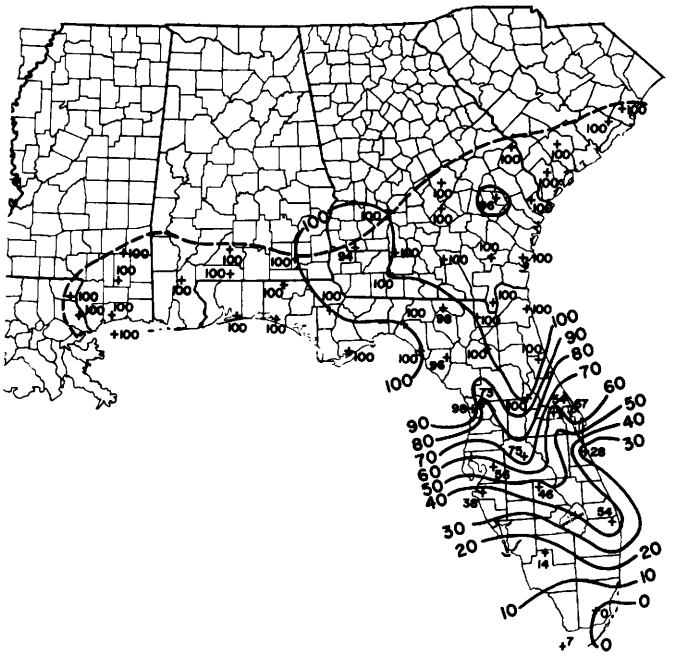


Figure 3.—Percent of trees having high myrcene (From Gansel and Squillace, In Press).

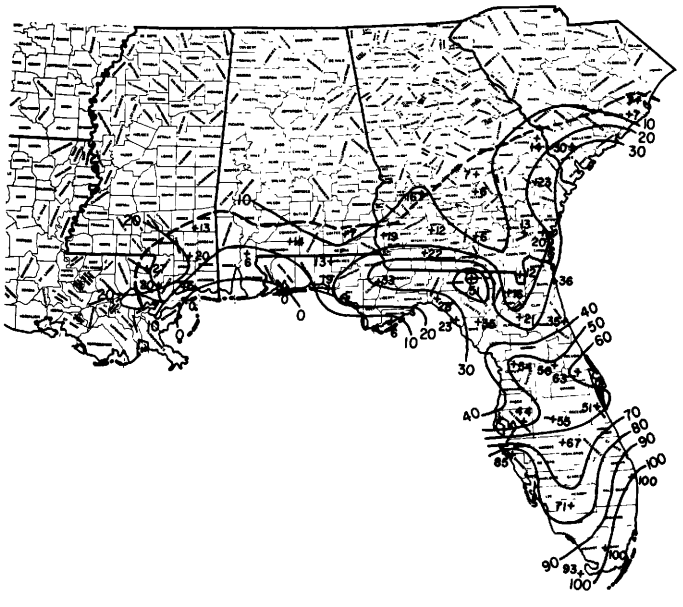


Figure 4.—Percent of trees having high limonene (From Gansel and Squillace, In Press).

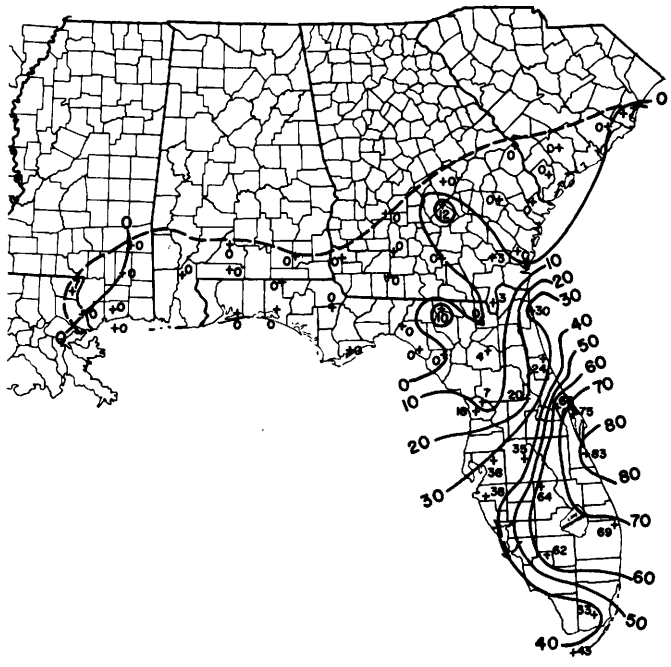


Figure 5.—Percent of trees having high β-phellandrene (From Gansel and Squillace, In Press).

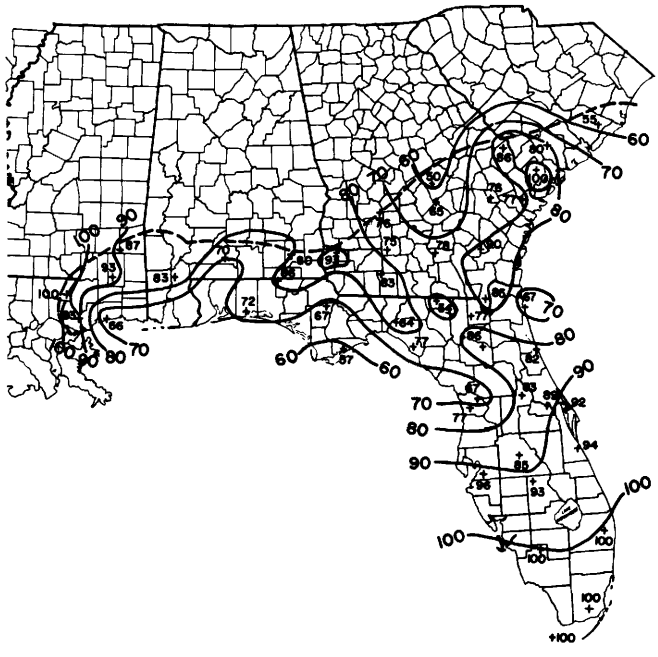


Table 4.—Average monoterpene composition in branch cortical oleoresin of 15 slash pine phenotypes. 1)

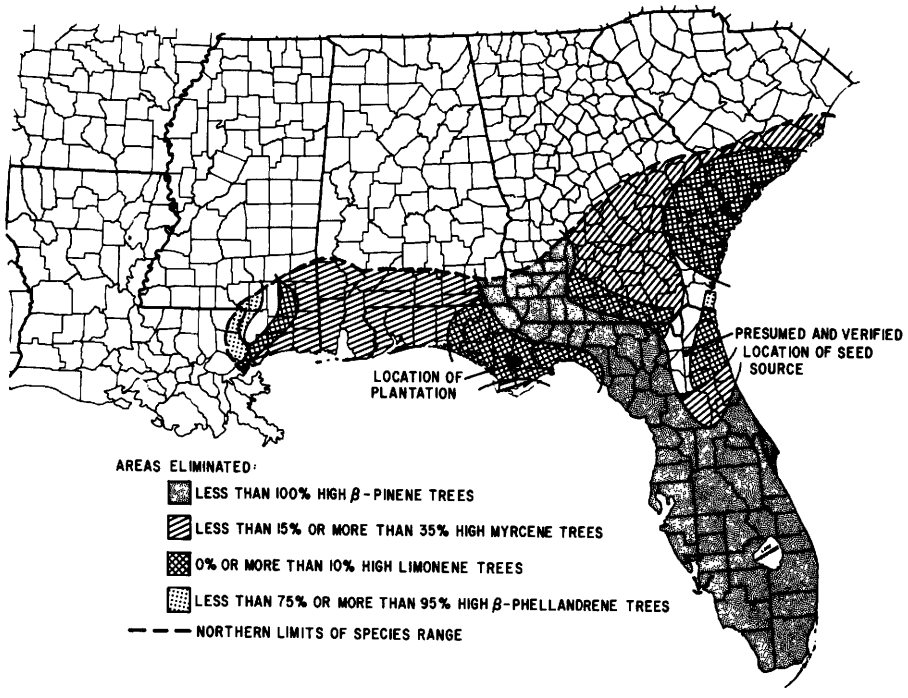
Phenotype 2)	Basis, trees	Composition 3)				
		α-pinene	β-pinene	Myrcene	Linonene	β-phellandrene
	Number	Percent				
BMLP	32	8.4	18.9	14.9	44.3	13.4
BMLp	6	11.8	21.2	15.8	49.8	1.3
BM1P	548	17.2	42.5	23.7	.5	15.8
BM1p	83	29.3	44.3	25.5	.1	.1
BmLP	66	10.3	25.3	2.5	52.5	9.8
BmLp	10	19.1	27.0	2.4	50.5	.9
Bm1P	1076	34.5	49.2	1.0	.6	14.2
Bm1p	269	46.1	52.2	.6	.3	.5
bMLP	40	6.2	1.2	18.6	53.0	20.8
bMLp	0	-	-	-	-	-
bM1P	60	16.8	3.1	38.9	.9	39.6
bM1p	1	44.0	1.0	55.0	.0	.0
bmLP	28	8.5	1.1	4.0	71.6	14.7
bmLp	3	8.7	1.0	4.0	86.3	.0
bm1P	9	60.0	5.7	.9	.3	31.9
bm1p	2	96.0	3.5	.0	.0	.0

1) Sixteen phenotypes are theoretically possible but no trees of the type bMLp occurred in the sample of 2233 trees

2) B, M, L and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

3) Small amounts of camphene and/or α-phellandrene frequently occur.

Figure 6.—Determination of probable origin of seed in a 40 year old Pinus elliottii plantation (See Table 3).



orchard seed, especially where the orchards are based on trees from different geographic origins. We recently had occasion to sample seedlings grown from wind-pollinated seed collected from a clone in a seed orchard, in two different years. Monoterpene composition data were remarkably similar for the 2 years (Table 6). Hopefully random seed from the whole orchard would likewise show such year-to-year constancy and this would be important for identification purposes.

Table 5.—Percentages of trees having high amounts of 4 monoterpenes in 3 adjacent P. elliottii plantations.

Plantation	β-pinene	Myrcene	Limonene	β-phellandrene
A	100	23	3	87
B	100	13	3	87
C	100	37	7	80
Average	100	24	4	85

Table 6.—Percentages of trees having high amounts of 4 major monoterpenes in progeny produced from wind-pollinated seed collected from a P. elliottii clone in a seed orchard in two years.

Monoterpene	Seed collected in :	
	1973 (Basis, 250 trees)	1974 (Basis, 296 trees)
β-pinene	93.6	93.2
Myrcene	13.6	13.5
Limonene	1.2	1.3
β-phellandrene	76.0	72 9

Identification of Relatives

Knowledge of the mode of inheritance of 4 major monoterpenes permits us to identify relatives in trees to about the same extent that relatives can be identified in human beings using blood types. Identification of ramets within clones is, of course, done rather easily. In several instances we have suspected that certain ramets had been mislabelled, on the basis of cone and seed characteristics, and then verified such suspicions by examining monoterpene composition. In some instances we were able to determine the proper clone. Identification of parents and progenies is, of course, less certain although in one instance we detected and corrected a serious labelling error in a progeny test.

Selfing and Wild Pollen Contamination in Orchards

An unusual situation in one of our demonstration seed orchards permitted us to make rough estimates of both the extent of selfing and the degree of wild pollen contamination. The orchard covered 5 acres (2 hectares) and all P. elliottii trees within 400 feet (122 meters) had been removed. It contained 9 clones, which were all related as either half sibs or full sibs. We knew monoterpene genotypes of all the clones (Table 7). One of

the clones, No. 5, was thought to be suitable for estimating the degree of selfing because 1/16 of its selfed progeny would be of the type bmlp which could not be produced from matings among clones in the orchard. Hence, wind-pollinated seeds were collected from it and monoterpene composition was determined on seedlings grown from them.

Table 7.—The genotypes of nine clones in an experimental slash pine orchard being used to estimate selfing and wild pollen contamination.

Clone Number	Genotype
1	BB Mm ll Pp
2	BB Mm ll Pp
3	BB mm ll Pp
4	BB Mm ll Pp
5	Bb mm ll Pp
6	BB Mm ll Pp
7	BB mm ll PP
8	Bb mm ll PP
9	BB Mm ll PP

Frequencies of the various phenotypes showed that 1.3 percent were of the type bmlp (Table 8). Hence, we could guess that approximately 16 x 1.3 = 21 percent of the progeny of clone 5 were selfs. However, we also computed expected frequencies, first assuming that all matings were crosses among trees in the orchard, and then also by assuming that all progeny were sired by contaminate pollen. The latter were made on the basis of known gene frequencies determined for the region in which the orchard was located.

Comparison of observed and expected frequencies strongly suggested that considerable wild pollen contamination is occurring in this orchard. All seedlings containing high limonene plus those of the type bMlP and bMlp are necessarily contaminants because they cannot be formed by any mating among orchard clones. Also, the correlation of observed frequencies with frequencies expected from orchard out-crosses is smaller than with frequencies expected from contamination. Note also that bmlp trees can be produced by contamination as well as by selfing. Hence, a better estimate of the degree of selfing might be $16(1.3 - .8) = 8.4$ percent and this agrees more closely with estimates of selfing that we have obtained in other orchards through use of chlorophyll-deficient seedlings as gene markers.

Thus, we believe that production of selfed seedlings in this orchard is low, but that contamination is high. Of course these results apply only to one orchard, but they demonstrate the possible utility of monoterpene composition.

Table 8.—Observed and expected phenotypic frequencies in 546 wind-pollinated progeny 1) of clone 5 in an experimental slash pine orchard average

Phenotype	Observed frequencies 1)	Expected frequencies if pollen is entirely: Self	Orchard outcross	Wild (contamination)
BMLP	0.000	0.0	0.0	0.002
BMLp	.000	.0	.0	.001
BM1P	.106	.0	.253	.095
BM1p	.017	.0	.065	.031
BmLP	.011	.0	.0	.013
BmLp	.002	.0	.0	.004
Bm1P	.578	.562	.569	.615
Bm1p	.220	.188	.077	.203
bMLP	.000	.0	.0	.000
bMLp	.000	.0	.0	.000
bM1P	.009	.0	.0	.004
bM1p	.004	.0	.0	.001
bmLP	.000	.0	.0	.000
bmLp	.000	.0	.0	.000
bm1P	.040	.18	.036	.023
bm1p	.013	.062	.0	.008
Sums	1.000	1.000	1.000	1.000

1) Averages of data from seed collections made in 1973 and 1974.

DISCUSSION

Several other uses of monoterpene composition in solving forestry problems have been suggested (Squillace 1976b). For example, relationships have been shown between monoterpene composition and insect and disease resistance. Such correlations permit indirect genetic selection for these traits. Monoterpene composition is well suited for studies of relationships between species and for identification of hybrids. A recent paper (Squillace, et al in press) suggests gene flow between P. caribaea and P. elliottii based on population analyses of monoterpene composition.

Utility of monoterpene composition will likely be enhanced by more modern gas chromatographs. A recent model, for example, provides for automatic injection of 35 samples, permitting around-the-clock analyses with a minimum of attendance. Thus, more intensive and extensive sampling will be feasible.

In short, monoterpene composition has proved to be very useful in many forest genetics studies and its use is likely to increase.

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SESSION PAPER
SEANCE I DOCUMENT 2
SITZUNG DOKUMENT

GEOGRAPHICAL VARIATION IN THE MONOTERPENES OF THE RESIN OF *Pinus contorta*

VARIATION GEOGRAPHIQUE DANS LES MONOTERPENES DE LA RESINE DU *Pinus contorta*

GEOGRAPHISCHE VARIATION IN DEN MONOTERPENEN VON *Pinus contorta* - HARZ

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SUMMARY

The percentage monoterpene composition of the shoot cortical resin of Lodgepole pine has been studied by gas-liquid chromatography, using material from several different sites in Britain grown from seed collected from a large number of provenances in north-west America. The objective was to assess the degree of biochemical variation occurring within and between provenances, as part of a breeding programme concerned with genetic variation within tree populations.

The resin monoterpene composition was found to be strongly correlated with geographical origin. Provenance from the outlying parts of the natural range tended to give the most highly specific and least variable monoterpene patterns, while towards the centre of the range a sample of trees from a given provenance tended to be a mixture of a small number of fairly distinct genotypes. Coastal populations were dominated by β-phellandrene. The pinenes increased clinally inland and towards the centre of the range, with β-pinene reaching high concentrations in the Puget Sound region of Washington. Limonene and Δ^3-carene were the most variable monoterpenes, and also showed clinal increases towards east central parts of the range, becoming maximal in the Rocky Mountains, Cascades, and Sierra Nevada. A distinct type of monoterpene pattern, characterised by high levels of the pinenes and of camphene, was restricted to two discrete and widely separated areas, in central British Columbia and in south-west Oregon; this pattern may have been a result of introgression between species.

Variations between replicate plots and between different sites in Britain were small, and the results showed that resin analysis of a sample of 25 trees was usually sufficient to allocate that sample to a provenance or a provenance group on the basis of the monoterpene composition.

RESUME

On a étudié par la chromatographie en phase gazeuse la composition pourcentage du profil monoterpénique de la résine tropicale de la pousse du pin de Lodgepole, à l'aide de matières premières provenant de différents endroits de Grande-Bretagne où on les faits pousser à partir de graines en provenance d'un grand nombre de régions du nord-ouest de l'Amérique.

L'objet de cette étude était d'évaluer le degré de la variation biochimique qui se produit dans une même provenance ou entre provenances, Cet exercice faisant partie d'un programme de culture s'intéressant aux variations génétiques des populations d'arbres.

On a trouvé que la composition des monoterpènes de la résine était en forte corrélation avec l'origine géographique. Les provenances des parties éloignées de la réserve naturelle ont eu tendance à donner les formations de monoterpènes les plus hautement spécifiques et les moins variables, alors que vers le centre de la réserve un échantillonnage d'arbres d'une provenance donnée a eu tendance à donner un mélange d'un petit nombre de génotypes assez distincts. Les populations côtières étaient dominées par le β-phellandrène. Les pinènes ont augmenté en fonction de l'inclinaison à l'intérieur des terres et vers le centre de la réserve, le β-pinène atteignant des concentrations élevées dans la région "Puget Sound" de Washington. Les monoterpènes les plus variables ont été le limonène et le Δ^3-carène, et ils ont aussi accusé des augmentations en fonction de l'inclinaison vers les parties centre-est de la réserve, atteignant leur maximum dans les Montagnes Rocheuses, les Cascades et la Sierra Nevada. Un type distinct de formation de monoterpènes, caractérisé par des niveaux élevés de pinènes et de camphène a été limité à deux zones discrètes et très éloignées, au centre de la Colombie britannique et dans le sud-ouest de l'Orégon; ce profil terpénique peut être le résultat d'un mélange des espèces.

Les variations entre parcelles repliées et entre les différents endroits de Grande-Bretagne ont été faibles; les résultats ont montré que l'analyse de la résine d'un échantillonnage de 25 arbres a généralement été suffisante pour identifier tel échantillon à telle provenance ou à tel groupe de provenance sur la base des profils monoterpéniques.

ZUSAMMENFASSUNG

Die Zusammensetzung der Monoterpen-Fraktion im Rindenharz von Pinus contorta wurde mit der gaschromatographischer Analysenmethode untersucht.

Das Untersuchungsmaterial stammte von einer Anzahl vereschiedener Standorte in Grossbritannien un war aus Samen von einer grossen Anzahl nordwest-amerikanischer Herkünfte gezogen. Ziel der Untersuchung war - als Teil-arbeit zu einem mit der genetischen Variation innerhalb von Baumpopulationen befassten Zuchtprogramm - den Grad der biochemischen Variation innerhalb und zwischen den Herkünften zu bestimmen.

Zwischen dem Harz-Monoterpenprofil und dem geographischen Ursprung wurde eine starke Korrelation festgestellt. Bei Herkünften von den Randgegenden des natürlichen Verbreitungsgebietes zeigte sich eine Tendenz in Richtung der am höchsten arteigenen und am wenigsten variablen Monoterpenprofile; andererseits tendierte gegen die Mitte des Verbreitungsgebietes hin eine Baumprobe aus einer bestimmten Herkunft in der Richtung einer Mischung von einer kleinen Anzahl ziemlich individuell ausgeprägter Genotypen. Unter den Küstenpopulationen war β-Phellandren vorherrschend. Im Binnenland und in Richtung auf die Mitte des Verbreitungsgebietes stiegen die Pinene in konti-nuierlicher Stufenfolge an, wobei die β-Pinen im Gebiet des Puget Sound von Washington hohe Konzentrationen erreichten. Limonen und Δ^3-Caren waren die Monoterpene von der grössten Variabilität; sie zeigten gleichfalls einen Anstieg in kontinuierlicher Stufenfolge gegen die östlichen Zentralgegenden des Verbreitungsgebietes hin und erreichten Höchstwerte in den Rocky Mountains, den Kaskaden und in der Sierra Nevada. Ein ausgeprägter Typ von Monoterpenprofil, der sich durch einen hohen Stand der Pinene und Camphen kennzeichnete, war auf zwei getrennte und weit von einander entfernte Gegenden - im Zentralgebiet von British Columbia und in Südwest-Oregon - beschränkt. Dieses Profil mag vielleicht als Folge einer Artenmischung aufgetreten sein.

Variationen zwischen Replikationsflächen und verschiedenen Standorten in Grossbritannien waren nur gering; die Ergebnisse zeigten, dass gewöhnlich die Harzanalyse einer Probe von 25 Bäumen genügte um die Probe auf Grund des Monoterpenprofile in eine Herkunft oder Herkunftstgruppe einzureihen.

GEOGRAPHICAL VARIATION IN THE MONOTERPENES OF THE RESIN OF Pinus contorta

INTRODUCTION

This work forms part of a research project to investigate the sources of biochemical variation in the coniferous tree species most widely planted in Britain. It was hoped the biochemical features of the resin would provide information on the degree of genetic varability within populations, and on the identification of material of doubtful or unknown origin. A further possibility was that the biochemical characteristics might be correlated with desirable physiological features, and might thus provide indirect selection criteria in a breeding programme.

The two species chosen for investigation were Sitka spruce (Picea sitchensis) and Lodgepole pine (Pinus contorta). Polyphenols of Sitka spruce and the resin monoterpenes of both species have been studied, but only the work on the resin of Lodgepole pine is described here.

The immediate aim was to investigate the extent of variation of the percentage monoterpene composition of the resin within and between provenances, and to determine whether sufficient significant geographical variation existed so that provenances or provenance groups could be identified on the basis of their resin monoterpene composition.

MATERIALS AND METHODS

The provenances examined were growing at five sites in Britain (TABLE 1), representing a variety of soil types, elevation, and ages of tree (from 4 to 19 years). The majority of provenances (65) came from a provenance collection at Solway Forest in south-west Scotland, which originated from seed collections made in north-west America in 1965. Additional provenances were sampled from the other four sites, a number of provenances being sampled from two or more sites. A few of the provenances had common origins but were growing at different elevations in America. Altogether, 85 different provenances were sampled, or 93 including origins of different elevation. The sample size was usually 25 trees per provenance plot.

The seed origins ranged widely over the natural distribution of Lodgepole pine, from Gustavus in the Alaskan panhandle to Mendocino and Eldorado in California, and from Queen Charlotte Islands and Vancouver Island eastwards to Cypress Hills in Saskatchewan. There were heavy

concentrations of provenances from the Skeena and Bulkley Rivers in west central British Columbia and from the southern interior of British Columbia.

The sampling procedure was to cut the apical 1 cm off a lateral shoot and to collect cortical resin from the cut surface in a capillary tube by suction from the mouth. Samples were stored deep frozen until analysis. The resin was dissolved in n-pentane immediately before analysis. About 0.5ul of the solution was injected into a Pye Unicam Series 104 gas-liquid chromatograph, fitted with a heated dual flame ionization detector. The column was 2.13 m glass packed with 10% polyethylene glycol 20M on 100-120 mesh Diatomite C-AW. Gas flow rates were: carrier (nitrogen), 45 ml/min; hydrogen, 45 ml/min; air, 700 ml/min. The injection temperature was 165°, the column oven was 140° isothermal, and the detector oven 200°. Monoterpenes were identified by comparison with known standards, and were quantified as the percentage contribution of each peak to the total monoterpenes present.

RESULTS AND DISCUSSION

1. Monoterpene patterns

Eight monoterpenes were usually present; the three constantly occurring components were α- and β-pinene, and β-phellandrene, while the two most variable components were Δ-3-carene and limonene. In addition to these five major components, smaller concentrations of camphene, α-terpinene, and terpinolene were usually present. It was observed that chromatograms generally fell into a relatively small number of distinct types, so that a classification system was adopted, using visual inspection, on the basis of the relative amounts of the five major components (TABLE 2). Primary division was by the relative proportions of the three constant components (Types A-D, with the proportion of β-phellandrene gradually decreasing, and the pinenes, especially β-pinene, increasing), while each of these primary types was subdivided on the basis of the presence or absence of significant amounts of Δ-3-carene and of limonene (Subtypes 1-4). A few additional rarely-occurring types of monoterpene pattern were defined, the most important of which was the pinene-camphene type, in which the chief features were the relatively high proportions of either or both of the pinenes, and more especially the relatively very high level of camphene compared with all other types.

Each chromatogram within a provenance was allocated to one of these basic pattern types, so that the characteristics and the variability of the monoterpene composition of the resin could be estimated. Although most provenances gave a range of different pattern types (see TABLE 5), it was found that certain types were characteristic of certain geographical regions, so that the resin composition was correlated to a large extent with the geographical origin of the provenances.

2. Geographical variation in monoterpene pattern

All the North Coastal provenances from Alaska and British Columbia, including those from Queen Charlotte Islands and the smaller islands to the north, gave a very characteristic type of pattern, Type A1, with β-phellandrene predominant and with insignificant amounts of Δ-3-carene and limonene. The degree of variation between trees was very samll. Date for the mean monoterpene percentage composition of the resin of a typical provenance within each provenance group are given in TABLE 3. Proceeding inland south-eastwards, two changes to this pattern occurred. First, the proportions of Δ-3-carene, β-pinene, and, rather later, of limonene, became progressively greater, so that in the Rocky Mountain region in southern Alberta and British Columbia any one or any combination of these terpenes could dominate the monoterpene pattern. Secondly, the variation between trees within a provenance became much greater. These two effects gradually became more prounced south-eastwards, so that there was a biochemical cline in this direction throughout British Columbia.

An important feature of the monoterpene patterns in Central British Columbia was that a discrete group of five or six provenances was characterised by a fairly high percentage of trees giving the rare pinene-camphene pattern, Type E. This provenance group appeared to exist as a band centred around Prince George on the upper Fraser River, and extending in a south-west to north-east direction from Anahim Lake to Wonowon, in the region of the upper Fraser and Peace Rivers. This region is adjacent to one of the two main introgression zones of Pinus contorta with Pinus banksiana (Jack pine), and since the type of pattern observed, with the pinenes predominating over all other monoterpenes, has been found to be characteristic of Jack pine, this may be additional chemical evidence for introgression. The pinene-camphene pattern was very distinct, and no intermediate forms were found between it and the more common type of Lodgepole pine pattern. Nevertheless, the trees showing

this pattern appeared morphologically to be normal Lodgepole pine, so that if the terpene pattern was due to introgression it is apparently a very sensitive method of detecting the phenomenon.

In the extreme eastern part of the natural range, the single provenance east of the Rocky Mountains was distinguished from the Rocky Mountain populations primarily by higher percentages and frequencies of occurrence of limonene.

The provenances from the Puget Sound region in Washington formed a well-defined group characterised by Type D1, with high levels of β-pinene, and low or insignificant levels of Δ-3-carene and limonene. Provenances from east and from west Vancouver Island gave distinct patterns. Those from the west were of the North Coastal type (Type A1), while the eastern samples formed part of a provenance group extending from the Vancouver region down the coastal strip of Washington and Oregon. This latter South Coastal group was dominated by Type B1. The distinction of east from west Vancouver Island is correlated with the ecological differences between the two sides of the island. Samples from Sooke in the southern-most part of the island tended to be intermediate between east and west types. On the mainland in the Vancouver area a complex situation exists, for representatives of the South British Columbian, South Coastal, and Puget Sound groups were all found within a relatively small area.

The Vancouver and South Coastal group could be clearly subdivided into northern and southern parts. In the northern part, which includes east Vancouver Island and all provenances down to the Columbia River, a substantial percentage of the trees contained Δ-3-carene; while in the southern part, which consists of the Oregon coastal strip down to North Bend, Δ-3-carene was relatively very low.

The two coastal provenances sampled from southern Oregon and northern California (Bandon and Del Norte County) were of particular interest, since they contained a high percentage of trees of the pinene-camphene type (Type E). The mean percentage occurrence of the type was about 30% within the group, nearly twice as high as that in the central British Columbian group; in one of the Del Norte replicate plots, 50% of the trees were of Type E. The high incidence of this type in a region remote from the present distribution of Jack pine must throw doubt on the introgression hypothesis as an explanation, although the two

provenances may represent a relict population after migration as a result of the extensive glaciations which have occurred in north America.

In the south-western extremity of the natural range there is an isolated population north of San Francisco. This Mendocino provenance was very distinct and homogeneous, consisting predominantly of Type A2, similar to the North Coastal type but with the addition of a high percentage of Δ-3-carene.

The remaining provenances were from the inland mountainous region running southwards from the Washington and Oregon Cascades to the Californian Sierra Nevada. The Cascade samples were characterised by Types A4 and B4, similar to the coastal provenances at similar latitudes but with the addition of high levels of Δ-3-carene and especially of limonene which reached its highest concentrations in this region. The Sierra Nevada provenance at Eldorado was quite unlike any other provenance, with two-thirds of the trees being of Type J, otherwise almost absent from the entire range, and the remainder of Type D. Thus the percentage of β-phellandrene was abnormally low, and β-pinene formed 50% of the total monoterpenes; Δ-3-carene and limonene were relatively high.

There was generally little or no variation in the monoterpene pattern distribution obtained from different replicate plots of a given provenance. Furthermore, the results obtained from the five different sampling sites in Britain showed that there was virtually no important site effect. The young trees at Bush in south-east Scotland were used in a preliminary trial for this work, and indicated some of the trends which have been substantiated from the provenance plantations at the other four sites. The details of the sampling intensity from these provenance experiments are given by provenance groups in TABLE 4, which also indicates the predominant pattern types within each group and the characteristic pattern types, i.e. the types which occur preferentially within that provenance group. The distribution of pattern types within each provenance group is shown in TABLE 5.

3. Variation of individual monoterpenes

If the percentage contributions of individual monoterpenes to the total monoterpenes are inspected over the natural range, several out-standing features are apparent. The pinenes reached high concentrations in the central and Puget Sound areas, but in fact α-pinene was at its highest

level in the two spectacularly disjunct locations of the pinene-camphene type, while β-pinene was highest on the Sierra Nevada. Δ-3-carene increased gradually from the north, becoming maximal in the Rocky Mountain region and in north California, while limonene behaved rather similarly but attained its peak on the Cascade range. β-phellandrene was maximal in North Coastal regions, declining clinally south-eastwards, while terpinolene increased in this direction, becoming maximal along the South Coastal strip.

These changes are reflected in the geographical distribution of the predominant pattern types (see TABLE 6), where the trend was from Type A in the north-west and to a lesser extent in the south, through Type B in central regions, to Type D in the Rocky Mountain and Puget Sound regions; this represents an increase in the pinenes, expecially β-pinene, towards the centre and east of the range, away from coastal areas. The geographical distribution of pattern subtypes illustrates the increasing incidence of Subtype 2 with Δ-3-carene towards east central parts of the range; Subtype 3 with limonene was similar in distribution but was excluded to a greater extent from coastal areas.

4. Provenance identification

The separation of the natural range of Lodgepole pine into 12 broad provenance groups on the basis of the resin monoterpene composition is in good agreement with the ecological and topographical subdivisions of the range. The survey has shown that it was the outlying populations which had the most specific and relatively invariate patterns. Towards the centre of the natural range, particularly in British Columbia, a given provenance would consist of trees giving several different pattern types, so that it was not possible to assign a single tree to a provenance or even to a provenance group on the basis of its monoterpene pattern; this can be seen from TABLE 5. Nevertheless, the contributions of the different pattern types as percentages of the trees sampled within a given provenance were usually found to be remarkably constant, so that it was often possible on this basis to assign a given population of trees to a provenance or to a provenance group. Thus this method of provenance identification is essentially statistical. The sample size required to allow a reasonably positive identification is dependent on the provenance group, since, as has been shown, some provenance groups are much more variable than others. In the most variable regions a sample size of about

25 trees has generally been found to be sufficiently informative.

It should be emphasised that the variability encountered is not random, but that each pattern type is a partial representation of the true genotype of the tree; so that in the more central areas of the range there is an admixture of various genotypes which are represented in a purer form on the outlying parts of the range.

For the purpose of comparing provenances, it has been found that comparison of classification by pattern types is more informative than comparison of mean monoterpene percentage values, since the former method takes into account the relative proportions of the different monoterpenes. However, additional information on the degree of variation of individual monoterpenes is valuable in comparisons, and a computer programme has been developed for generating scatter diagrams for the percentage data for each monoterpene by provenances. These scatter distributions tend to be bimodal, trimodal, or further polymodal, as would be expected from patterns which tend to fall into a relatively small number of more or less discrete types, and the relative importance of the different modes can be informative in the comparison or identification of provenances.

TABLE 1 DETAILS OF SAMPLING SITES AND DATES.

LOCATION	AGE (yr)	ELEVATION (m)	SOIL TYPE	DATE SAMPLED	Nº OF PROVENANCES SAMPLED
Bush Estate S. E. Scotland	5	180	brown earth	May 1975	18
Solway Forest, S.W. Scotland	7	12	deep basin peat	Sept 1975	65
Thetford Forest, E. England	8	38	sand with flints	March 1976	31
Glentress Forest, S.E. Scotland	12	275	deep blanket peat	May 1976	4
Stenton Forest, S.E. Scotland	(4,6), 19	130	loam (sand/clay)	Aug 1976	14

TABLE 2 CLASSIFICATION OF TYPES OF MONOTERPENE PATTERNS.

This method is based on relative peak heights, estimated visually from chromatograms.

Primary divisions

A	β-phellandrene	>>	β-pinene	~	α-pinene
B	β-phellandrene	>	β-pinene	>	α-pinene
C	β-phellandrene	~	β-pinene	>	α-pinene
D	β-pinene	>	β-phellandrene	>	α-pinene
E	pinene-camphene type				
J	β-pinene	>	α-pinene	≥	β-phellandrene

Each division is subdivided as follows:

Subdivisions

1	Δ-3-carene and limonene insignificant
2	Δ-3-carene present
3	limonene present
4	Δ-3-carene and limonene present

TABLE 3 MEAN MONOTERPENE PERCENTAGE COMPOSITION OF SHOOT CORTICAL RESIN OF A TYPICAL PROVENANCE WITHIN EACH PROVENANCE GROUP

PROVENANCE GROUP	TYPICAL PROVENANCE	NO. OF TREES	MEAN PERCENTAGE COMPOSITION							
			α-pinene	camphene	β-pinene	Δ-3 carene	α-terpinene	limonene	β-phellandrene	terpinolene
N. Coastal	Annette Is.	48	4.4	0.4	4.9	1.2	1.5	3.0	82.0	2.5
Skeena & Bulkley Rivers	Cedarvale	102	4.5	0.3	9.3	9.0	1.9	5.3	65.9	3.9
Central Brit. Columbia (B.C.)	Wonowon	119	11.7	2.3	17.9	8.6	1.7	8.8	44.1	4.9
South Brit. Columbia (B.C.)	Mt. Ida	49	5.1	0.4	18.6	16.8	2.0	7.4	44.0	5.7
Rocky Mts.	Crowsnest	94	4.6	0.1	20.5	24.3	2.1	6.5	35.7	6.3
E. of Rockies	Cypress Hills	96	3.9	0.1	14.8	26.8	2.3	10.9	34.3	6.9
Vancouver & S. Coastal	Warenton	48	4.8	0.3	14.0	1.2	1.1	2.7	68.0	7.9
S.W. Oregon	Del Norte	93	10.6	3.6	7.4	7.8	1.9	2.8	59.9	6.0
Mendocino	Mendocino	49	3.5	0.2	5.4	24.8	2.5	2.9	51.3	9.4
Puget Sound	Rainier	112	6.6	0.4	33.8	2.5	0.9	2.0	50.0	3.7
Cascades	Klamath	74	4.2	0.2	12.6	14.5	2.3	12.0	47.3	6.9
Sierra Nevada	Eldorado	39	7.6	0.1	49.9	13.9	1.9	9.2	14.7	2.9

TABLE 4 DETAILS OF PROVENANCE GROUPS

PROVENANCE GROUP	NO. OF PROVENANCES	TOTAL NO. OF REPLICATES	TOTAL NO. OF TREES	PREDOMINANT PATTERN TYPES	CHARACTERISTIC PATTERN TYPES
N. Coastal	10	13	319	A1	A1
Skeena & Bulkley Rivers	29	33	846	A1, B1	
Central B. C.	7	18	437	B1, B2	E4, E2
South B.C.	15	16	387	B2, B4	
Rocky Mountains	2	5	117	B2, D2	B2, D2
East of Rockies	1	4	96	B4, D4	B4
Vancouver & S. Coastal	15	19	453	B1, A1	B1
S.W. Oregon	2	7	166	B1, A1, A2	E1, E2
Mendocino	1	2	49	A2	A2
Puget Sound	5	11	286	D1, B1	D1, (C1)
Cascades	5	11	275	B4, A4	A4, B4
Sierra Nevada	1	2	39	J4, D4	J4, D4
TOTAL: 12	93	141	3470		

TABLE 5 PERCENTAGE COMPOSITION OF PATTERN-TYPES OF PROVENANCE GROUPS

PROVENANCE GROUP	A 1	A 2	A 3	A 4	B 1	B 2	B 3	B 4	C 1	C 2	C 3	C 4	D 1	D 2	D 3	D 4	E 1	E 2	E 3	E 4	J 1	J 2	J 3	J 4	OTHER
N. Coastal	72	13	3	1	6	3	1			1															
Skeena & Bulkley Rivers	24	14	10	6	16	13	4	6	1	1			1	1			1								
Central B.C.	11	6	5	4	14	12	6	10	2	1		1	3	2	1	2	3	5	3	5					5
South B.C.	3	4	2	5	10	20	5	18	2	4	2	3	2	8	3	9		1		1					
Rocky Mts.	1	2	2	2	3	25	3	12	2	5		7	1	21	5	8									3
E. of Rockies	1	4		9		10	5	38		1		9		7	2	13									
Vancouver & S. Coastal	21	6			43	11	1	1	5	1			4	2											
S. W. Oregon	17	17		2	20	8	1	2	1				2	1			14	13	1	1					
Mendocino		88		6		6																			
Puget Sound	6				22	3		1	9	3			45	8	1		1								
Cascades	8	8	7	15	9	12	4	26	1	1	1	1	1	1		3									
Sierra Nevada														3	8	23						13	21	33	

TABLE 6 PERCENTAGE COMPOSITION OF PATTERN-TYPES OF PROVENANCE GROUPS, SUMMARISED BY TYPES AND BY SUBTYPES

PROVENANCE GROUP	TYPES							SUBTYPES			
	A	B	C	D	E	J	OTHER	1	2	3	4
N. Coastal	89	10	1					79	17	3	1
Skeena & Bulkley Rivers	54	40	2	3	1			43	29	14	13
Central B. C.	26	41	4	7	16		4	33	27	16	24
South B. C.	13	53	11	21	1			17	37	11	35
Rocky Mts.	6	43	14	34		1	3	6	55	10	29
E. of Rockies	15	53	10	22				1	23	7	69
Vancouver & S. Coastal	32	56	6	6				78	20	2	1
S.W. Oregon	37	31	1	3	29			55	39	1	5
Mendocino	94	6							94		6
Puget Sound	6	26	12	54	1			82	14	2	1
Cascades	37	52	4	5				20	23	12	45
Sierra Nevada				33		67			15	28	56

SESSION PAPER
SEANCE I DOCUMENT 3
SITZUNG DOKUMENT

VARIATION OF GUM TURPENTINE BETWEEN PROVENANCES OF *Pinus caribaea* Morelet AND *P. oocarpa* Schiede IN CENTRAL AMERICA

VARIATIONS DES RESINES A TEREBENTHINE SUIVANT LES PROVENANCES DE *Pinus caribaea* Morelet ET *P. oocarpa* Schiede EN AMERIQUE CENTRALE

VARIATIONEN DES TERPENTINHARZES NACH VERSCHIEDENEN HERKÜNFTEN VON *Pinus caribaea* Morelet UND *P. oocarpa* Schiede IN ZENTRALAMERIKA

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This paper includes extracts from a full report in preparation for Silvae Genetica to be co-authored by the following collaborators in the research project described herein:- B. Keeble (Tropical Products Institute, London) and G. Chaplin, A. Greaves, R.H. Kemp and J. Lee (Commonwealth Forestry Institute, Oxford)

SUMMARY

Gas chromatographic analysis of 40 terpenes from stem xylem oleoresin of mature trees in natural populations of Pinus caribaea Morelet and P. oocarpa Schiede was undertaken as part of a taxonomic and genecological study of the inter- and intra-specific variation of the two species; in addition it was undertaken to determine the feasibility of (a) predicting the terpene composition of an untested natural population and (b) identifying the natural source of an exotic plantation.

Distributions of most terpenes overlapped between populations and species and it would be impossible to identify absolutely the source of an individual tree. However, on the basis of means for 12 terpenes of 20-30 trees per population (6 P. caribaea; 15 P. oocarpa) it was possible to recognise differences between the species and trends of variation between populations. (Samples of fewer than 10 trees in a further 21 populations tended to confuse the picture. P. caribaea var. bahamensis differed from the mainland variety in having low α-pinene and high β-phellandrene.) Univariate and multivariate statistical analysis and geometrical cluster analysis indicated major differences between high altitude/low rainfall and coastal/high rainfall populations, especially in P. caribaea; in P. oocarpa, although the relations between terpene composition and characters of the source location (latitude, longitude, altitude and rainfall) were not so clearcut, it was possible to distinguish several populations some of which have unexpectedly good growth in exotic trials, e.g. Mountain Pine Ridge, Belize and Yucul, Nicaragua.

The major terpenes of value for prediction and identification include α-pinene, β-pinene, Δ-3-carene, myrcene, β-phellandrene, α-terpinolene, allo-ocimene, longifolene, anethole and one compound as yet unidentified (peak "15-unknown").

These results have value in determining the extent of natural populations for seed collection and gene conservation. They have potential for identifying the natural origin of exotic plantations and this is being tested with samples from such exotics for which the source is known.

RESUME

L'analyse gaz-chromatographique a été effectuée pour 40 terpènes de l'oléorésine de tiges matures prises dans quelques populations naturelles de Pinus caribaea (Morelet) et P. oocarpa Schiede dans le cadre d'une étude taxonomique et génécologique de la variation entre et à l'intérieur des deux espèces; elle a aussi été conçue pour évaluer les possibilités (a) de prédire la composition terpénique d'une source naturelle non examinée et (b) d'identifier l'origine naturelle d'une plantation exotique.

Les courbes de distribution de la plupart des terpènes se chevauchent entre les populations et les espèces et il serait impossible d'identifier absolument l'origine d'un arbre individuel. Cependant, sur la base des moyennes de 12 terpènes chez 20-30 arbres par peuplement (6 de P. caribaea; 15 de P. oocarpa) on pourrait apercevoir des différences entre les essences et des tendances systématiques de variation entre des populations. (Les échantillons de moins de 10 arbres pris dans 21 autres populations tendraient à confondre la situation.) Il y a une grande différence entre P. caribaea var. bahamensis et l'autre variété; la variété bahamensis contient une faible proportion de α-pinène et une forte proportion de β-phellandrène.

Les analyses statistique (uni- et multi-variable) et de groupement montraient une grande différence entre les populations de haute altitude et d'une pauvre pluviosité et les populations littorales où la pluviosité est faible, particulièrement chez P. caribaea; chez P. oocarpa, quoique les relations entre la composition terpénique et la latitude (ou longitude, altitude, pluviosité) n'étaient pas assez manifestées, on pourrait distinguer plusieurs populations; quelques unes montrent une bonne croissance parmi les essais de provenances exotiques, par exemple Mountain Pine Ridge, Belize et Yucul, Nicaragua.

Les terpènes les plus importantes pour la prédiction et l'identification incluent α-pinène, β-pinène, Δ-3-carène, myrcène, β-phellandrène, α-terpinolène, allo-ocimène, longifolène, anethole et une terpène non encore identifiée ("15-unknown").

Ces résultats sont valables pour un examen des limites des populations naturelles en vue de la récolte des graines et de la conservation génétique. Il y a une grande potentialité pour l'identification de l'origine naturelle des plantations exotiques et on va examiner cette hypothèse parmi les échantillons de certaines parcelles dont l'origine est bien connue.

ZUSAMMENFASSUNG

Es wurde eine gaschromatographische Analyse an 40 Terpinolen des Stammxylembalsamharzes von haubaren Bäumen in natürlichen Populationen von Pinus caribaea Morelet und Pinus oocarpa Schiede als Teil einer taxonomischen und genekologischen Untersuchung in die inter- und intra-spezifische Variation der beiden Arten ausgeführt; ausserdem wurde die Bestimmung der Durchführbarkeit mit Bezug auf a) die Vorausbestimmung der Terpinolzusammensetzung einer ungetesteten natürlichen Population und b) auf die Identifikation der natürlichen Herkunft eines exotischen Pflanzenbestandes, in Angriff genommen.

Die Verteilungen der meisten Terpinole überlagerten sich zwischen Populationen und Arten, und es ist nicht möglich, die Herkunft eines individuellen Baumes zu identifizieren. Dennoch war es auf Grund eines Durchschnittswertes für 12 Terpinole von 20-30 Bäumen pro Population (6 Pinus caribaea; 15 Pinus oocarpa) möglich, Unterschiede zwischen den Arten und Tendenzen zur Variation zwischen den Populationen zu erkennen. (Proben von weniger als 10 Bäumen in weiteren 21 Populationen verundeutlichten das Gesamtbild. P. caribaea var. bahamensis unterschied sich von der Festlandsart durch einen niedrigen α-Pinene und einen hohen β-Phellandren.) Univariate und multivariate statistische Analyse und geometrische Gruppenanalyse wiesen wesentliche Unterschiede zwischen grosser Höhenlage/niedrigem Niederschlag und küstennahen/hohen Niederschlag Populationen auf, besonders bei P. caribaea; bei P. oocarpa, obwohl die Beziehungen zwischen Terpinolverbindung und Charakteristika des Herkunftsortes (Breitengrad, Längengrad, Höhe und Niederschlagsmenge) nicht so eindeutig zur Verfügung standen, war es möglich, zwischen meheren Populationen zu unterscheiden, von denen einige, während exotischen Probeanpflanzungen, unerwartet gutes Wachstum aufzeigten, z.B. Mountain Pine Ridge, Belize und Yucul, Nicaragua.

Die wichtigen Terpinole zur Bestimmung und Identifizierung sind α-Pinen, β-Pinen, Δ-3-Caren, Myrcen, β-Phellandren, α-Terpinolen, Allo-Ocimen, Longifolen, Anethole und eine Verbindung soweit noch nicht identifiziert ("15-unknown").

Die Ergebnisse sind für die Bestimmung der Ausmasse von natürlichen Populationen mit Bezug auf die Sameneinsammlung und Generhaltung wertvoll. Sie bieten potentiellen Wert für die Identifikation der natürlichen Herkunft von exotischen Pflanzenbeständen, und dies wird im Moment mit Proben von solchen exotischen Arten getestet, dessen Herkunft bekannt ist.

VARIATION OF GUM TURPENTINE BETWEEN PROVENANCES OF *Pinus caribaea* Morelet AND *P. oocarpa* Schiede IN CENTRAL AMERICA

INTRODUCTION

Two species of pines, *Pinus caribaea* Morelet and *P. oocarpa* Schiede, indigenous to tropical Central America are important as natural forests and as exotic plantation species. In their natural ranges both species have been used for timber and for resin production. As an exotic *P. caribaea* particularly has been planted on a large scale, mainly for pulp and paper, in many tropical countries while *P. oocarpa* has promise for timber and for pulp on selected sites in these same countries. International demand for seed now exceeds supply and cooperative efforts are being made by bilateral and mutilateral agencies to develop *in situ* and *ex situ* conservation stands and improved breeding populations.

Despite the wide use of these species it is only relatively recently that attention has been paid to intra-specific variation and the importance of seed source for exotic plantations (see *e.g.* Lückhoff, 1964; Barrett and Golfari, 1962; Nikles, 1966). The taxonomic affinities of the species and their varieties is by no means finalised although the 3-needled *P. caribaea* with its varieties *bahamensis*, *caribaea* and *hondurensis* (Barrett and Golfari, 1962) is generally believed distinct from the 5-needled *P. oocarpa* of which some Mexican sources originally classified as var. *ochoterenai* are now believed equivalent to *P. patula* var. *longepedunculata* (Styles, 1977). The geographic limits of the natural ranges of these several taxa are not known exactly but in some areas typical *P. caribaea* and typical *P. oocarpa* are sympatric; morphological characters of cones and needles suggest that hybrids may occur although none have been produced artificially.

A programme for the exploration, conservation, evaluation and utilization of the genetic resources of the two species has been initiated by the Commonwealth Forestry Institute, Oxford (CFI), with moral and financial support from FAO (1), IUFRO (2), the UK Government and the Governments of most Commonwealth countries (see e.g. Kemp and Burley, 1977). The programme includes international provenance trials in which some 50 populations are being tested in over 300 locations in more than 30 countries (Kemp, 1973a,b).

OBJECTIVES OF GUM TURPENTINE ANALYSIS

In support of this programme a research project was established jointly between CFI and the Tropical Products Institute, London (TPI), to examine gum turpentine from the xylem of mature trees in natural forests throughout the natural range of P. caribaea and P. oocarpa on the Central American mainland (3).

The objects of the research included the following:-

1. Study of taxonomic relationships and intra-specific variation

Although P. caribaea and P. oocarpa are recognized as valid species the ranges of variation in classical taxonomic characters are large both within and between populations. Terpenes hold promise for description of variation patterns and clarification of taxonomic affinities by chemotaxonomy.

2. Study of genetic variation within populations

Even when taxonomic relationships are known the silviculturist and tree breeder should be interested in the extent of genetic variation in both natural populations (to estimate the effect of inbreeding, isolation and hybridization) and in plantations (to determine the effects of varying levels of natural and artificial selection). The more commonly assessed production variables such as height or volume of trees are continuous (metric) characters that are strongly affected by environment and they may not be assessable until late in a commercial rotation.

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(1) FAO : Food and Agriculture Organization of the United Nations
(2) IUFRO : International Union of Forestry Research Organizations
(3) One sample of P. caribaea var. bahamensis from High Rock, Grand Bahama, was included for comparison; Gansel and Squillace (1976), Nikles (1966) and Squillace and Nikles (1977) also refer to Bahamas material.

Constituents of oleoresin can frequently be evaluated in young material and may have more direct genetic control, thus facilitating more precise estimation of juvenile-mature correlations (see e.g. Squillace, 1971; Baradat et al., 1972).

3. Identification of seed source

Using a combination of information from these studies it may be possible to identify (t_0 "fingerprint") the geographic origin (provenance) of a plantation or a batch of seed. This is particularly important in tropical pines for which several tons of seed are shipped internationally each year; many hundreds of thousands of hectares have been established often without adequate identification of seed source (see Jones and Burley, 1973); this makes it impossible to obtain further seed of good sources and to avoid bad sources for future plantings.

Since the relative proportions of terpenes in some species have been shown to be under strong genetic control, the origin of a given exotic plantation may be identified on the basis of similarity between its terpene pattern and the pattern of variation within and between natural populations. (This assumes small environmental effects and little change due to selection in the nursery and field stages of the exotic plantation; a check on this is currently being made in exotic commercial stands of one known origin of P. caribaea from the Mountain Pine Ridge, Belize, in six countries.)

4. Location of commercial sources of oleoresin

In addition to its strict research applications the project had the objective of identifying sources of pinene isolates and other terpenes of value in the international market; although the market is not buoyant at present there is no doubt that in the current energy crisis natural resources will increase in value, particularly compounds that are difficult to synthesise (e.g. β-pinene). Sources may be either individual trees that can be propagated vegetatively in seed orchards, or natural stands that can be tapped in situ or used as the source of seed for plantations.

5. Relation to earlier work

Preliminary studies showed considerable differences between populations of *P. caribaea* and *P. oocarpa* (Green, Keeble and Burley, 1974, 1975). The present work, which includes only part of the comprehensive report to be submitted to Silvae Genetica, extends the field sampling, intensifies the statistical analysis, and develops a multivariate, "fingerprinting", identification procedure.

METHODS

Random, healthy, dominant trees were sampled in natural populations of *P. caribaea* and *P. oocarpa* throughout the natural range in Central America. One sample of *P. caribaea* var. *bahamensis* from Bahamas was also included for comparison but no samples of *P. caribaea* var. *caribaea* from Cuba were available. The numbers of trees sampled and the locations of all samples are shown in Table 1A for populations represented by less than 10 trees and Table 2A for populations with over 10 trees, usually 20-30. The approximate locations are illustrated in Figures 1 and 2. Most subsequent statistical analyses refer to data from populations with over 10 trees.

Stem xylem oleoresin was collected in screw-top vials inserted into trees near ground level during the seed collection season (January-March for *P. oocarpa*, May-July for *P. caribaea*) and reference herbarium specimens were also collected. Preliminary studies had shown no significant effects due to age (over 20 years) or size of tree, or season of year (*cf.* the variation of monoterpenes within trees of *P. elliottii* Engelm. observed by Franklin, 1976).

The vials filled with resin in 2-3 days or else were artificially filled with distilled water or glass beads to expel air and minimise oxidation. They were air-freighted to TPI, London, for laboratory analysis by the techniques described by Green, Keeble and Burley (1974); turpentine samples were prepared by steam distillation and the composition was analysed with a Hewlett-Packard gas chromatographic apparatus with automatic injection and an Infratronics integrator. Up to 40 peaks appeared in the gas chromatograms and 23 compounds were tentatively identified by comparison of retention times with those of authentic samples. In some cases positive identification was made by mass spectrometry and infra-red spectroscopy.

At the CFI, Oxford, data were analysed on the Oxford University Computing Laboratory's ICL 1906A computer. Univariate analyses of variance indicated 12 terpenes for which significant differences appeared between populations; the data for these 12 terpenes were subjected to multivariate analysis. The basic data comprised the content of each terpene expressed as a percentage of the total terpene content. Most analyses to date have used untransformed values; several of the distributions were non-normal with many zero values and, although this is unimportant for principal component analysis, it is important in the discriminant analysis currently in progress and various transformations are being examined. Initial canonical analyses, using raw data and logarithms of raw data (+ 0.001 to remove zeros), in fact have not proved different.

RESULTS AND DISCUSSION

1. Univariate analysis

Based on data from all samples, the differences between species were small but significant for several terpenes (β-phellandrene***; allo-ocimene**; 15-unknown*; longifolene***) (see Figure 3). Population means are given in Tables 1B and 2B (respectively for less than 10 trees and more than 10 trees per sample).

In P. oocarpa less than 61% of the variation in terpene content was explained by multiple linear regression on source characters except for longifolene which was related positively to altitude and negatively to rainfall (R^2 = 82% for samples with over 10 trees, 61% for all 28 populations) - see Table 3.

In P. caribaea there were too few samples (5 continental and 1 insular source) to permit multiple regression analysis but simple linear regression of terpene content on each source variable in turn (Table 4) showed that the Bahamas source contributed largely to the latitudinal and longitudinal effects except for Δ-3-carene, myrcene, 15-unknown and longifolene (shown by the similarity of R^2% values for analyses with and without Bahamas data). Regardless of latitude and longitude, however, significant effects were attributable to altitude (positive) and rainfall (negative) for myrcene, allo-ocimene and anethole; this suggests a distinction between dry, inland sites at high altitudes and wet, coastal and insular sites at low altitudes.

Despite the strong correlations observed between altitude and both myrcene and allo-ocimene in P. caribaea the number of samples was considered too few to generalise the result over the whole range of the species or to identify the source of an unknown sample. Further sources will be examined. For P. oocarpa, on the other hand, although more sources were sampled, the relationships between individual terpene constituents and source variables were not considered close enough to permit prediction of the properties of an untested source nor identification of the source of an analysed sample. The use of information on all variables together was therefore considered desirable.

2. Multivariate analysis

The simplest form of multivariate examination is a geometrical clustering of raw data with no statistical distance analysis (Figure 4); a mean Euclidean distance cluster analysis of means of all samples with over 10 trees emphasised several points:-

a) Repeated samples in different years were very similar (three samples from La Lagunilla, Guatemala).

b) Three mainland sources of P. caribaea were more similar to sources of P. oocarpa than to other P. caribaea samples confirming the known overlap of the distributions of morphological characters in these species.

c) The Pimientilla, Honduras, La lagunilla, Guatemala, Las Mangas and Yucul, Nicaragua, sources of P. oocarpa were distinct from each other and from the main group.

d) The P. caribaea var. bahamensis material was widely different from all other samples.

The most suitable form of preliminary multivariate analysis is principal component analysis of the data for 12 terpenes using means of all samples pooled within each species. The contributions of the first six roots to total variation are shown in Table 5A.

There is a clear difference between species with only 3 roots necessary in P. caribaea but 5 in P. oocarpa to explain 90% of the total variation among all 12 terpenes. The contributions of individual terpenes to the first three vectors are shown in Table 5B.

For P. oocarpa (Table 5B1) the first vector is heavily weighted for α-pinene (negative), Δ-3-carene, α-terpinolene and anethole (positive); this applies to samples with more than 10 trees, less than 10 trees and all combined. The situation is more confused for vectors 2 and 3, however, depending upon which set of samples is used; overall β-phellandrene is the most important contributor to vector 2 and longifolene to vector 3. There are thus six terpenes in P. oocarpa that have value in source identification; the regression of principal component scores on source characteristics suggested that principal component 1 was unrelated to source (R^2 = 40% with four independent variables), principal component 2 was significantly related to latitude, longitude and altitude (73%), and principal component 3 was significantly related to rainfall (64%).

In P. caribaea only six sources have over 10 trees but they total 146 trees; α-pinene (positive) and β-phellandrene (negative) are the most important contributors to vector 1 (although myrcene and allo-ocimene were the most closely related to source characters by univariate regression analysis). Vector 2 is heavily weighted for myrcene, allo-ocimene and, marginally, Δ-3-carene. The third vector is weighted mainly for α-pinene.
The use of data from eight sites with less than 10 trees confuses the interpretation. For P. caribaea therefore a slightly different set of six terpenes have value in source identification:-

	P. oocarpa	P. caribaea
Vector 1	α-pinene Δ-3-carene α-terpinolene anethole	α-pinene β-phellandrene
Vector 2	β-phellandrene	myrcene allo-ocimene Δ-3-carene
Vector 3	longifolene	β-pinene

The regression of principal component scores on individual source characteristics shows that the second component is closely related to altitude and rainfall of the source (Table 6); this would be expected from the weighting of the second component in P. caribaea which is largely for myrcene and allo-ocimene.

Overall this suggests that laboratory analysis of new samples, discrimination analysis of existing data, and classification of new data could justifiably be restricted to these ten terpenes.

The principal component scores for original samples can be used to typify the various sources without the need for normal distributions or transformations (Figures 5A-F). It is clear that four populations of P. oocarpa are distinct from each other and from the remainder as a group. The repeatability of the samples lends confidence to this assertion but confidence limits per se cannot be placed on the population scores; further treatment of the data now in progress includes canonical analysis to develop a distance measure (estimating the significance of differences between populations) and a discriminant function (facilitating the classification of new samples). Preliminary canonical analysis confirms the distinctness of the four P. oocarpa populations.

The immediate practical significance of these results is two-fold. Firstly, the unknown source of a plantation (of which there are many in the tropics) could, on the basis of six terpenes from even this limited sample, be attributed with reasonable confidence; in the case of P. oocarpa it could be attributed to a specific area while in the case of P. caribaea var. hondurensis it could be attributed to an environmental category.

Secondly, the distinctness of some populations is of interest in relation to field growth. Hitherto most seed for plantations has been derived from Mountain Pine Ridge, Belize, (P. caribaea) and Mexico (P. oocarpa). In early growth (2-4 years) in many countries collaborating in the international provenance trials (Kemp. 1973a,b), the populations of P. oocarpa from Mountain Pine Ridge, Belize, and Yucul, Nicaragua, perform consistently well whereas material from La Lagunilla, Guatemala, performed consistently badly and Las Mangas, Nicaragua, was very variable. The natural populations in Central America were not always geographically separated from each other although they are being rapidly eroded by felling; it would be highly desirable to determine, through terpene analysis, the genetic limits of each population, particularly that from Yucul, Nicaragua, for purposes of seed collection and genetic conservation.

3. Gene frequency

Single gene control of several terpenes has been demonstrated in some pine species (e.g. Baradat et al., 1972; Squillace, 1971). With the present sample size of mainly 20-30 trees per population, it was not possible to test statistically the frequency distributions of data within each population; an attempt was made to identify on frequency histograms a cut-off point (for "highness" versus "lowness") in each of the 12 terpenes (Table 7). Trees were allocated to high or low classes; the population frequency of high content was not related to geographic distribution. In the case of α-pinene, β-pinene, Δ-3-carene and anethole this may be because the distribution was multimodal reflecting multigenic action.

CONCLUSIONS

Although it was possible to separate consistently 40 terpene compounds by gas-liquid chromatography of steam-distilled xylem oleoresin, only 12 of these differed significantly between populations within species when expressed as a percentage of total terpene content and eight contributed most of the variation. (Since only 12 out of 40 were considered in statistical analysis there were strictly no problems of correlation by constraint to 100%). The two species differed in content of some terpenes but the intra-specific ranges of variation always overlapped.
The species differed also in the terpene components that accounted for most of the variation. Generally both species have high α-pinene contents while P. caribaea has high β-phellandrene; some populations of P. oocarpa have high Δ-3-carene; populations of both have high longifolene.

Although part of the inter-population variation of individual terpenes could be explained by location and environmental factors, the patterns of variation were not clearly systematic (i.e. not closely related to latitude or altitude, for example) so that no precise predictions could be made of the properties of untested sources.

Individual populations could be characterised by principal component analysis; since most exotic plantations are most likely to have originated from one or other of the areas sampled, it should be possible to identify unknown sources unless major changes in the population genotype have occured through silvicultural and natural selection in the exotic condition.

Because of the great variation between individual trees within populations, small numbers of sample trees are unreliable indicators of population means; 20-30 trees appear desirable as a minimum. It is certainly not possible to identify unequivocally the source of an individual tree.

Table 1. Data for populations sampled by less than 10 trees

1A. Source data

Species	No. of Samples	Site Identity number	Country	Site	Latitude (N)	Longitude (W)	Altitude (m)	Rainfall (mm)
P. oocarpa	2	P.O. 53	Honduras	Los Limones	14.03	86.42	700	663
"	6	P.O. 1	Nicaragua	Las Camelias	13.46	86.18	900	1500
"	2	P.O. 20	Nicaragua	San Rafael	13.15	86.10	1200	1362
"	3	P.O. 57	Guatemala	Chichicastenango	14.55	91.06	2050	
"	1	P.O. 24	Guatemala	Huehuetenango	15.13	91.32	1700	1036
"	6	P.O. 15	Guatemala	Bucaral	15.01	90.09	1100	800-900
"	5	P.O. 21	Honduras	Siguatepeque	14.32	87.50	1100	1247
"	7	P.O. 9	Honduras	Zamorano	13.58	86.59	1100	1120
"	1	P.O. 16	Honduras	Valle de Angeles	14.07	87.04	1300	920
"	1	P.O. 37	Honduras	Campamento	14.33	86.35	650	1200
"	4	P.O. 54	Mexico	Uruapan	19.25	102.01	1500	
"	6	P.O. 55	Mexico	Playitas	18.30	102.40	1600	
"	1	P.O. 65	Mexico	San Cristobal	16.45	92.57	1300	
P. caribaea	6	P.C. 6	?	?	?	?	?	?
"	8	P.C. 4	Nicaragua	Laguna del Pinar	12.13	83.42	10	4200
"	4	P.C. 8	Honduras R.	Guanaja Island	16.27	85.54	75	2300
"	2	P.C. 18	Honduras R.	Danli-Yuscaran	14.00	86.52	650	
"	5	P.C. 14	Honduras R.	Culmi	15.05	85.37	500	1325(+)
"	2	P.C. 36	Honduras R.	Campamento	14.33	86.35	650	1200
"	1	P.C. 35	Honduras R.	Zamorano	14.01	87.02	800	1120
"	5	P.C. 20	Belize	Mountain Pine Ridge	17.00	88.55	400	1558-2064

1B. Percentages of 12 terpenes intotal terpene content

Site identity number	α-pinene	β-pinene	Δ^3 carene	myrcene	limonene	β-phellandrene	p-cymene	α- terpinolene	allo-ocimene	15-unknown	longifolene	anethole
P.O. 53	66.00	7.50	1.50	0.40	0.80	13.40	0.05	0.15	0.55	0.30	6.55	1.00
P.O. 1	78.43	6.43	0.92	0.00	0.63	0.45	0.04	0.06	0.53	0.35	7.63	1.70
P.O. 20	5.35	0.00	72.10	0.00	0.45	0.75	0.25	4.30	0.65	0.35	7.20	4.70
P.O. 57	47.33	4.77	23.90	0.00	0.47	0.63	0.00	1.63	0.77	0.50	13.57	3.07
P.O. 24	73.10	4.80	0.80	0.00	0.40	1.30	0.05	0.05	0.90	0.60	14.00	1.20
P.O. 15	63.12	3.53	13.50	0.00	0.50	0.20	0.03	0.93	0.72	0.48	11.07	2.58
P.O. 21	61.92	3.64	14.92	0.04	0.50	1.66	0.05	1.09	0.64	0.42	9.90	1.96
P.O. 9	75.59	6.21	0.70	0.00	0.51	0.34	0.00	0.10	0.67	0.50	10.34	1.90
P.O. 16	81.40	4.20	0.60	0.00	0.50	0.10	0.10	0.00	0.60	0.50	8.90	0.80
P.O. 37	57.50	1.80	1.70	0.60	0.80	27.10	0.20	0.20	0.60	0.30	6.30	1.30
P.O. 54	78.48	0.98	0.58	0.08	1.43	3.58	0.08	0.3	0.53	0.55	9.68	0.00
P.O. 55	80.73	1.00	5.48	0.07	0.95	2.27	0.00	0.83	0.17	0.22	3.72	1.38
P.O. 65	81.60	0.70	3.70	0.00	0.40	0.10	0.10	0.00	0.70	0.50	8.70	0.10
P.C. 6	72.78	3.78	1.22	0.48	0.87	15.48	0.00	0.11	0.07	0.07	0.70	3.17
P.C. 4	53.54	3.20	6.79	0.61	1.01	22.26	0.06	0.64	0.66	0.35	6.94	2.11
P.C. 8	53.15	3.30	13.03	0.48	0.90	17.50	0.05	1.18	0.55	0.28	5.90	1.55
P.C. 18	62.90	2.65	1.10	0.30	0.80	13.60	0.20	0.15	0.50	0.40	7.85	2.90
P.C. 14	68.58	2.94	1.00	0.12	0.86	7.42	0.26	0.04	0.50	0.34	7.18	2.94
P.C. 36	74.33	4.40	0.95	0.20	0.80	5.65	0.10	0.10	0.40	0.25	5.30	2.85
P.C. 35	51.60	2.10	1.50	0.60	0.90	30.10	0.20	0.30	0.50	0.30	6.50	3.20
P.C. 20	50.80	2.64	1.86	0.74	1.00	38.52	0.01	0.04	0.00	0.09	1.64	1.94

Table 2. Data for populations sampled by more than 10 trees

2A. Source data

Species	No. of Samples	Site Identity number	Country	Site	Latitude (N)	Longitude (W)	Altitude (m)	Rainfall (mm)
P. oocarpa	19	P.O. 39	Honduras	Villa Santa	14.12	86.25	900	1320(a)
"	26	P.O. 27	Honduras	Zamorano	14.02	87.03	1100	1120
"	21	P.O. 22	Guatemala	La Cumbre	15.02	90.13	1300	
"	29	P.O. 19	Guatemala	La Lagunilla	14.42	89.57	1300	936
"	33	P.O. 28	Honduras	Zamorano	14.02	87.03	1300	1120
"	50	P.O. 26	Belize	Mountain Pine R.	17.00	88.55	400	1811(a)
"	27	P.O. 41	Honduras	San Juan	14.24	88.23	1300	800
"	29	P.O. 18	Nicaragua	Yucul	12.54	85.47	900	2586
"	35	P.O. 14	Nicaragua	Las Mangas	12.50	86.18	950	922
"	21	P.O. 42	Honduras	Pimientilla	14.54	87.30	700	1134
"	94	P.O. 44	Guatemala	Pueblo Viejo	15.22	91.36	1800	1036
"	30	P.O. 31	Nicaragua	Dipilto	13.43	86.32	1150	1000
"	14	P.O. 47	Nicaragua	Cusmapa	13.17	86.39	1250	1474
"	24	P.O. 19	Guatemala	La Lagunilla I	14.42	89.57	1300	936
"	26	P.O. 19	Guatemala	La Lagunilla II	14.42	89.57	1300	936
P. caribaea	15	P.C. 21	Nicaragua	Santa Clara	13.48	86.12	700	1150
"	15	P.C. 13	Honduras	Los Limones	14.03	86.42	700	663
"	30	P.C. 3	Nicaragua	Alamicamba	13.34	84.17	25	2900
"	30	P.C. 23	Belize	Melinda	17.01	88.20	20	2138
"	28	P.C. 10	Belize	Las Lomitas	16.28	88.33	30	2326
"	28	P.C. 38	Grand Bahama	High Rock	26.40	78.12	10(a)	1670

(a) = approximately

2B. Percentages of 12 terpenes in total terpene content

Site identity number	α-pinene	β-pinene	Δ-3-carene	myrcene	limonene	β-phellandrene	p-cymene	α-terpinolene	allo-ocimene	15-unknown	longifolene	anethole
P.O. 39	71.31	7.51	3.27	0.02	0.49	1.14	0.00	0.29	0.67	0.32	10.18	1.95
P.O. 27	69.85	7.28	4.83	0.00	0.47	0.57	0.00	0.42	0.68	0.60	9.74	3.10
P.O. 22	80.42	3.50	0.57	0.00	0.44	0.15	0.00	0.15	0.59	0.55	8.77	2.30
P.O. 19	19.27	2.84	51.33	0.00	0.39	0.46	0.06	3.24	0.95	1.19	13.87	2.81
P.O. 28	70.30	5.98	4.64	0.03	0.46	1.53	0.00	0.36	0.67	0.61	10.52	2.24
P.O. 26	84.55	4.38	1.63	0.01	0.56	1.31	0.01	0.06	0.10	0.21	2.67	2.98
P.O. 41	71.59	5.83	5.85	0.01	0.43	0.77	0.00	0.33	0.00	0.96	11.50	1.35
P.O. 18	18.30	11.18	51.73	0.00	0.37	0.70	0.11	2.85	0.00	0.51	7.20	5.36
P.O. 14	46.10	7.88	22.20	0.05	0.56	4.74	1.38	0.16	0.75	0.56	11.14	2.67
P.O. 42	63.65	15.47	2.66	0.05	0.60	5.22	0.00	0.17	0.00	0.69	8.59	1.45
P.O. 44	73.29	2.24	3.51	0.01	0.78	0.31	0.00	0.21	1.03	0.55	13.63	1.40
P.O. 31	74.70	3.71	4.11	0.00	0.46	0.21	0.00	0.33	0.89	0.44	10.95	2.48
P.O. 47	79.93	6.35	0.88	0.00	0.44	0.29	0.00	0.06	0.74	0.32	8.34	1.36
P.O. 19	25.48	1.88	49.41	0.00	0.40	0.37	0.00	3.30	1.03	0.55	12.94	2.73
P.O. 19	16.00	1.56	60.02	0.00	0.41	0.43	0.00	3.97	0.86	0.46	11.16	3.12
P.C. 21	60.87	3.13	3.50	0.45	0.79	14.72	0.08	0.23	0.44	0.24	5.34	2.03
P.C. 13	64.11	4.02	1.78	0.40	0.56	18.28	0.04	0.15	0.61	0.28	6.24	2.05
P.C. 3	68.43	3.87	1.28	0.02	0.86	17.55	0.00	0.01	0.02	0.47	4.72	1.49
P.C. 23	63.25	3.39	1.53	0.00	0.71	25.27	0.00	0.00	0.00	0.21	2.37	1.78
P.C. 10	68.38	4.74	1.36	0.00	0.82	17.32	0.05	0.16	0.00	0.17	2.94	1.74
P.C. 38	36.39	2.63	2.31	0.00	0.20 /2	53.99	0.15	0.45	0.01	0.01	0.13	1.36
F /1	42.96***	12.67***	43.78***	42.97***	1.32 /2	41.10***	12.84***	43.05***	69.78***	3.05***	20.77***	14.10***

/1 F = variance ratio $\frac{\text{Between provenance variance}}{\text{Within provenance variance}}$ *** = Significant at 0.1% level of probability

/2 Limonene is included because highly significant differences were detected between populations with less than 10 trees.

TABLE 3

Constant, coefficients of regression, and coefficient of multiple determination (R^2%) for regression of terpene proportion on site data for P. oocarpa.

Terpene	/1	A. 16 populations with > 10 trees: Constant	Latitude	Longitude	Altitude	Rainfall	F	R^2%	B /2: R^2%
α- pinene	1	1645.0**	36.09**	-24.61**	0.07*	-0.01	3.9*	61	28
	2	1535.0**	35.86**	-23.58**	0.08*	-	4.8*	57	-
β- pinene	1	57.0	-1.04	-0.34	-0.005	0.0005	2.7	52	34
	2	13.2**	-	-	-0.006*	-	6.2*	32	-
Δ- 3 - carene	1	-1435.0*	-29.98**	21.90**	-0.06	0.02	3.2	56	20
	2	-1309.0*	-29.71**	20.73*	-0.07*	-	3.6*	50	-
Myrcene	1	0.2	-0.004	-0.0003	-0.00003	-0.00002	1.4	35	27
	2	-	-	-	-	-	-	-	-
Limonene	1	0.9	0.05	-0.01	0.00008	-0.00002	0.5	16	57
	2	-	-	-	-	-	-	-	-
β-phellandrene	1	-14.2	-0.97	0.42	-0.005*	-0.002	2.8	53	27
	2	3.9*	-	-	-0.002	-	4.3	25	-
p - cymene	1	-7.9	-0.38*	-0.17	-0.001	-0.0003	2.3	48	29
	2	-	-	-	-	-	-	-	-
α- terpinolene	1	-82.8*	-1.52*	1.22*	-0.03	0.001	2.0	44	18
	2	-	-	-	-	-	-	-	-
Allo-ocimene	1	-0.6	-0.04	0.17	0.0005	-0.0002	2.2	47	41
	2	-0.2	-	-	o.0007*	-	8.9*	41	-
15 - Unknown	1	-5.2	-0.14	0.09	-0.0002	-0.0002	1.0	29	16
	2	-	-	-	-	-	-	-	-
Longifolene	1	-21.4	-1.38	0.58	0.003	-0.003*	11.3**	82	61
	2	7.1*	-	-	0.005**	-0.002*	16.1**	73	-
Anethole	1	-33.2	-0.81	0.54	-0.002	-0.002*	3.6*	59	47
	2	0.8	-	-	-	0.001**	9.3**	42	-

/1 = all variables; 2 = stepwise elimination of non-significant variables

/2 = all 28 populations including those with less than 10 trees - included here only to demonstrate the loss of relationship (in all except limonene) caused by poor sampling.

Table 4. Coefficient of determination (r^2%) and significance of simple linear regression of terpene content on source characteristics for P. caribaea from sites with over 10 trees (+B = including, -B = excluding Bahamas material)

Terpene	Latitude		Longitude		Altitude		Rainfall	
	+B	-B	+B	-B	+B	-B	+B	-B
α- pinene	84*	1	76*	2	3	47	7	51
β- pinene	32	7	46	6	0	13	7	7
Δ- 3 - carene	0	19	6	2	38	55	36	35
Myrcene	24	37	4	6	99***	99***	68*	82*
Limonene	75*	0	62	6	0	31	17	60
β- phellandrene	96**	54	71*	23	19	25	0	5
Paracymene	56	8	54	2	1	48	16	38
α- Terpinolene	56	10	55	2	1	53	19	44
Allo-ocimene	21	32	4	5	96**	96**	76*	89*
15-unknown	69*	47	16	87*	3	1	10	13
Longifolene	81*	78*	28	43	57	68	12	41
Anethole	44	1	49	8	70*	77	44	94**

Table 5. Principal component analysis for 12 terpenes

A. Contribution of first six roots

Root	Percentage of variation accounted for			
	P. oocarpa		*P. caribaea*	
	>10 trees	<10 trees	>10 trees	<10 trees
1	37.3	35.2	55.1	41.3
2	22.4	26.6	29.8	32.2
3	18.0	14.9	6.6	19.0
4	9.9	10.3	5.0	3.2
5	4.8	5.8	3.5	2.7
6	3.3	3.4	-ve	1.2
Number required to account for 90%	5	5	3	3

Table 5 (continued)

B. Loadings for individual terpenes (Scaled eigenvectors) for first three vectors (only values exceeding 0.8 are considered significant)

1. P. oocarpa

Terpene	>10 trees (15 sites)			<10 trees (13 sites)			All sites combined		
	Vector 1	Vector 2	Vector 3	Vector 1	Vector 2	Vector 3	Vector 1	Vector 2	Vector 3
α - pinene	-0.87			-0.94			-0.95		
β - pinene		0.83							
Δ-3-carene	0.94			1.00			1.00		
myrcene		0.81				0.96		0.99	
limonene									
β - phellandrene		1.00				1.00		1.00	
p - cymene		0.80				0.95			
α - terpinolene	1.00			0.98			0.97		
allo-ocimene						0.99			1.00
15-unknown					1.00				
longifolene			1.00		0.99				
anethole				0.96			0.83		

Table 5 (continued)

2. P. caribaea

Terpene	> 10 trees (6 sites)			< 10 trees (8 sites)			All sites combined		
	Vector 1	Vector 2	Vector 3	Vector 1	Vector 2	Vector 3	Vector 1	Vector 2	Vector 3
α- pinene	1.00							1.00	
β- pinene			1.00			1.00			-0.82
Δ- 3 - carene		0.88						-0.88	
myrcene		1.00		1.00					
limonene	0.90			0.92					
β- phellandrene	-0.99			0.91			-0.86		
p- cymene	-0.87								1.00
α- terpinolene	-0.87							-0.98	
allo-ocimene		0.94			1.00		0.88		
15 - unknown	0.85				0.92		0.85		
longifolene	0.85				0.94		1.00		
anethole									

Table 6. Coefficient of determination (r^2%) and significance of simple linear regression of principal component scores on source characters (for sites with over 10 trees of P. caribaea (+B = including, -B = excluding Bahamas)

Component	Latitude +B	Latitude -B	Longitude +B	Longitude -B	Altitude +B	Altitude -B	Rainfall +B	Rainfall -B
1	95**	42	69*	60	11	0	1	1
2	3	27	0	2	87**	96**	81*	82*
3	1	19	8	42	0	0	6	7

Table 7. Subjective decision on cut-off points for high and low concentrations of selected terpenes

	P. oocarpa		P. caribaea	
	Highness	Lowness	Highness	Lowness
α - pinene	> 40	≤ 30	> 70?	≤ 70
β - pinene	-	-	-	-
Δ - 3-carene	> 20	≤ 10	-	-
Myrcene	> 0	0	> 0	0
Limonene	-	-	> 0	0
β - phellandrene	> 5	≤ 5	-	-
Paracymene	> 2	< 1	> 2	< 1
α - terpinolene	> 2	≤ 1	> 0	0
Allo-ocimene	> 0	0	> 0	0
15-unknown	-	-	-	-
Longifolene	> 20	≤ 20	> 10	≤ 8
Anethole	-	-	-	-

Figure 1. Location of samples, P. oocarpa

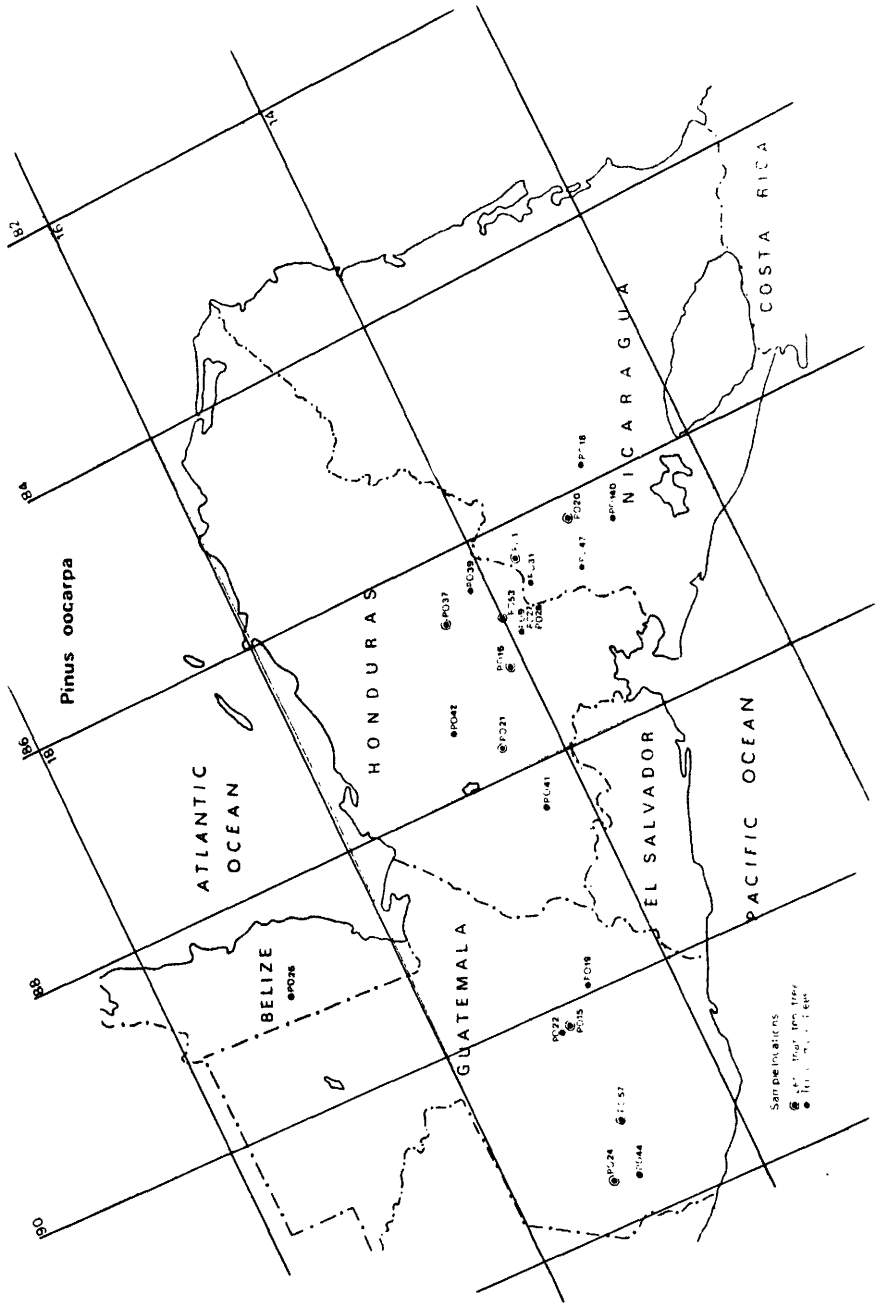


Figure 2. Location of samples, P. caribaea
(PC 38, Bahamas, not shown; latitude 26° 40'; longitude 78° 12')

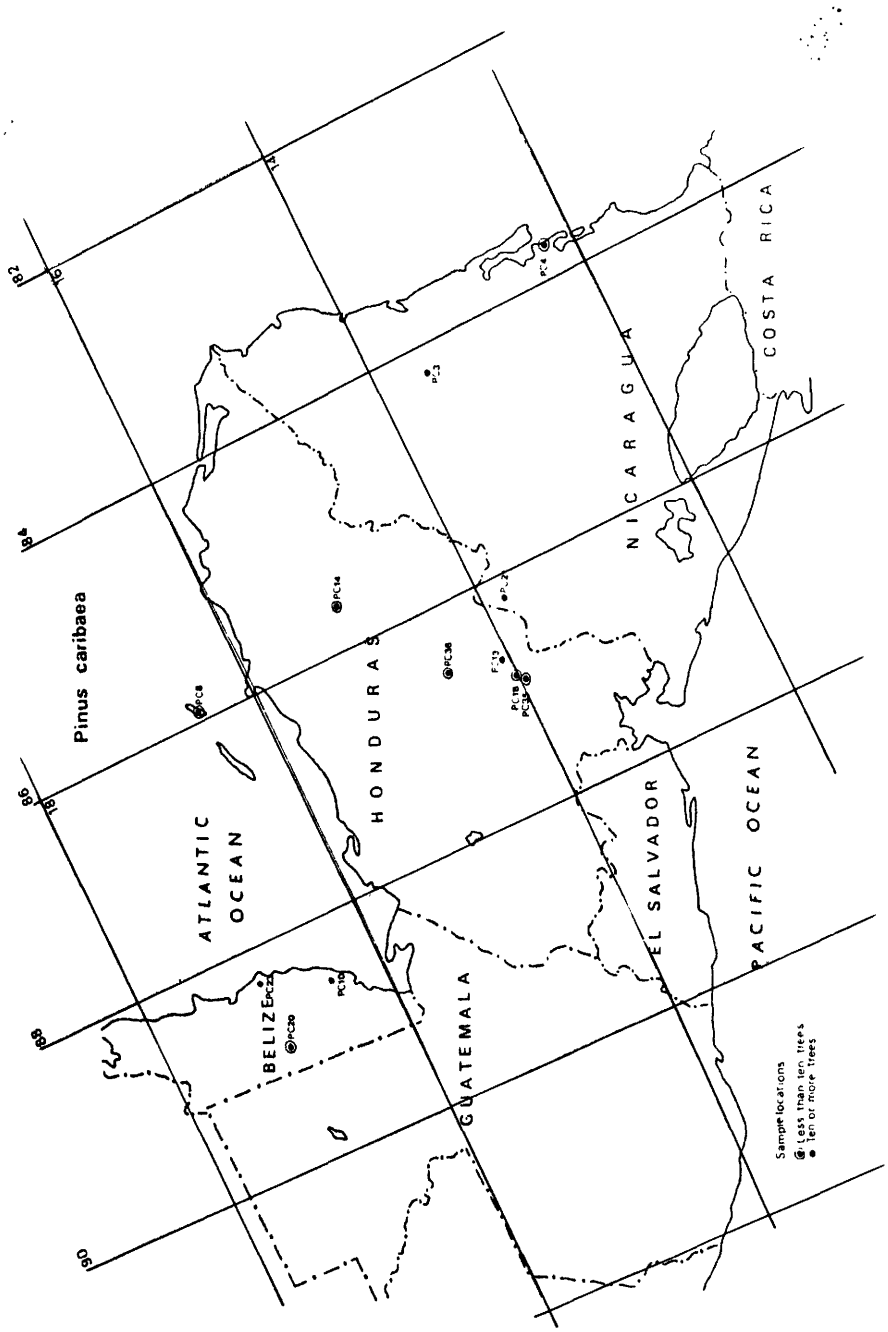


Figure 3. Comparison of species mean content for 12 terpenes: Mean, standard error of mean, and range for P. oocarpa (from 28 sites) and P. caribaea (from 14 sites); these include sites represented by less than 10 trees

Figure 3.

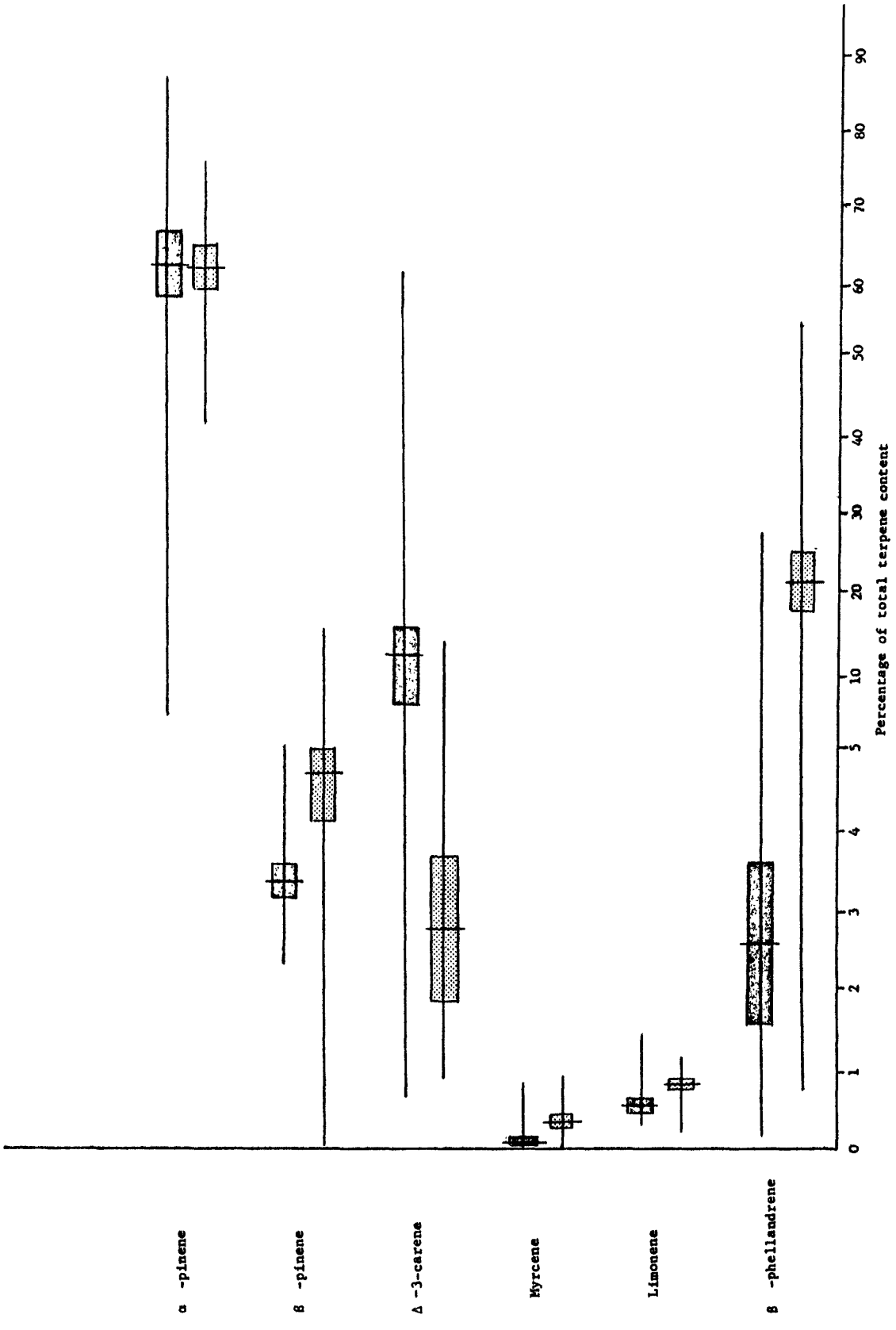

α -pinene
β -pinene
Δ -3-carene
Myrcene
Limonene
β -phellandrene
0
1
2
3
4
5
10
20
30
40
50
60
70
80
90
Percentage of total terpene content

Figure 3 continued

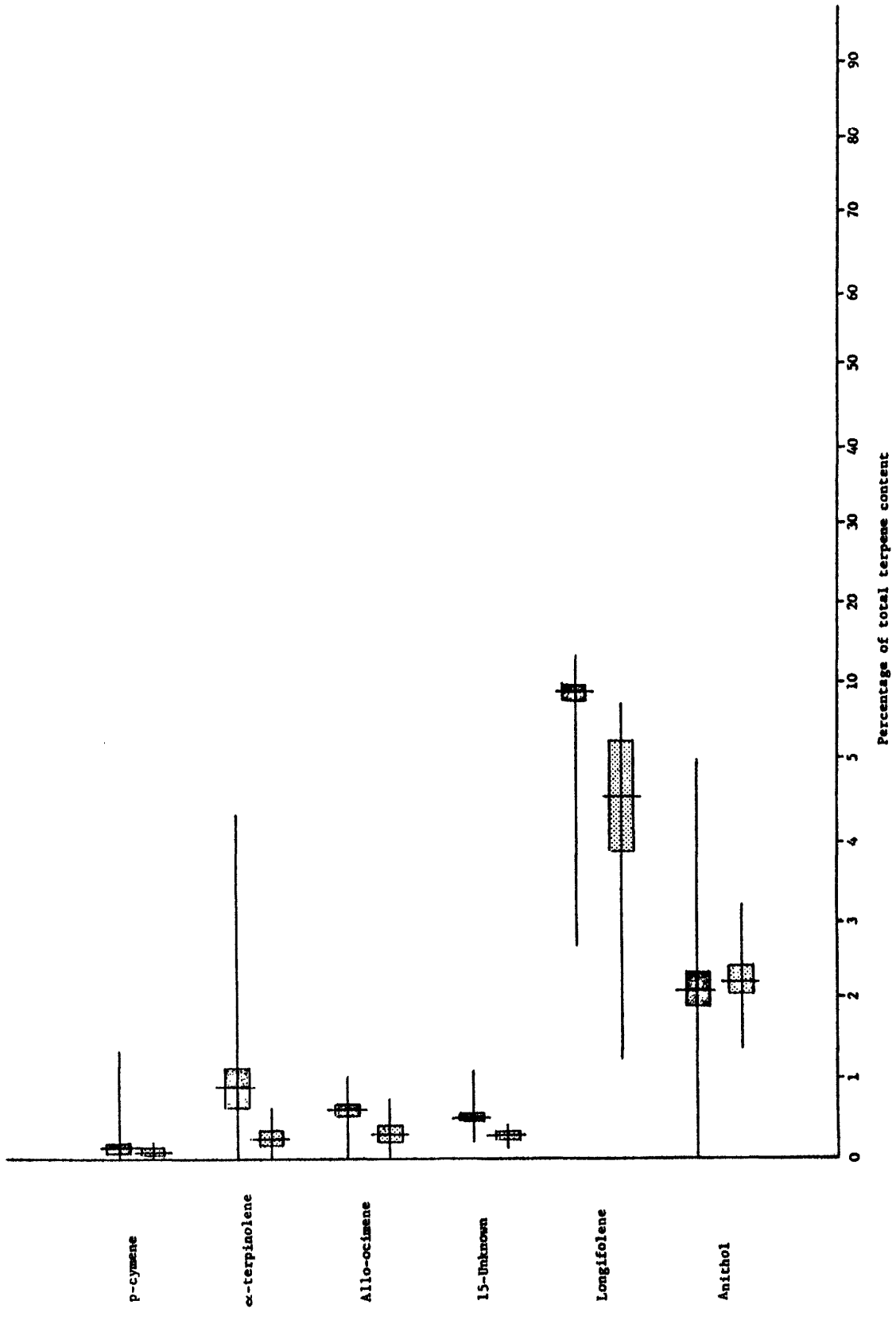
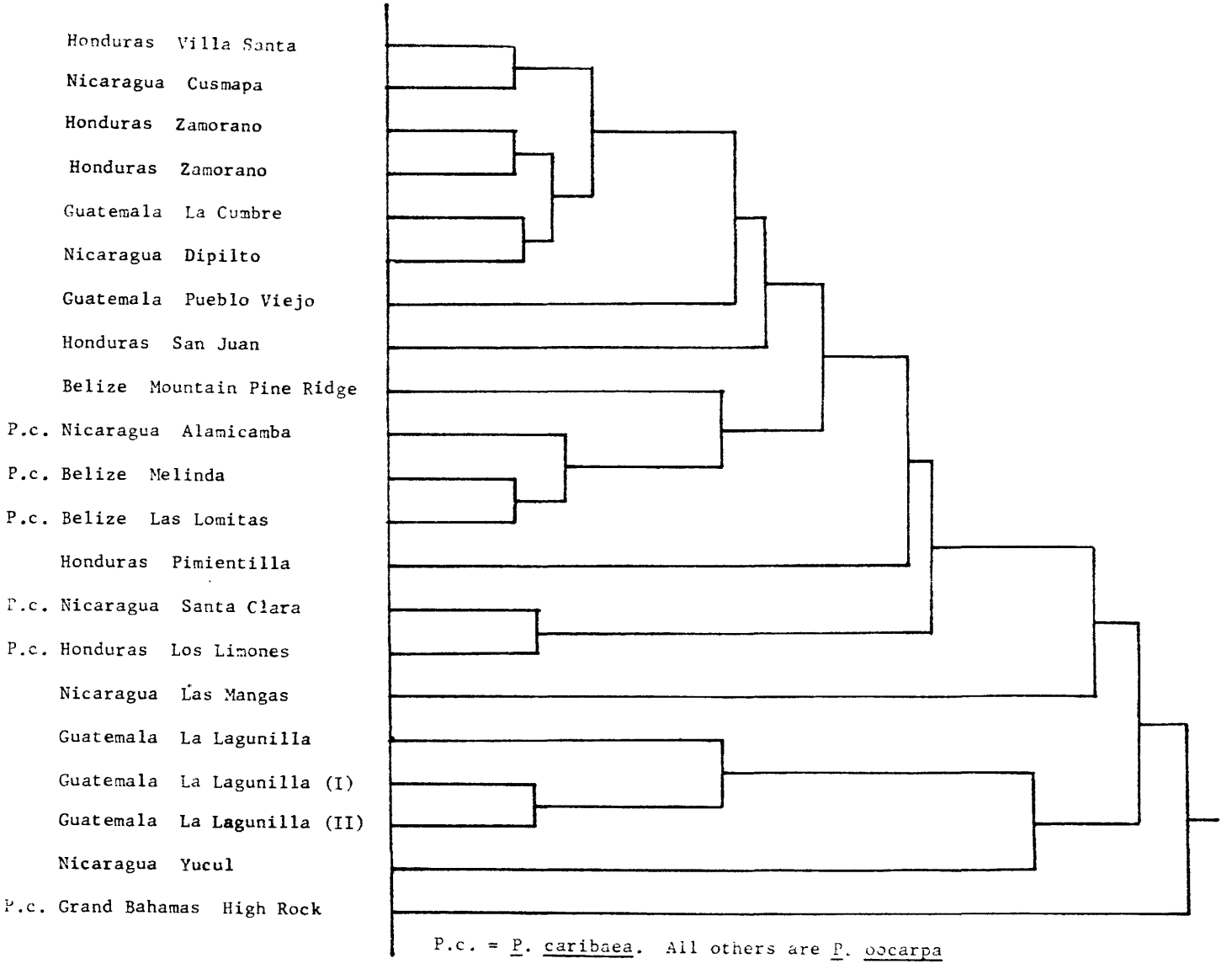


Figure 4. Dendrogram from cluster analysis of 12 terpenes from sites with 10 or more samples



P.c. = P. caribaea. All others are P. oocarpa

Figure 5. Relationships between pairs of principal components scores for sources with over 10 trees

P. oocarpa	*P. caribaea*
A. PC1 vs PC2	D. PC1 vs PC2
B. PC1 vs PC3	E. PC1 vs PC3
C. PC2 vs PC3	F. PC2 vs PC3

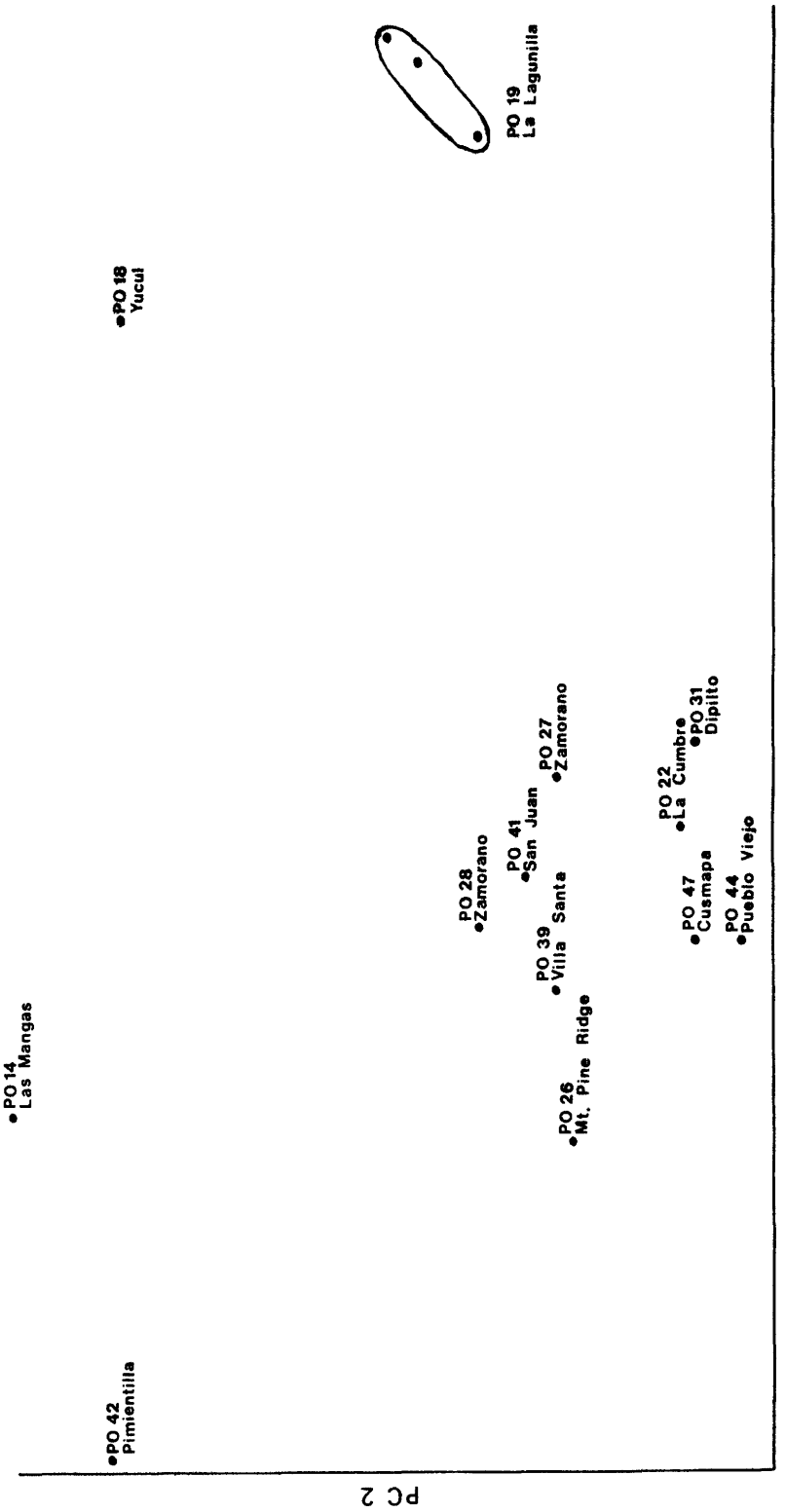
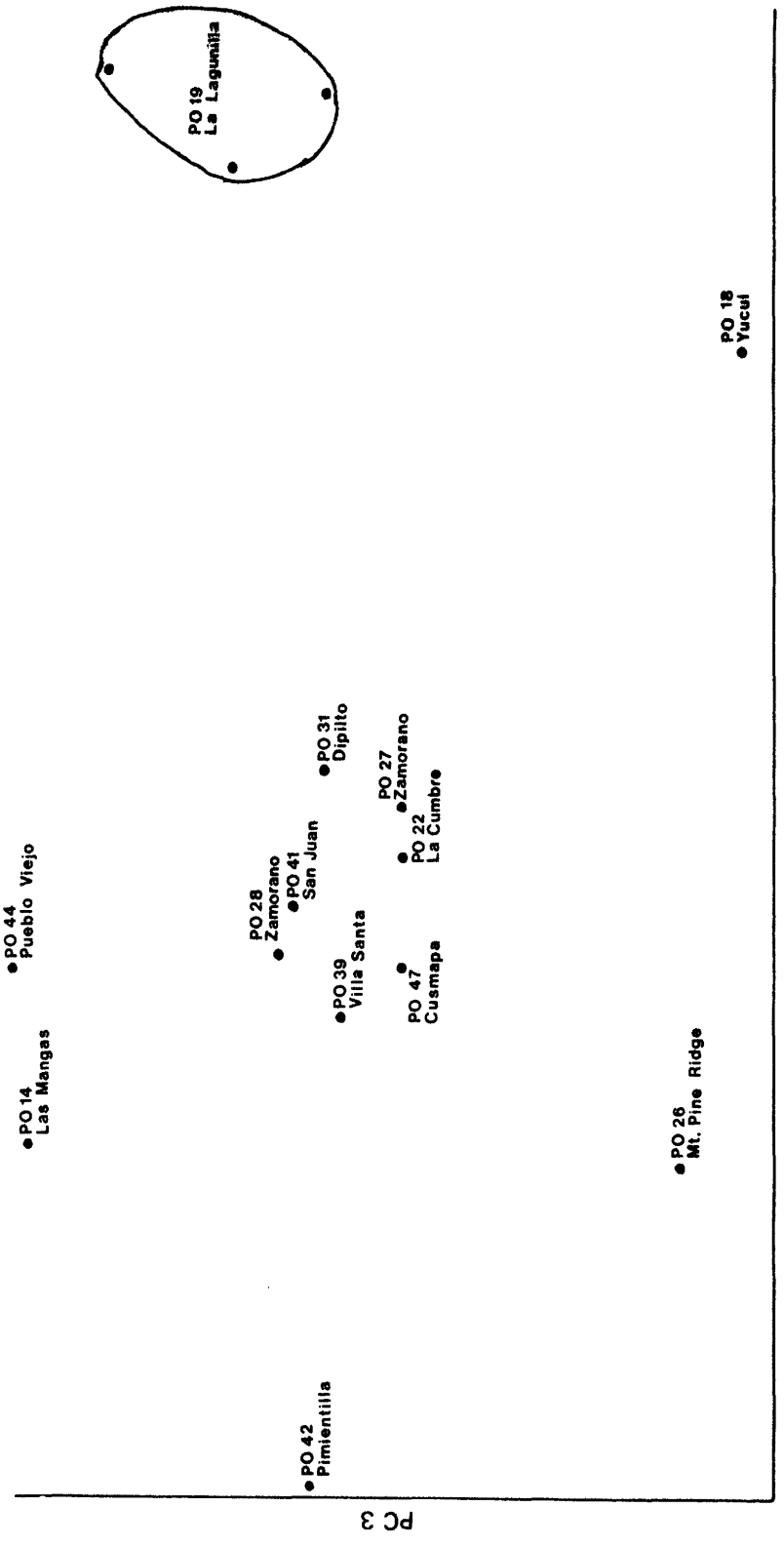

PO 19
La Lagunilla
PO 18
Yucul
PO 14
Las Mangas
PO 42
Pimientilla
PO 28
Zamorano
PO 41
San Juan
PO 27
Zamorano
PO 39
Villa Santa
PO 26
Mt. Pine Ridge
PO 22
La Cumbre
PO 31
Dipilto
PO 47
Cusmapa
PO 44
Pueblo Viejo
PC 1
PC 2

Figure 5A.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La Laguniila
PO 18
Yucul
PO 31
Dipilto
PO 27
Zamorano
PO 22
La Cumbre
PO 41
San Juan
PO 28
Zamorano
PO 44
Pueblo Viejo
PO 39
Villa Santa
PO 47
Cusmapa
PO 14
Las Mangas
PO 26
Mt. Pine Ridge
PO 42
Pimientilla
PC 1
PC 3

Figure 5B.

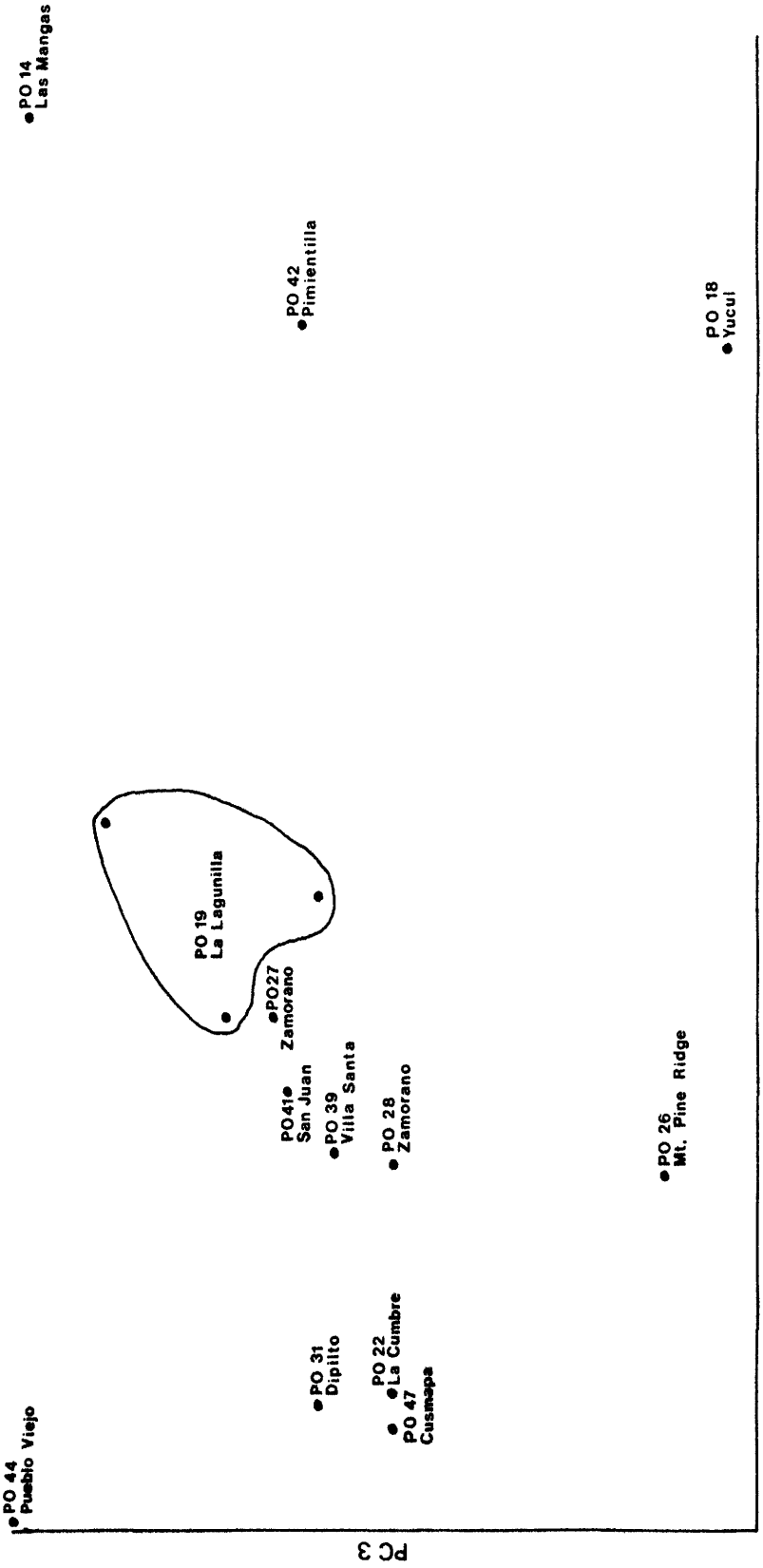
PO 14
Las Mangas
PO 42
Pimientilla
PO 18
Yucul
PO 19
La Lagunilla
PO27
Zamorano
PO41
San Juan
PO 39
Villa Santa
PO 28
Zamorano
PO 26
Mt. Pine Ridge
PO 31
Dipilto
PO 22
La Cumbre
PO 47
Cusmapa
PO 44
Pueblo Viejo
PC 3
PC 2

Figure 5C.

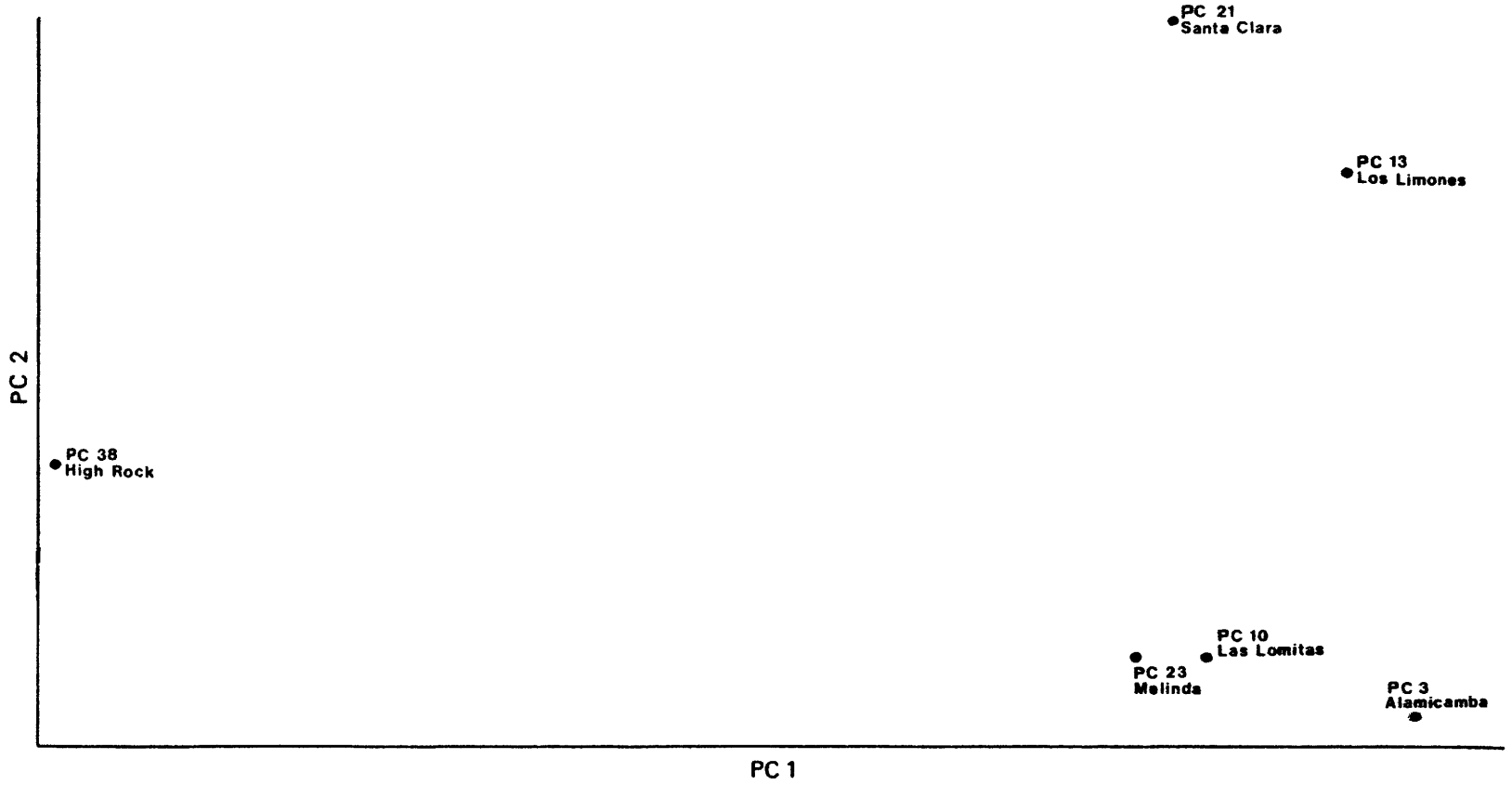
PC 21
Santa Clara
PC 13
Los Limones
PC 38
High Rock
PC 10
Las Lomitas
PC 23
Melinda
PC 3
Alamicamba
PC 2
PC 1

Figure 5D.

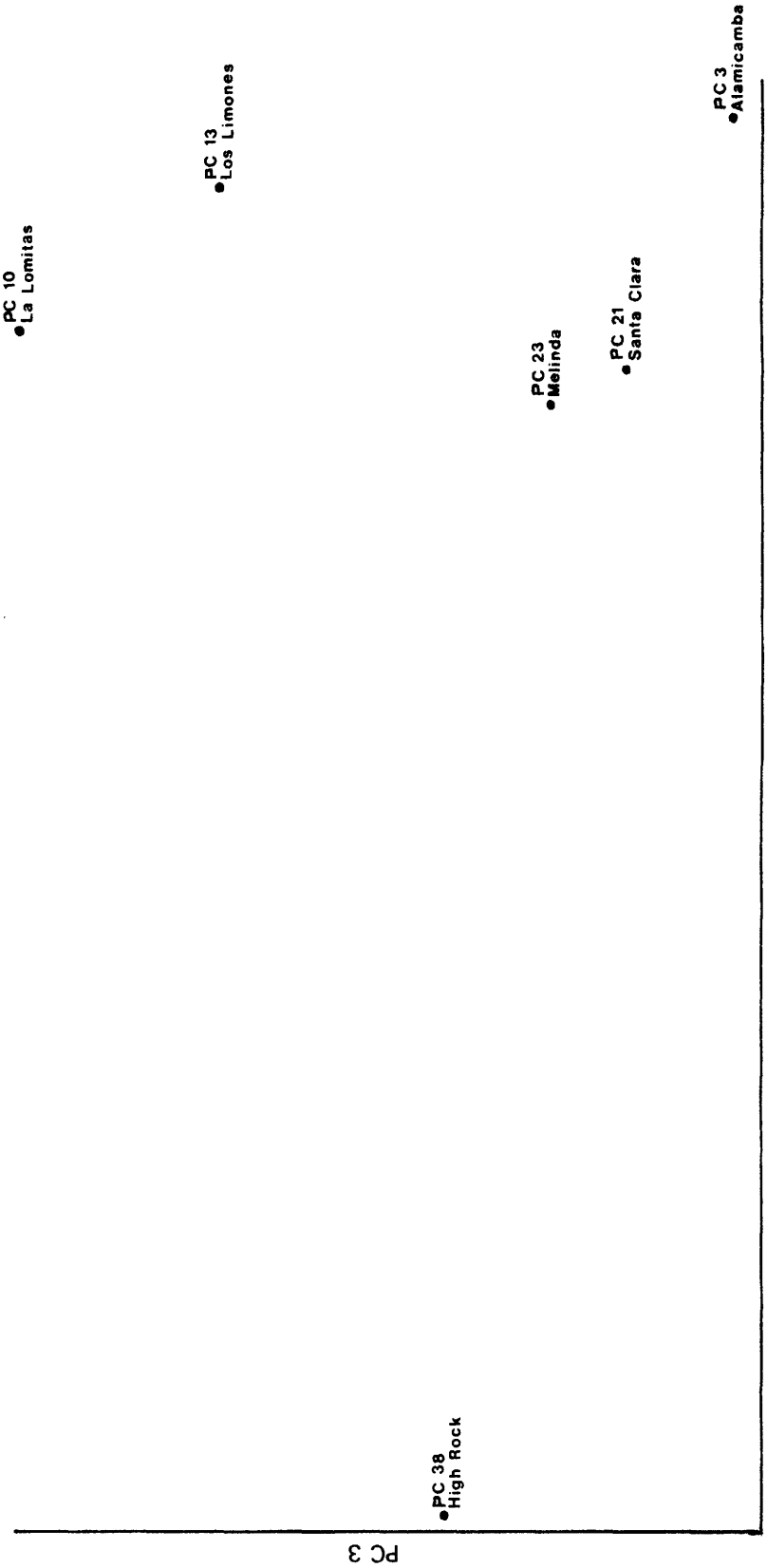
PC 10
La Lomitas
PC 13
Los Limones
PC 3
Alamicamba
PC 23
Melinda
PC 21
Santa Clara
PC 38
High Rock
PC 1
PC 3

Figure 5E.

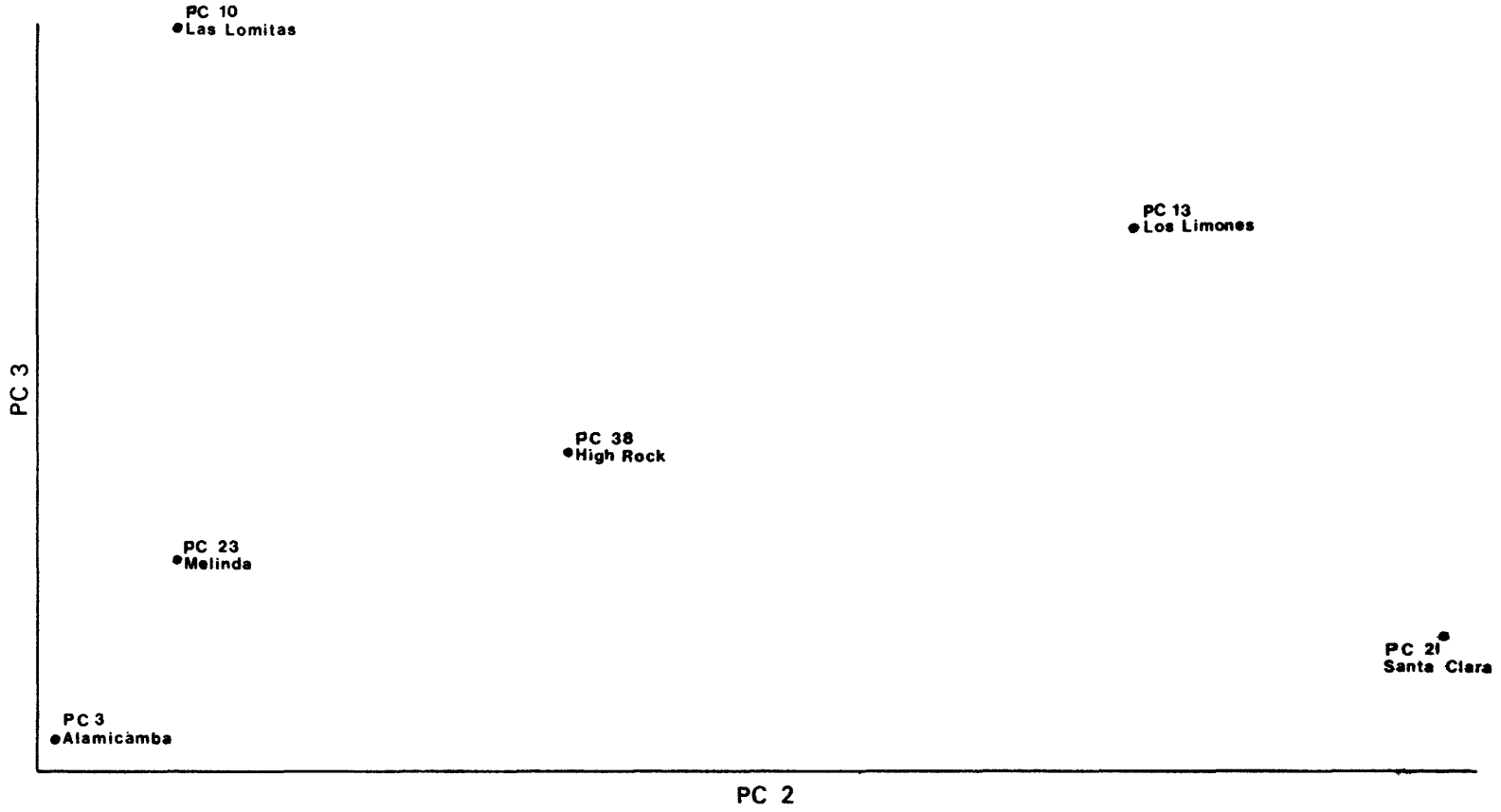
PC 10
Las Lomitas
PC 13
Los Limones
PC 38
High Rock
PC 23
Melinda
PC 21
Santa Clara
PC 3
Alamicamba
PC 3
PC 2

Figure 5F.

SESSION		PAPER	
SEANCE	I	DOCUMENT	4
SITZUNG		DOKUMENT	

UTILISATION DES TERPENES COMME OUTIL EN GENETIQUE FORESTIERE

USE OF TERPENES AS A TOOL IN FOREST GENETICS

ANWENDUNG DER TERPENE ALS HILFSMITTEL IN DER FORSTGENETIK

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RESUME

Les terpènes représentent un remarquable moyen d'étude en génétique forestière pour trois raisons principales. Leur analyse chromatographique et leur détermination physicochimique sont des méthodes rigoureuses et parfaitement reproductibles. Ensuite les gènes contrôlant leur synthèse semblent avoir un mode d'action analogue chez les diverses espèces. Enfin leurs variations saisonnières sont faibles dans certains tissus comme les tissus corticaux alors que les variations entre espèces, entre provenances et entre individus peuvent être considérables.

Nous avons montré chez le Pin maritime le contrôle monogénique du 3-carène, du myrcène et pour la première fois celui du longifolène et du caryophyllène. Le type et le degré de dominance d'un allèle sur l'autre sont variables selon le terpène. Un cas de linkage a pu être mis en évidence.

Les profils terpéniques permettent une bonne discrimination entre provenances de Pin maritime. La comparaison des fréquences géniques doit permettre de préciser les relations phylogénétiques entre les diverses provenances de l'espèce et de tester l'homogénéité génétique à l'intérieur des provenances. C'est ainsi que l'étude de la variabilité des fréquences de l'allèle C^+ (3-carène) a déjà permis de conclure à l'absence de variabilité infraspécifique du Pin maritime à l'intérieur du massif landais.

Il est parfois difficile de déterminer le génotype d'un individu par son phénotype. Le problème de l'interprétation des histogrammes est donc discuté.

Les applications des terpènes en génétique forestière sont multiples. Ils permettent d'identifier des provenances d'origine inconnue (peuplements ou graines) et de contrôler l'authenticité du matériel végétal (test variétal). Par ailleurs on peut, en utilisant les gènes mis en évidence, décrire les lois de croisements en verger à graines. Enfin il serait possible de créer à volonté, par sélection et hybridation, des arbres présentant les "profils terpéniques" les plus intéressants soit par leur production gemmère soit pour leur résistance aux attaques de certains insectes.

SUMMARY

Terpenes represent a convenient means of studying forest genetics for three major reasons. Their analysis by gas chromatography and their chemical identification is accurate and reproducible. The genes controlling their

synthesis seem to present an identical behaviour in the different species. Their seasonal variations are small in certain tissues such as cortical tissues whereas the variations between species, provenances and individuals may be very great.

In the maritime pine, we have shown the monogenic control of 3-carene, myrcene, and for the first time longifolene and caryophyllene. The type and the degree of dominance of one allele on another is variable according to the terpene. An example of linkage has been shown.

The terpene profiles give a good discrimination between provenances of maritime pine. The comparison of gene frequencies will permit the determination of the phylogenetic relationship between the various provenances of the species and will estimate the genetic homogeneity within provenances. For example, from the study of the variability of the frequencies of the C^+ allele (3-carene) we have been able to deduce the absence of infraspecific variability of maritime pine within the Landes forest.

In some cases the determination of the genotypes of an individual using its phenotype is difficult. Thenthe interpretation of histograms is discussed.

The applications of terpenes in forest genetics are numerous. They are a means of identification of provenances from unknown origins (seeds or stands) and a means of control of the authenticity of the plant material (varietal test). Furthermore using the demonstrated genes one can describe the laws of crossing in a seed orchard. Finally it would be possible to create as required, by selection and hybridization, those trees presenting the most advantageous "terpene profiles" either for their gum turpentine production or their resistance to certain insect attacks.

ZUSAMMENFASSUNG

Die Terpene stellen ein bemerkenswürdiges Mittel für die Untersuchung der Forstgenetik dar, und zwar für 3 hauptsächliche Gründe. Ihre chromatographische Analysis und ihre physiko-chemische Bestimmung sind unerlässliche und vollkommen reproduzierbare Arbeitsweisen. Weiter scheinen die diese Synthese kontrollierenden Gene eine Wirkungsweise zu üben, die bei den verschiedenen Arten ähnlich ist. Letztens sind die jahreszeitlichen Änderungen in irgend welchen Geweben wie die Rindegewebe schwach ; gegensätzlich können die Veränderungen zwischen Arten, zwischen Herkünften und Individuen beträchlich sein.

Wir haben bei der Meerstrandkiefer die monogenetische Kontrolle des 3-Karens, des Myrzens u.erstmalig des Longifolens und des Karyophyllens gezeigt. Typ und Grad der Dominanz eines Alleles über den anderen sind je mit dem Terpen veränderlich. Ein Beispiel des "linkage" ist klargelegt worden.

Die terpenischen Profile ermöglichen eine gute Differenzierung zwischen Herkünften der Meerstrandkiefer. Die Vergleichung der genischen Frequenzen sollte es ermöglichen, die phylogenetischen Verbindungen zwischen den verschiedenen Herkünften der Art näher zu bezeichnen, und die genetische Homogeneität innerhalb der Herkünfte zu prüfen.

So hat die Untersuchung der Variabilität der Frequenzen des C^+ Alleles (3-Karen), erlaubt, auf die Abwesenheit der intraspezifischer Veränderung der Meerstrandkiefer innerhalb Südwest-Frankreichs Grundwald zu schliessen.

Manchmal ist est schwierig den Genotyp eines Individuums durch seinen Phenotyp kennzuzeichnen. Die Frage der Auslegung der Histogrammen wird also erörtert.

Die Anwendungen der Terpene in der Forstgenetik sind vielfach. Sie ermöglichen die Identifizierung der Herkünfte die einen unbekannten Ursprung (Bestände oder Samen) haben, und sie ermöglichen die Echtheit des pflanzlichen Materials (Abart-Test) nachzuprüfen. Anderseits, indem man die klargestellten Gene anwendet, ist es möglich, die Gesetze der Kreuzungen im Samenanlagen zu geben.

Zuletzt hätte man die Möglichkeit, durch Auslese und Hybridierung nach Beliebe Bäume zu erzeugen, die sei es wegen ihres Harzertrages sei es wegen ihres Widerstandes entgegen den Angriffen von irgend einen Insekten die interessantesten Profile anbieten.

UTILISATION DES TERPENES COMME OUTIL EN GENETIQUE FORESTIERE

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INTRODUCTION

Les résultats que nous présentons sur le Pin maritime et surtout la méthodologie mise en oeuvre sont, pour une large part, transposables à toute espèce résineuse. En effet, un terpène est un constituant parfaitement identifiable par les méthodes physico-chimiques et la plupart des terpènes se retrouvent chez tous les conifères, en des proportions certes très variables. Par ailleurs, les recherches entreprises jusqu'à présent ont mis en évidence des gènes au mode d'action analogue chez les diverses espèces étudiées (Pinus monticola (1), Pinus elliottii (2, 3), Pinus palustris (4), Pinus pinaster (5, 6, 7), Pinus silvestris (8), Picea abies (9)...).

De ce fait, les résultats des travaux de biochimie et de génétique biochimique conduits sur une espèce peuvent en grande partie être utilisés pour faire progresser d'autres programmes de recherches. Squillace (10) a récemment mis à jour l'ensemble des connaissances sur les espèces résineuses.

Nous nous proposons donc de faire le point des résultats acquis en ce domaine sur le Pin maritime, d'offrir ensuite quelques solutions pour résoudre les problèmes d'interprétation des résultats, de suggérer enfin les multiples applications que peuvent offrir les terpènes dans la pratique forestière.

1 - RAPPEL DES RESULTATS ACQUIS SUR LE PIN MARITIME

1.1. Choix du matériel végétal

1.1.1. Les terpènes des différents organes

La composition terpénique des essences d'un même arbre est plus ou moins complexe selon les organes (11).

Le spectre chromatographique des essences de feuilles de Pin maritime (fig. 1a) révèle une soixantaine de constituants et nous en avons déterminé 48 avec certitude : carbures monoterpéniques et sesquiterpéniques, composés oxygénés divers, principalement des esters, mais aussi des alcools, aldéhydes, cétones (11). Il est possible de séparer les carbures des composés oxygénés (fig. 1b) ; la chromatographie laisse alors apparaître deux groupes bien différenciés de carbures : les monoterpènes ($C_{10}H_{16}$) et les sesquiterpènes ($C_{15}H_{24}$).

Les essences présentes dans les pousses portant ces aiguilles sont beaucoup plus simples. Elles comportent peu (tissus extérieurs au bois) ou très peu (bois) de composés oxygénés ; les carbures sesquiterpéniques sont aussi moins abondants et moins complexes (fig. 1c, d). Il est à remarquer que les essences du bois des pousses comportent simplement 2 monoterpènes très majoritaires ; elles rappellent les térébenthines obtenues par gemmage. La présence presque exclusive des pinènes dans les essences de bois les rend peu intéressantes pour les études de génétique quantitative.

Par contre, les essences des tissus extérieurs au bois ("tissus corticaux") comportent un large éventail de carbures terpéniques dont la variabilité justifie une étude approfondie de leur transmission héréditaire.

Les feuilles, si elles apportent pour les études chimiotaxinomiques un matériel intéressant du fait de la très grande variété des composés terpéniques élaborés, ne sont pas favorables aux recherches de génétique quantitative. En effet, il faudrait alors tenir compte des interactions physiologiques multiples qui peuvent intervenir entre les diverses classes de terpènes. En outre, des variations saisonnières peuvent se produire, plus marquées au niveau des aiguilles, physiologiquement plus actives.

C'est pour ces diverses raisons que nous avons choisi les "tissus corticaux" comme matériel d'étude ; tous les résultats présentés portent sur les essences présentes dans ces tissus.

1.1.2. Variations saisonnières

La figure 2 montre les variations de la concentration en 3-carène (a), myrcène (b), limonène (c), pinènes (d, e) et sesquiterpènes (f) dans les tissus corticaux au cours de la première année de croissance (13). Pendant la période de croissance intense (de mai à août) la composition des essences se modifie ; ce sont les teneurs en 3-carène, myrcène et limonène qui sont le plus affectées. Par la suite les pourcentages de chaque terpène restent (14) remarquablement stables jusqu'au durcissement du rhytidome des pousses.

Les études portent donc sur les tissus corticaux des pousses jeunes ayant terminé leur croissance et dont la composition, stable, peut caractériser un individu.

1.2. Variations individuelles

Le profil terpènique des tissus corticaux manifeste une forte variabilité d'un individu à l'autre. Les 3 clones présentés sur la figure 3 montrent des différences notables pour leurs concentrations en 3-carène, myrcène, limonène, longifolène et caryophyllène. Il est à remarquer que le 3-carène et le longifolène peuvent être absents de façon presque complète.

1.3. Etude de l'hérédité des terpènes

Les études portant sur le mécanisme de la transmission héréditaire des terpènes ont utilisé des familles de pleins-frères relativement jeunes (7 ans au plus). Le caractère adulte de l'aptitude à la synthèse de chaque terpène a été chaque fois vérifié (existence de bonnes corrélations entre parents et descendants).

1.3.1. Identification des génotypes dans les familles

Pour le 3-carène, la discrimination sur la base des concentrations dans les tissus corticaux entre arbres "pauvres" et "riches" est évidente : les teneurs en 3-carène des premiers sont inférieures à 2 % tandis que celles des seconds sont supérieures à 15 %. Pour tous les autres terpènes la discrimination des génotypes doit être effectuée d'après les histogrammes des concentrations à l'intérieur des différentes familles. La figure 4 montre les histogrammes correspondant à la ségrégation du longifolène.

1.3.2. Test de l'hypothèse du contrôle monogénique pour différents types de croisements entre génotypes

Nous avons montré un contrôle monogénique pour le 3-carène (5), le myrcène (6), le longifolène et le caryophyllène (7), dans différentes catégories de croisements. Le tableau 1 compare les fréquences attendues et observées dans différents croissements, pour la concentration en myrcène.

1.3.3. Type et degré de dominance

Selon la dominance d'un allèle sur l'autre on peut distinguer 3 situations différentes :

(a) dominance modérée de la richesse

Cette situation est rencontrée pour le 3-carène. Les génotypes hétérozygotes sont plus voisins des génotypes homozygotes riches que des homozygotes pauvres (fig. 5a).

(b) Hérédité pratiquement additive

Le myrcène et le longifolène ont une quasi-absence de dominance pour l'allèle de richesse comme le montre la figure 5b.

(c) Forte dominance de la pauvreté

Une forte dominance de l'allèle de pauvreté a été rencontrée dans le cas du caryophyllène. C'est une situation rare dans le domaine de la génétique des terpènes (fig. 5c).

1.3.4. Relations de linkage entre gènes

Toutes les liaisons entre les 4 loci mis en évidence n'ont pu être étudiées. Un seul cas de linkage a pu être montré entre les loci C (3-carène) et M (myrcène) avec un taux de recombinaison de 0.10 (6).

1.4. Variabilité infraspécifique

1.4.1. Ensemble de l'aire naturelle

Un premier échantillonnage (14) portant sur un petit nombre d'arbres représentant l'ensemble de l'aire naturelle du Pin maritime a donné les résultats suivants, regroupés sur la figure 6.

Le 3-carène se révèle un excellent critère de discrimination des provenances. En effet, d'après ce critère de comparaison, les Pins étudiés se séparent en 3 classes :

- taux de 3-carène pratiquement nul : Maroc, Maures, Esterel
- faible taux de 3-carène, inférieur à 8 % : Corse et Portugal
- teneur élevée en 3-carène, supérieure à 16 % : Landes et Espagne.

Le myrcène est présent dans les tissus corticaux de 7 provenances étudiées à un taux voisin de 20 % ; la provenance marocaine se particularise en atteignant seulement 1,2 % de myrcène.

Ces résultats préliminaires devront être confirmés par d'autres analyses portant sur un grand nombre d'arbres par zone géographique. Ces nouvelles analyses faites arbre par arbre permettront de caractériser chaque provenance par des fréquences géniques, comme nous l'avons déjà fait pour le Massif landais.

1.4.2. Etude approfondie d'une zone géographique : le Massif landais

Nous avons analysé 400 arbres appartenant à dix zones différentes réparties sur l'ensemble du Massif landais.

L'étude de la variabilité des fréquences de l'allèle C^+ (3-carène) dans les différentes zones montre que l'on peut attribuer à l'ensemble des peuplements prospectés une seule origine : l'ancienne population autochtone implantée sur le littoral aquitain (15).

Ces résultats permettent d'envisager l'utilisation des terpènes à la fois pour la discrimination des populations et des individus. La possibilité de déterminer par son phénotype le génotype d'un individu suggère également une autre application : l'étude des lois de croisements en peuplement ou en verger à graines. Cependant cette détermination n'est pas toujours facile : dans certains cas, les histogrammes des concentrations en terpènes ne permettent pas de définir avec précision les limites entre génotypes.

2. LE PROBLEME DE L'INTERPRETATION DES HISTOGRAMMES

Sur les histogrammes d'une même population, certaines classes sont nettement séparées ; cependant des chevauchements plus ou moins importants dans leur distribution peuvent rendre malaisée la séparation des génotypes. L'amplitude de ces chevauchements dépend à la fois du degré de dominance d'un allèle sur l'autre et de la concentration en terpènes autres que le composé considéré. Il existe en effet des corrélations entre terpènes dues aussi bien au mode d'expression des concentrations en pourcentages qu'à des relations physiologiques.

On peut alors recourir à deux moyens différents pour préciser les limites entre génotypes : l'étude des concentrations chez des individus connus grâce à l'étude de leur descendance, ou la construction d'histogrammes séparés regroupant des individus triés selon leurs génotypes pour des gènes contrôlant d'autres terpènes.

2.1. Exemples d'histogrammes

La figure 7 représente les histogrammes de 400 Pins maritimes landais pour le 3-carène (fig. 7-a), le myrcène (fig. 7-b), et le longifolène (fig. 7-c).

En ce qui concerne le 3-carène, la séparation entre génotypes C-/C- et C+/C- n'offre aucune difficulté car leurs distributions ne se recouvrent pas. Par contre, la distinction entre génotypes C+/C- et C+/C+ n'est pas évidente.

Dans le cas du longifolène et du myrcène, le chevauchement entre génotypes affecte aussi bien les limites entre génotypes Lo-/Lo-, M-/M- et Lo+/Lo-,

M+/M- que celles qui séparent Lo+/Lo-, M+/M- de Lo+/Lo+, M+/M+.

2.2. Définition de la limite entre génotypes d'après la distribution des concentrations chez les individus de génotypes connus par l'étude de leur descendance

Cette méthode est utilisable lorsque les chevauchements entre génotypes sont de faible amplitude. Par exemple, on connaît pour le 3-carène, la concentration maximum rencontrée pour le génotype C+/C- (38,7 %) ainsi que la concentration minimum d'un génotype C+/C+ (39,6 %). On peut donc grossièrement considérer comme C+/C- tout arbre dont la concentration en 3-carène (exprimée en % de monoterpènes) est inférieure à 39 % ; tout arbre dont la concentration est supérieure à 39 % sera classé parmi les génotypes C+/C+.

2.3. Prise en considération d'autres terpènes pouvant modifier l'expression d'un génotype

Lorsqu'il est possible, comme pour le 3-carène, d'identifier sur un histogramme les classes de génotypes, on peut pratiquer ensuite une subdivision de l'histogramme d'un autre terpène = histogrammes des pauvres en 3-carène (C^-/C^-), des hétérozygotes (C^+/C^-) et des homozygotes C^+/C^+. La fig. 8 représente ainsi les histogrammes des concentrations en myrcène correspondant à ces 3 catégories. La distinction des 3 génotypes pour la synthèse du myrcène devient alors relativement aisée parmi les arbres C^-/C^- (fig. 8 a) ; elle reste difficile pour tous les phénotypes riches en 3-carène (fig. 8 b et c). L'augmentation du taux de 3-carène réprime les concentrations en myrcène ; la répression est d'autant plus forte que la teneur en myrcène est plus élevée. Il s'ensuit un "tassement" des histogrammes qui rend difficile la discrimination des génotypes. Pour éliminer cet effet, il faudrait établir un modèle qui rende compte des interactions entre ces deux terpènes. On serait alors en mesure de construire un histogramme global où les trois classes apparaîtraient clairement.

3. QUELQUES EXEMPLES D'APPLICATION

Du fait de leur fort contrôle génétique, les terpènes constituent un outil efficace pour la discrimination des populations ou d'individus. La connaissance précise du mode d'hérédité de certains d'entre eux permet d'affiner la caractérisation des génotypes. En outre, certaines applications comme l'étude des lois de croisements sont rendues possibles grâce à l'utilisation comme gènes marqueurs de certains des allèles mis en évidence.

3.1. Applications immédiates

3.1.1. Identification de provenances d'origine inconnue

Les études de chimiotaxinomie faites sur différentes espèces résineuses font apparaître une variabilité du profil terpénique qui peut être soit discontinue (Pinus pinaster (4)), soit clinale (Pseudotsuga menziesii (16), Pinus elliottii (17), Abies lasiocarpa (18)). Une meilleure description de la variabilité est atteinte si les populations locales de provenances sont caractérisées par des fréquences géniques.

A partir de la description de la variabilité d'une espèce, il est ensuite relativement aisé de caractériser tout peuplement d'origine inconnue sur lequel on se proposerait de récolter des graines. Il est également possible d'identifier des jeunes plants en étudiant les terpènes à manifestation précoce (3-carène, myrcène). Ceci offre aussi un moyen pour contrôler l'origine des graines après obtention de jeunes plants issus de ces graines.

3.1.2. Etude des lois de croisements

Il peut être important de vérifier, en verger à graines (de clones ou de semis), que la loi de croisements ne s'écarte pas trop d'un schéma panmictique. En particulier un taux d'auto-fécondation ou des croisements trop fréquents entre arbres apparentés peuvent diminuer considérablement la valeur de la graine produite. L'analyse de descendants en pollinisation libre d'arbres de génotypes connus, échantillonnés dans le verger, peut permettre de répondre à cette question. Les conclusions peuvent ensuite orienter les éclaircies génétiques : par exemple élimination des clones présentant une trop forte tendance à l'auto-fécondation.

3.2. Applications à plus long terme

Dans la mesure où la concentration en certains terpènes serait liée à des caractères économiquement intéressants, on pourrait envisager une sélection indirecte utilisant la connaissance du génotype des arbres. Dans le cas du Pin maritime, les premiers résultats encourageants dans ce domaine concernent la résistance à Dioryctria splendidella qui serait plus grande chez les arbres riches en limonène (19).

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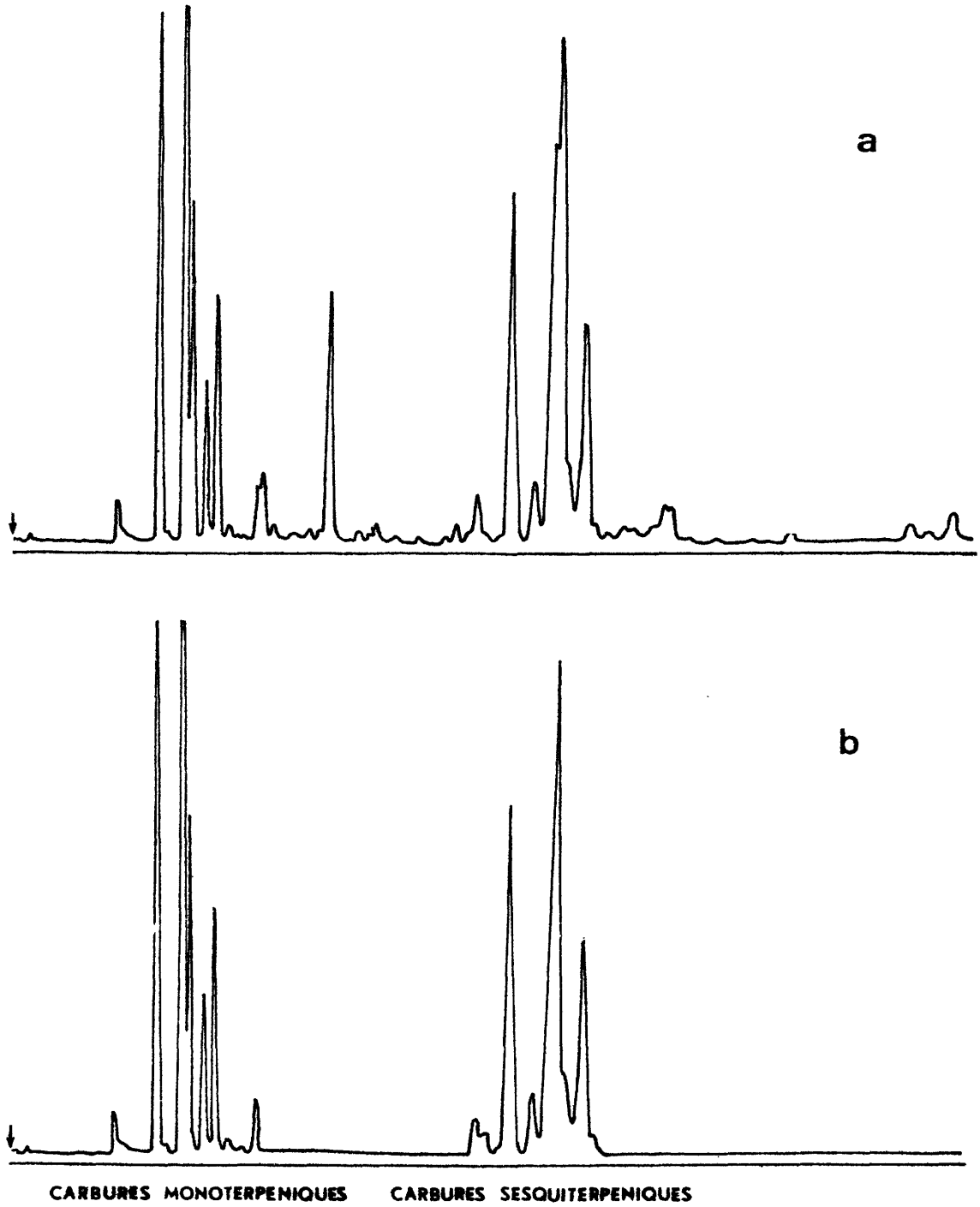
ANNEXE 1

Fig. 1a Analyse par chromatographie en phase gazeuse d'une essence totale d'aiguilles de Pin maritime.

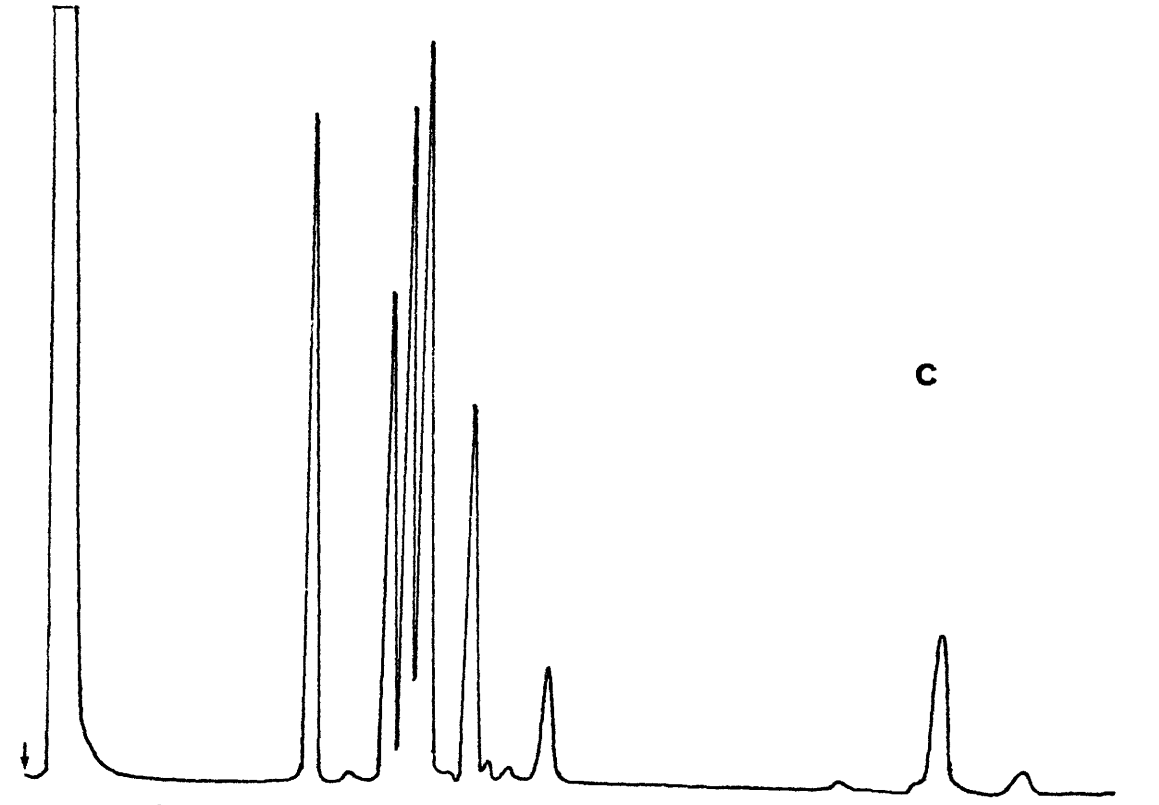
Fig. 1b Analyse des carbures monoterpéniques et sesquiterpéniques présents dans la même essence d'aiguilles (après un traitement éliminant les composés oxygénés).

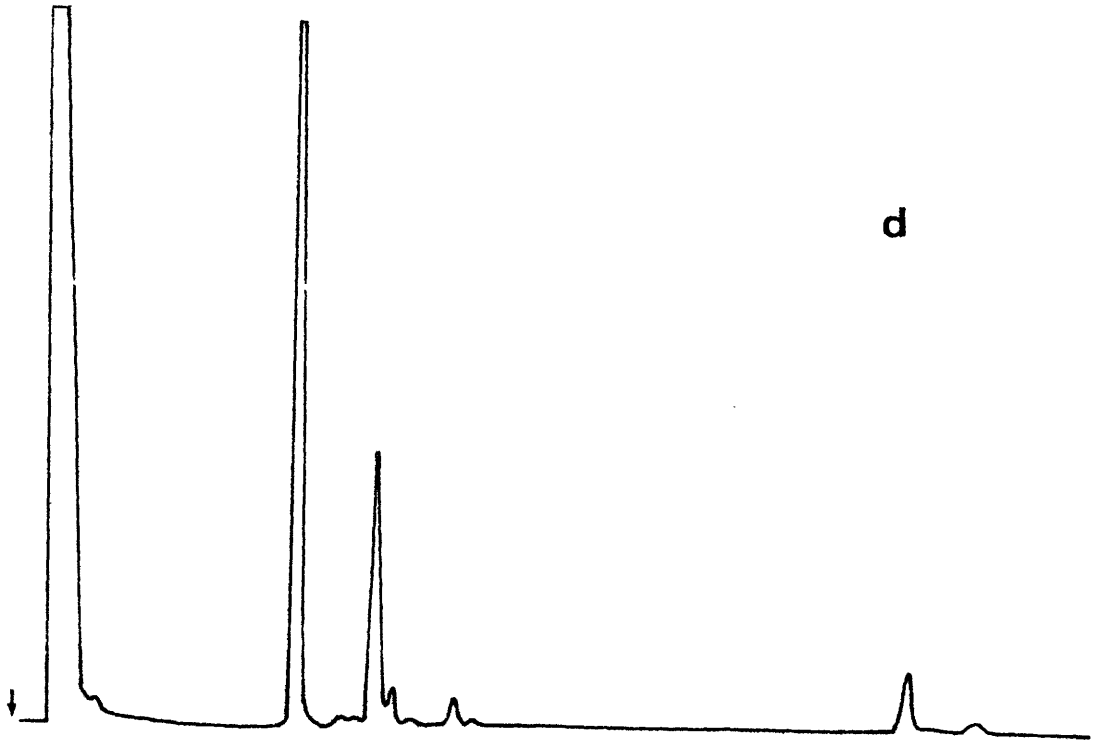


ANNEXE 2

Fig. 1c Analyse par chromatographie en phase gazeuse des carbures monoterpéniques et sesquiterpéniques d'une essence de tissus extérieurs au bois.

Fig. 1d Chromatogramme d'une essence de bois.

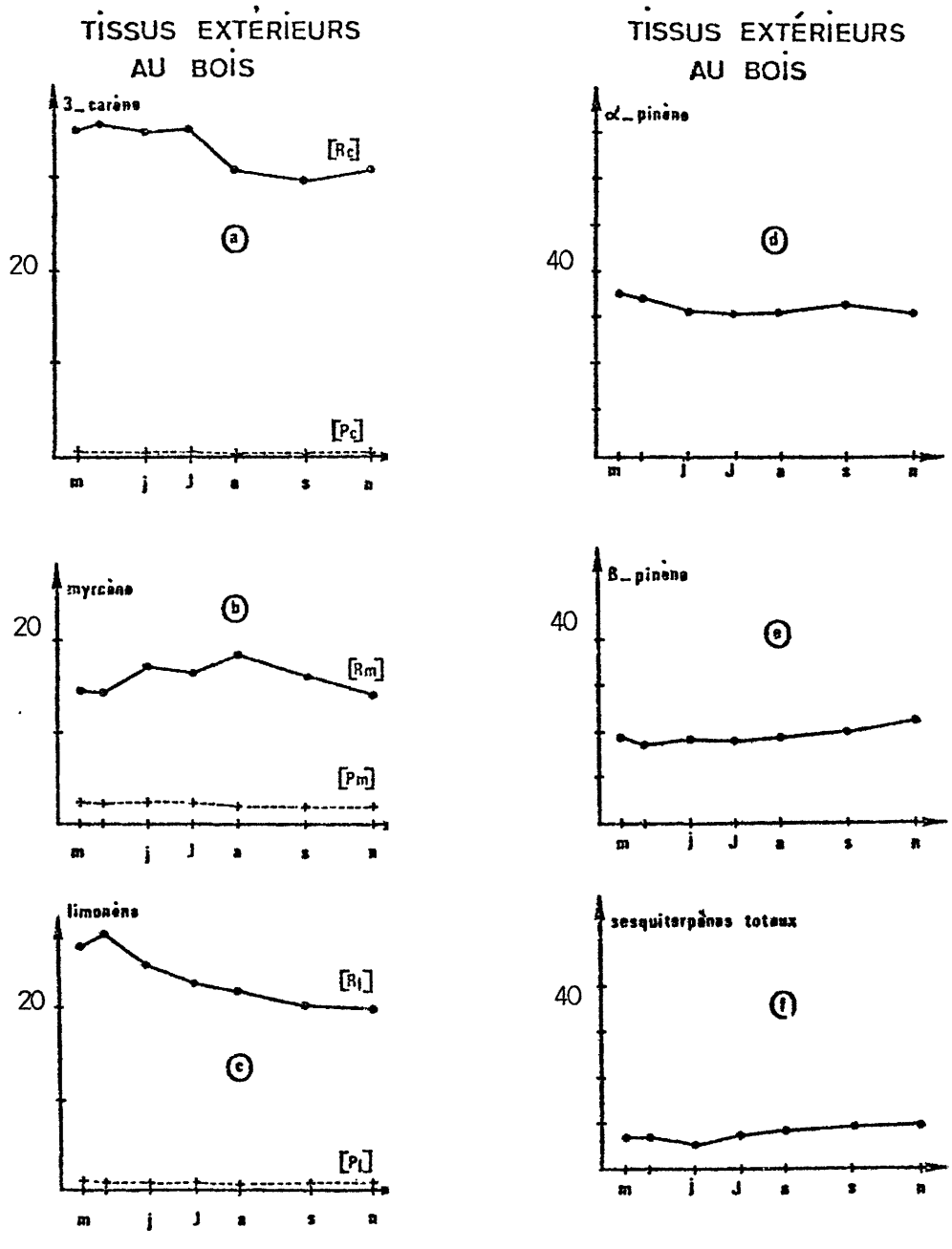




ANNEXE 3

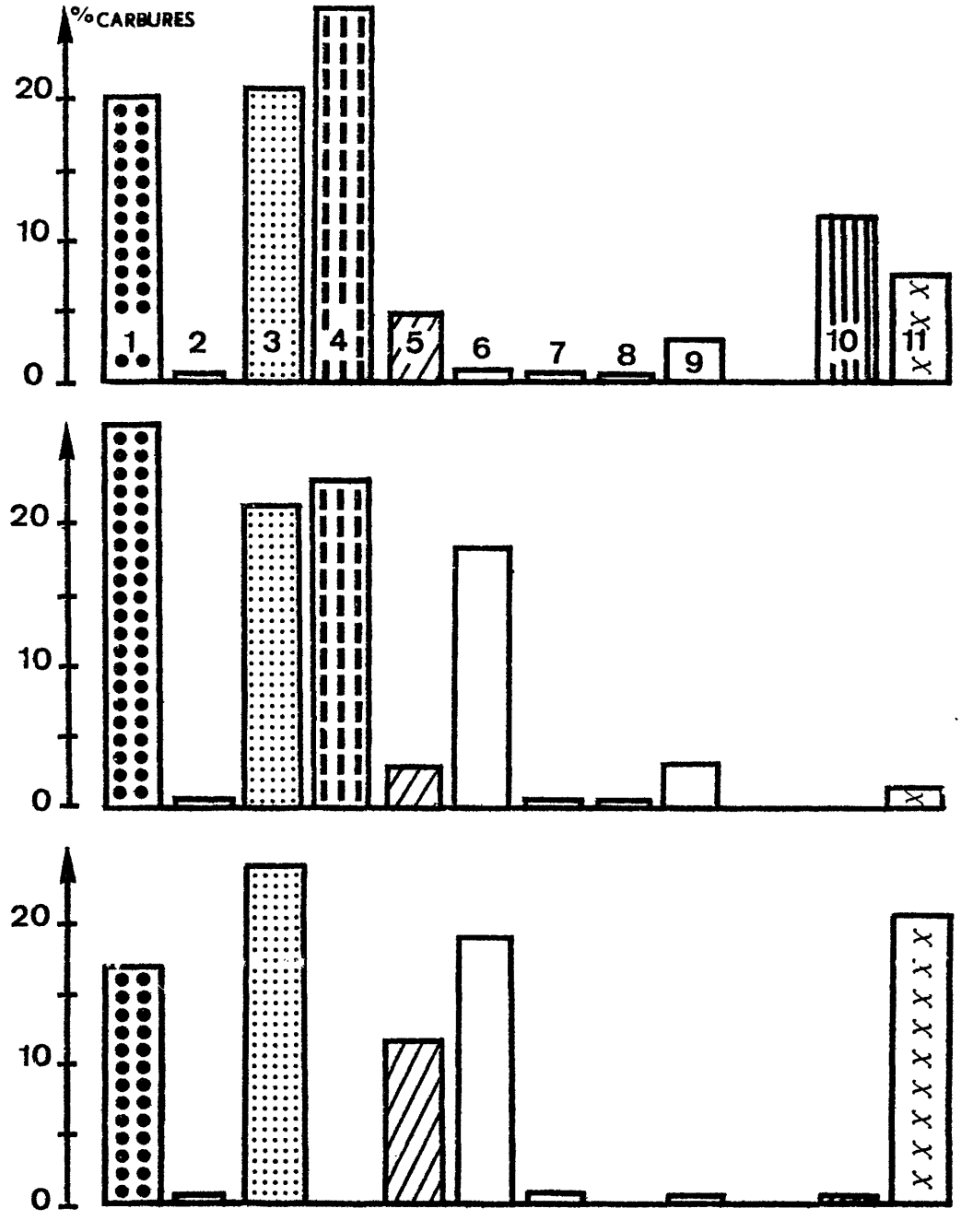
Fig. 2 Variations de la concentration des divers carbures dans les tissus corticaux au cours de la première année de croissance des pousses.

a - 3-carène
b - myrcène
c - limonène
d - α-pinène
e - β-pinène
f - sesquiterpènes



ANNEXE 4

Fig. 3 Histogrammes de répartition des carbures monoterpéniques (dans l'ordre α-pinène (1), camphène (2), β-pinène (3), 3-carène (4), myrcène (5), limonène (6), β-phellandrène (7), trans-β-ocimène (8) terpinolène (9)) et des carbures sesquiterpéniques (caryophyllène (10) et longifolène (11)) dans les tissus extérieurs au bois de 3 clones de Pin maritime.



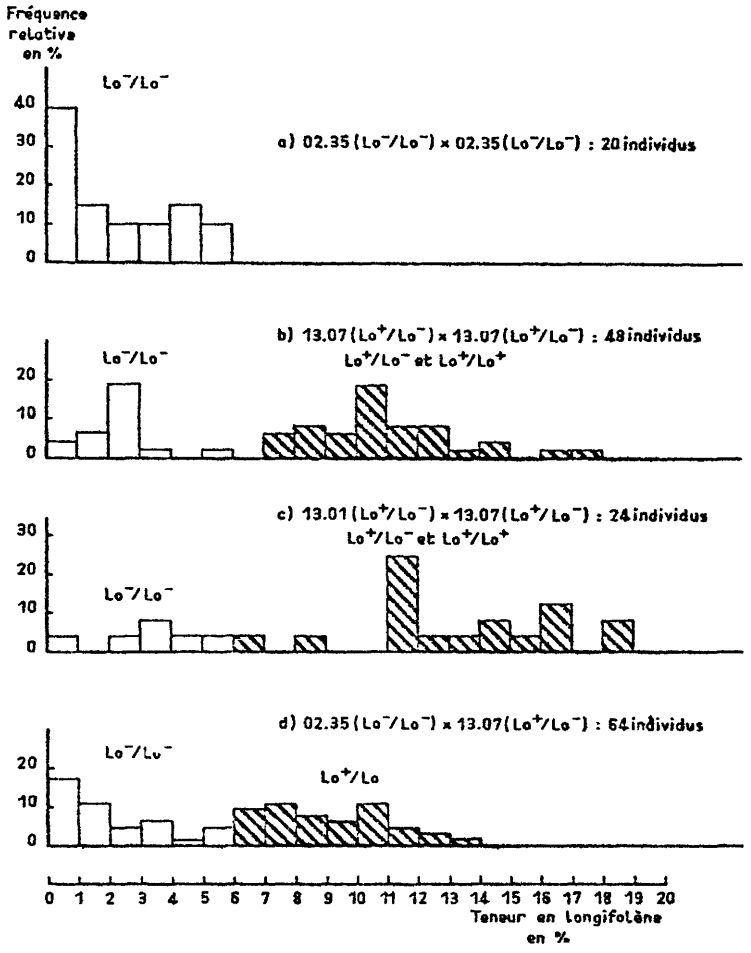
ANNEXE 5

Fig. 4 Répartition des teneurs en longifolène dans 4 familles de pleins-frères

a. chez 20 plants issus du croisement 02.35 x 02.35 (autofécondation)
b. chez 48 plants issus du croisement 13.07 x 13.07 (autofécondation)
c. chez 24 plants issus du croisement 13.01 x 13.07
d. chez 64 plants issus du croisement 02.35 x 13.07

Aire hachurée : distribution des plants de phénotype (R)

Les classes représentent des intervalles de 1 p. 100 depuis 0 jusqu'à la teneur maximale.



ANNEXE 6

Tableau I Comparaison des fréquences théoriques et des fréquences observées dans 4 types de croisements, pour la concentration en myrcène (258 observations).

Types de croisements		nombres théoriques [R]	nombres théoriques [P]	nombres observés [R]	nombres observés [P]	Test χ^2 (1 dl)
M — / M — × M — / M — (1) : 31.15 × 31.15		0	30	0	30	/
M — / M — × M + / M — (2) : 00.11 × 00.01		32	32	32	32	0
M + / M — × M + / M — (4)	00.14 × 13.08	48	16	50	14	0,31 : 0,75 > p > 0,50
	00.01 × 13.08	30	10	28	12	0,53 : 0,50 > p > 0,25
	00.14 × 51.02	30	10	23	17	6,53 : 0,025 > p > 0,01
	00.14 × 13.08 00.01 × 13.08	78	26	78	26	0
M + / M — × M + / M + (5) : 01.46 × 00.01		20	0	20	0	/

ANNEXE 7

Fig. 5 Comparaison des valeurs des concentrations moyennes des 3 génotypes illustrant les types et degrés de dominance rencontrés.

Fig. 5a Dominance modérée de la richesse - Exemple du 3-carène

$a = 22,19$ $\quad \frac{d}{a} = 0,29$
$d = 6,46$

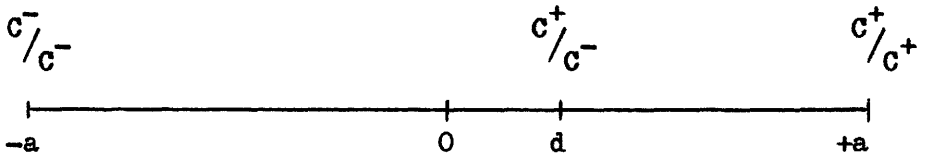


Fig. 5b Hérédité pratiquement additive - Exemple du myrcène

$a = 12,9$ $\quad \frac{d}{a} = -0,12$
$d = -1,6$

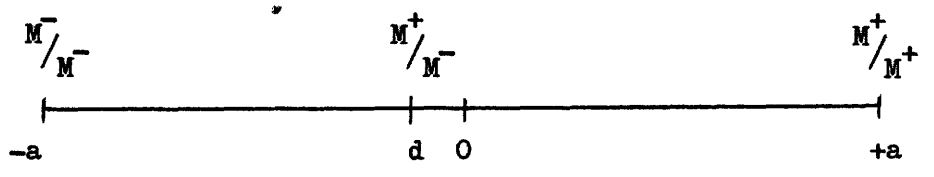
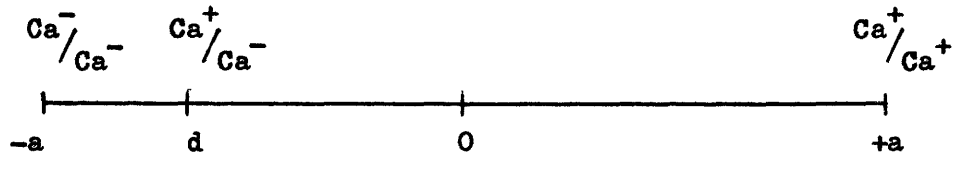


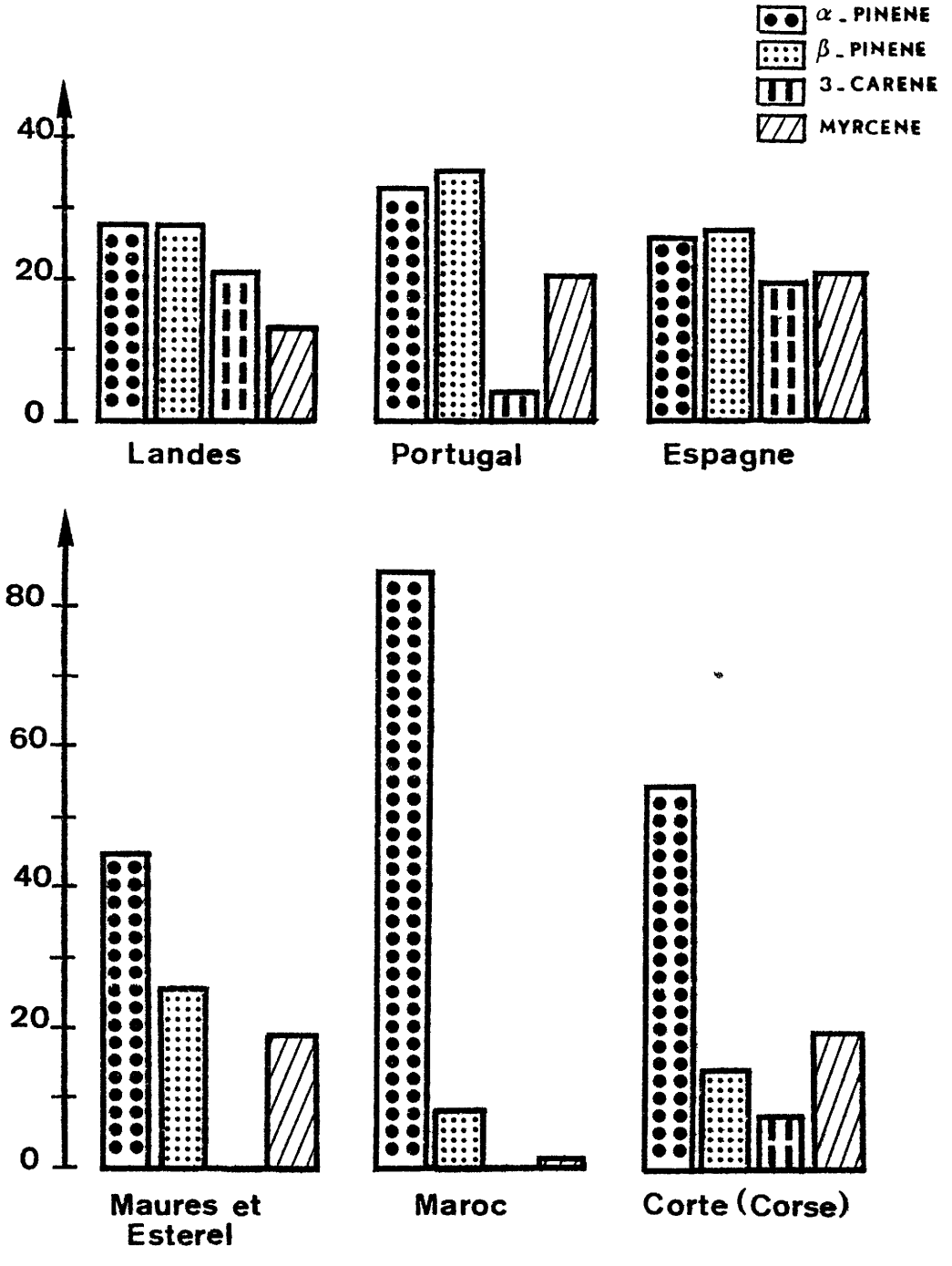
Fig. 5c Dominance de pauvreté - Exemple du caryophyllène

$a = 7,18$ $\quad \frac{d}{a} = 0,70$
$d = 5,02$



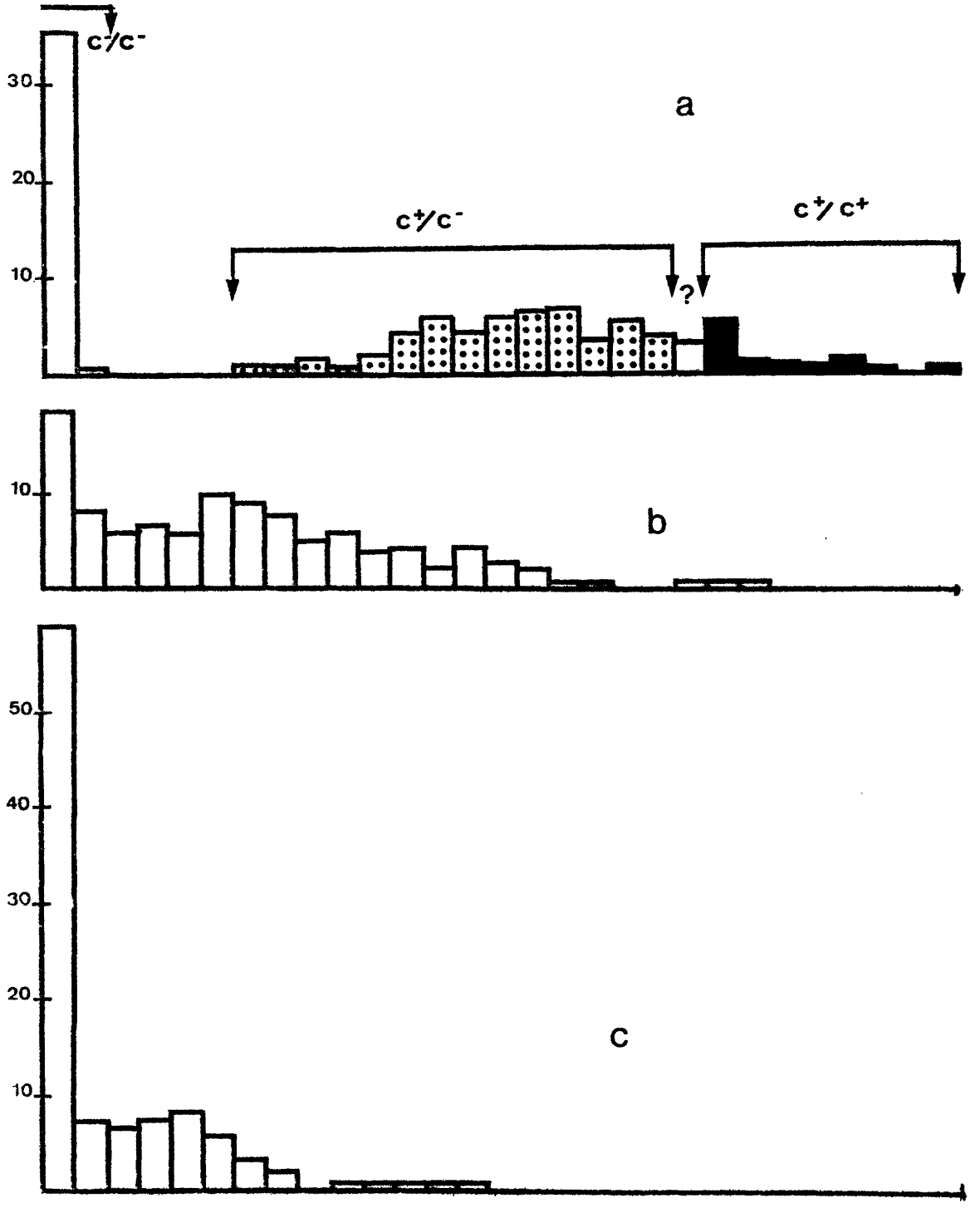
ANNEXE 8

Fig. 6 Histogrammes de la répartition de l'α-pinène, du β-pinène, du 3-carène et du myrcène dans les tissus extérieurs au bois de 6 provenances de Pin maritime.



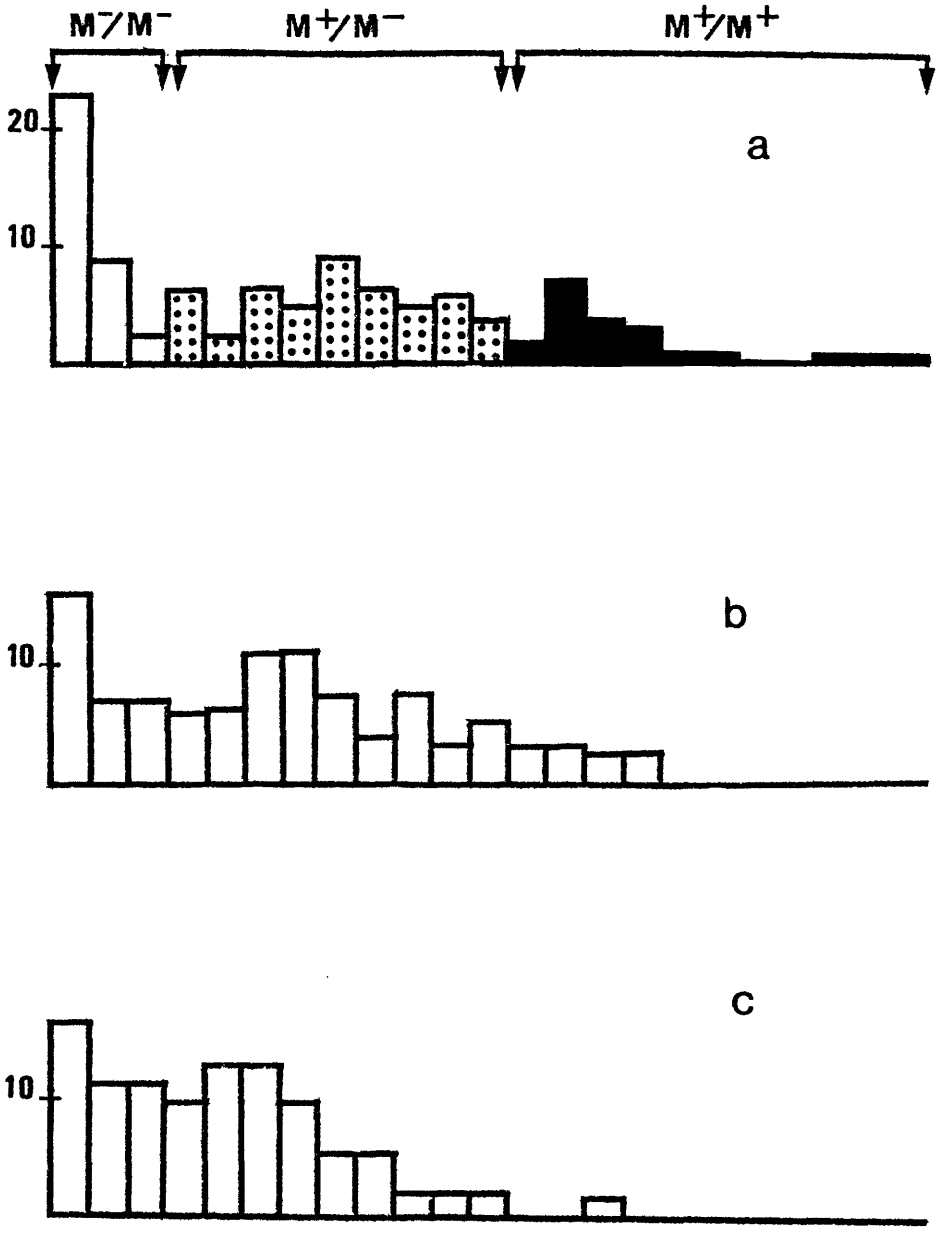
ANNEXE 9

Fig. 7 Histogrammes des concentrations en 3-carène (a), myrcène (b) et longifolène (c) des tissus corticaux de 400 Pins landais (classes de 2%).



ANNEXE 10

Fig. 8 Histogrammes des concentrations en myrcène correspondant aux trois génotypes possibles pour le 3-carène : C^-/C^- (a), C^+/C^- (b) et C^+/C^+ (c).



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FOREST ISOZYME STUDIES IN UMEÅ SWEDEN

LES ETUDES DES ISOZYMES FORESTIERS A UMEÅ, SUEDE

STUDIEN ÜBER FORSTLICHE ISOZYME IN UMEÅ, SCHWEDEN

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SUMMARY

The forest isozyme programme of the Umeå group, Sweden, is presented. Some important prestudies before the use of needles as a source of information are suggested. Studies on the mode of the inheritance both for needles and macrogametophytes (endosperms) are described for the two species of our interest, namely Picea abies and Pinus sylvestris. Linkage studies between 13 allozyme (allelic isozymes) loci of Pinus sylvestris are announced.

Variation within populations is discussed in terms of "average heterozygoti" according to NEI and ROYCHOUDHURY (1974). Some recent calculations of this measure comprising 11 Swedish stands of Picea abies are performed. Among other forces which are creating genotypes in our stands, pollen distribution and gamete selection are illustrated by some preliminary results from one stand of Pinus sylvestris in northern Sweden. Inter-populations variation is estimated as clinal variation of gene-and genotype frequencies, adaptive significance and genetic distances. Calculations according to these measures based on results from Swedish stands of Picea abies and Pinus sylvestris are presented. Good possibilities to distinguish introduced seed sources are demonstrated.

Some applications of allozyme studies in seed orchards are pointed out. The bases for these applications are mainly the use of rare or unique allozymes. Recent studies evaluating the net effect of open pollination in a Swedish seed orchard are briefly referred to.

Finally, a brief discussion concerning future studies of correlations between phenotypical traits and allozymes is presented.

RESUME

Le programme isozyme forestier du groupe d'Umeå, Suède, est présenté. Quelques études préliminaires importantes sont suggérées avant l'usage des aiguilles comme source d'information. Des études sur le caractère de l'héritage des aiguilles et des macrogamétophytes (endospermes) sont référées au sujet des deux espèces de notre intérêt, le Picea abies et le Pinus sylvestris. Des études de combinaison entre 13 allozymes (allelic isozymes) loci du Pinus sylvestris sont annoncées.

La variation à l'intérieur des populations est discutée dans des mots d'"hétérozygosité moyenne" d'après NEI et ROYCHOUDHURY (1974). Quelques calculs récents de cette mesure comprenant 11 populations suédoises de Picea abies sont effectuées. Parmi d'autres forces créant des génotypes dans nos populations, la distribution du pollen et la sélection "gamétique" sont illustrées par quelques résultats préliminaires d'un peuplement de Pinus sylvestris du nord de la Suède. La variation des populations est comme une variation clinale des fréquences gene- et genotypes, une signification adaptive et une distance génétique. Des calculs selon ces mesures fondées sur les résultats des peuplements suédois de Picea abies et Pinus sylvestris sont présentés. De bonnes possibilités de distinguer les populations introduites sont exposées.

Quelques applications des études allozymes des plantages à graines sont démontrées. Les fondements de ces applications sont avant tout l'usage des allozymes rares ou uniques. Des études récentes évaluant l'effet net de la pollinisation libre d'un plantage à graines suédois sont brièvement référées.

Finalement une discussion sommaire concernant les études futures des corrélations entre les traits phenotypicaux et les allozymes est présentée.

ZUSAMMENFASSUNG

Das Programm der forstlichen Isozym-Gruppe in Umeå, Schweden, wird in diesem Rapport vorgestellt. Einige wichtige Vorstudien vor dem Gebrauch von Nadeln als Informationsquelle sind vorgeschlagen. Vererbungsstudien für Nadeln und Macrogametophyten werden für Picea abies und Pinus sylvestris referiert. Kopplungsstudien zwischen 13 allozymen (allele Isozymen) Loci von Pinus sylvestris werden behandelt.

Die Variation innerhalb von Populationen wird diskutiert in Termen von dem erwarteten "Durchschnittswert von Verhältnis der Heterozygoten", von NEI und ROYCHOUDHURY (1974) beschrieben. Einige neue Berechnungen von diesen Verhältnissen sind an 11 schwedischen Beständen von Picea abies durchgeführt und werden berichtet. Beispiele von Kräften, die die Genotypen in unseren Beständen schaffen, sind Pollenverbreitung und Selektion der Gameten. Einige Ergebnisse eines nordschwedisches Pinus sylvestris-Bestandes illustrieren diese Kräfte.

Die Variation zwischen Populationen ist als klinale Variation von Verhältnissen der Gene und Genotypen, Adaption und genetischer Entfernung geschätzt. Berechnungen von diesen Massen sind auf Ergebnisse von schwedischen Beständen der Picea abies und Pinus sylvestris basiert. Gute Möglichkeiten für die Identifizierung der eingeführten Bestände werden demonstriert.

Einige Beispiele von Allozymstudien in Samenplantagen werden auch berichtet. Als Basis für diese Beispiele dienen hauptsächlich die Verwendung von selten vorkommenden Allozymen. Neue Studien, die den Reinertrag von freier Pollination in einer schwedischen Samenplantage bewerten, werden kurz referiert.

Schliesslich wird eine kurze Diskussion über zukünftige Studien von Korrelationen zwischen verschiedenen Phänotypen und Allozymen vorgetragen.

FOREST ISOZYME STUDIES IN UMEÅ, SWEDEN

Introduction

Among forest geneticists the lack of knowledge about basic genetical mecanisms of forest trees is a well-known and embarrassing fact. This insight in the year 1968 forced me inspired by and together with Professor Bertil Rasmuson at the Department of Genetics, University of Umeå, Sweden, to start a project which aimed to evaluate the isozyme technique for forest genetical purposes. Our intensions were preliminary based on the exciting possibilities for identification of single trees. Today the forest isozyme group in Umeå consists of two additional persons, Associate Professor Marianne Rasmuson, population geneticist and doctorand Kenneth Lundkvist, both at the Department of Genetics, University of Umeå. Furthermore, we have a very active supporting board consisting of three persons at the Department of Forest Genetics, Swedish College of Forestry. They are Professor Gösta Eriksson, Assistant Professor Inger Ekberg, both in Uppsala and Associate Professor Dag Lindgren, Stockholm, all of them with a solid interest in those basic forest genetical mechanisms which have created and are creating the genetical structure of our forests. Our group in Umeå is working mainly with *Picea abies* and *Pinus sylvestris* along five main-roads:

1. Development of the isozyme technique and sampling.
2. Studies concerning inheritance and linkage.
3. Studies of variation within populations.
4. Studies of variation between populations.
5. Applications in seed orchards.

1. Development of the isozyme technique and sampling

When starting there is always a need to adapt the isozyme technique to species and tissue for the analysis. If needles are used some additional questions about sampling arises. Therefore our first approach to this technique comprised studies concerning:

1.1 biochemical methods of electrophoresis and isozyme staining.

1.2 season for collection of needles in relation to stability of isozyme patterns

1.3 where in a tree to collect needles

1.4 isozyme pattern in relation to environment

1.1 Methods for studies of esterases (EST), peroxidases (PEROX), leucine-amino-peptidasen (LAP) and glutamate-oxalate-transaminases (GOT) were established at our laboratory. All these enzyme systems, but especially the two first mentioned, were subjected to studies according to points 1.2, 1.3 and 1.4.

1.2 The suitable season for the collection of needles was determined for *Picea abies* and *Pinus sylvestris*. The study revealed a clearcut recommendation to harvest needle material during the dormancy period. During other periods the isozyme bands turned out to appear and disappear relative to the state of development. This seems to be true for all the four enzyme systems except LAP, which is stable all the year round. During the dormancy period, however, only PEROX showed somewhat unstable patterns. Together with the toxicity of substrates for the staining of PEROX the instability forced us to disregard these isozymes in the future. Before this decision was made we found illustrative inherited patterns in controlled crosses of PEROX.

1.3 The question of where in the crown of the tree to sample needles, was studied for both species. No indications of changed configurations of patterns in any position could be traced. The staining intensity, however, was highest to the south and for *Picea abies* especially at the top of the tree. This could of course be interpreted as a higher metabolic level on the sunny side of the tree.

1.4 The LAP- and GOT-patterns from parent and progeny in spite of growing in quite different environments correspond very well. The Mendelian inheritance was convincing. For EST, however, some unexpected types were found. Because of this a study of EST-pattern in relation to environment was carried out. Genetically identical material as grafted clones are placed in different seed orchards and clone banks. Three such different environments were visited and needles from two different grafts of 16 clones were harvested at each place. The needle samples were numbered from 1 to 96 and analysed for EST-pattern. The task was to group the numbers only according to the EST-pattern. In 94 out of 96 cases a correct grouping was arrived at (RASMUSON and RUDIN, 1971). Our conclusion was that the patterns ought to be sufficiently reliable for many genetic studies.

To use seeds instead of needles facilitates the use of the isozyme technique in many respects. Three are represented by studies according to the points 1.2-1.4 which can be omitted. Further discussion of the advantages with analyses of needles and endosperms, respectively, is found in another current paper by the author (RUDIN, 1976).

2. Studies concerning inheritance and linkage

The demand of checking of inheritance is quite different for use of endosperms (macrogametophytes) and needles as tissue for genetical investigations. The haploid endosperm tissue needs a test of 1:1 segregation of heterozygotes but also what looks like homozygotes because they may carry silent (no band) allels. Such segregation studies on seeds collected from a single tree ought to accompany all studies of natural stands if the purpose of the investigation is to characterize the adult stands in terms of genetical parameters. The reason for this is that we have found that 7 out of 13 loci represented by 9 trees out of 31 show significant (on at least 5% level) deviation from 1:1 segregation (RUDIN, in preparation). Based on these data we have so far found that the average risk of getting a significant deviation from 1:1 segregation is 3%.

Combined studies of inheritance using both endosperms and needles of controlled crosses from the same trees have been performed for four loci GOT-A, GOT-B, LAP-A and LAP-B in *Pinus sylvestris*, (RUDIN, 1975: RUDIN, in press) and for one locus of acid phosphatases (PHOS) in *Picea abies* (LUNDKVIST, 1975). Mendelian inheritance is convincingly demonstrated in these three papers. The same is true for two papers demonstrating the inheritance of locus EST-A, EST-B and EST-C in *Pinus sylvestris* (RUDIN and RASMUSON, 1973) and of LAP-B in *Picea abies* (LUNDKVIST, 1974 a). Both papers refer only to the analysis of needles of controlled crosses.

In order to evaluate allozyme variation within and between stands in a correct manner it is necessary to know if the loci studied are representative of the genome. Therefore linkage studies are important to carry out. There is an excellent opportunity to make linkage studies using the haploid endosperm (macrogametophytes) tissue of conifers. There is a mini pilot study on linkage of three loci of *Picea abies* published by LUNDKVIST (1974 b). No linkage was found between the three loci.

We are just now preparing a publication with the title: Linkage studies in *Pinus sylvestris* L. using allozymes in macrogametophytes. It comprises linkage studies based on 13 loci and 60 pairs of these (figure 1). The source of information has been at least 31 double heterozygous trees. Of these 23 trees come from one stand in northern Sweden. The remaining ten originates from one stand in southern Sweden and seed orchards in central and northern Sweden. Results from the stand in northern Sweden and the other trees point clearly to one linkage group consisting of four loci. This comprises two alcoholdehydrogenase loci, ADH-A and -B, very closely linked, -- LAP-B -- GOT-B in the order mentioned. There are indications of another linkage group consisting of two loci. This is found among trees in the northern stand and comprises EST-EB -- EST-EC. Finally from one tree (Kosta 4) in the stand in southern Sweden one linkage group is derived between LAP-A and ADH-EB. The presences of this linkage group is checked but not found in the stand in northern Sweden. The chromosome arrangement in south Sweden therefore seems to differ in relation to that found in northern Sweden.

3. Studies of variation within populations

The efficiency of the breeding strategy for forest tree breeders very much depends on knowledge about genetic structure of natural stands. In stands with wide genetical variation the opportunities to make great progress by selection are of course greater than in stands with very narrow variation.

In order to guide the choise of appropriate stands for the preservation of gene resources in forestry it is necessary to have a relatively quick method for the estimation of the genetic variation within stands. These are only two important examples of applications of the allozyme technique.

If allozyme studies should be an efficient tool for these purposes two important demands must be made. As pointed out above available loci should represent a major part of the genome but there should also be a proper method for calculations of the variability. Most allozyme studies, including our own have operated with far too few loci. However it is possible to trace single tendencies even with few loci. Until now we have not found the ideal method for the measurement of genetic variability by isozyme analysis. One of our group DAG LINDGREN is attempting to establish such a method.

3.1 The genetic variation in 11 populations of *Picea abies* in Sweden has been investigated by means of isozyme analysis. In this study, based on needle analysis only four loci were available. We have not yet sufficient information about linkage between them. In spite of this such drastic events as the introduction of Baltic populations to the central Sweden and German populations to the south of Sweden are easy to trace in our results. The "average heterozygosity" according to NEI and ROY-CHOUDHURY (1974) can be expressed by the formula:

$$H = \Sigma h_j / r$$

$$h_j = 1 - \Sigma x_i^2 \quad \text{for j-th locus}$$

r = number of loci

x_i = frequency of the i-th-allele

The average heterozygosity has a pronounced higher value for the introduced populations. The highest values are those of the Baltic populations (.407 and .413). Compared to the average value of pure Swedish stands (.342), they are significantly higher (figure 2). There also is a striking difference between the German stand with an average heterozygosity of .386 and the Baltic stands. The difference may be explained by the fact that the latter were introduced to Sweden during the 18th century. Therefore those stands are the first generation of provenance hybrids while the German stand was introduced during the 19th century and has not yet got their genomes mixed up with Swedish genomes in this case (cf LUNDKVIST and RUDIN in preparation).

A statistical analysis of variance based on four isozyme loci shows that 97 % of the total variance found in the eight Swedish stands are to be found within stands. The same tendency (99 %) is found among five stands of *Pinus sylvestris* originating from North to South Sweden. They were analysed for five loci (EST-B, GOT-A, GOT-B, LAP-A and LAP-B) two of which are linked. In spite of these results single allels show a clearcut clinal variation for both species (cf chapter 4).

3.2 In order to gain knowledge about the basic mechanisms which create the genetic structure within populations of *Pinus sylvestris*, one seed-tree stand in northern Sweden (Gårdstjärn) has been chosen for model studies.

In the experimental area we have the following studies in progress:

3.2.1 Comparison between needle and endosperm analysis

3.2.2 Spatial distribution of genotypes

3.2.3 Composition of father population

3.2.4 Gamete selection

3.2.5 Mating system = proportion of self-fertilization

3.2.6 Natural selection

3.2.7 Linkage (see chapter 2)

We shall only give some short comments on the above mentioned points.

3.2.1 A pilot comparison between endosperm and needle estimated gene frequencies comprising 46 trees and four loci common for the two kinds of tissues reveals the following. The average deviation between endosperm and needle estimation is .010 frequency units. The discrepancy increases with the rarity of an allele to .023 for an allele frequency in the order of .033 for GOT-A1. Isozyme bands indicating GOT-A1 and A2 lie close to each other. In such a situation the endosperm analysis is the most reliable method to use. For gene frequencies more than .10 the discrepancy seems to be negligible.

3.2.2 There seems to be a special grouping of some alleles in the experimental area. The rare allels fit for such a study seem to have a dispersion in the direction of the prevailing of winds during the pollen shedding.

3.2.3 A study of some fathers in the father population can be made by tha aid of rare alleles in the pollen cloud. Trees with rare or possibly unique alleles are located on a map covering a central part of the experimental area two hectares of size. Mother trees for harvest of open pollinated seeds are chosen representatively over the central part of the area. These are used as indicator trees for rare alleles studied. The two year old plants grown from these seeds are isozyme analysed. An example from such a study is presented in figure 3. It shows that the tree No´s 110, 152, 148, 150, 132 carries the rare allele LAP-A3 in a heterozygous condition (carrier tree). No other rare alleles are polluted within 80 m from any indicator tree. Trees No´s 110, 154, 107, 140, 104, 121, 136 and 133 are indicator trees, which have produced seeds to 100-120 plants from each tree. The area of the filled circle of the mother tree sign is proportional to the recatch of the allele studied, in other words to the frequency of this allele in the progeny. An unfilled ring indicates no recatch at all. The figure demonstrates that the distribution of the allele LAP-A3 is highly uneven over the testplot. The summed results of four rare alleles indicate the same situation and to that possibly a pollen migration in the main direction NE for distances above roughly 20 metres. This direction roughly concords with the spatial distribution of rare alleles in the trees themselves.

3.2.4 Gamete selection is studied on the female side in the macrogametophytes (endosperms). During linkage studies it was discovered that there was a lack of certain allele combinations. This tendency was accentuated in the crossing over fraction. For example LAP-B3 and GOT-B22 cooperate very badly with each other in a female gamete. The survival lies in an intervall of .45-.65 relative to other combinations of the alleles. The viability seems to be restored if any of these alleles are replaced.

3.2.5 The proportion of self-fertilization is estimated by the use of rare and unique alleles too. For example LAP-A1 in the heterozygous constitution of tree No. 136 is unique in the area investigated. The recovery of this allele by tree No. 136 itself is manifested by the homozygote LAP-A1/A1. The frequency of such homozygotes was found to be .04. If there is a 1:1 segregation of alleles studied this figure should be divided by .25 because the share of LAP-A1/A1 should be of this proportion after self-fertilization. A slight deviation from a 1:1 distribution is consistently found between LAP-A1 in relation to LAP-A2 in the ratio of .58:.42. Therefore the frequency of LAP-A1/A1 in this case should be divided by .29 instead of .25. This correction results in an estimated proportion of self-fertilization of .14. Albinos were found in the open pollinated progeny from another tree. Calculations on these albinos give a proportion of self-fertilization of .17.

This share is somewhat higher than reported from other investigations e.g. KOSKI (1970). In his study two particular pines in a full densed stand (350 trees/ha) in central Finland showed a recovery of radioactivated pollen of 6.8% and 18.1% in crowns of the trees themselves. Our stand has a density of only 10-18 trees/ha. This may be the explanation of the higher level of our results keeping in mind that our results represent the net effect of the pollination and should be lower than those presented by KOSKI.

Another method for the calculations of the proportion of self-fertilization will also be applied to our material. This method is based on a data-simulated probability for each tree to be pollinated by surrounding-trees and by itself, using a maximum likelihood estimation according to BROWN et al (1975).

3.2.6 Natural selection will be tested in two steps. Firstly by comparison of gene- and genotype frequencies of nursery grown progeny with young self-reforestrated plants at a maximum five years old sampled beneath the adult trees. Secondly by comparing the latter plants with the adult trees. These tests are not yet available for the Gårdstjärn area, but comparisons comprising step two have been performed for four other stands. They originate from South, Central and North Sweden. Only one stand in South Sweden points to a significant (on 5 % level) difference between the young and adult generation. In this southern area of Sweden, *Pinus sylvestris*, originating from Germany frequently was introduced during the 18th and 19th century. Therefore it is not surprising that stands in this area have not reached an equilibrium.

4. Studies of variation between populations

If the purpose is to study variation between populations it must be emphasized that those allozyme loci should be chosen for calculations which have a sufficient amplitude of clinal variation in relation to traits of interest. We are studying this variation along three main lines namely:

4.1 Clinal variation of gene-and genotype frequencies

4.2 Adaptive significance of genotypes

4.3. Genetic distance

4.1 A strong tendency of clinal variation from North to Central Sweden was found among the above mentioned eleven Swedish stands of *Picea abies*. This tendency is most pronounced for the most frequent alleles of two loci LAP-A and acid phosphatases -A (PHOS). In *Pinus sylvestris* only one clear tendency of clinal variation from North to Central Sweden is found among five investigated stands. The tendency is manifested in the most frequent but one allele in the EST-B locus (R^2 = .81).

4.2 The adaptive significance will be tested by a stepwise regression analysis where we intend to put in such parameters as latitude, longitude, altitude, exposition and site quality of investigated stands as independent variables and gene- and genotype frequencies as dependent. Such an analysis comprising the three first mentioned independent variables for the eleven Swedish stands of *Picea abies* revealed a stronger correlation between a combined expression of latitude and altitude on the one side, and the most frequent allele of LAP-A on the other, than found for metrical distances only (R^2 = .77 and .72 respectively). More investigated stands of course are needed in order to get a better evaluation of adaptive significance. We are now trying to reach this.

4.3 Calculations of genetic distance are informative in many cases. It gives a condensed profile of a stand in relation to others. If a genetic distance between adjacent stands is surprisingly high it ought to be fruitful to look for adaptive significance of introduced populations. Another application of this measure together with an expression of the proportion of homozygotes is that these ought to give hints of which provenances could be chosen for provenance crosses.

That is, if stands or likely individuals show both phenotypical and allozyme mirrored indications on inbreeding depression and simultanously the genetic distance between them is of a sufficient magnitude a cross between them ought to give maximum genetic gain in terms of heterosis.

Our calculations of genetic distance according to NEI (1975) of the above mentioned 11 stands of *Picea abies* points to a clearcut distinction for the introduced populations. The average genetic distance between Swedish stands is .014 $\pm$ .009, between Baltic and Swedish stands .021 $\pm$ .015, and between German and Swedish stands .050 $\pm$ .026. A closer study of table 1 indicates that stand No. 5 might have been moved from South to Central Sweden. In spite of the fact that this stand has a longer average geographic distance of 80 km to the southern stands than to the northern ones,

the average genetic distance to stands in the north is .019 ± 0.13 and the average to stands in the south is .006 ± .002. Another interpretation concerning stand 5 is that environmental conditions for this stand are better than expected and that selection has favoured "southern" trees.

For *Pinus sylvestris* the situation seems to be similar to that of *Picea abies*. Our above mentioned pilot study comprising three stands from the north and two stands in the south of Sweden indicates the following tendency. Average genetic distance according to NEI (1975) among the three northern stands is .0019 ± .0006 between the two stands in the south .0103 and between the north and the south group .0054 ± .0019. These measures at this stage however are very rough because although five loci having been used for these calculations only two of them are of real value for such calculations. This is due to the fact that two of the loci are linked and two others have gene frequencies close to 1.0.

The interpretation of these results could however be that the natural invasion from Central Europe togehter with the introduction of seeds from different seed sources in Germany during the 18th and 19th century to the south of Sweden has increased the genetic distance of these stands to our Central and North Swedish stands, which immigrated from the north. On account of the introduction of seeds from different sources to the south of Sweden the genetic distance should be greater also between stands in southern than in northern Sweden.

5. Applications in seed orchards

One important base for these studies is the opportunity to use clones in the seed orchard which distinguish isozymetic from the others (cf RUDIN, 1976; RUDIN and LINDGREN in preparation). If a clone in one locus carries one or two alleles unique to the orchard there are possibilities to trace the contribution of this special clone to the seed formation.

The use of this method for studies concerning the net effect of open pollination in a seed orchard is pointed out. In this way the impact from one clone is calculated by the aid of two rare alleles. The impact equal to the frequency of the rare alleles found in open pollinated progeny of an orchard in Sweden (Nedansjö), was found to be .036 and .025 respectively These values should be compared to the expected impact of .040 from each of the 25 clones in the orchard.

Proportion of self-pollination can be calculated in the same manner as pointed out in point 3.5. In the absence of such unique alleles for the estimation of the inbreeding situation another method is suggested. By collecting open pollinated seeds from a clone and by counting plants which by means of isozyme patterns show a documented cross pollination, it is possible to get rough information about tendencies of self-fertilization. An application of this method was made by analysing individuals derived from open pollination and harvested at the top and the bottom of crowns of grafts in a seed orchard (Nedansjö). By comparing the frequency of individuals which can only be a result of cross pollination on the two levels it should be possible to trace differences in the amount of spontaneous self-fertilization. All clones in an orchard are not fit for such calculations.

Therefore a Diagnostic Value ($0 < DV \leq 1$) for all clones, which serve as a seed source, must be computed. The clones at Nedansjö were analysed for four loci and a DV between .40 on 1.00 were obtained for the 17 harvested clones. Only progeny from clones with a DV > .6 were analysed. From each level a total of 500 individuals was analysed. The preliminary difference between the high and the low level was found to be 11.17 per cent fewer documented cross-pollinations at the low level. This may indicate a higher proportion of self-fertilization at the lower levels.

Studies of the frequency of successful interprovenance crosses in a provenance-crossing seed orchard is available also by using rare alleles. On the same basis control of different crossing techniques and controlled crosses may be performed.

Finally the above mentioned publication in preparation - Isozyme studies in seed orchards - comprises a brief discussion concerning future studies of correlations between phenotypical traits and allozymes.

It is pointed out that this is an interesting and important, but laborious, subject. For instance, correlations between isozyme genotype and wood production, resistance to fungi and hardiness may be studied. Von WEISSENBERG (1976) has discussed the effectiveness of indirect selection using gene markers. WEIR et al (1972) measured fitness values for locus pairs in ten separate generations of barley. Four loci were available for this study. The results indicate that single locus selection estimates bear little relationship to two locus estimates. Their conclusion is that complex epistatic selective forces operate in that population. This and other studies of *Avena barbata* strongly indicate that natural selection acts to structure the genome (CLEGG et al, 1972; HAMRICK and ALLARD, 1972) into coadapted gene complexes. This might also be true for forest trees. If so, it is an encouraging fact for studies of natural populations by the aid of allozymes (allelic isozymes). This is so because the probabilities to catch one locus by an isozyme locus in a coadapted gene group are much greater than if single gene selection is pronounced.

On the other hand this fact makes it somewhat more difficult to study associations between characters of interest and isozyme loci by progeny from orchards because these coadapted gene complexes are broken up in the artificial population taken from orchards. In this situation the most efficent way of looking for associations between isozyme profiles and phenotypic characteristics is to make selections for different traits in full sib families in order to keep the genetical background as isogenic as possible.

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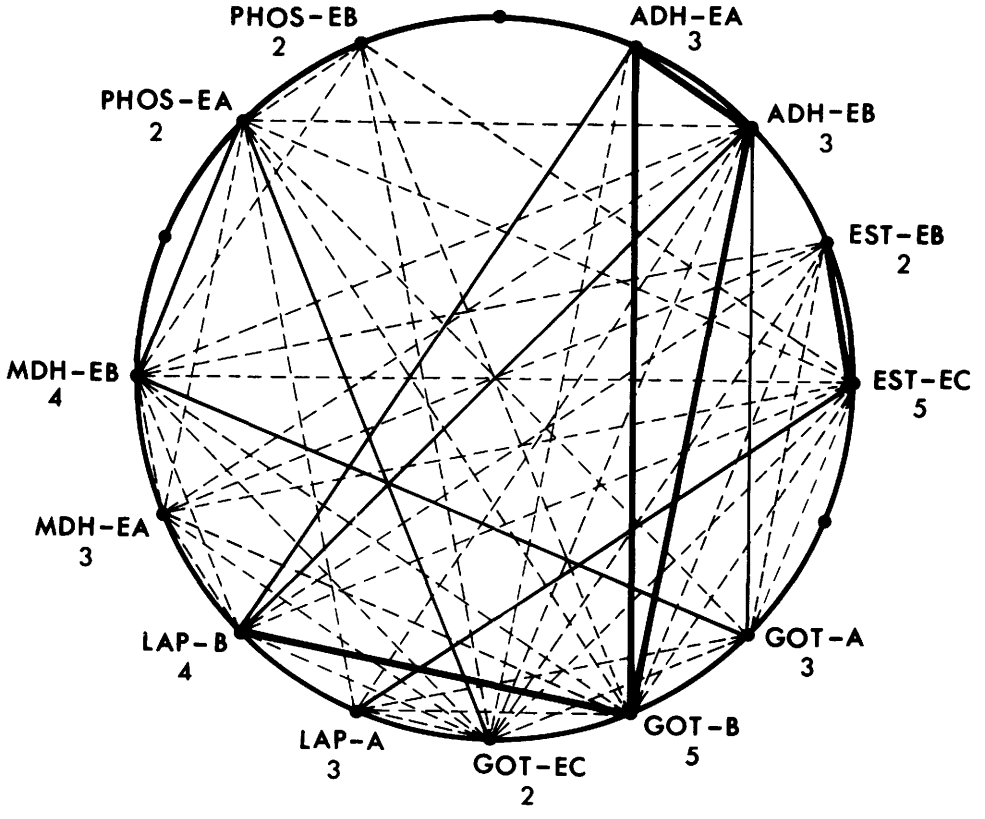
Figure 1. Schematic illustration of linkage studies between isozyme loci of *Pinus sylvestris* from one stand (Gårdstjärn) in northern Sweden. Lines designate linkage (significant on 5 % level in a χ^2_L -analysis).



Abbreviations of loci studied

ADH = Alcohol DeHydrogenases
EST = ESTerases
GOT = Glutamate-Oxalate-Transaminases
LAP = Leucine Amino Peptidases
MDH = Malate DeHydrogenases
PHOS = acid PHOSphatases
-A = a fast migrating region in the gel
-B = a medium ,,
-C = a slow ,,
-E = locus found in endosperms only

——— = Linkage from at least two trees
——— = ,, ,, one ,,
- - - - = ,, tested but not significant
Figures designate number of alleles for each locus

Figure 2. Locations of the eleven investigated populations of *Picea abies* and their "average heterozygosity" according to NEI and ROYCHOUDHURY (1974) (from LUNDKVIST and RUDIN 1976).

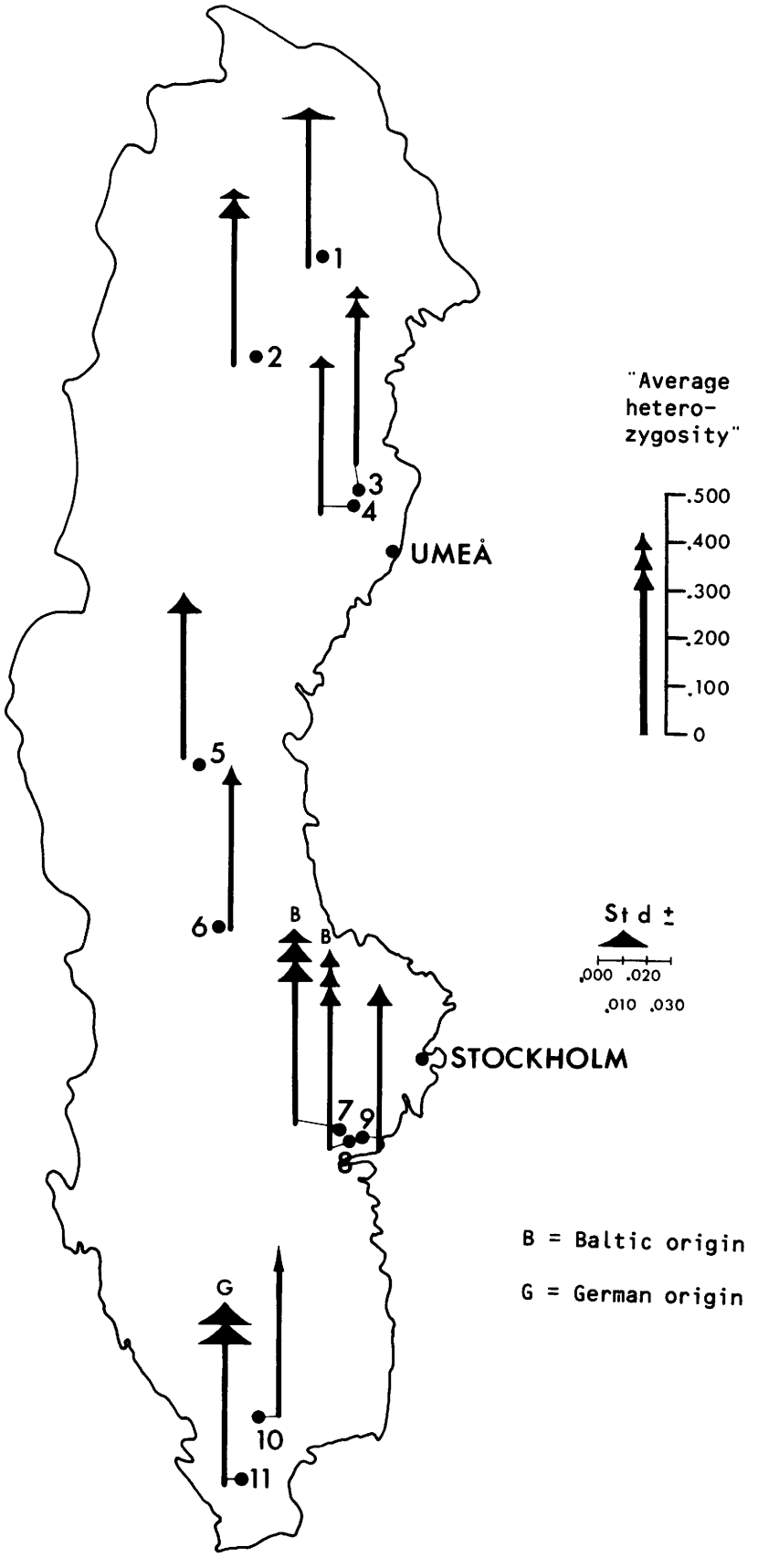
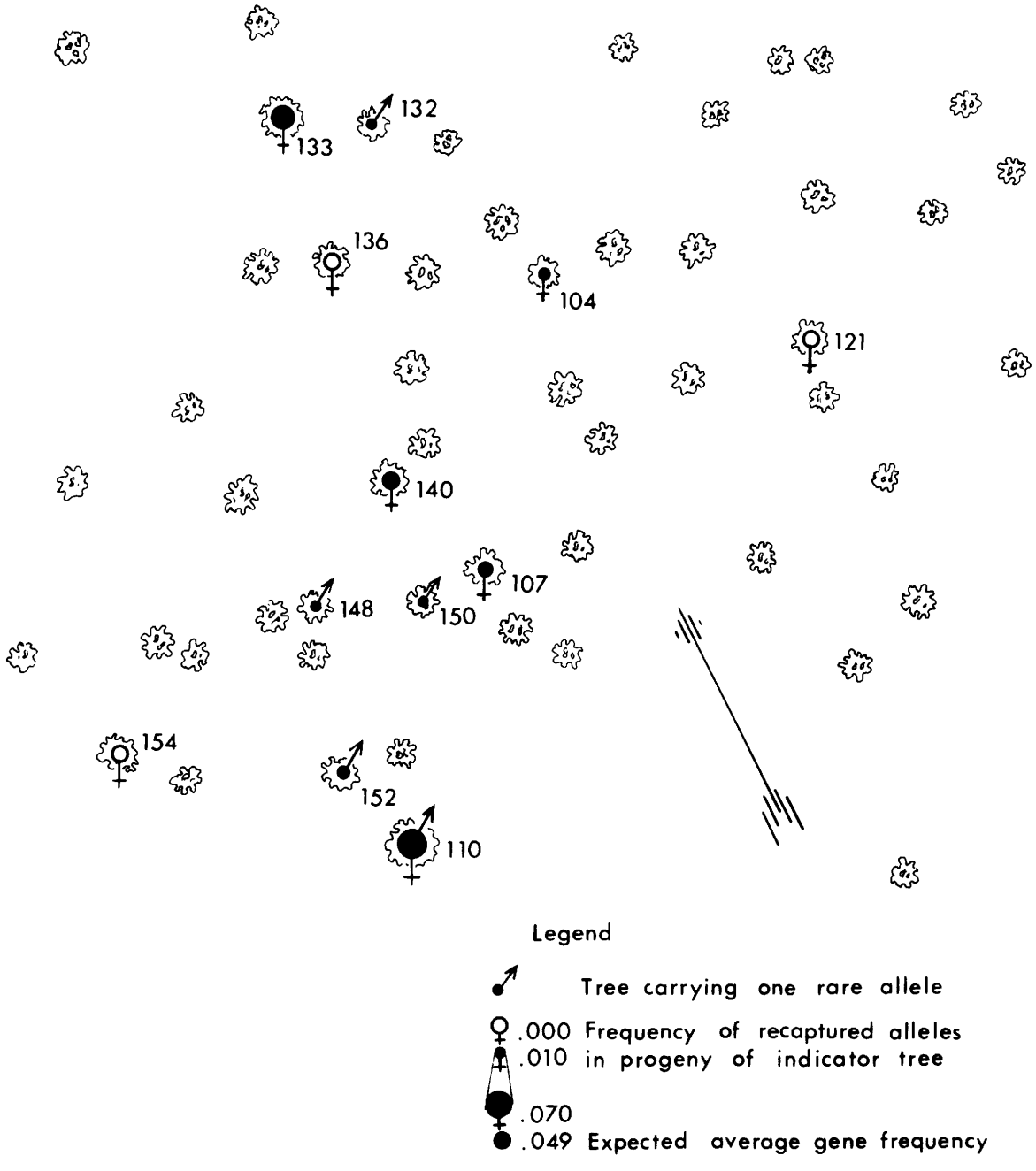


Figure 3. Effective fertilization capacity of the rare allele LAP-A3 on the experimental plot at Gårdstjärn in Northern Sweden.



Stand nr	Latitude	1 66°30'	2 65°53'	3 64°28'	4 64°24'	5 62°26'	6 61°09'	7 59°06'	8 58°57'	9 58°57'	10 56°31'	11 56°01'
1	66°30'	-										
2	65°53'	.019	-									
3	64°28'	.017	.013	-								
4	64°24	.012	.009	.002	-							
5	62°26'	.039	.012	.012	.013	-						
6	61°09'	.028	.018	.014	.014	.008	-					
7	59°06'	.063	.037	.030	.032	.012	.013	-				
8	58°57'	.044	.026	.018	.018	.013	.011	.005	-			
9	58°57'	.031	.011	.013	.011	.007	.010	.013	.009	-		
10	56°31'	.035	.014	.010	.011	.004	.005	.010	.008	.006	-	
11	56°01'	.111	.054	.064	.063	.034	.050	.020	.031	.035	.033	-

Table 1. Values of genetic distance (D) between the 11 Swedish populations of *Picea abies* calculated over 4 loci. Stand No. 7 and 8 are of Baltic origin and No. 11 of German origin. (From LUNDKVIST and RUDIN 1976).

SESSION		PAPER	
SEANCE	II	DOCUMENT	6
SITZUNG		DOKUMENT	

IDENTIFICATION OF FOREST SEED ORIGIN BY MEANS OF ISO - ENZYME GENE FREQUENCIES

L'IDENTIFICATION DE L'ORIGINE DES SEMENCES FORESTIERES BASEE SUR LES FREQUENCES DES ISO - ENZYMES DES GENES

IDENTIFIZIERUNG DER HERKUNFT DES FORSTLICHEN SAATGUTS MIT HILFE DER ISO - ENZYM GENFREQUENZEN

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SUMMARY

In forestry, methods are required which are capable of exactly distinguishing individual provenances, and subsequently, identifying the origin of unknown material. However, the various experimental procedures so far introduced as "early tests" are not applicable in all cases, and have repeatedly led to doubtful results. Therefore, a new method has been developed, which is based only on data of the population-specific gene pool. These data consist of gene frequencies determined at six gene loci, which are coding for isoenzymes of three different enzyme systems (EST, LAP, SAP). These polymorphic isoenzyme systems function as so-called gene markers.

Testing this method with seed samples of 15 Norway spruce provenances from Sweden, Finland, Poland and Germany it could be shown that the occurrence and frequency of individual isoenzyme genes enable a clear differentiation of the species in its natural range to be made. Thus, a sufficient characterization of these populations was possible with the aid of the gene frequency distributions. The data obtained may allow for the subsequent identification of unknown material. For general application of this method, additional isoenzyme gene loci must be included in the investigation.

RESUME

Les méthodes expérimentales utilisées jusqu'ici pour identifier les provenances de graines forestières n'étaient pas valables dans tous les cas et ne fournissaient pas toujours des résultats sûrs. On a donc été amené à mettre au point une nouvelle méthode permettant de déterminer sans ambiguité toutes les provenances de graines forestières. Cette méthode est basée sur l'étude des groupements de gènes qui sont spécifiques de chaque population. Il est possible de chiffrer la fréquence de différents gènes - répartis sur 6 loci - en étudiant le polymorphisme des isoenzymes.

Cette nouvelle méthode a été testée sur 15 provenances d'épicéas de Suède, Finlande, Pologne, Allemagne; on a ainsi montré que d'après la présence et la fréquence de certains gènes, il était possible de différencier avec certitude les provenances aussi bien pour l'ensemble de l'aire que pour les différentes zones de dispersion. Les provenances étant ainsi caractérisées avec précision, il est également possible d'identifier sans ambiguité des échantillons de graines dont la provenance était inconnue.

ZUSAMMENFASSUNG

In Forstwissenschaft und praktischer Forstwirtschaft werden Methoden benötigt, die verschiedene Herkünfte genau unterscheiden können und die weiterhin eine Identifizierung unbekannter Herkünfte ermöglichen. Da die bislang eingeführten experimentellen Verfahren (sog. Frühtests) nicht immer anwendbar sind und auch wiederholt keine gesicherten Resultate gewährleisten, wurde nun eine neue Methode erarbeitet. Diese selektive Methode verwendet nur Daten des jeweils Populations-spezifischen Genpools, d.h. die entscheidenden Parameter sind einzelne Gene und deren Häufigkeiten. Diese Gene konnten anhand von Analysen bei verschiedenen Isoenzym-Systemen identifiziert werden. Bei der hier vorliegenden Untersuchung wurden insgesamt 6 Genloci bei 3 polymorphen Isoenzym-Systemen (EST, LAP, SAP) analysiert.

Bei der Erprobung dieser Methodik an 15 Fichtenherkünften aus Schweden, Finnland, Polen und Deutschland zeigte es sich, dass anhand von Vorkommen und Häufigkeit einzelner Gene eine klare Herkunfts-Differenzierung im gesamten Areal wie auch in den einzelnen Verbreitungsgebieten möglich ist. Die dadurch sich ergebende exakte Charakterisierung dieser Herkünfte sollte auch eine nachfolgende Identifizierung unbekannter Samenproben gewährleisten. Bei einer allgemeinen Verwendung dieser Methode zur Analyse zahlreicher Herkünfte sollten noch weitere Isoenzym-Genloci in die Untersuchung eingeschlossen werden.

IDENTIFICATION OF FOREST SEED ORIGIN BY MEANS OF ISOENZYME GENE FREQUENCIES

INTRODUCTION

Forest research work, and especially all types of provenance research and utilization, needs methods capable of identifying the origin or the provenance of the reproductive material of forest trees. Thus, it would be of great advantage if such methods, ("laboratory early tests"), could already be applied to seeds or very young seedlings, in order to obtain data before cultivation as to the origin or locality of the provenance. Later on, the "field early tests" performed with young plants should verify initial indications as to the origin of the respective seed lot, and furthermore, should provide information on the future development of those characters important for forestry.

However, nearly all experimental procedures so far introduced as laboratory early tests, are based on the analysis of quantitative characters, which are controlled by many genes and modified by environmental factors. Since many of these characters were found to show significant variation only between populations from areas with very different environmental factors, e.g. cold and moderate climate, the applicability of such tests was restricted. For example, provenances from different localities, but with similar climatic conditions, could not be separated. Additionally, it could not be ascertained in each case, as to whether the variation observed in a character was due to genetic variability or to environmental fluctuation only. In general, the methods so far employed in forestry are not reliable enough to allow for a clear characterization of most of the provenances of a tree species, and subsequently, for an identification of unknown seed or seedling material from these provenances. Therefore, it is necessary to develop a selective method capable of characterizing each natural forest tree population, so that a subsequent distinction and identification of provenances will commonly be possible.

One of the most specific attributes of each natural population is its gene pool, i.e. its integrated gene composition, which has been developed through natural selection in a specific manner depending on the relevant

factors of its environment. Consequently, the data of its gene pool, i.e. genes and their frequencies, should enable a sufficient characterization of a natural forest tree population to be made. Hence, the search must be for those characters which are directly related to single gene loci and therefore can be designated "gene markers".

The isolation of such gene markers is nowadays possible, even in forest trees, by the application of modern biochemical methods, as for example has been demonstrated by the successful analyses of isoenzyme systems (see FERET and BERGMANN 1976). By means of electrophoretic procedures, numerous isoenzyme and protein systems can be analyzed, their variation patterns traced, and their genetic control identified. Since several isoenzyme systems have also been detected in the endosperm tissue of dry seeds, the identification of the different genes and the estimation of their provenance-specific frequencies can already be performed with untreated seed lots.

In the following report, I want to demonstrate how different provenances of Norway spruce (Picea abies) are being characterized by means of the gene (allele) frequencies at six isoenzyme gene loci with the aim of reaching the possibility of assigning an unknown seed sample to its original provenance.

MATERIAL

The test material investigated consisted of seed lots from 15 Norway spruce provenances which are located in Sweden, Finland, Poland and Germany. The geographical data for these provenances are given in Table 1. Each provenance sample consisted of 2-3000 seeds and was collected from 20 or more trees. Of each sample, 200 seeds per enzyme system were analyzed, since previous tests showed that this quantity was sufficient for providing representative values.

The experimental procedures (enzyme extraction, electrophoretic separation, isoenzyme staining) and the genetic analyses of the isoenzyme variation have been described in detail in the following publications (BERGMANN 1973, 1974; FERET and BERGMANN 1976).

Table 1

Geographical description for 15 spruce provenances

Provenance	Region/Country	Lat.°	Long.°	Altitude
Kolari	Länsipohja (North Finland)	67°16'	23°51'	150 m
Pihtipudas	Suomenselkä (Central Finland)	63°17'	25°27'	160 m
Tuusula	Salpausselkä (South Finland)	60°21'	24°59'	50 m
Kåbdalis	Norrbotten (North Sweden)	66°11'	20°03'	410 m
Sörliden	Norrbotten (North Sweden)	65°49'	19°14'	450 m
Bergsjö	Hälsingland (Central Sweden)	62°01'	16°58'	60 m
Västanfors	Västmanland (South Sweden)	59°57'	15°49'	120 m
Kartuzy	Gdansk (North Poland)	54°23'	18°08'	200 m
Tarnawa	Waldkarpaten (South-east Poland)	49°05'	22°52'	750 m
Westerhof	Harzvorland	51°45'	10°08'	200 m
Zwiesel-Ost	Bayerischer Wald	49°03'	13°15'	900 m
Hanselehof	Neustadt (Südl. Schwarzwald)	47°52'	8°18'	1250 m
Eschenmoos	Schluchsee (Südl. Schwarzwald)	47°49'	8°07'	1150 m
Seegatterl	Reit i. W. (Chiemgauer Alps)	47°41'	12°28'	1000 m
Ruhpolding	Chiemgauser Alps	47°45'	12°39'	900 m

RESULTS

I. Genetic variation at six isoenzyme gene loci

Of all isoenzyme systems so far investigated in our laboratory, the esterases (EST), leucine aminopeptidases (LAP) and acid phosphatases (SAP) were found to exhibit the greatest variability in nearly all regions of the natural Norway spruce range, so that these threee systems appear to be primarily suitable for provenance characterization. The variation patterns of each isoenzyme system are controlled by two polymorphic gene loci, whereby the degree of variability results from the number of alleles occurring at each distinct gene locus. While the two EST loci possess only two or three alleles, four or five allelic types were observed at the LAP and SAP loci. A compilation of all isoenzyme gene loci and alleles used in this investigation is found in Table 2.

TABLE 2 - COMPILATION OF ISOENZYME LOCI AND ALLELES

Isoenzyme loci	Alleles
EST-A	EST-A_1, -A_2
EST-B	EST-B_1, -B_2, -B_3
LAP-A	LAP-A_1, -A_2, -A_3, -A_4
LAP-B	LAP-B_1, -B_2, -B_3, -B_4
SAP-A	SAP-A_1, -A_2, -A_3
SAP-B	SAP-B_1, -B_2, -B_3, -B_4, -B_5

II. Distribution of isoenzyme-gene frequencies within the provenances.

All isoenzyme loci are characterized by a considerabl variation, i.e. they possess, with a few exceptions, two or more alleles in the provenances investigated. Only the EST-A locus exhibits monomorphism in four cases. The distribution of allele frequencies of the six isoenzyme loci in the 15 spruce provenances is shown in Table 3. It can easily be seen that the generally predominant alleles of all gene loci are present in all populations, whereas only the more infrequent alleles are absent in several cases. However, the frequency distribution patterns of the different provenances are not identical, and in several cases, they differ widely, indicating the

existence of very dissimilar gene pools. In particular, both LAP loci and the SAP-B locus reveal large differences in allele frequencies between individual provenances.

The SAP-B system is characterized by a marked environmentally-dependent variation, because two alleles (SAP-B_1, -B_2) are closely correlated with cold climate and two others (SAP-B_3, -B_4) with moderate climate. The frequencies of the alleles SAP-B_1 and SAP-B_2, as well as SAP-B_3 and SAP-B_4, were combined (Table 3) in order to obtain larger and more pronounced values, since no essential frequency differences could be found between these allelic types, respectively.

III. Genetic differentiation of the spruce provenances

A clear characterization and subsequent identification of an arbitrary provenance is based on the capability of genetically differentiating most or all populations under study. This requires a system capable of separating the whole tree range into different regions and subdividing each region into individual provenance zones or provenances. Such a system is here represented by the different gene frequency distributions.

The provenances here investigated can primarily be divided into two main groups; one forms the Scandinavian spruce region and the other the Central European region. These two regions differ significantly in two gene pool data -1) all Scandinavian populations so far studied lack the LAP-B_4 allele, whereas it is always observed in European spruce stands; 2) the allele LAP-A_1 predominates in the northern populations, but in all other provenances, its frequency is lower than that of LAP-A_2 (Table 3). A further partition of the Scandinavian spruce range is possible, since several gene loci were found to exhibit a clinal variation of their allele frequencies. This dependence upon gradually changing climatic factors from the north to the south is a characteristic property of the EST-A and EST-B systems, and especially of the SAP-B system, where the allele group SAP-B_1/B_2 occurs with frequencies of 70 or more per cent in northern provenances and only with about 5 per cent in southern stands (Table 3).

The differentiation of the Central European spruce range does not reach the same extent as in Scandinavia, i.e. there is a lack of pronounced geographical division in horizontal direction, however, the genetic variation pattern

mostly results from differences in vertical direction. This is demonstrated by the fact that the greatest differentiation generally exists among provenances originating from very different elevations. In particular, the allele frequencies of the SAP-B locus characterize the different altitudinal zones. The provenance Westerhof, for example, possesses the allele group SAP-B_1/B_2 with a frequency of about 5 per cent, whereas other populations from higher elevations reveal frequency values of 20 or more per cent (Zwiesel-Ost/900 m: 23%, Seegatterl/1.000 m : 27% - Table 3). Furthermore, differences in frequency distributions of other izoenzyme genes help to discriminate even those populations which are located in similar or adjoining areas.

DISCUSSION

The investigation reported here should contribute to the efforts in developing a selective method for provenance distinction and identification. With the aid of biochemical procedures, it was possible to identify several isoenzyme systems, which could be utilized as gene markers. The gene frequency distributions at these izoenzyme loci enable a first differentiation of the provenances under study. It could be demonstrated that the spruce range is divisible into different regions according to several significant allele differences. Including all deviations between the individual gene frequency distributions, it must be possible to distinguish all provenances here selected.

However, the general application of this method implies the addition of further isoenzyme gene loci, thus increasing the number of gene pool data. After analyzing most of the provenance zones of a tree species, by the method here described, individual populations should become distinguishable. Subsequently, the assignment of unknown material to one of the analyzed provenances (provenance indentification) can be performed with great precision, since the allele frequency distributions at each isoenzyme gene locus of both samples must coincide statistically.

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Herkunft	EST - LOCI					LAP - LOCI							
	EST A		EST B			LAP A				LAP B			
	A_1	A_2	B_1	B_2	B_3	A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4
Kolari		100	80.1	19.9		53.6	31.7	14.7		65.2	34.8		
Pihtipudas	5.6	94.4	74.8	21.0	4.2	53.8	30.8	8.0	7.4	59.3	36.8	3.9	
Tuusula	6.7	93.3	66.1	29.0	4.9	52.6	35.0	8.1	4.3	64.4	32.3	3.3	
Kåbdalis	24.3	75.7	69.5	30.5		43.4	38.9	13.3	4.4	42.9	49.3	7.8	
Sörliden	16.3	83.7	65.6	31.4	3.0	48.1	32.1	14.1	5.7	45.0	47.8	7.2	
Bergsjö	5.6	94.4	62.7	37.3		46.9	36.1	10.4	6.6	50.5	41.6	7.9	
Västanfors	4.1	95.9	54.4	45.6		46.7	37.1	7.4	8.8	51.8	39.1	9.1	
Kartuzy	4.2	95.8	58.5	41.5		43.3	56.7			53.3	26.8		19.9
Tarnawa	8.3	91.7	66.7	33.3		24.2	65.9	4.7	5.2	63.1	29.9		7.0
Westerhof	4.8	95.2	54.8	41.2	4.0	49.2	50.8			69.0	19.3		11.7
Zwiesel-Ost	11.6	88.4	56.4	43.6		40.9	56.1		3.0	64.5	25.7	5.5	4.3
Hanselehof	3.3	96.7	52.6	42.1	5.3	33.7	56.3	3.3	6.7	65.7	23.5		10.8
Eschenmoos		100	60.9	39.1		33.3	60.0	3.3	3.4	50.2	29.7		20.1
Seegatterl		100	41.7	38.7	19.6	40.0	53.2	3.2	3.6	69.8	13.9	6.5	9.8
Ruhpolding		100	45.0	36.2	18.8	42.1	50.6	3.5	3.8	63.6	22.1	6.9	7.4

Table 3

Allele frequencies at six isoenzyme loci in 15 spruce provenances (in %)

Table 3 cont.

Herkunft	SAP - LOCI					
	SAP A			SAP B		
	A_1	A_2	A_3	B_1+B_2	B_3+B_4	B_5
Kolari	32.2	67.8		53.5	42.9	3.6
Pihtipudas	37.5	62.5		19.2	80.0	
Tuusula	33.9	62.9	3.2	4.8	95.2	
Kåbdalis	37.5	58.3	4.2	78.0	15.3	6.7
Sörliden	27.1	68.0	4.9	74.2	20.2	5.6
Bergsjö	36.3	60.0	3.7	48.1	51.9	
Västanfors	39.0	54.6	6.4	30.4	66.2	3.4
Kartuzy	33.3	63.1	3.6	30.0	70.0	
Tarnawa	38.8	61.2		33.3	66.7	
Westerhof	34.1	65.9		4.5	95.5	
Zwiesel-Ost	30.2	66.1	3.7	23.4	76.6	
Hanselehof	26.7	73.3		22.6	77.4	
Eschenmoos	28.8	71.2		19.7	80.3	
Seegatterl	28.7	67.5	3.8	26.8	73.2	
Ruhpolding	32.1	64.3	3.6	18.9	81.1	

SESSION PAPER
SEANCE II DOCUMENT 7
SITZUNG DOKUMENT

EXPERIENCES OF IDENTIFYING GENOTYPES USING THE ISOENZYME TECHNIQUE.

EXPERIENCES EN MATIERE D'IDENTIFICATION DES GENOTYPES BASEE SUR LE TECHNIQUE ISOENZYMIQUE.

ERFAHRUNGEN DIE AUF DEM GEBIET DER IDENTIFIZIERUNG DER GENOTYPEN MIT HILFE DER ISOENZYMTECHNIK GEMACHT WORDEN SIND.

H-J MUHS

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SUMMARY

With the aid of examples of isoenzyme patterns from Saccharomyces cerevisiae, Drosophila melanogaster and the results from studies with some forest trees, the value as well as the limits and possibilities of the isoenzyme technique are demonstrated. A scheme is given summing up some modifying factors which possibly influences the expression of a gene controlling an isoenzyme pattern.

RESUMÉ

La valeur ainsi que les limites de la technique des isoenzymes sont explicitiés au moyen de schémas de concentrations d'isoenzymes de Saccharomyces cerevisiae et de Drosophila melanogaster ainsi qu'au moyen de résultats d'études entreprises sur quelques arbres forestières. Un modèle résume l'influence possible de certaines facteurs sur la représentativité d'un gène liée au shéma d'un isoenzyme.

ZUSAMMENFASSUNG

Anhand von Beispielen einiger Isoenzymmuster von Saccharomyces cerevisiae und Drosophila melanogaster sowie einige Ergebnisse von Untersuchungen mit Forstpflanzen wird die Brauchbarkeit ebenso wie die Grenzen und Möglichkeiten der Isoenzymtechnik dargelegt. Ein Schema wird gegeben, in dem mögliche Ursachen von Modifikationen zusammengestellt werden, die die Expressivität eines für ein Isoenzym codierendes Gen beeinflussen.

EXPERIENCES OF IDENTIFYING GENOTYPES USING THE ISOENZYME TECHNIQUE

In 1968 the isoenzyme technique was introduced into the laboratory of the Institute at Schmalenbeck by the author, who had learned this technique from Rasmusson and Rudin at Umeå/Sweden. Since that time many experiments on different subjects using this technique have been carried out. The author has worked mainly with Drosophila melanogaster and during his stay at the Institute of Forest Botany at the University of Freiburg from 1971 to 1976, with poplar, Norway spruce, and Douglas-fir. At the Institute of Forest Botany he had the chance of introducing the isoenzyme technique into yeast genetics, which was well established there.

Isoenzymes of the alcoholdehydrogenases of yeast have been extensively dealt with by M. von Ciriacy, to whom the author is indebted for the data from yeast genetics.

The objective of most of the studies was the exact identification of the individual or genotype.

This was true for the studies with forest trees. There, mostly needles or leaves were used, to check the variation of isoenzyme patterns when influenced by various environmental factors.

In other studies an exact identification was the objective, for example as with the studies on population genetics with Drosophila.

The aim of this brief paper is to summarize some outstanding examples of isoenzyme patterns which may lead to misinterpretation, if the causes of variation are not known. Here, examples of yeast (Saccharomyces cerevisiae) and Drosophila melanogaster are included, to demonstrate that the genetical source of variation is very difficult to explain in some cases. As yeast and Drosophila are also eukaryotes, such genetical variation may also occur in higher plants like forest trees. The type of variation is qualitative and in special cases even quantitative. A few examples of forest trees presented here mostly show quantitative variation. Although the genetic control of isoenzyme patterns is not known, the patterns have been studied in relation to the different physiological stages of the cell or tissue caused by different factors. Variation of this type is

troublesome when trying to identify the "genotype".

Finally a simplified scheme will be discussed showing the different factors which modify the expression of the gene that controls the isoenzyme pattern. The scheme will illustrate the limits as well as the possibilities of the isoenzyme technique as applied to forestry problems.

Since the participants of the symposium are familiar with the main principles of the isoenzyme technique there is no need to explain it. But it should be pointed out once more that this technique is a most powerful tool, even if the following examples may raise some doubts about its practical value because of the many interpretations given. It should be borne in mind that the various ways of interpreting the various cases are a measure of the sensitivity of the technique and thus its value.

For the general application of this technique, see Feret and Bergmann (1976).

The examples presented are divided into three groups according to the subjects investigated. The first group contains yeasts.

1. The wild-type isoenzyme pattern of the alcoholdehydrogenase of yeast (Saccharomyces cerevisiae) consists of up to nine bands, four of them in the anodal region of the gel and five of them near the origin, (Figure I). (Nomenclature and all figures of ADH from yeast, in some cases modified, after von Ciriacy, 1975).

1.1 ADH I and ADH II are native enzymes, while ADH II' is probably a conformational isoenzyme to ADH II; i.e. ADH II modified by e.g. substances associated with it, and thus changing its electrophoretic mobility. Isoenzymes of this type, which appear mostly as subbands of a main band are obviously not rare. Their recognition and identification as conformational isoenzymes is rather difficult. Genetical as well as biochemical methods are needed. With regard to the interpretation of the isoenzyme pattern, conformational isoenzymes are easily misinterpreted as native enzymes controlled by a different gene from that gene controlling the main band.

1.2 The isoenzymes of m-ADH are probably conformational isoenzymes, too. The band containing the native enzyme cannot be identified. All five bands seem to be conformational (Ciriacy, 1975)possibly associated with fragments of structural proteins or membranes. The enzyme is located in the mitochondria but is controlled by the gene of the nucleus. The homogenizing method heavily influences the pattern of the m-ADH. If the mitochondria are not disturbed by homogenizing, no bands can be obtained. In some cases only two or three bands (probably the bands located in the middle) can be stained. If "Alcoa" instead of glass beads is added before homogenization, strongly stained bands can be obtained. This pattern of the m-ADH can easily be misinterpreted as representing native enzymes controlled by two alleles of the same locus, so that the three bands in the mid position are suggested as hybrid enzymes of a tetrameric structure as postulated by Fowler et al. (1972).

1.3 The isoenzyme pattern shown in Fig. I can only be obtained if the cells grow in a glucose-free medium. Then the ADH II (and the ADH II') and the ADH I/II bands are not repressed by glucose. If a glucose containing medium is used, these bands are repressed and no staining can be detected in the zymogram. After the glucose has been consumed, these bands become derepressed again.The ADH I band can be stained all the time since the enzyme is constitutive.

Misinterpretation may occur if the repression of ADH isoenzymes is not known and if extracts of both repressed and derepressed cells are used in the same study.

1.4 The study of the segregation ratios by tetrad analysis shows that all three loci controlling the ADH I, ADH II and m-ADH are unlinked and that the ADH I/II band must consist of a hybrid-enzyme controlled by the allele ADR 2 of one locus (ADR 2) and the allele ADC of the other locus (ADC). V. Ciriacy (1975) shows additional criteria for this hypothesis.

The far right pattern in Fig. III containing the hybrid band ADH I/II is difficult to explain because (1) the staining intensity of the hybrid band is usually twice as great as that of the corresponding homomeric isoenzymes, and (2) three hybrid bands are expected instead

of one, since the ADH is a tetrameric enzyme. The right interpretation obviously is :- the hybrid enzymes consisting of three and one, or one and three, sub-units of both enzymes cannot come into existence probably because of the steric structures. The reduced activity of the existing hybrid enzyme may have the same exlanation. Misinterpretation of the above pattern as dimeric rather than tetrameric is possible, if the enzyme structure is not known. In the case in point the enzyme structure is known and can be demonstrated (see below).

1.5 The ADR 2 locus can be shown to be the structural gene controlling the ADH II isoenzymes. Different alleles for the ADR 2 locus have been found by v. Ciriacy (1975).

Case (1) : Two codominant alleles control two isoenzymes with different electrophoretic mobility : ADH II F and ADH II S. In heterozygotes, 3 hybrid enzymes exist. The staining intensity is consistent with the probability of a free aggregation of the two different sub-units to form tetrameric enzymes (1 : 4 : 6 : 4 : 1), (see Fig. IV left).

Case (2) : The allele adr 2 - f 2 is a recessive allele not able to form sub-units to aggregate with sub-units of the ADR 2 S allele. The heterozygote therefore contains only one band and cannot be distinguished from the dominant homozygote.

Case (3) : The allele adr 2 - f 13 is a recessive allele forming an inactive sub-unit which aggregates with the sub-unit formed by the ADR - S allele. Three intergenic hybrid enzymes exist, but the total activity of all isoenzymes including the active homomeric one, is only about 25%. V. Ciriacy (1975) presented the following interpretation :- sub-units aggregate freely while the hybrid enzymes have reduced activity because of the interaction of the sub-units (see Fig. IV). Misinterpretations are not possible, except for case (2) (see below). These three cases are presented to demonstrate the different types of alleles.

1.6 In addition to the structural gene ADR 2 controlling the ADH II isoenzymes, another locus controls the expression of the structural gene and thus the appearance of these isoenzymes. V. Ciriacy found different alleles (controlling wild type, low or no activity) and characterized this locus as having a regulatory function with regard to the ADR 2 locus. In special cases, the isoenzyme pattern controlled by the wild type or recessive allele of the ADR 1 locus cannot be distiguished from that controlled by the wild type or recessive allele of the ADR 2 locus (structural gene), see case (2) in 1.5.

Misinterpretation may be as follows :- the recessive allele of the regulatory gene has the same effect as the recessive allele of the structural gene, and this can only be distinguished by additional genetical analysis.

2. Some examples from Drosophila melanogaster may demonstrate that the interpretation of isoenzyme patterns from higher organisms is not any easier. The first two examples deal with leucine aminopeptidases (LAP), while the last deals with unspecific esterases.

2.1 Codominant alleles of the LAP, D locus are shown in Fig. V, left. The heterozygote contains both bands DF and DS but with about half the intensity of that of the homozygotes. The total activity adds up to about the same value as that of the homozygotes. The enzyme structures are suggested to be monomeric, thus hybrid enzymes do not exist.

On the right of the figure, a suggested recessive homozygote DO/DO (without any staining in the D region) and a heterozygote DO/DS, with half the staining intensity of that of the homozygote, are shown. The reduced staining intensity of the heterozygote may be due to a dosage effect. As the recessive homozygote DO/DO has a very strong detrimental effect (probably most insects die during pupation), segregation ratios are not in accordance with simple Mendelian ratios. Therefore, the suggestion of the recessive allele could not be proved.

Misinterpretation may occur, if the recessive homozygote suggested, cannot be found. Then the heterozygote may be interpreted as homozygous for an allele controlling an isoenzyme with equal electrophoretic mobility but with reduced activity which produces a detrimental effect.

2.2 Fig. VI presents some isoenzyme patterns which occur in addition to that shown in Fig. V. These patterns are rare and homozygous strains for one of the patterns could not be obtained, because of a possible detrimental effect caused by reduced activity. The occurrence of the heterozygote DF/DS with low activity, individuals showing only a trace of staining in the DF region, and the absence of the E and F bands suggests that a complex regulation may be responsible for these patterns. So far, no plausible interpretation exists. The occurrence of these isoenzymes with reduced activity is rather inconvenient, if the LAP-D locus is analysed or the gene frequencies of the LAP-D alleles in populations are investigated.

2.3 At the Est - 6 locus coding for unspecific esterases, three codominant alleles exist - 6F, 6S and $6F^h$. Their corresponding isoenzymes have respectively, high, low and high electrophoretic mobility; the isoenzymes 6F and $6F^h$ have equal mobility. Therefore, the latter two isoenzymes cannot be distinguished by electrophoresis. They differ in heat sensitivity (MacIntyre & Wright, 1966) and thus heat treatment of the extracts or the unstained gels can distinguish between them.

Misinterpretation may occur if the extracts or gels are exposed to more or less high temperatures for a longer time, because the heat sensitive isoenzyme will lose its activity.

3. With higher plants, especially perennials like forest trees, additional difficulties have to be considered. Different physiological stages occur during the life cycle and during seasons etc. even in the same organs. Thus we have to ask: how do different physiological stages of the cells/tissue influence the isoenzyme patterns in such a way that misinterpretation may be possible? In Fig. VII some of the principle factors causing

physiological changes are listed. We know that for instance age and differentiation (tissue - specific isoenzyme patterns), diseases, hormones and herbicides do influence the isoenzyme pattern, in some cases very markedly. The author is working with Douglas-fir (poplar and Norway spruce) using leaves from clones to test the influence of different sites and seasons on the peroxidase isoenzymes, (esterase patterns were not reproducible at all times, because some plants suffered too much from the extreme site conditions chosen for this study).

Briefly the results can be summarized as follows :

(a) Effects of site seemed to be rather small in this investigation. Differences in patterns and staining intensities of the bands between different sites seemed to be more influenced by climatic factors than by soil factors, except that nutrient-deficient soils resulted in poorly stained patterns.

(b) Effects of season were large. During the year certain bands appeared and disappeared and staining intensities varied extremely. Best patterns could be obtained in winter and spring (from end of December to May). During June to August peroxidase patterns were rather weak and sometimes could hardly be identified, especially with those extracts from dry sites.

(c) The effect of climatic factors could not be investigated because of the lack of a phytotron. But during extremely high temperatures or thundery conditions, weak and tailed bands could be observed, which could not be identified in all cases.

Although it is rather difficult to distinguish between the different influencing factors, the main factor seems to be the physiological condition of the plant. Plants in visibly poor condition i.e. with yellowish and short needles, resulted in weak and tailed isoenzyme bands. The factors causing the bad conditions seem to be less important, for example extremely hot weather has the same or nearly the same effect as dryness or a deficit of nutrients.

Thus, when identifying genotypes by the isoenzyme technique, we must look for well-conditioned material if misinterpretation is to be avoided.

4. To summarise the possible modifying factors which could influence the expression of a gene controlling an isoenzyme pattern, in Fig. VIII a tentative scheme is given which may demonstrate the complexity and interaction of different factors. The scheme does not cover all possibilities.

The transcription of the structural gene coding for an isoenzyme pattern is probably regulated by some other genes. This process is denoted in the scheme as regulation of the transcription. By this, direct regulation is meant, (for instance by the operator) excluding other factors like repression which have an extra position in the scheme. The expression of a gene may be codominant, dominant, partially dominant, recessive or it can be influenced by the different modifying factors in a more or less quantitative way in so far as the amount and activity of the isoenzymes controlled which can be detected as an isoemzyme pattern are concerned.

Some genetical factors acting at the molecular level modify the expression. Suppression may have an influence in a rather complex way, e.g. a mutation of a gene coding for the RNA polymerase may be lethal or may result in suppression. In this case all loci are concerned and additional effects via epistasis can be expected. Repression is a way of regulating the gene activity, e.g. by specific molecules, substrates or products of the enzyme, by influencing directly or via epistasis the amount or activity of the enzyme. Here we have to include regulation by hormones and other substances.

Although suppression and repression have epistatic effects they have been included, since they occupy key positions where the expression of isoenzyme patterns is concerned - regulation by regression is the most important. Besides these factors, others are also known to result in epistatic effects such as intergenic interaction of two or more structural loci (see the intergenic

hybrid enzymes in Figs. II and III). Epistatic effects may modify the isoenzyme pattern directly as well as in relation to the amount and/or activity of the isoenzymes.

The regulation directly influences the different physiological characteristics of the cell, tissue or organ which are thus the expression of developmental differentiation, tissue specificity, stress, climatic and edaphic impacts and others (see Fig. VII). In many cases the amount and activity of the isoenzymes are affected, whereby usually allelic ones react in the same way and non allelic ones possibly in a different way. In some cases different physiological stages may have an influence on the isoenzyme pattern via the enzymes' properties, e.g. causing aggregation into polymeric units or disassociation into non-active sub-units.

Properties of the isoenzymes, especially the sub-unit structure, are of great importance for the interpretation of the pattern. But their specificity to the artificial substrates mostly used for staining, heat stability and other properties may also influence the pattern. Enzymes, which are associated with other enzyme-forming systems or with structural proteins or membranes, are able to induce conformational isoenzymes.

Besides the molecular genetical processes like suppression and repression, cytogenetical factors are also involved since they have a modifying effect on isoenzyme patterns. A changing of the serial gene system, for instance translocation and inversion, should not have any effect unless the regulation of the gene activity is disturbed. In such cases recessive mutation of the structural gene under study can be simulated.

The degree of ploidy is usually of low importance if only one allele exists. But if different alleles are present, the changing of the degree of ploidy by meiotic division or fertilization is the most effective way of analyzing the isoenzyme pattern (see Figs. I - V). Higher degrees of ploidy originating from endomitosis which can be found in the tissues of different higher plants may have no effect on the isoenzyme pattern. If the higher degrees of ploidy resulted from fertilization additional real alleles could occur.

When two or more nuclei per cell are present, the number of additional homologous alleles increases and may have the same effect as with higher degrees of ploidy. This can be of interest when working with certain fungi.

Finally, the methodical factors should be mentioned since they can have influences ranging from slight modifications to the destruction of the isoenzymes.

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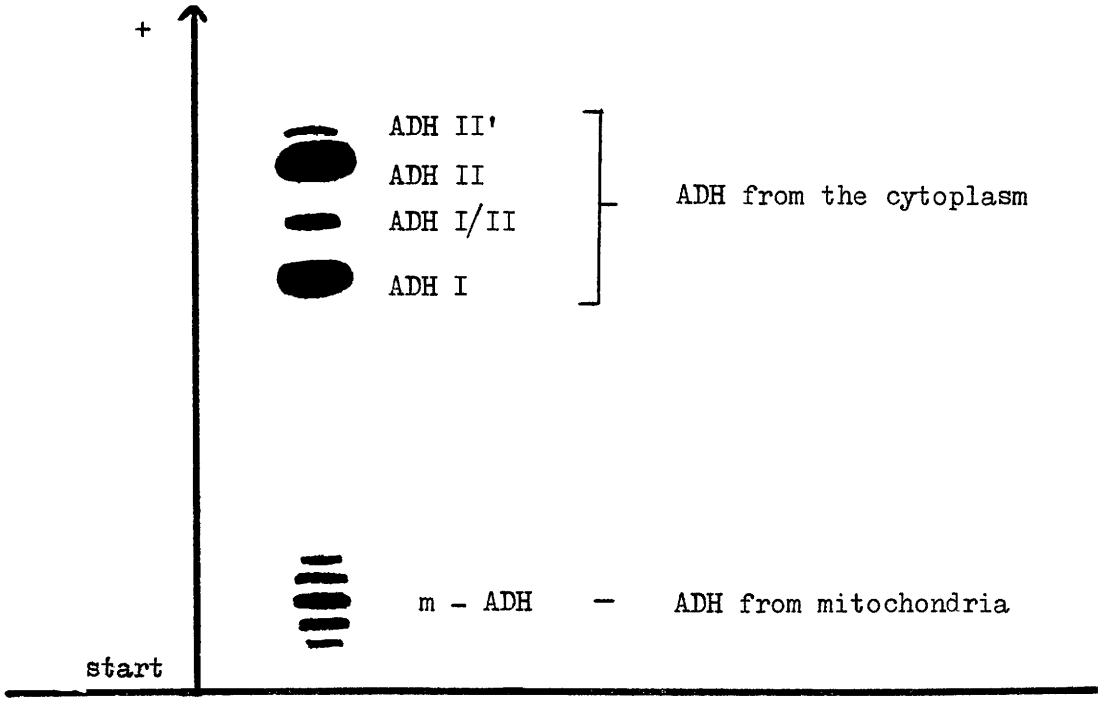
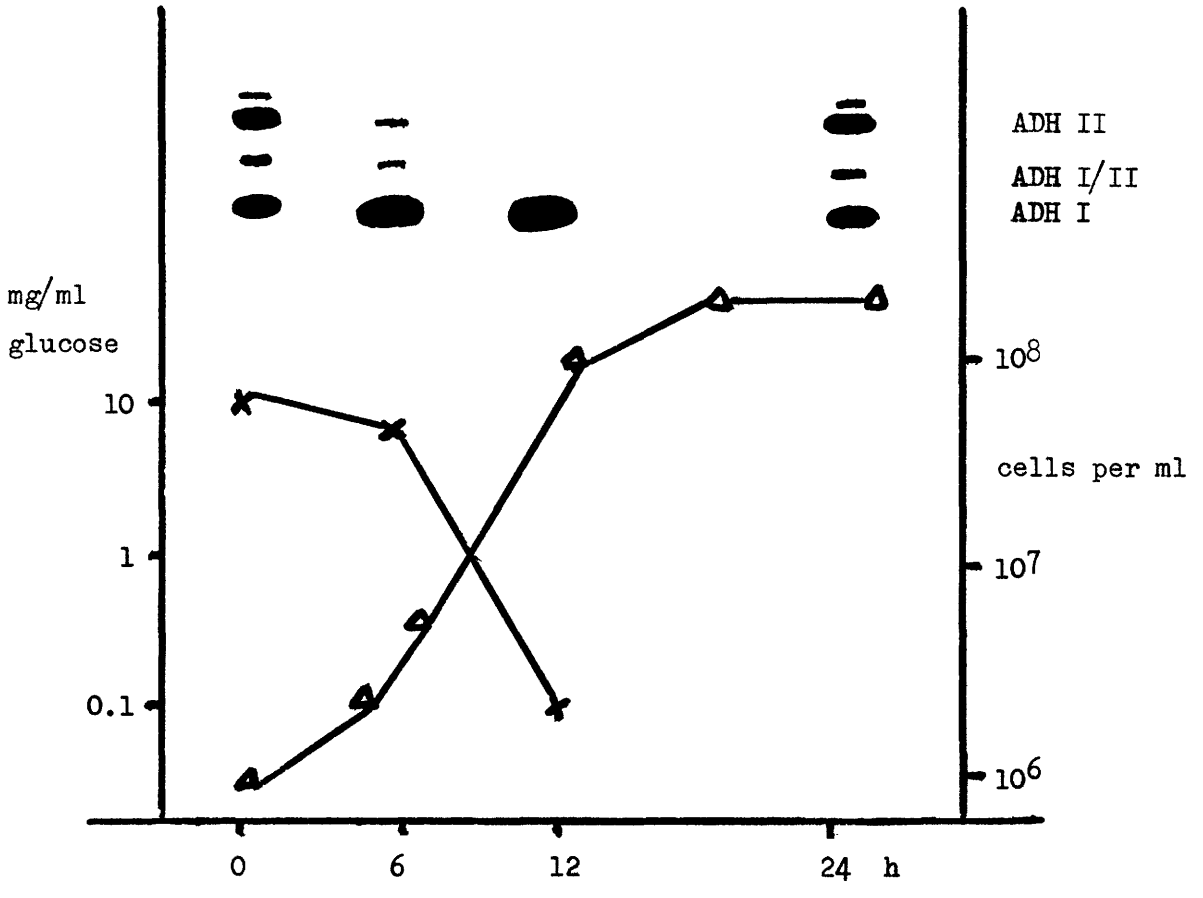
MacIntyre, R.J., and Th. R. F. Wright, 1966 : Responses of esterase 6 alleles of Drosophila melanogaster and D. simulans to selection in experimental populations. Genetics 53, 371 - 381.

Fig. I. Wild-type isoenzyme pattern of the alcoholdehydrogenase (ADH) of yeast. (after v. Ciriacy, 1975)



a) haploid and diploid cells have identical patterns.
b) ADH II' is a conformational isoenzyme to ADH II.
c) ADH I/II is a hybridenzyme, see Figs. II and III.
d) m - ADH bands appear in up to 5 bands, weakly stained.

Fig. II. Repression and Derepression of the ADH II by glucose, wild-type pattern. (after v. Ciriacy, 1975).

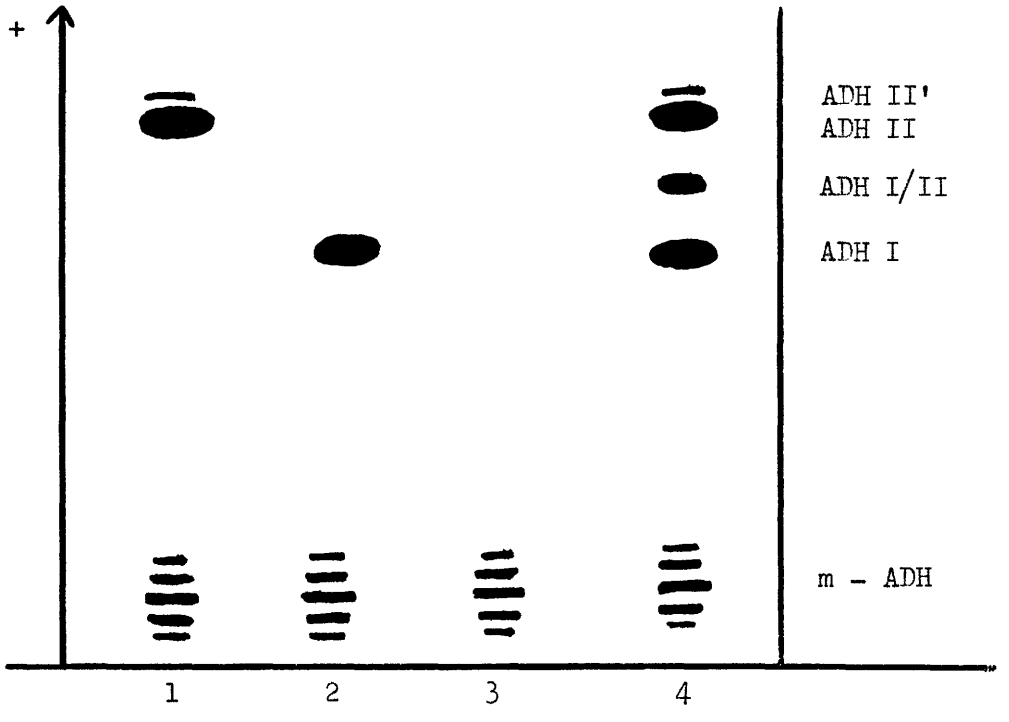


Start at 0 h with derepressed cells.

After 6h staining intensity of repressed ADH II and ADH I/II isoenzymes are rather low and are missed at 12h.

After 24h cells are derepressed, ADH II and ADH I/II show activity.

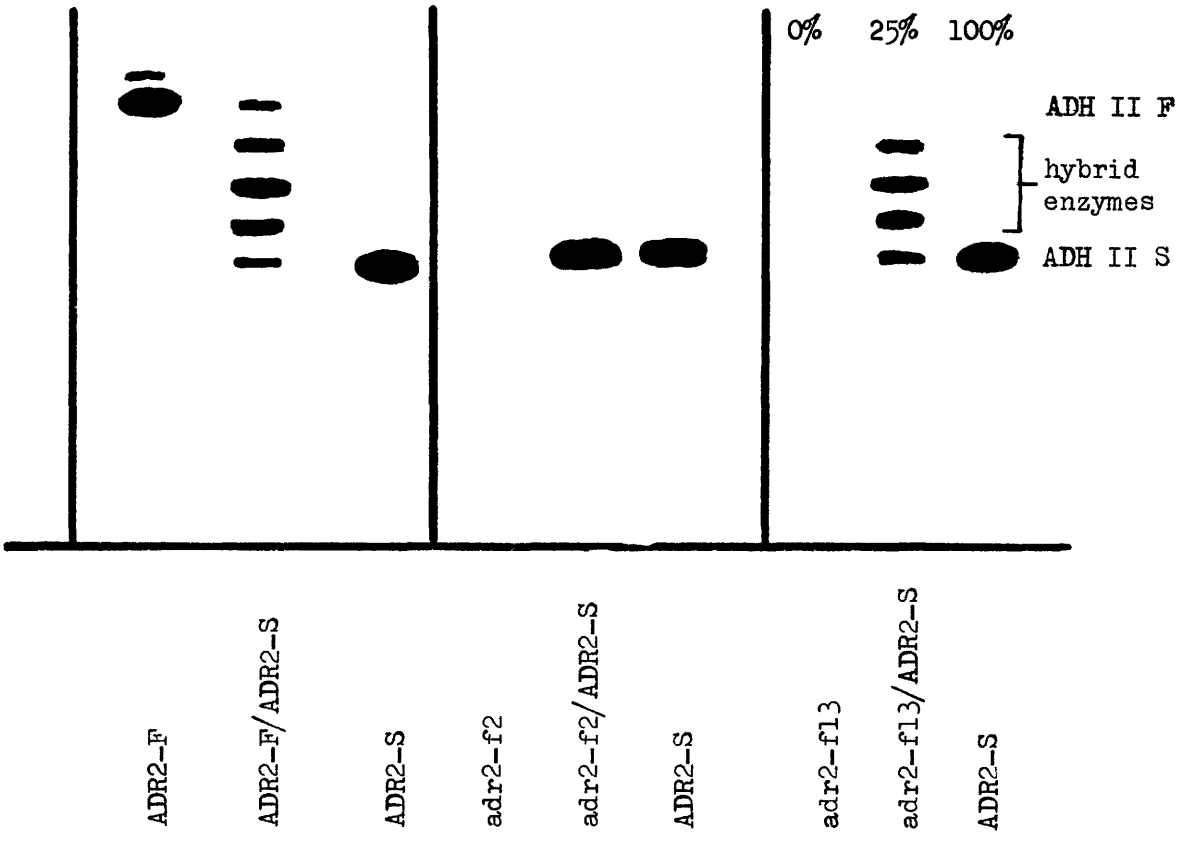
Fig. III. Segregation of ADH bands, example of a tetrad analysis, haploid cells. (after v. Ciriacy, 1975).



1 = genotype: ADR 2, adc, ADM
2 = genotype: adr 2, ADC, ADM
3 = genotype: adr 2, adc, ADM
4 = genotype: ADR 2, ADC, ADM

ADR = dominant allele controlling ADH II and ADH II' bands.
adr = recessive allele of the same locus.
ADC = dominant allele controlling ADH I band.
adc = recessive allele of the same locus.
ADM = dominant allele controlling the m - ADH.
ADH I/II is an intergenic hybrid enzyme.

Fig. IV. Codominant and recessive alleles of the ADR 2 locus. (after v. Ciriacy, 1975)



Homozygotes and haploids of the genotype have identical patterns.

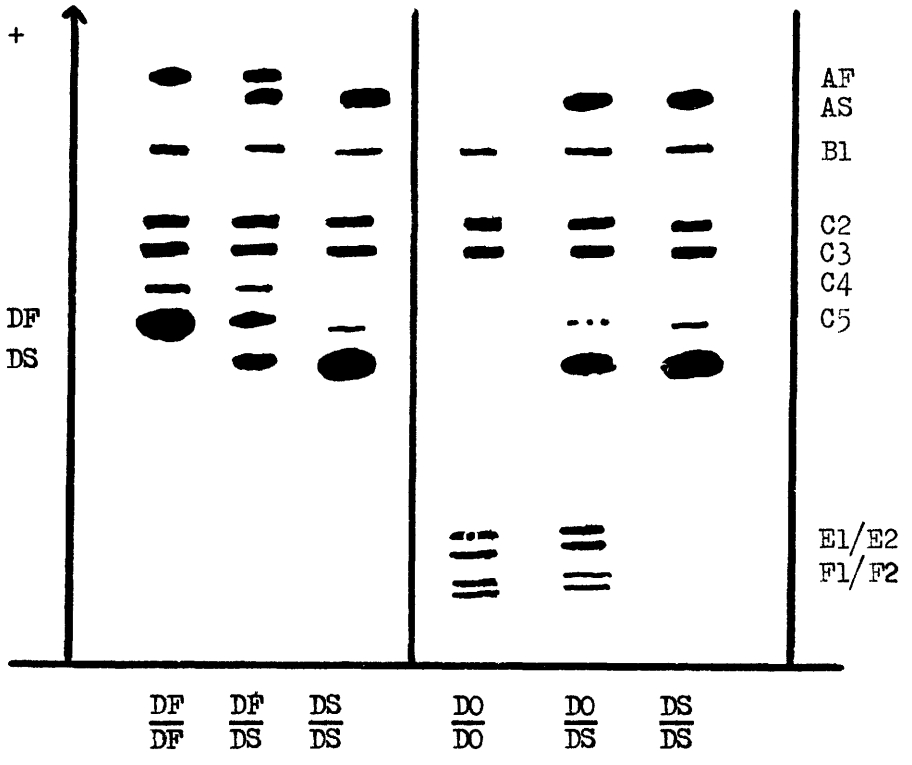
In addition, all genotypes have recessive alleles concerning the ADC and ADM locus.

adr2-f2 is a recessive allele not able to form sub-units which aggregate with sub-units of the ADR2-S allele.

adr2-f13 is a recessive allele forming inactive sub-units which aggregate with sub-units of the ADR2-S allele.

Total activity of the heterozygote is about 25%.

Fig. V. Drosophila melanogaster, codominant and recessive alleles of the LAP-D locus.



DF and DS are codominant alleles controlling the isoenzymes DF resp. DS.

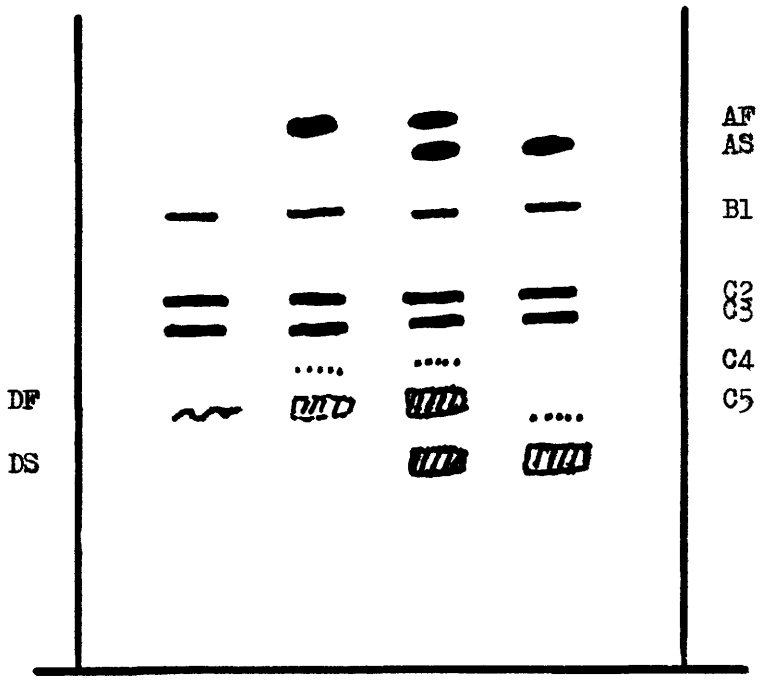
DO is probably a recessive allele.

The heterozygote DO/DS shows half the staining intensity of that of the homozygote DS/DS. This may be due to a dosage effect.

Bands C4 and C5 are probably conformational band to DF resp. DS; DO shows no conformational band.

Bands E1, E2, F1, F2 usually appear if total activity is low.

Fig. VI. Drosophila melanogaster, LAP-D isoenzymes with reduced activity.



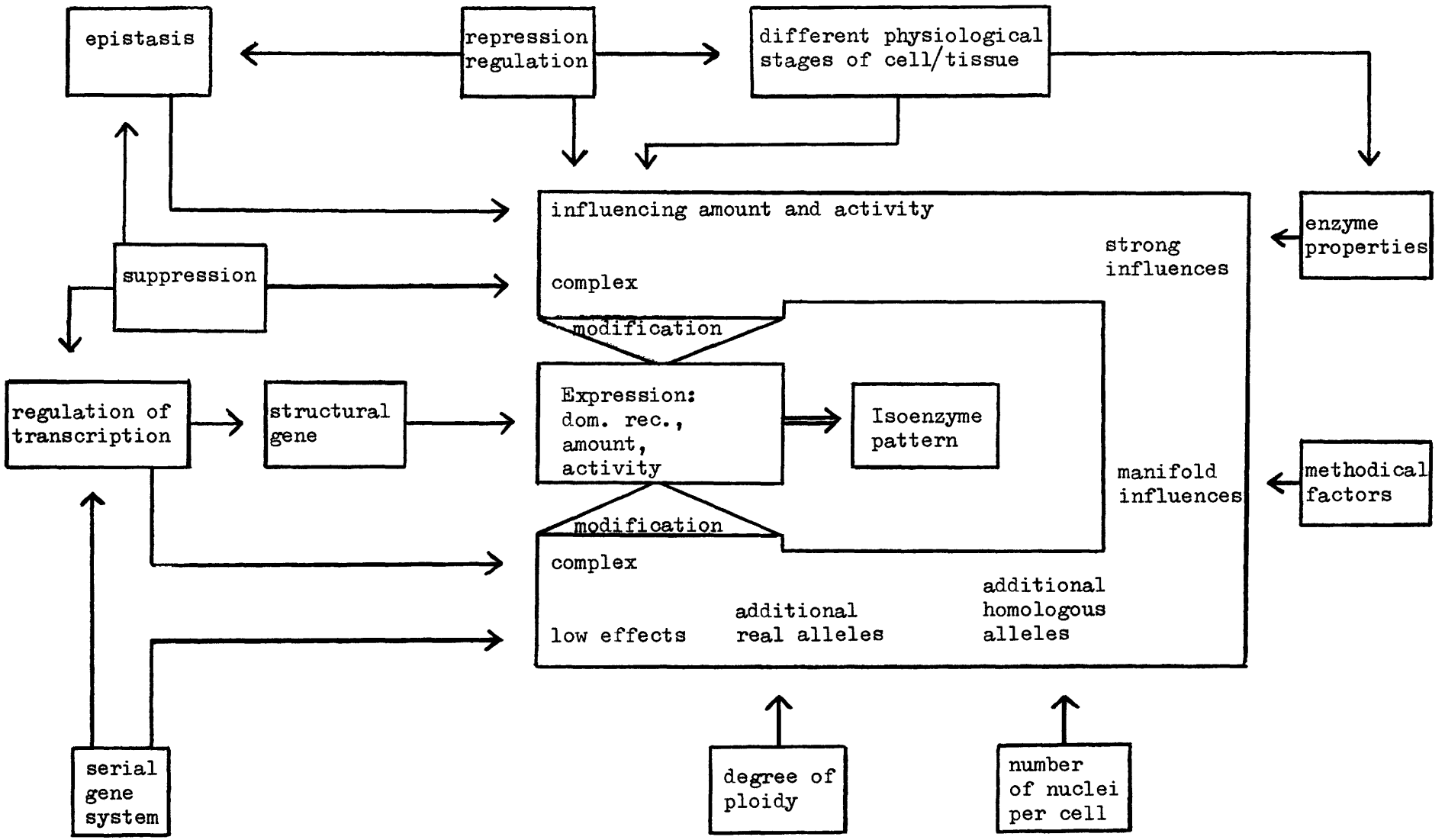
The isoenzymes DF and DS have reduced staining intensity, mostly far less than half of that of the homozygotes. On the left only a trace of staining could be detected.

The conformational bands C4 and C5 could only be seen in some cases.

Fig. VII. Factors which may influence the physiological characteristics of cells, tissues or organs of higher plants (list not complete)

1) Ontogeny, differentiation, age
2) Season of the year, possibly time of day
3) Nutritional conditions, climate, site conditions
4) Diseases, pests, air and water pollution
5) Hormones, herbicides
6) Secondary substances of the plants and other substances

Fig. VIII. Factors which may influence the expression of a gene controlling an isoenzyme pattern.



SESSION PAPER
SEANCE II DOCUMENT 8
SITZUNG DOKUMENT

ETUDE DES ISOZYMES DE LA GLUTAMATE OXALOCETATE TRANSAMINASE CHEZ LES DIFFERENTES SOUS-ESPECES DE Pinus nigra - Arnold

STUDY OF THE GLUTAMATE OXALOCETATE TRANSAMINASE ISOENZYMES IN THE DIFFERENT SUB-SPECIES OF Pinus nigra - Arnold

STUDIE DER ISOENZYME GLUTAMAT-OXALOACETAT-TRANSAMINASE IN DEN VERSCHIEDENEN SUBSPEZIES VON Pinus nigra - Arnold

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RESUME

A l'aide des isozymes des glutamate-oxaloacetate-transaminases on a étudié dans un but de taxonomie expérimentale l'espèce collective Pinus nigra Arn.regroupant 4 sous espèces (laricio, clusiana, nigricans, pallasiana) parfois difficiles à différencier. Par électrophorèse sur gels d'amidon d'endospermes haploides de graines dormantes issues de 163 descendances maternelles on a identifié 4 loci regroupant respectivement 2,3,6 et 3 allèles. L'essai a ensuite porté sur 40 provenances inégalement réparties entre les 4 sous espèces. L'analyse des résultats, quoiqu'encore très partielle puisque ne portant que sur les fréquences alléliques du locus regroupant 6 allèles, semble indiquer que ce système pourrait permettre une bonne séparation des deux sous espèces nigricans et pallasiana. Les clusiana constituent un ensemble assez hétérogène à l'inverse des laricio corsicana. Par contre nous ne parvenons pas à une bonne discrimination entre les laricio de Corse et ceux de Calabre. Faute d'avoir pu mettre en évidence des bandes très caractéristiques de chaque sous espèce le dépouillement des résultats nécessite l'intervention d'un outil mathématique important. La possibilité d'étendre des études de ce type à des contrôles de certificats génétiques de graines forestières est discutée.

SUMMARY

The collective species Pinus nigra Arn., is composed of four sub-species: laricio, clusiana, nigricans, and pallasiana which are often difficult to distinguish from each other. For the purposes of experimental taxonomy, glutamate oxaloacetate transaminase isozymes have been studied. Four loci composed respectively of 2, 3, 6 and 3 alleles have been identified after the starch gel electrophoresis of dormant seed haploid endosperm belonging to 163 half sib progenies. The same process has then been applied to 40 provenances unequally distributed between the 4 sub-species. The results, although incomplete because they only involve the allele frequencies of the 6-allele locus, seem to indicate that this method can be used to distinguish between the pallasiana and nigricans sub-species. The clusiana sub-species constitutes a fairly heterogeneous group contrary to the laricio corsicana sub-species. On the other hand we have not been able yet to discriminate properly between the corsican and calabrican laricio types. Since the bands have not always been very characteristic of each sub-species, it has become necessary to use advanced mathematical means to analyse the results. Finally the possibility of extending studies of this type to the forest tree seed genetic certification controls is discussed.

ZUSAMMENFASSUNG

Die Art Pinus nigra Arn. Wird insgesamt in 4 Subspezies unterteilt: laricio, clusiana, nigricans und pallasiana. Diese sind oft nur schwer voneinander zu unterscheiden. In dieser Untersuchung wird die Brauchbarkeit des Isoenzyms Glutamat - oxaloacetat - Transaminase als Hilfsmittel der experimentellen Taxonomie untersucht. Im haploiden Endosperm ruhender Samen von 163 Halbgeschwisterfamilien werden mit Hilfe der Stärke-Gel-Elektrophorese 4 unterschiedliche Loci identifiziert, für die 2, 3, 6 und 3 Allele nachgewiesen werden. Mit dem gleichen Verfahren sind 40 Herkünfte untersucht worden, welche die 4 Subspezies in unterschiedlicher Anzahl vertreten. Die vorläufigen Ergebnisse, die bisher nur die Allelhäufigkeiten des 6 Allel-Locus umfassen, deuten daraufhin, dass mit dieser Methode die Subspezies pallasiana und nigricans unterschieden werden können. Die Subspezies clusiana ist im Gegensatz zur Subspezies laricio corsicana eine ziemlich heterogene Gruppe. Andererseits sind die Autoren nicht in der Lage gewesen sauber zwischen den Laricio-Typen corsicana

und calabrica zu unterscheiden. Es war notwendig, fortschrittlichere mathematische Methoden zur Analyse der Ergebnisse heranzuziehen, weil die Bänder nicht immer sehr charakteristisch für die einzelnen Subspezies waren. Die Möglichkeiten, solche Studien für die genetische Zertifizierung forstlichen Saatgutes anzuwenden, werden diskutiert.

ETUDE DES ISOZYMES DE LA GLUTAMATE OXALOACETATE TRANSAMINASE CHEZ LES DIFFERENTES SOUS-ESPECES DE Pinus nigra Arn.

Note préliminaire

A la suite du meeting IUFRO de Göttingen (juillet 73) consacré à la génétique biochimique appliquée aux arbres forestiers, nous avons entrepris dans le cadre de la Station d'Amélioration des Arbres Forestiers (Institut National de la Recherche Agronomique) quelques études préliminaires sur les possibilités d'utilisation des isozymes.

L'objet de cette communication est donc de faire le point de l'état d'avancement de nos travaux dans ce domaine et non de donner des résultats définitivement acquis. Ceux-ci nécessitent encore certaines confirmations. En fait ces résultats partiels sont extraits d'un travail de thèse de doctorat de 3ème cycle actuellement poursuivie dans notre laboratoire par Monsieur BIKAY - BIKAY.

INTRODUCTION

L'espèce collective Pinus nigra Arnold occupe une aire à la fois vaste et très morcelée allant de la péninsule ibérique à la Crimée, d'une part, des Alpes autrichiennes aux contreforts de l'Atlas d'autre part. C'est dire que les variations dans l'écologie de l'espèce sont très grandes et que, jointes à l'existence d'un grand nombre d'isolats, une forte différenciation génétique a du s'opérer. Tout ceci explique une systématique assez confuse de cette espèce. FUKAREK (1958) [1] a proposé une classification qui semble maintenant admise par la plupart des auteurs. D'après ARBEZ (1971) on peut distinguer quatre sous-espèces :

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(1) cité par ARBEZ (1971)

- la sous espèce clusiana CLEM ex ARIAS qui comprend les formes du secteur occidental et méridional (Cévennes, Pyrénées, Espagne et Afrique du Nord).
- la sous-espèce laricio POIRET qui comprend les formes géographiques de Corse, de Calabre et de Sicile.
- la sous-espèce nigricans HOST qui regrouperait les formes d'Autriche, de Yougoslavie et d'Italie centrale et dans laquelle on classe aussi habituellement les populations de Grèce, Roumanie et Bulgarie.
- la sous-espèce pallasiana LAMB qui occupe le secteur oriental, en Turquie d'Asie, en Crimée et à Chypre.

La frontière entre nigricans et pallasiana demeure assez confuse, et dans la pratique il est souvent bien difficile de différencier des plants de ces deux sous-espèces.

Pinus nigra est actuellement très utilisé comme espèce de reboisement tant de protection que de production dans tous les pays de l'Europe moyenne et méridionale, notamment en France (DEBAZAC 1971). Or, compte tenu de la grande variété des milieux dans lesquels il est utilisé, une parfaite connaissance du matériel de base (niveau sous-espèce, mais aussi niveau provenance) est indispensable. Aussi un certain nombre d'études de taxonomie expérimentale ont-elles été entreprises. ARBEZ (1971 et 1974) a utilisé successivement des caractères de morphologie des aiguilles puis de composition monoterpénique à partir desquelles des conclusions intéressantes ont été apportées.

L'objet du présent travail était donc double : d'une part compléter au niveau des sous-espèces la taxonomie expérimentale par l'utilisation des isozymes, d'autre part, dans la mesure du possible, parvenir à une différenciation et une caractérisation d'un certain nombre de populations à l'intérieur de chaque sous-espèce. De nombreuses études récentes, notamment BERGMANN (1971 - 1973) montrent que les isozymes offrent de larges possibilités dans ce domaine. En ce qui concerne Pinus nigra, il semble d'après RUDIN (1976) que seul NIKOLIC (1974) ait étudié cette espèce à l'aide des isozymes de la Leucine aminopeptidase.

MATERIEL ET METHODES

Pour tester un grand nombre de populations d'origines géographiques très variées, la technique de BARTELS (1971) et de BERGMANN (1971) à partir de l'endosperme haploïde des graines de gymosperme, nous est apparue la mieux adaptée. Nous avons donc comparé 40 provenances réparties de la façon suivante entre les différentes sous-espèces :

- Laricio de Corse : 6 provenances représentées chacune par 10 à 30 descendances maternelles
- Laricio de Calabre : 6 provenances dont une (Bocchigliero) représentée par 10 descendances maternelles
- Clusiana : 7 provenances
- Pallasiana : 9 provenances
- Nigricans : 12 provenances dont 2 (Badaroux et Sonnerup) correspondent à des peuplements artificiels.

La figure 1 montre la localisation géographique de ces différentes provenances.

Nous avons disposé en outre, sous forme de 10 à 16 descendances par provenance , de deux provenances de clusiana 1 provenance de nigricans et 1 provenance de pallasiana.

Les lots de graines étaient conservés, parfois depuis plusieurs années à + 4°C en chambre froide. Dans tous les cas les facultés germinatives étaient encore très bonnes au moment de l'analyse enzymatique. La dissection et l'extraction des graines étaient réalisées d'après la méthode exposée à Göttingen par BERGMANN : Section longitudinale de la graine, élimination des téguments et de l'embryon, broyage de l'endosperme dans des micromortiers en présence d'un tampon phosphate 0,05 (pH 7,5) additionné de 5mM d'acide ascorbique et 5mM de cysteine. L'extrait est absorbé sur une mèche de papier whatman n°3 (6mm x 4mm) disposée dans chaque alvéole, et que l'on laisse imprégner pendant environ 1 h à + 4°C avant de l'introduire dans le gel. Chaque provenance est représentée par environ 100 graines.

La séparation électrophorétique des glutamates oxaloacétate transaminases (GOT) est effectuée sur un gel à 12% d'amidon, pH 8,2 d'après ASHTON et BRADEN (1961) modifié par RUDIN (1974). Le gel est coulé sur des lames de verre (7,5 x 2,5 cm). Les mèches contenant l'extrait des différentes graines sont insérées dans des petites fentes individuelles (évite les diffusions par capillarité d'un échantillon à l'autre) de façon à ce que 1 cm les sépare du bord cathodique du pont. Ceci nous permet d'observer partiellement la migration cathodique. Après 30 minutes de précourant les mèches sont retirées du gel et la migration se poursuit pendant 90 minutes. L'ensemble est effectué à voltage constant avec un courant mesuré aux deux extrémités d'un gel de 5,2 volts par cm. La migration se fait dans un réfrigérateur à + 4°C. La coloration est réalisée selon la méthode de SCHWARTZ et al (1963) modifiée par RUDIN (1974) (100 ml de tampon phosphate 0,1M à pH 7,4 ; 0,5 mg de pyridoxal -5- phosphate; 229,3 mg d'acide L-aspartique ; 65 mg d'acide cètoglutarique; 200 mg de Fast blue BB salt).

Les résultats sont observés et les distances de migration sont mesurées après une incubation d'une heure à 37°C à l'obscurité. La conservation éventuelle des électrophorégrammes ainsi obtenues se fait à l'aide d'une solution de fixation contenant du glycerol et de l'eau (1:1).

RESULTATS ET DISCUSSION

A partir des différentes descendances maternelles dont nous disposions (163 au total pour les 4 sous-espèces), nous avons pu établir une carte précise des différentes bandes rencontrées présentant une activité enzymatique. Nous avons ainsi pu définir 4 zones appelées A.B.C.D. Les zones A et B correspondent à des migrations anodiques, la zone D à une migration cathodique, et la zone C présente une faible migration anodique ou cathodique selon les cas. Dans ce dernier cas, la comparaison des bandes rencontrées dans les endospermes d'un même arbre montre cependant qu'il s'agit bien d'une même zone. Pour chacune de ces zones, un certain nombre de phénotypes ont été mis en évidence qui se répartissent selon un rapport 1:1 à l'intérieur d'un même individu. On peut donc raisonnablement penser qu'il s'agit à chaque fois d'allèles d'un même locus.

On dispose donc de 4 loci présentant respectivement 2, 3, 6 et 3 allèles différents. La figure n° 2 donne une représentation schématique des variants électrophorétiques de ces 4 zones. Les allèles B_o, C_o et D_o, ne présentent aucune activité enzymatique ("Allèles silencieux").

En fait, la zone A située vers des R_f de 0,4 présente peu d'intérêt au niveau des provenances puisque l'allèle A_1 n'est apparu qu'exceptionnellement dans un certain nombre de provenances (2 laricio corsicana, 2 clusiana, 4 pallasiana). Il pourrait par contre servir de marqueur individuel pour certains arbres.

Dans le tableau n° 1 nous ne donnons que les résultats concernant les zones B et C qui semblent les plus intéressantes. L'observation de ce tableau ne révèle malheureusement pas l'existence de véritables bandes caractéristiques de l'une ou l'autre sous-espèce. On remarque toutefois que l'allèle C_3 qui n'est jamais très abondant sauf chez les nigricans, mais pratiquement présent dans toutes les provenances, est totalement absent chez les pallasiana sauf Ayancik (n° 30).
On note enfin que systématiquement l'allèle B_1 est nettement dominant chez les laricio calabrica et les clusiana alors que pour les autres sous-espèces on trouve des représentations plus équilibrées bien que généralement favorables à B_1. Dans presque tous les cas une importante variabilité intra sous-espèce se fait jour.

Pour analyser ces résultats nous avons d'abord eu recours à des calculs de distances génétiques d'après la méthode proposée par BERGMANN (1974), mais, compte tenu des effectifs, le tableau des résultats demeure d'interprétation peu aisée.
Monsieur MILLIER, de la station de Biomètrie du C.N. R.F. (Institut National de la Recherche Agronomique) a bien voulu procéder à une analyse de nos résultats à l'aide d'un programme d'analyse factorielle des correspondances. Les calculs fondés sur des distances de 2 correspondent à une notion proche de celle des écarts génétiques [1].
Au moment de la rédaction de ce rapport, nous ne disposons pas de la totalité des résultats. Nous nous limiterons ici à l'analyse effectuée sur la zone C.

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(1) MILLIER (communication personnelle)

Le tableau n° 2 donne les paramètres d'intervention des différentes variables dans chacun des trois principaux facteurs explicatifs, ainsi que le pourcentage de variabilité expliquée par chacun de ces facteurs.

Variables	Facteurs et % de variabilité expliquée		
	n° 1 50,05%	n° 2 27,18%	n° 3 13,41%
c_0	0,262	1,199	1,566
c_1	0,323	0,454	0,197
c_2	0,848	- 0,665	0,063
c_3	0,568	0,850	- 0,620
c_4	- 0,860	- 0,156	- 0,067
c_5	- 0,375	0,874	1,432

Tableau n° 2 - Analyse factorielle des correspondance : Coefficients d'intervention des différentes variables dans les 3 principaux facteurs explicatifs.

Nous donnons, d'autre part, dans les figures n° 3 et n° 4 la situation des différentes provenances en fonction de ces 3 facteurs, ce qui conduit à 2 plans explicatifs combinant les facteurs n° 1 et n° 2 d'une part, les facteurs n° 1 et n° 3 d'autre part. La situation des variables apparaît aussi dans ces deux systèmes d'axes.

L'observation de ces figures révèle un certain nombre de points intéressants à souligner. Le plus important est sans doute la bonne séparation qui est réalisée entre l'ensemble des provenances nigricans et l'ensemble des pallasiana. Seules les provenances GRAN (n° 49) et AYAN (n° 30) se trouvent très proches, d'ailleurs dans une zone marginale pour l'une et l'autre sous-espèces. Si ces résultats se confirment, cela permettrait une différenciation jusqu'alors difficile tant à l'aide de critères morphologiques (ARBEZ 1971) que de critères de composition terpénique (ARBEZ 1974). L'ensemble nigricans paraît relativement homogène et il est intéressant de noter que les deux peuplements artificiels SONN (n° 50) et BADA (n° 51) s'inscrivent bien dans le nuage de points correspondant.

L'ensemble pallasiana est moins homogène. Par ailleurs, les clusiana sont éclatés en 3 zones dans le plan explicatif x= 2 ; y = 1, même si un meilleur groupement apparaît dans le plan x= 3; y= 1. Il convient cependant de noter que, aussi bien GAGN (n° 26) que AINS (n° 22) constituent des isolats ce qui peut expliquer leur caractère original dans l'ensemble de l'aire. La similitude existant entre GAGN (n° 26) et NAVA (n° 27), les deux provenances les plus éloignées dans notre échantillon de clusiana, demeure cependant étonnante. Par contre les 4 autres provenances, géographiquement proches présentent entre elles de grandes analogies. L'ensemble laricio corsicana semble très homogène, ce qui ressortait déjà nettement du tableau des résultats, mais se trouve dans une zone où l'on rencontre aussi des laricio calabrica et quelques provenances marginales de pallasiana et clusiana. Seul TART (n°03) s'éloigne curieusement du noyau central, et en particulier de TAME (n° 04) dont il n'est pourtant géographiquement séparé que par une crête. Des différences d'exposition, mais aussi la certitude que des incendies ayant pû modifier différemment la composition génétique de ces populations ont pu intervenir dans ces deux provenances, les plus basses en altitude de tout notre échantillon, peuvent expliquer ce résultat (ARBEZ, communication personnelle). Enfin l'observation des laricio calabrica révèle une scission très nette en 2 groupes alors que toutes ces provenances sont géographiquement très voisines. Ceci nous a conduit à suspecter la validité de certains résultats obtenus. En effet, une partie des analyses a été réalisée en 1975 alors que toutes les autres l'ont été 8 mois plus tard en 1976. Il est possible qu'une modification dans la technique, ou plus vraisemblablement dans la qualité des produits soit intervenue entre 1975 et 1976 qui ne permette pas une bonne comparaison entre ces deux groupes de provenances. Nous tentons actuellement d'identifier les causes de ces divergences.

Il faut toutefois reconnaître que dans l'immédiat notre comparaison porte sur une seule zone, la zone C, ce qui est logiquement insuffisant pour permettre une bonne discrimination. Une analyse simultanée sur la zone B et la zone C ainsi que sur les associations rencontrées entre les différents allèles de ces deux zones pourra sans doute affiner notre analyse.

D'autre part, le choix de la zone C, à faible migration et où donc l'appréciation des R_f est difficile est sans doute assez critiquable. Néanmoins les résultats obtenus semblent cohérents.

CONCLUSIONS

Nous considérons que le travail exposé ici est véritablement exploratoire et nous sommes bien conscients des nombreuses imperfections qu'il présente. Il a surtout constitué pour nous l'occasion de se familiariser avec une technique qui paraît offrir de grandes possibilités en génétique forestière. Ces premiers essais nous conduisent à faire un certain nombre de remarques sur son utilisation, au moins en matière de taxonomie et de réalisation de tests variétaux applicables aux règlementations en vigueur sur les transerts de semences forestières.

Tout d'abord, la méthodologie mise en oeuvre est moins aisée qu'il n'apparaît a priori et semble donc ne pouvoir être utilisée que par des laboratoires nettement spécialisés. En outre, la reproductibilité, dans l'état actuel des choses, n'est pas toujours parfaite ce qui pourrait constituer une limite à son utilisation, en particulier dans la réalisation de tests variétaux. Dans ce sens, il pourrait être instructif de tester un même matériel dans différents laboratoires pour mieux cerner les limites et les possiblités d'application de cette technique.

Par ailleurs, le choix du système enzymatique est un élément déterminant pour lequel on est malheureusement guidé que par d'autres exemples similaires trouvés dans la littérature, ou à l'occasion de contacts personnels entre chercheurs, ou enfin par une certaine habitude du laboratoire. Comme d'autre part chaque nouveau système enzymatique exploré dans un laboratoire nécessite une bonne période de mise au point, on peut se demander si, toujours dans l'hypothèse de tests variétaux, une certaine spécialisation de chaque laboratoire dans un nombre limité de systèmes enzymatiques ne serait pas envisageable? Il est probable que les prochaines années verront une standardisation souhaitable de ces techniques, supprimant quelques unes des réserves que nous émettons.

Enfin, l'exploitation des données recueillies par l'analyse peut présenter certaines difficultés. S'il est possible dans le cadre d'un travail de recherche d'avoir recours à une analyse du type de l'analyse factorielle des correspondances que nous avons utilisée, ceci demeure un outil mathématique difficile à mettre en oeuvre dans le cadre d'un simple laboratoire d'analyse. Il faut donc pouvoir mettre en évidence soit des différences de fréquences allèliques très notables, soit la présence de bandes caractéristiques pour aboutir à une clef de détermination suffisamment simple pour être appliquée dans la pratique.

Il reste que les autres utilisations, notamment par le généticien forestier, paraissent riches de possibilités comme en témoigne le grand nombre des articles récents cités par RUDIN (1976). Ceci prouve que les isozymes peuvent constituer des marqueurs génétiques très précis. Comme de nombreux autres laboratoires, nous attendons aussi beaucoup des possibilités de mise en évidence de liaisons bien nettes entre certaines caractéristiques isoenzymatiques et les caractéristiques physiologiques ou technologiques sur lesquels s'effectue la sélection. Sans doute une meilleure connaissance des enzymes intervenant dans certains mécanismes physiologiques fondamentaux, ainsi que des progrès en biochimie permettant leur mise en évidence sont-ils encore nécessaires.

REMERCIEMENTS

Nous tenons à exprimer notre gratitude aux Professeurs S. URGENC, R. MORANDINI, J. TORNERO et K. HOLZER grâce à qui nous avons obtenu des graines de descendances maternelles ayant nécessité de leur part des récoltes particulières. Nous remercions aussi le Dr. Dag. RUDIN qui nous a donné de précieux conseils lorsque nous avons entrepris cette étude. Nous remercions aussi tout spécialement Monsieur MILLIER qui a bien voulu analyser très rapidement nos résultats. Nous n'oublions pas non plus Mademoiselle Pierrette CAPELLI pour son assistance technique efficace dans toutes les phases de cette étude.

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	Provenance	Abré - viation	n°	Fréquence des différentes bandes (en %)								
				B_0	B_1	B_2	C_0	C_1	C_2	C_3	C_4	C_5
LARICIO CORSE	Aitone	AITO	01		40,4	59,6		3,4	0,7	7,5	84,3	4,1
	Noceta	NOCE	02		58,2	41,8	0,8	11,2	1,5	3,0	86,6	
	Tartagine	TART	03		72,6	27,4	1,1	32,9	2,3	9,1	54,5	
	Tartagine Melaja	TAME	04		59,7	40,3		9,1	7,8	1,3	81,9	
	Vizzavone	VIZZ	05		62,6	37,4		9,0	3,0	2,4	83,7	1,6
	Valdoniello	VALD	06		71,8	28,2		6,8	1,0	1,4	89,5	1,4
LARICIO-CALABRE	Grancia	GR 61	11		86,9	13,1		13,1	86,9			
	Machia del la Tavola	MA TA	12		73,2	26,8		8,1	76,8	15,1		
	Trenta Coste	TR CO	13		69,9	30,1		6,8	91,7	1,4		
	Catanzaro	CATA	14		72,3	27,7		3,8	4,8	5,7	78,1	7,6
	Gallopane	GALL	15	5,2	69,1	25,7		3,5	6,9	3,5	70,5	15,6
	Bocchigliero	BOCC	16		70,9	29,1		5,8		1,2	89,5	3,5
CLUSIANA	Los Carrascales	LO CA	21	0,8	78,3	20,9	20,0	23,3	10,8	27,7	18,4	
	Ainsa	AINS	22		76	24	2,0	3,0	1,0		94,0	
	Pinar Algarbe	PIAL	23	0,9	74,9	24,2	8,6	36,2	7,8	17,2	18,1	12,1
	Fresneda	FRES	24		80,3	19,7	8,6	44,4	10,3	20,5	11,9	4,3
	Zafrilla	ZAFR	25	2,2	76,8	21,0	15,7	42,1	9,5	5,3	9,5	17,9
	Gagnières	GAGN	26		62,4	37,6	3,2	21,5	58,1	8,6	7,6	1,1
	Nava Hondona	NAVA	27		78,3	21,7	1,3	26,6	72,0			

Tableau n° 1 (rectifié) - Fréquence d'apparition des différentes bandes pour les zones B et C pour l'ensemble des provenances .

	Provenances	Abréviation	N°	Fréquence des différentes bandes (en %)								
				Zone B			Zone C					
				B_0	B_1	B_2	C_0	C_1	C_2	C_3	C_4	C_5
PALLASIANA	Ayancik	AYAN	30		64,5	35,5		12,6	83,6	3,8		
	Alacam	ALAC	31		47,4	52,6	1,0	8,3			90,7	
	Troodos	TROO	32		57,0	43,0		8,6			91,4	
	Crimée III	CRIM III	33		51,1	48.9	1,1	5,5	54,4		38,9	
	Crimée IV	CRIM IV	34		55,2	44,8	2,3	12,7	43,6		41,4	
	Crimée V	CRIM V	35		42,7	57,3		8,4	53,1		38,5	
	Köprücek	KOPR	36	2,9	63,8	33,3		9,8	54,9		35,3	
	Acili Bogazi	ACBO	37	1,1	47,8	51,1		34,0	19,1		46,8	
	Yilanli	YILA	38		56,3	43,7	2,1	12,5	35,5		50,0	
NIGRICANS	Grabenweg	GRAB	40		59,3	40,7		46,9	21,0	32,1		
	Murfatlar	MURT	41		67,1	32,9		38,6	30,0	22,9	8,5	
	Rupea	RUPE	42	1,0	63,3	35,7		20.4	30,7	44,9	4,0	
	Singiorgiu de Padure	SPAD	43		61,8	38,2		16,0	33,0	46,9	4,3	
	Thassos	THAS	44		45,4	54,6		11,0	20,3	45,3	23,3	
	Villetta-Barrea	VIBA	45	2,0	83,5	14,5		16,5	3,1	51,5	28,9	
	Bad Fichau	BADF	46		18,5	81,5		38,2	28,9	32,9		
	Studenica	STUD	47		71,9	28,1		12,4	40,5	44,9	1,1	1,1
	Tara-Zlatibor	TAZL	48		45,8	54,2		23,8	47,4	28,9		
	Gradeski andek	GRAN	49		52,6	47,4	1,7	13,6	84,8			
	Sonnerup (1)	SONN	50		68,0	32,0		31,0	1,0	32,0	36,0	
	Badaroux (1)	BADA	51		59,9	40,1		32,2	38,9	21,0	7,8	

Tableau n°1 bis - Fréquence d'apparition des différentes bandes pour les zones B et C pour l'ensemble des provenances.

(1) peuplements artificiels.

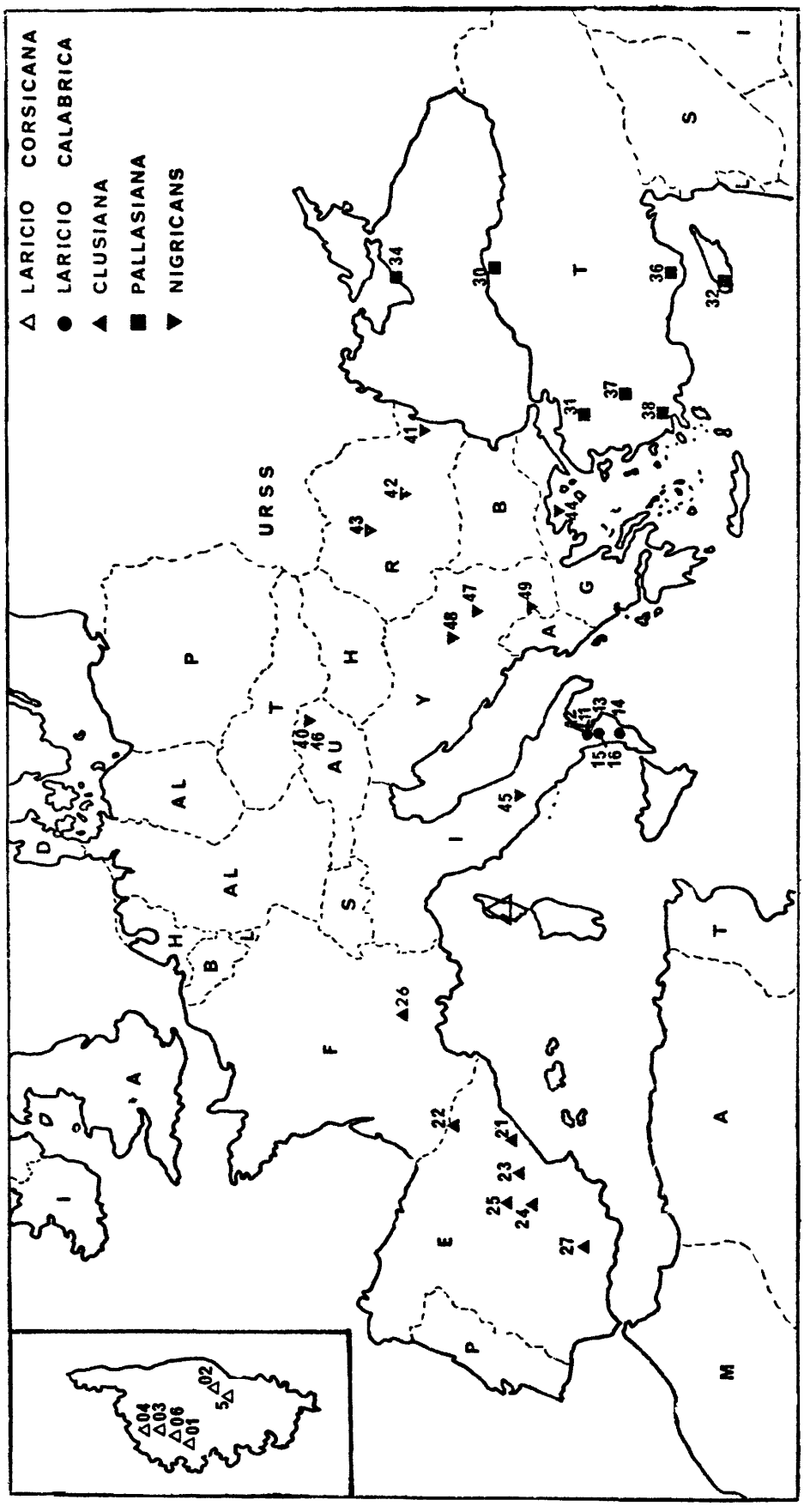

Fig. 1 - Localisation géographique des provenances utilisées (les numéros des provenances renvoient au tableau 1).

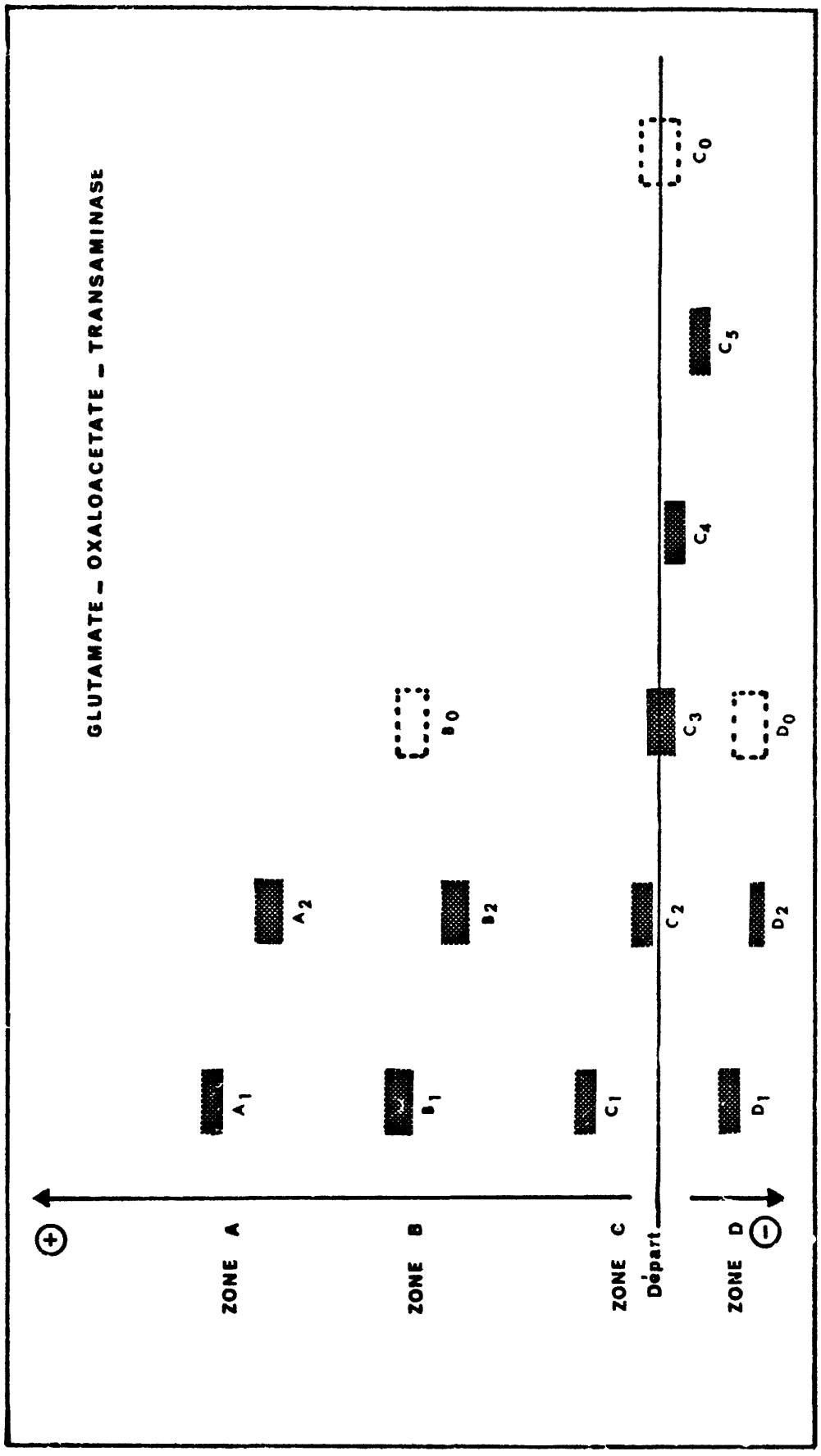

Fig. 2 Carte schématique des variants électrophorétiques des 4 zones de la G.O.T. d'endosperme de Pinus nigra

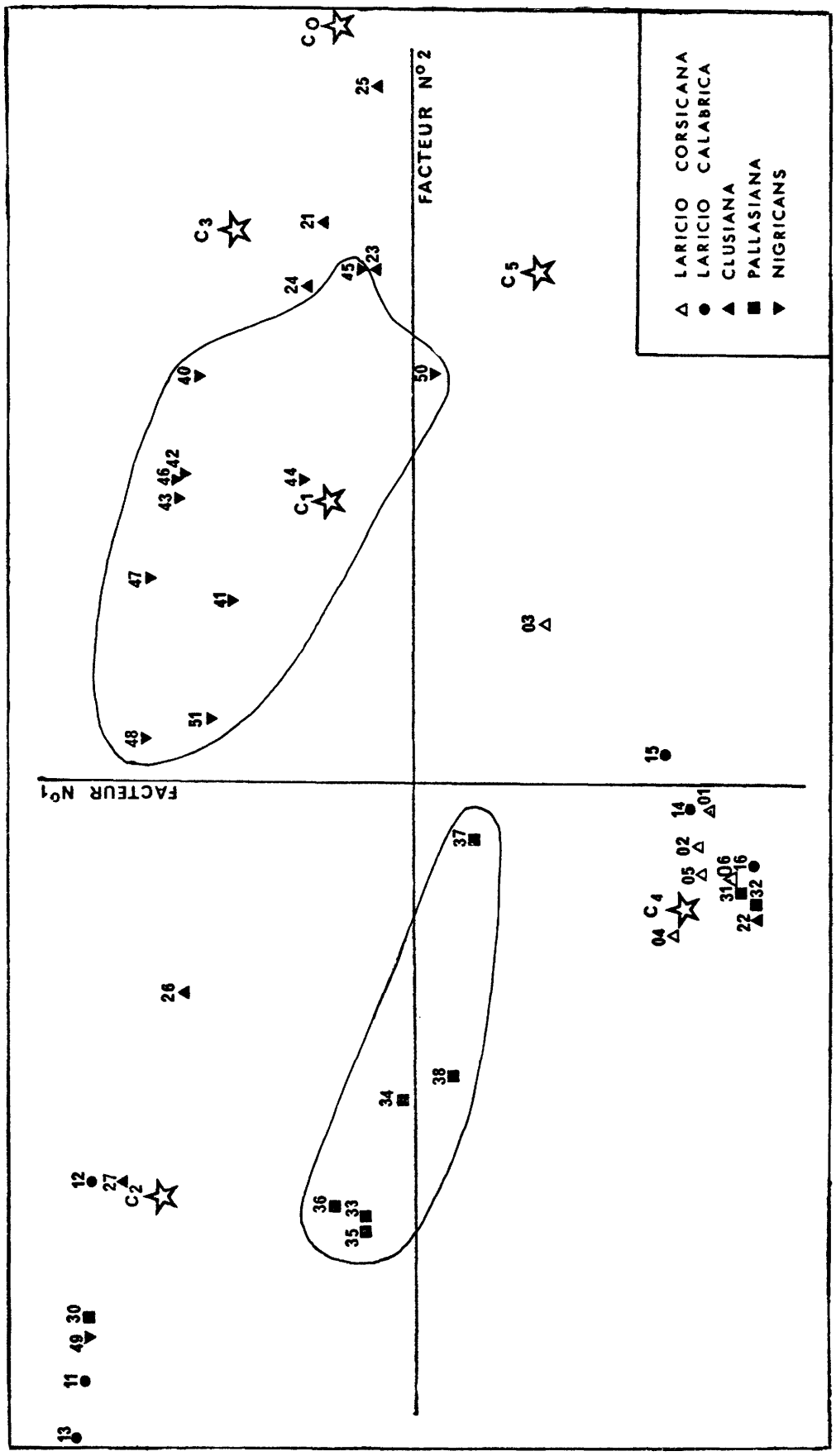

Fig. 3 - Analyse factorielle des correspondances -
Situation des provenances en fonction des facteurs explicatifs n° 1 et n° 2
(explique globalement 77% de la variabilité)

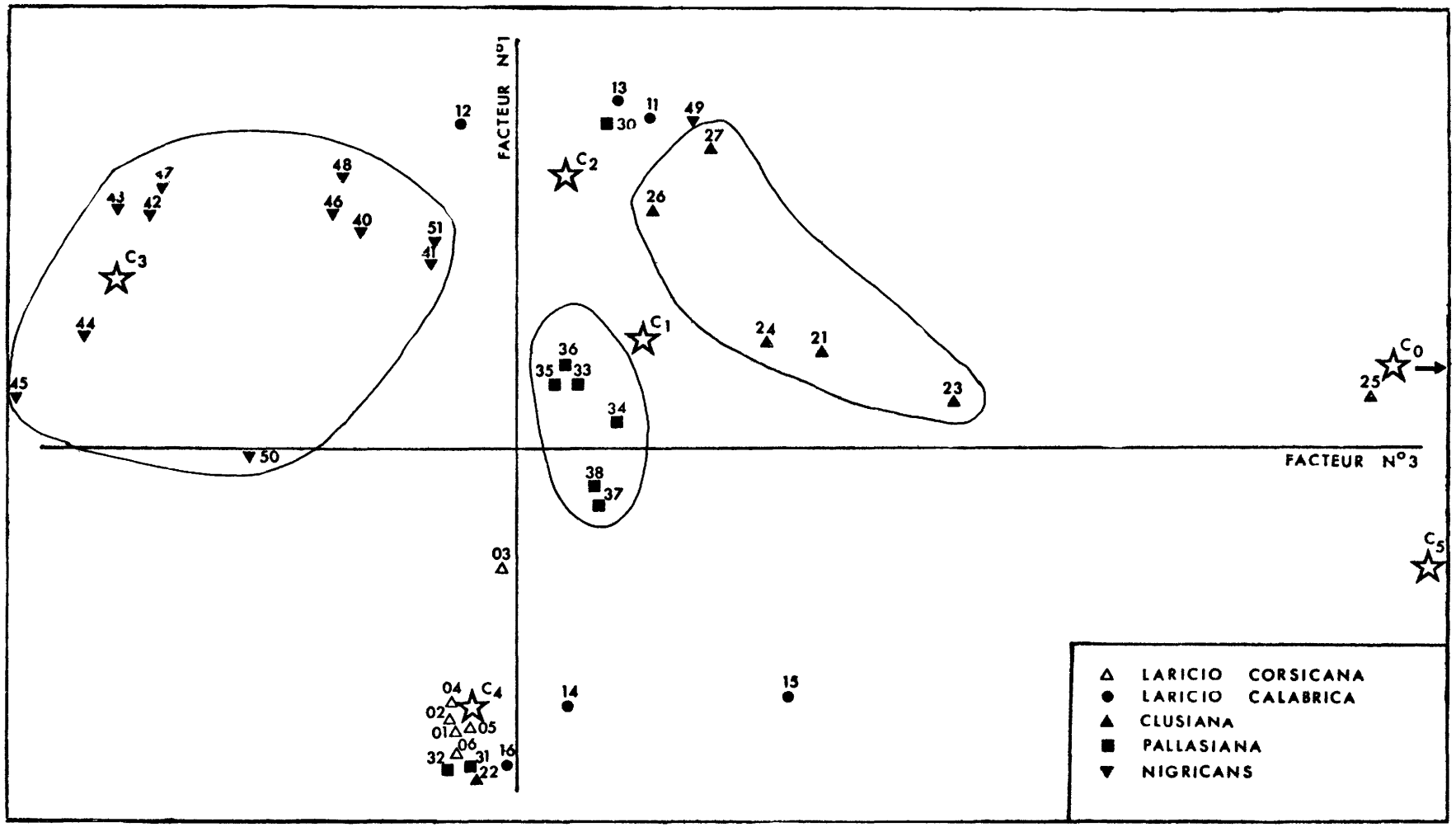

Fig. 4 - Analyse factorielle des correspondances-
Situation des provenances en fonction des facteurs explicatifs n° 1 et n° 3
(explique globalement 63,5% de la variabilité)

SESSION PAPER
SEANCE III DOCUMENT 9
SITZUNG DOKUMENT

THIN LAYER CHROMATOGRAPHY OF FLUORESCENT PHENOLIC COMPOUNDS IN NEEDLES: A REVIEW OF CURRENT ACTIVITIES IN Picea.

LA CHROMATOGRAPHIE EN COUCHES MINCES DE DERIVES PHENOLIQUES FLUORESCENTS D'AIGUILLES DE RESINEUX: UNE REVUE DES ACTIVITES COURANTES CHEZ Picea.

DIE DÜNNSCHICHTCHROMATOGRAPHIE AN FLUORESCIERENDEN PHENOLDERIVATEN IN NADELN: ZUSAMMENFASSUNG DER AKTIVITÄTEN BEI Picea.

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SUMMARY

The objectives of our programme are

A Use of thin layer chromatography (TLC) of fluorescent phenolic compounds for identification purposes - e.g. identification of clones, families, provenances, species and hybrids.

B Investigation of the relationship between species of Picea.

C Studies of phenotypic and genetic correlations between chromatographic data and physiological traits of importance in practical tree breeding.

A quantitative method of measuring fluorescent itensity of specified TLC-spots in one-dimensional chromatograms was used. In Danish environments reasonably stable fluorescence intensities for each of the 5 spots occurred outside the growing season, in different age-classes and in different levels in the crown. Genotypes represented by clones showed a near-perfect stability within the region of NE-Sjælland.

The method has been utilized in species comparisons between 23 Picea species. Although a highly significant between-species variation could be registered, the variation pattern contradicted strongly an a priori hypothesis of the relationship between species. A general trend of stronger concentrations of species with northern and interior distribution areas was evident. Similar geographic trends were registered between different geographic origins of Picea abies and Picea sitchensis.

A number of quantitative genetic analyses have been carried out with parents represented by ortets or ramets and offspring from open and controlled crosses (including selfings). The combined evidence of these analyses indicated, that we operate with traits of high broad sense heritabilities controlled by a few major genes and with certain dominance deviations. There were no indications of severe selections pressure

operating on the investigated spots in the mild Danish and Southern Swedish invironments. In contrast to this, there were indications of severe selection pressure operating in South Finland. Here the selection seemed to favour phenotypes with strong fluorescent intensities in spots A and B. Otherwise we have not been able to find correlations between breeding goals and our TLC-spots.

RESUME

Notre programme cherche à atteindre les objectifs suivants :

A L'application de la chromatographie en couches minces (CCM) à des dérivés phénoliques fluorescents, visant à une identification, par exemple de clones, de familles, de provenances, d'espèces et d'hybrides.

B Des recherches sur les relations de parenté entre les espèces Picea.

C Des études de corrélation phénotypes et génétiques entre des données chromatographiques et des qualités physiologiques, importantes à la pratique de l'amélioration des arbres.

Pour mesurer quantitativement les intensités de fluorescence de 5 dérivés de phénol, nous sommes servis de chromatogrammes à une dimension.

Dans les conditions de végétation danoises, l'intensité de fluorescence des 5 dérivés de phénol est assez stable en dehors de la période de croissance, pour différentes générations et à différents niveaux de la couronne. Des génotypes représentés par des clones montrent une stabilité de fluorescence presque idéale dans le Nord-Est de Seeland.

Cette méthode comparative a été employée pour 23 espèces de Picea. Bien que nous ayons trouvé une variation hautement significative entre les espèces, la manière dont elle varie va à l'encontre d'hypothèses formulées a priori sur les rapports de parenté entre les espèces. Une tendance

générale à une plus importante concentration apparaît clairement pour les espèces qui se répartissent dans les régions du nord et de l'intérieur du pays. En ce qui concernent les Picea abies et les Picea sitchensis, nous avons enregistré des tendances analogues, à lier à la géographie.

On a procédé à des analyses génétiques quantitatives sur des parents, représentés par des ortets ou des ramets, et sur les descendants issus de croisements contrôlés ou libres (y compris d'autofécondation). De l'ensemble de ces analyses il se dégage que nous opérons sur des grands traits de caractères transmissibles contrôlés par un petit nombre de gènes majeurs avec certains écarts de dominance. On n'observe aucune indication de forte pression sélective en relation avec la douceur du climat danois et de celui du Sud de la Suéde. Par contre, il y a des indications de pressions importantes au niveau de la sélection dans le Sud de la Finlande. Là la sélection semble favoriser des phénotypes se signalant par une forte intensité de fluorescence des taches A et B. Autrement, nous n'avons pas pu trouver de corrélations entre l'amélioration des arbres recherchés et nos taches-CCM.

ZUSAMMENFASSUNG

Die Zielsetzung des Projektes ist :

A Anwendung der Dünnschichtchromatographie (DC) an fluorescierenden Phenolderivaten zur Identifikation von beispielsweise Klonen, Familien, Proveniensen, Arten u. Hybriden.

B Untersuchung der Beziehungen zwischen den Piceaarten.

C Studien über phänotypische und genetische Korrelationen zwischen chromatographischen Daten und physiologischen Eigenschaften, welche für die praktische Züchtung von Wichtigkeit sind.

Für die quantitative Messung der Fluorescenzintensitäten von 5 Phenolderivaten sind eindimensionale Chromatogramme angewendet worden.

Unter dänischen Wuchsbedingungen ist der Inhalt von den 5 Phenolderivaten in den Piceanadeln hinreichend stabil, a) ausserhalb den Wachsperioden, b) in den verschiedenden Altersklassen und c) in den verschiedenen Zweigkränzen.

Genotypen, durch Klone repräsentiert, zeigen eine beinahe ideale Fluorescenzstabilität in Nordseeland.

Die Methode ist zu Artsvergleichungen zwischen 23 Piceaarten angewendet worden. Obgleich hochsignifikante Variationen zwischen den Arten gefunden wurden, ist das Variationsmuster in grossem Widerspruch mit a priori Hypothesen über die Beziehungen zwischen den Arten. Eine generelle Tendenz steigender Fluorescenzintensität (höhere Konzentration) in Arten mit nördlichen und inlandklimatischen Verbreitungsgebieten ist augenscheinlich. Ähnliche geografisch abhängige Tendenzen sind innerhalb von Picea abies und Picea sitchensis registriert worden.

Eine Anzahl von quantitativ-genetischen Analysen wurden ausgeführt mit Eltern (Orteten und Rameten), und Abkommenschaft von freien und kontrollierten Pollinierungen (inklusive Selbstungs). Eine Zusammenfassung dieser Analysen indiziert, dass wir mit Eigenschaften hoher Erblichkeit im weitern Sinne (h^2_{bs}) operieren, die von wenigen major genes kontrolliert werden und einer gewissen Dominanz unterliegen. Es gibt keine Indikation für die Annahme eines Selektionsdruckes in der Fluorescenzintensität der untersuchten Phenolderivate in dem milden Klima Dänemarks und Südschwedens. Im Gegensatz hierzu ist ein deutlicher Selektionsdruck in Südfinnland anzunehmen.

Die Selektion scheint Phänotypen, die grosse Fluorescenzintensität in den Flecken A und B aufweisen, hervorzuziehen. Andererseits sind wir jedoch nicht in der Lage gewesen, Korrelationen zwischen Veredlungszielen und unseren DC-separierten Flecken zu erzielen.

THIN LAYER CHROMATOGRAPHY OF FLUORESCENT PHENOLIC COMPOUNDS IN NEEDLES: A REVIEW OF CURRENT ACTIVITIES IN Picea.

OBJECTIVES

The objectives of our current project are :

A Utilization of thin layer chromatography (TLC) of fluorescent phenolic compounds for identification purposes - e.g. identification of clones, families, provenances, species and hybrids.

B Investigation of the relationship between species of Picea.

C Studies of phenotypic and genetic correlations between chromatographic data and physiological traits of importance in practical tree breeding.

CHEMICAL METHODS

Our methods have been described in detail earlier (Kaufmann et al 1974). Here is given only summarized descriptions.

Extraction : 5.00 g frozen (- 20°C) needles, cut into 5 mm long pieces, were boiled with 20 ml water for three hours. The plant material was filtered and washed with a few ml of water. The water extract was further extracted with n-butanol to free the phenolic constituents, stilbenes and their glycosides from disturbing tannins. The butanolic phase was separated and filtered.

Chromatography : For thin layer chromatography, cellulose plates prefabricated by Fa. Merck, Darmstadt were used. The 10 l needle extracts which were analysed were applied with a constriction pipette. One-dimensional chromatograms were used and the mobile phase was the supernatant of the mixture butanol/acetic acid/water in the proportion 80/20/100.

Scanning : The separated stilbenes were measured by a Zeiss TLC-scanner. The principle is that the separated compounds on the cellulose plates are excited by UV-light and their fluorescence intensity is recorded

quantitatively and compared to the standard curve of a known fluorescent compound, fluorescein. The intensities of different fluorescent compounds are expressed in terms of g fluorescein.

PROJECT DESCRIPTION

The general structure of the project is presented in Fig. 1.

For specified spots A, B, C, D and E we have carried out most of the activities in the project. Reasonably stable fluorescence intensities for each of the 5 spots occur outside the growing season, in different age-classes and in different levels in the crowns. Genotypes represented by clones show a near perfect stability within the region of NE-Sjælland (Kaufmann et al 1974). On the basis of this evidence, we have been reasonably successful for identification purposes. Our investigations of correlations to breeding goals have not yet given us clear-cut results. There are, however, indirect indications of correlations to frost hardiness.

REVIEW OF REGISTERED GENETIC VARIATION

As a consequence of our simple one-dimensional chromatography and the available quantitative measuring technique - the TLC-scanner - we have applied quantitative genetic methods in the analysis of our observations.

Species comparisons

Typical samples from 5-10 trees from each of 23 Picea species growing in Denmark and in Göteborg's Botaniska Trädgård in Sweden, have been analysed in an attempt to study the evolutionary relations between Picea species. Because 5 spots are involved, a multivariate discriminant analysis has been carried out with the help of the BMD 7M stepwise discriminant analysis programme (Dixon 1965). The final canonical analysis is presented graphically in Fig. 2. In this graph, alike species should be plotted in the neighbourhood of each other and vice versa. The result is controversial. Although a highly significant variation could be registered

between species, the variation pattern contradicts strongly with an a priori hypothesis about the relationship between species - e.g. from evidence of ability to hybridize. Furthermore, tendencies of stronger concentrations of species with northern and interior distribution areas are evident. Similar trends are registered between provenances within Picea abies. Further statistical analysis of this material is required.

Provenance variation

A special study of the geographic variation in Picea sitchensis has been carried out on two-year old material originating from the IUFRO International Ten-Provenance Experiment. 10 plants from each of 10 provenances were analyzed. A significant variation occurred in two of the five investigated spots. Part of the registered between-provenance variation in spot A is of a clinal nature, another part of the between-provenance variation in spot A and all the between-provenance variation in spot D seems to be of another, possibly ecotypic nature. The registered cline in spot A shows a gradual decrease of fluorescent intensity from north to south, (Fig. 3).

These observations confirm - at least for spot A - the impressions obtained in the species comparisons. In some way strong concentrations in spot A are associated with genetic adaptation to sites with a cold climate.

Clonal variation

Clonal variation within Picea abies has been demonstrated at an early stage of the programme. Fig. 4. (Kaufmann et al 1974) shows for each spot a graphic comparison of 20 year old ramets from 20 clones growing on two different sites in NE-Sjælland. The soil conditions are very different on these two sites (site 1 wet meadow, site 2 high moraine). In fact only minor deviations occur from complete stability of genotypes in these two environments. These investigations have been used to estimate broad-sense heritabilities within Picea abies in these environments. On individual trees within NE-Sjælland we ended up with $h^2_{b.s.}$ ranging from 0,98 (spot A) to 0.85 (spot E).

Family variation and parent-offspring regressions

At present we are performing a number of quantitative genetic analyses using parents represented by ortets or ramets and offspring from open and controlled crossings.

In Fig. 5 parent clones and offspring after open pollination of the ortets can be seen (Fussingö material). In Fig. 6 parent clones (represented by mid-parent values) and offspring after controlled crossings are presented (Ekebo material). The Fussing and the Ekebo material originate from Germany, although it has grown one generation in Denmark and Southern Sweden respectively. Age of material 15-25 years old.

In Figs. 7-9 it is attempted to correlate old ortets with 6-year old progenies after selfing and open pollination of the ortets. This material originates direct from an autochtone Southern Finnish stand (Tuusula).

A summary of the performed quantitative analyses are presented in table 1. For the German-origin material growing in Denmark and Southern Sweden the general trends seem reasonably simple.

Comparing total phenotypic mean and variances for parents and offspring, we find these parameters to be of the same magnitude. The expected smaller variances between presumed HS-families that between FS-families are confirmed for spot A, B and C. Correspondingly, larger variances are registered within HS-families than within FS-families. In many cases we find lack of variance-homogenity within both HS- and FS- families. Nearly all parent-offspring regressions are registered as significant, deviating from 0 and of the expected positive sign. The expected steeper slopes of the mid-parent-offspring regressions are registered and compared to the female-parent-offspring regressions.

In many of the tested parent-offspring regressions, only part of the variation between progenies could be explained by the regression, a significant source remained around the regression line in an apparent random pattern.

The combined evidence of the analyses of this particular material indicates that we operate with traits of high heritabilities controlled by a few major genes and with certain dominance deviations. The alikeness of the clones originating from old plus-trees and their offspring, indicates that no serious selection pressure on the investigated traits has operated in these environments.

In the Finnish material some striking contrasts and similarities to this situation are evident.

A Mean fluorescence intensity of the whole material is approximately 10 times greater than that of the German origin material growing in South Scandinavia.

B Comparing total phenotypic mean and variance for parents and offspring, we find higher means and smaller variances in parents compared to offspring.

C The strong inbreeding ($F = 0.5$ in the self-pollination generation) apparently does not influence the mean value compared to the open-pollination progenies.

D We can register a slight increase in total phenotypic variance when moving from open pollination progenies to self-pollination progenies.

E Comparing variances between and within families in the open pollination material - we register the expected higher within-family than between-family variances. In the self-pollination material we find variances of roughly the same magnitude or slightly higher variances within than between families.

F Compared to the German-origin material growing in Denmark and Southern Sweden, we find diminished variance in the offspring.

G Nevertheless we often find lack of variance-homogenity within families.

H All parent-offspring regressions are non-significant, although of the expected positive sign. An obvious statistical reason for this is the lack of sufficient variation between parent trees for spots A and B.

I For all 3 investigated spots A, B and C we find a significant correlation between offspring after self-pollination and open pollination (Figs. 7-9).

These observations confirm our earlier indications from provenance investigations and from the cited German-origin parent-offspring material in Denmark and Southern Sweden except for one important point. The narrowing down of the genetic variance amongst old parent trees at the strong end of the scale, indicates that a selection pressure might operate in these environments. As a working hypothesis, we can postulate that only individuals with high fluorescence intensities survive and compose the dominant trees in the mature stand. Because of dominance deviations, and lack of homozygosity, we still observe segregation in the offspring in this Southern Finnish spruce population (Fig. 10).

ATTEMPTS TO CORRELATE TLC-DATA TO BREEDING GOALS

A practical difficulty in this type of investigations, is that our breeding programme in Picea abies is so young, and that we have not yet accumulated sufficient knowledge of the long term field performance of e.g. clones or their offspring.

In table 2 we have tried to gather some information from limited early clone and progeny tests and also information of relative wood density from clones and 35-year old plus trees. The statistical method we have used is linear multiple regressions, the dependent variable being our breeding goals, the independent variables being the 5 spots, A, B, C, D and E. As can be seen, the results in this first attempt are not particularly promising.

If more material were available, this primitive approach of linear dependences should be supplemented by testing if certain equilibria between spots could be used as a prediction of favourable genotypes in certain environments. From a biochemical point of view, the approach of equilibria, rather than linear increases of concentrations, seems more realistic. Nevertheless, the working hypothesis put forward in chapter 5.4 about a simple correlation between fluorescence-intensity of spots A and B and ability to survive and grow into mature trees in cold environments, might be worthwhile to test experimentally. Whether it is a question of survival as a young tree, or of sustained growth in cold environments, we cannot say at present. The first possibility could be tested in young tree field experiments and/or in a phytotrone.

ACKNOWLEDGEMENTS

The project has partly been financed by the Danish "Statens jordbrugs- og veterinærvidenskabelige Forskningsråd". The laboratory work had been carried out by Margit Hansen and Birgit Pelle. Material from Finland has kindly been supplied by Prof. Max. Hagmann. The Ekebo-material has been collected in cooperation with Martin Werner, Inst. för Skogsförbättring, Sweden.

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Dixon, W.J. : BMD Biomedical Computer Programs, Los Angeles, 1965

Kaufmann, Uwe, Wellendorf, H. and Hansen, M : Thin layer chromatography of fluorescent phenolic compounds in needles. Degree of genetic control in Picea abies L., Forest Tree Improvement 8, 1974.

Fig. 1. STRUCTURE OF PROJECT

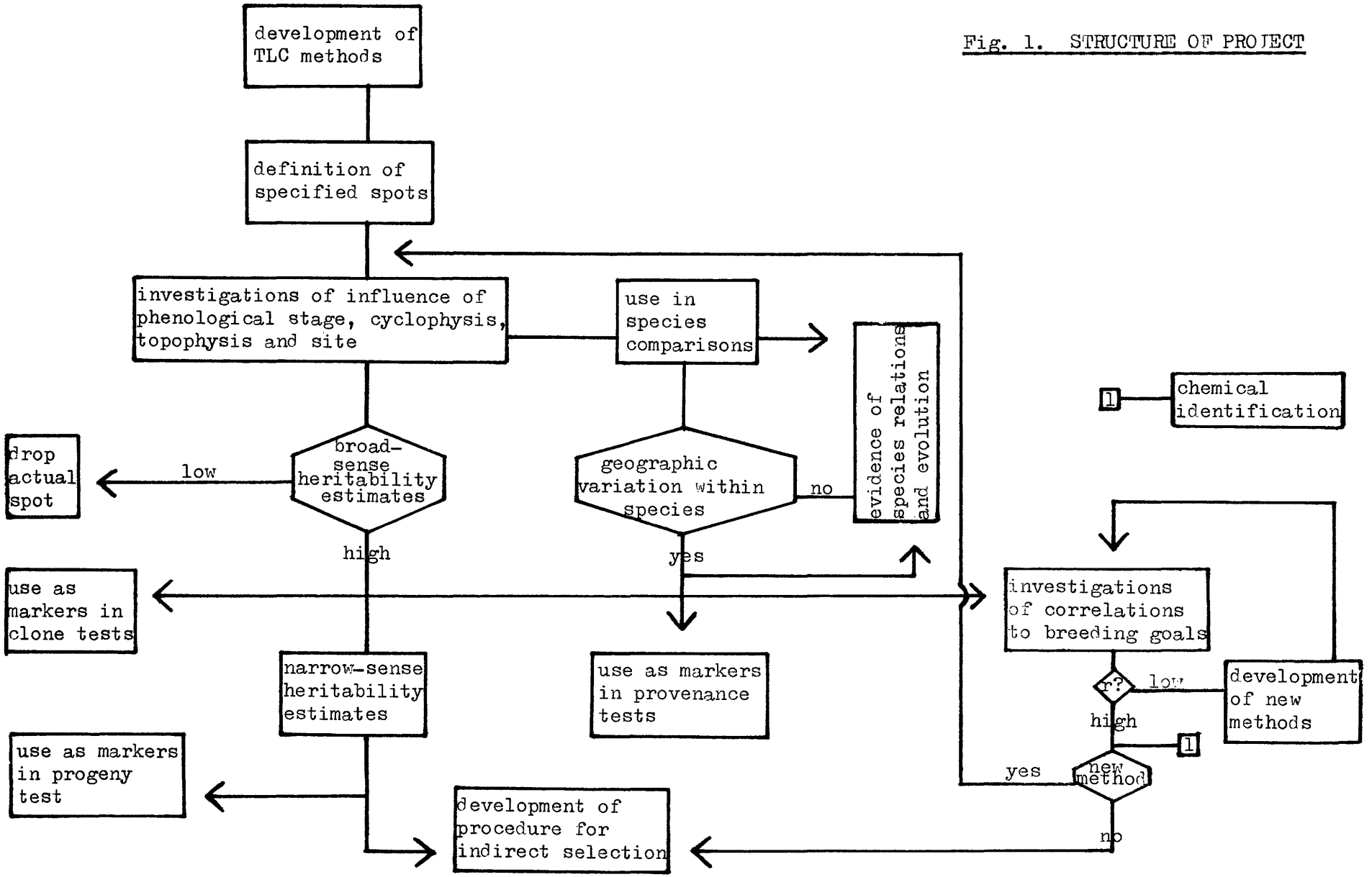


Fig. 2. MULTIVARIATE CANONICAL ANALYSIS OF 23 PICEA SPECIES (6 - 25 years old material)

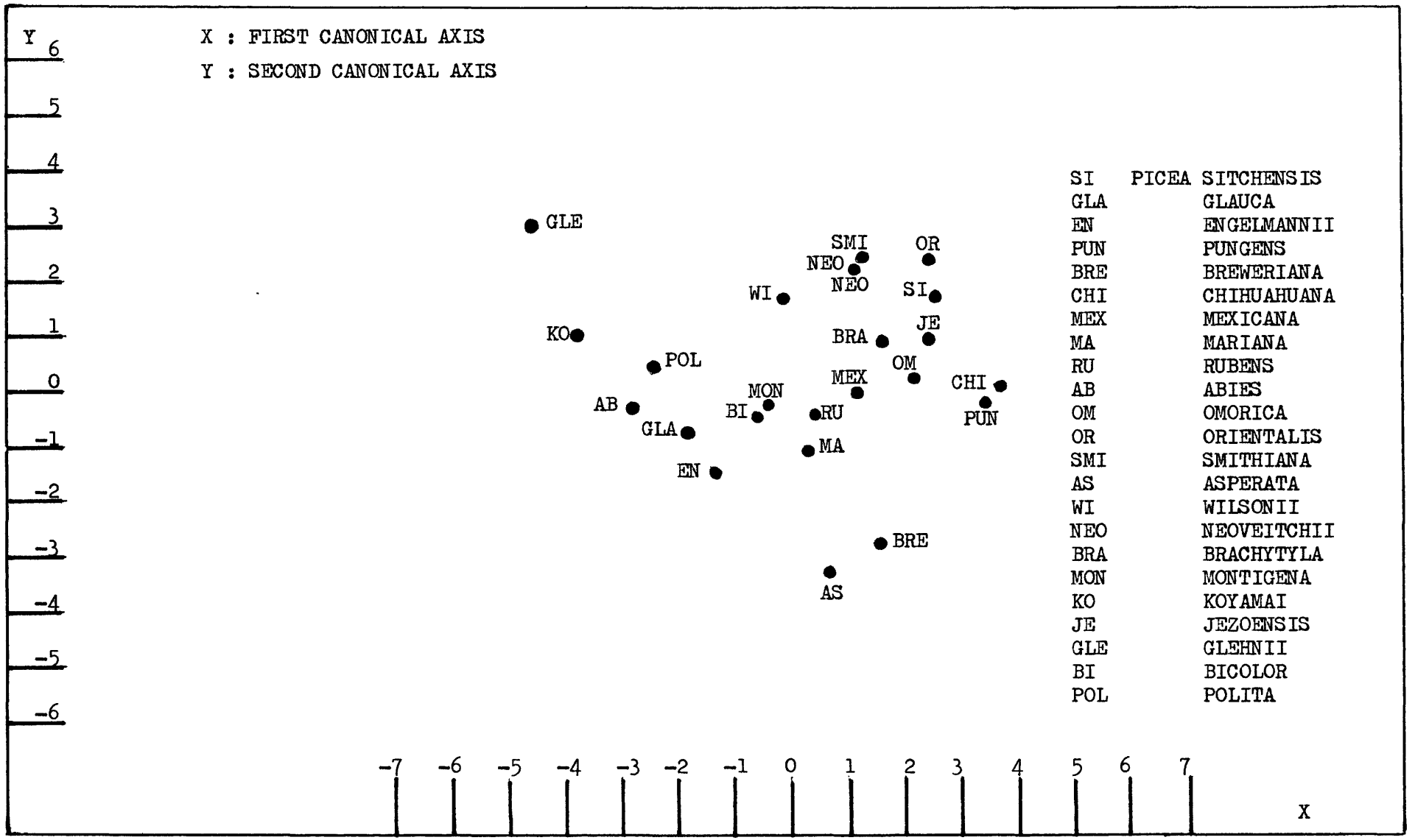
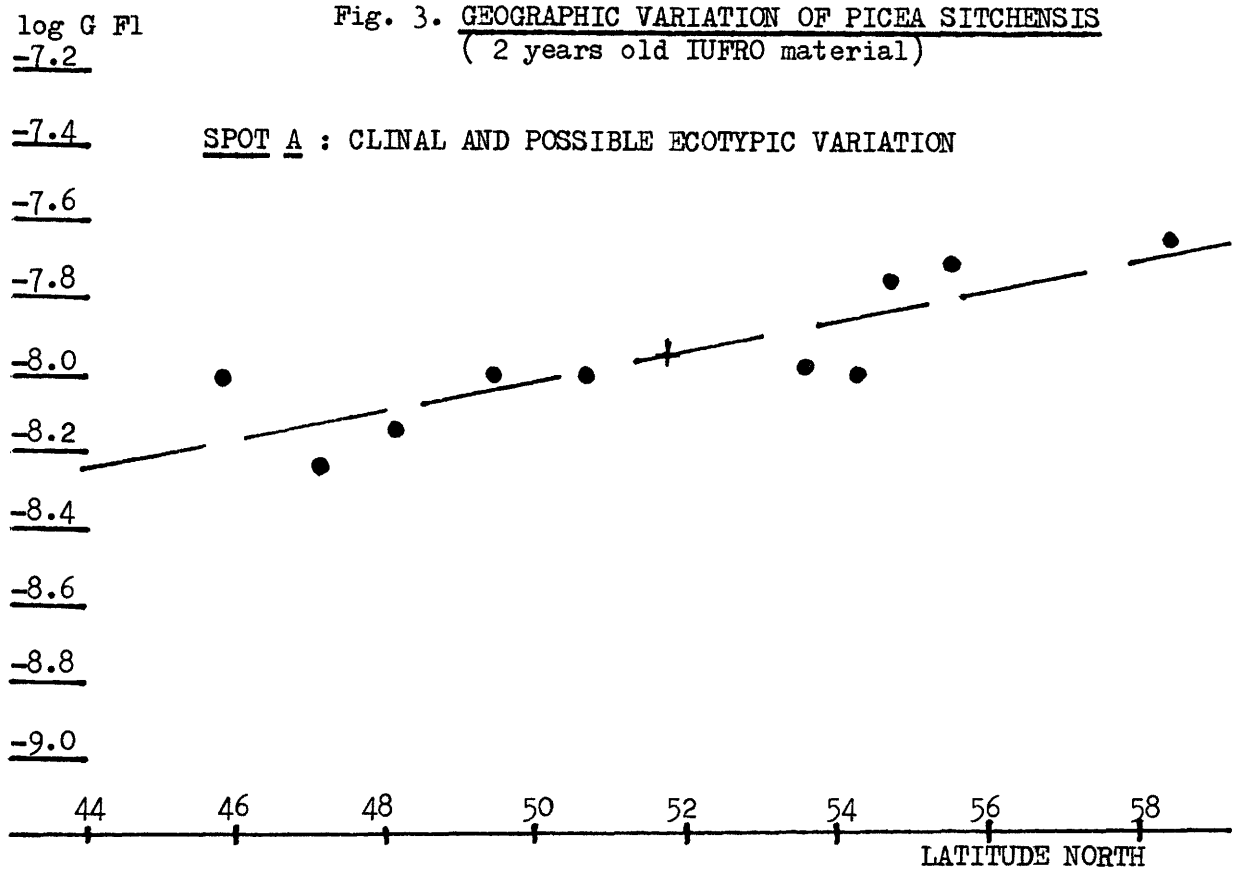


Fig. 3. GEOGRAPHIC VARIATION OF PICEA SITCHENSIS
(2 years old IUFRO material)



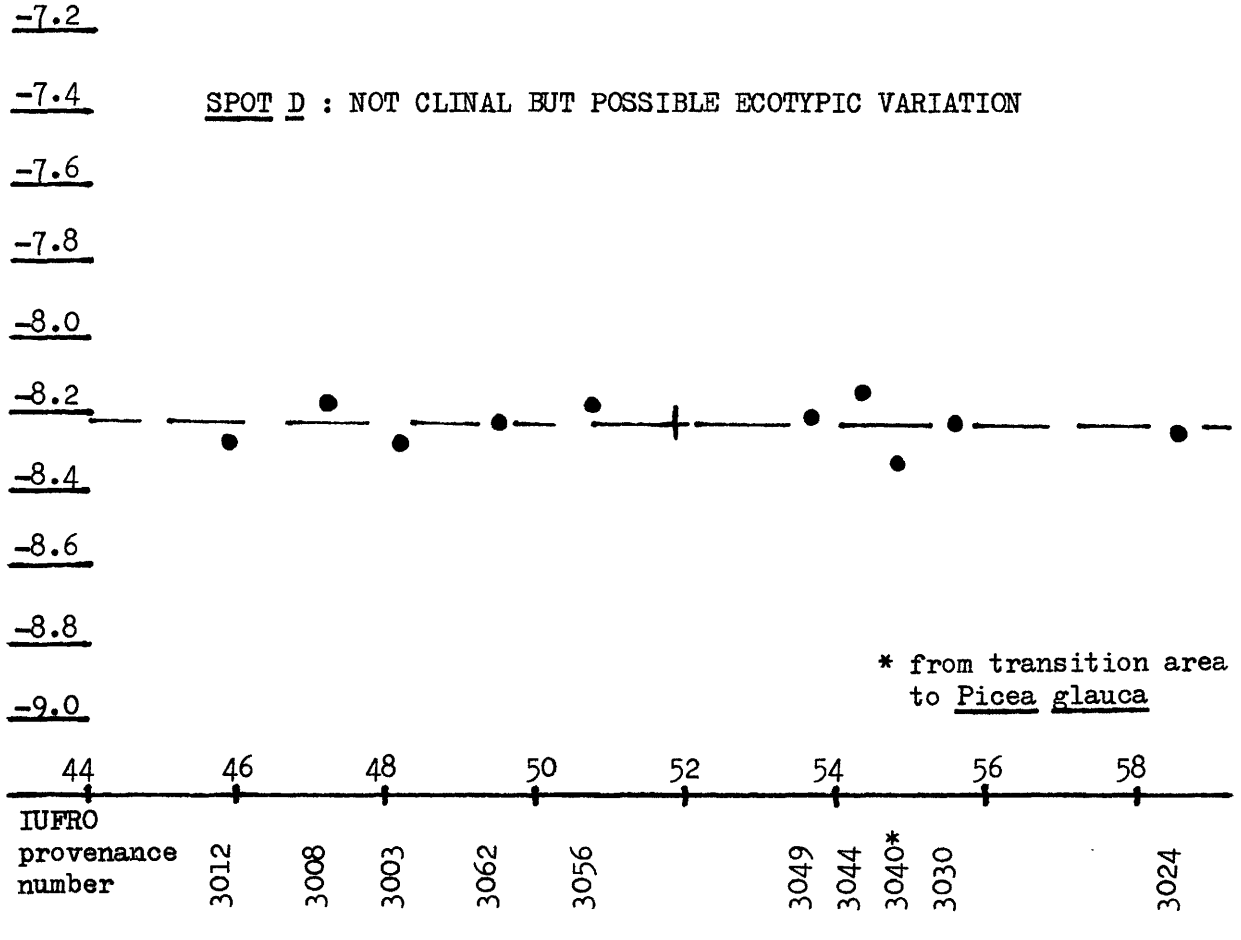


Fig. 4. GRAPHIC COMPARISON OF 20 YEAR OLD RAMETS FROM 20 CLONES GROWING ON TWO VERY DIFFERENT SITES IN NE-SJAELLAND.

THE DASHED LINE INDICATES EQUALITY, i.e. WHAT SHOULD BE EXPECTED IN THE CASE OF COMPLETE RESEMBLANCE OF THE CLONES GROWING ON THE TWO SITES.

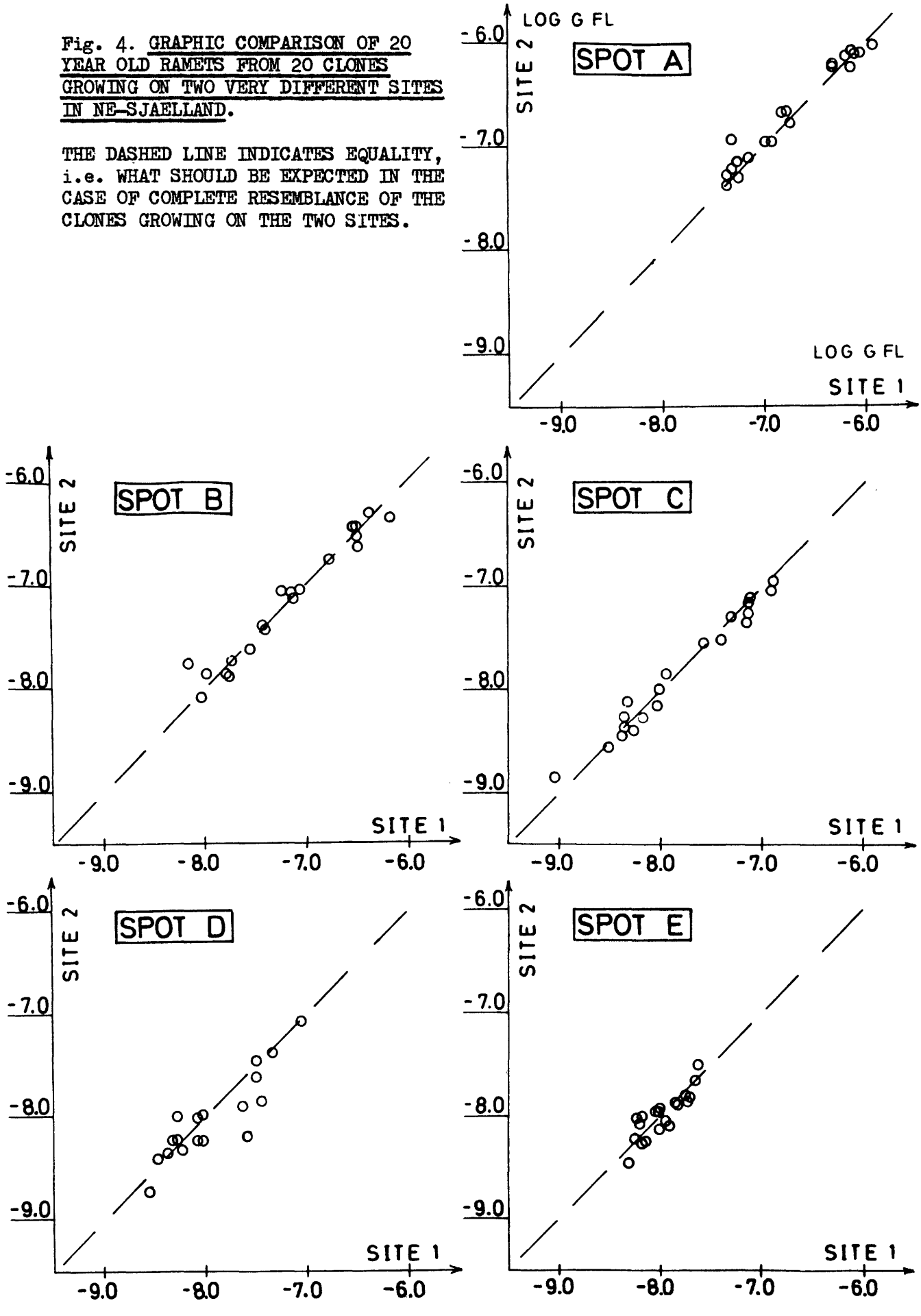


Fig. 5. PARENT CLONES AND OFFSPRING AFTER OPEN POLLINATION OF ORTETS. 20 YEAR OLD MATERIAL OF GERMAN ORIGIN (FUSSINGØ).

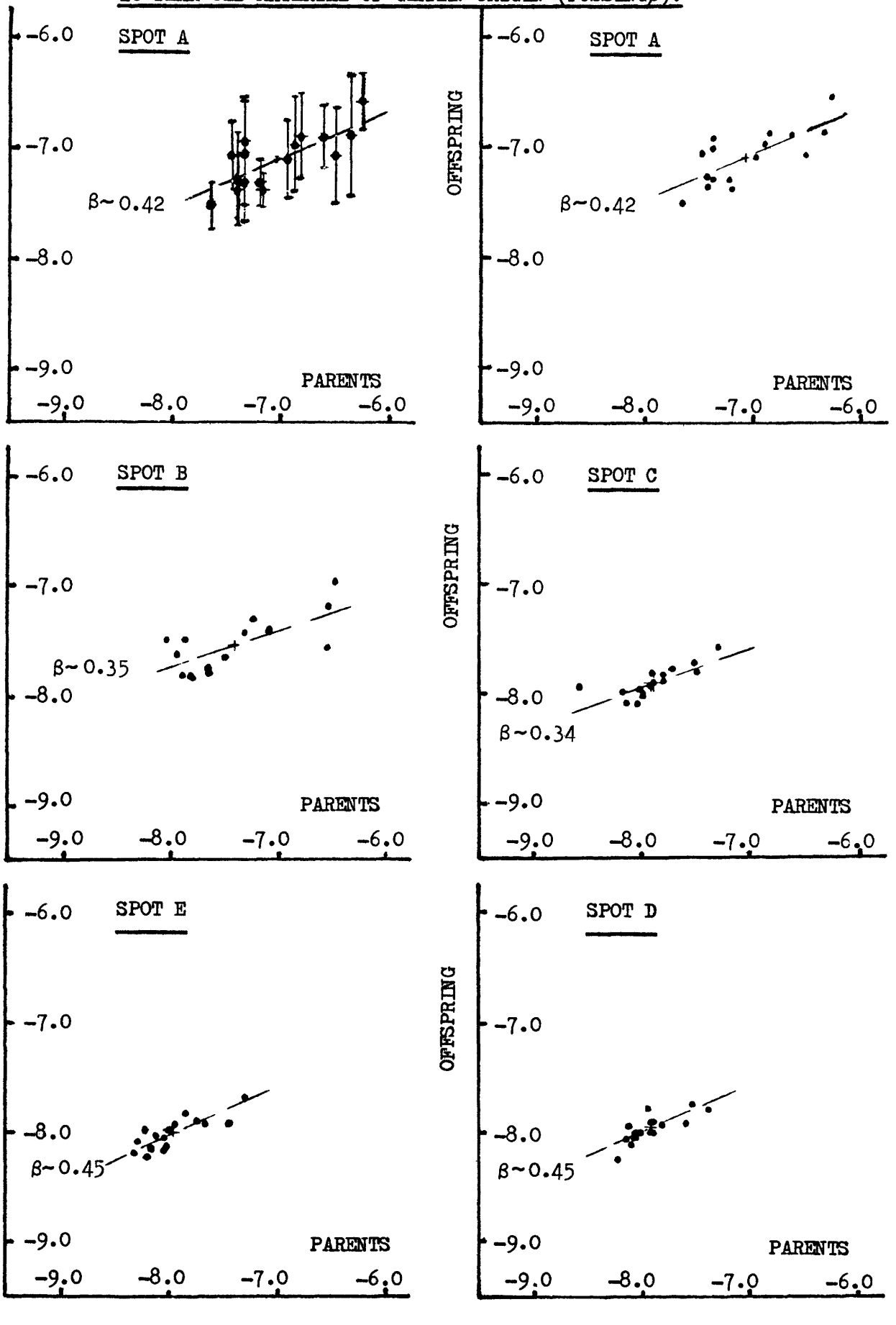


Fig. 6. PARENT CLONES (REPRESENTED BY MID-PARENT VALUES) AND OFFSPRING AFTER CONTROLLED CROSSINGS. 20 YEAR OLD MATERIAL OF GERMAN ORIGIN (EKEBO)

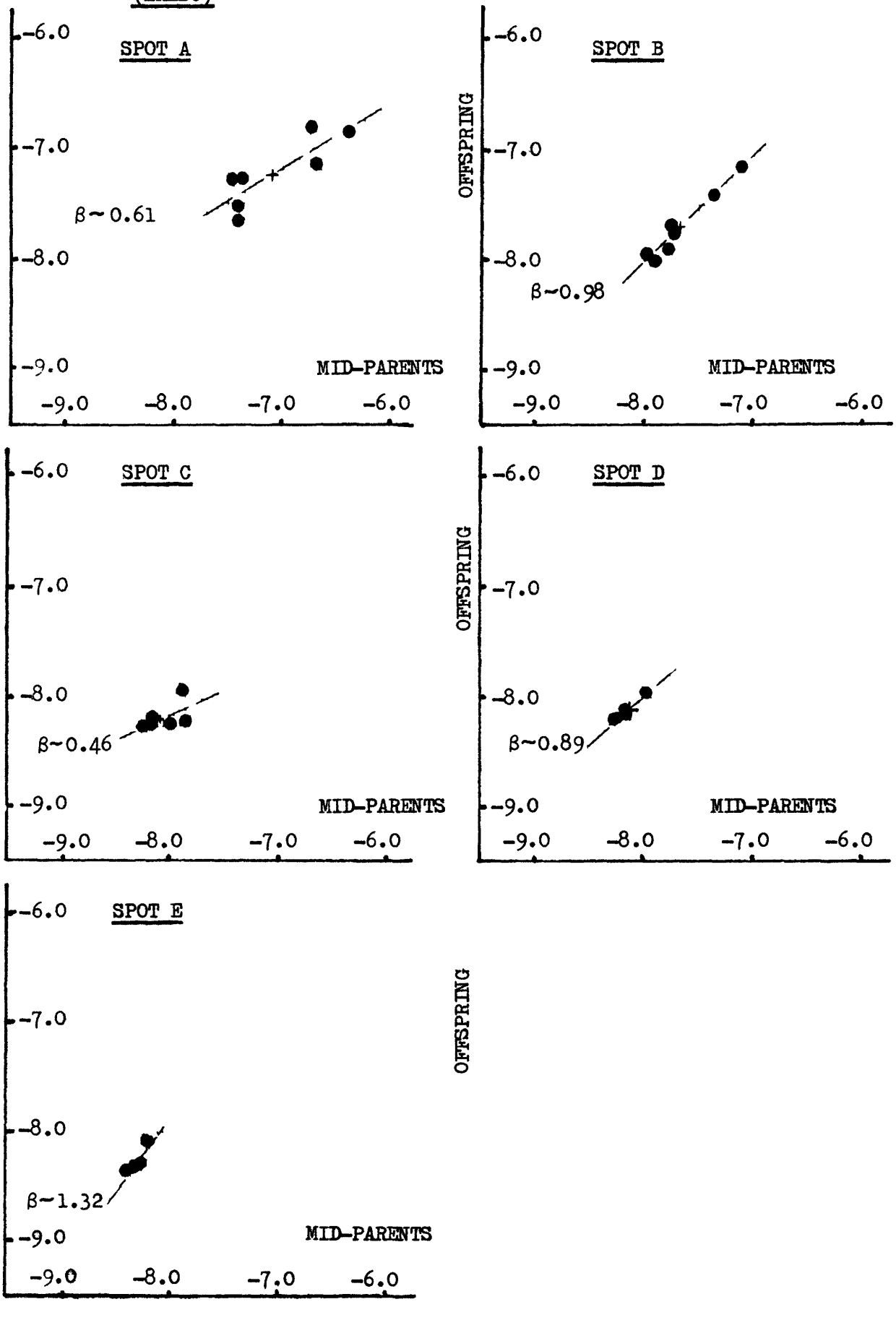
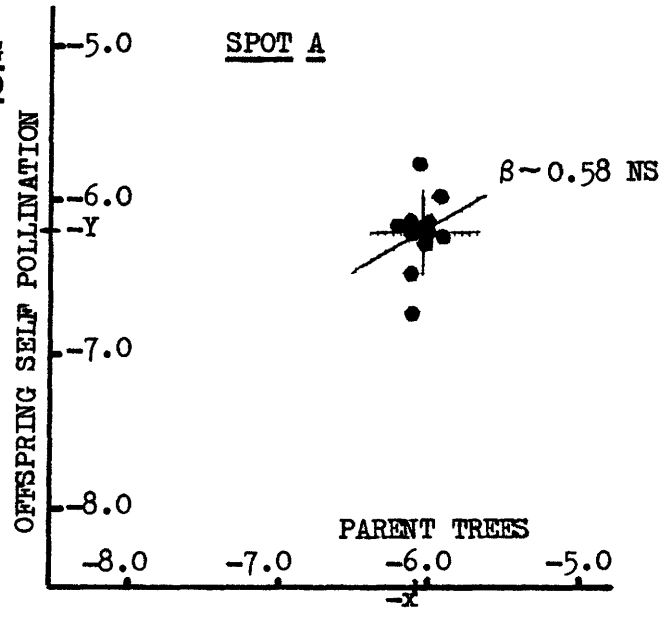
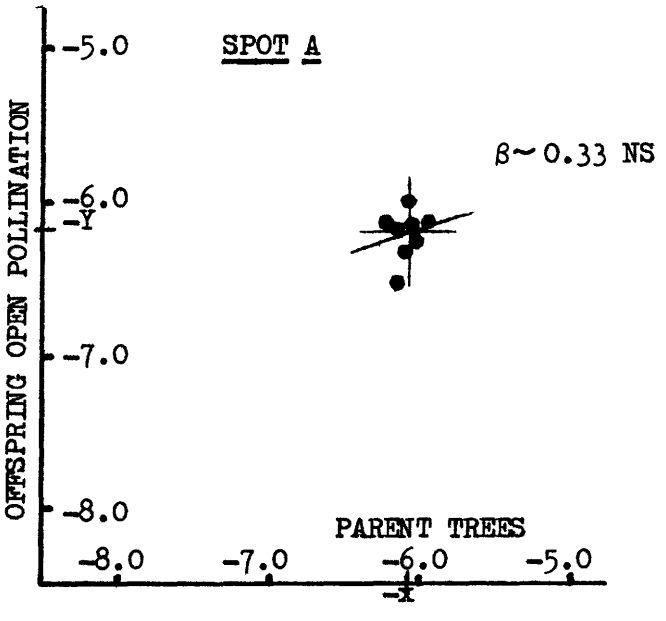


Fig. 7. PARENT TREES AND OFFSPRING AFTER SELF-POLLINATION AND AFTER OPEN POLLINATION.

SPOT A. MATURE PARENT TREES AND 6-YEAR OLD OFFSPRING OF AUTOCHTONE SOUTHERN FINNISH SPRUCE POPULATION.





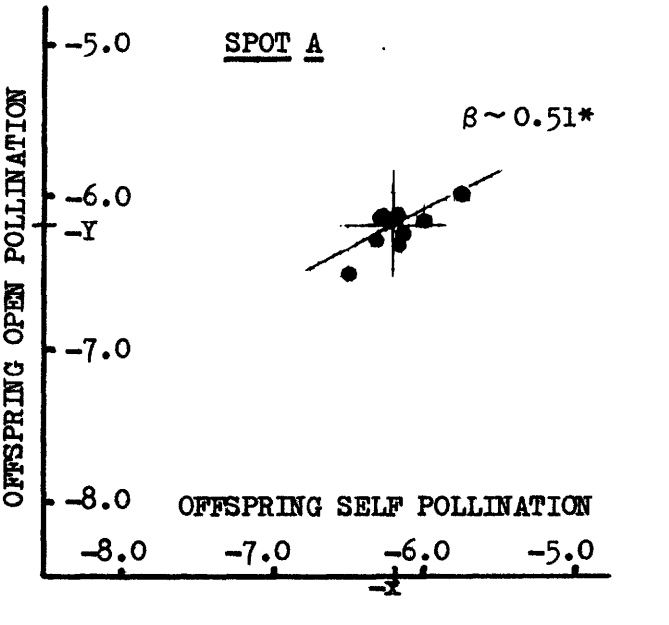


Fig. 8. PARENT TREES AND OFFSPRING AFTER SELF-POLLINATION AND AFTER OPEN POLLINATION.

SPOT B. MATURE PARENT TREES AND 6-YEAR OLD OFFSPRING OF AUTOCHTONE SOUTHERN FINNISH SPRUCE POPULATION.

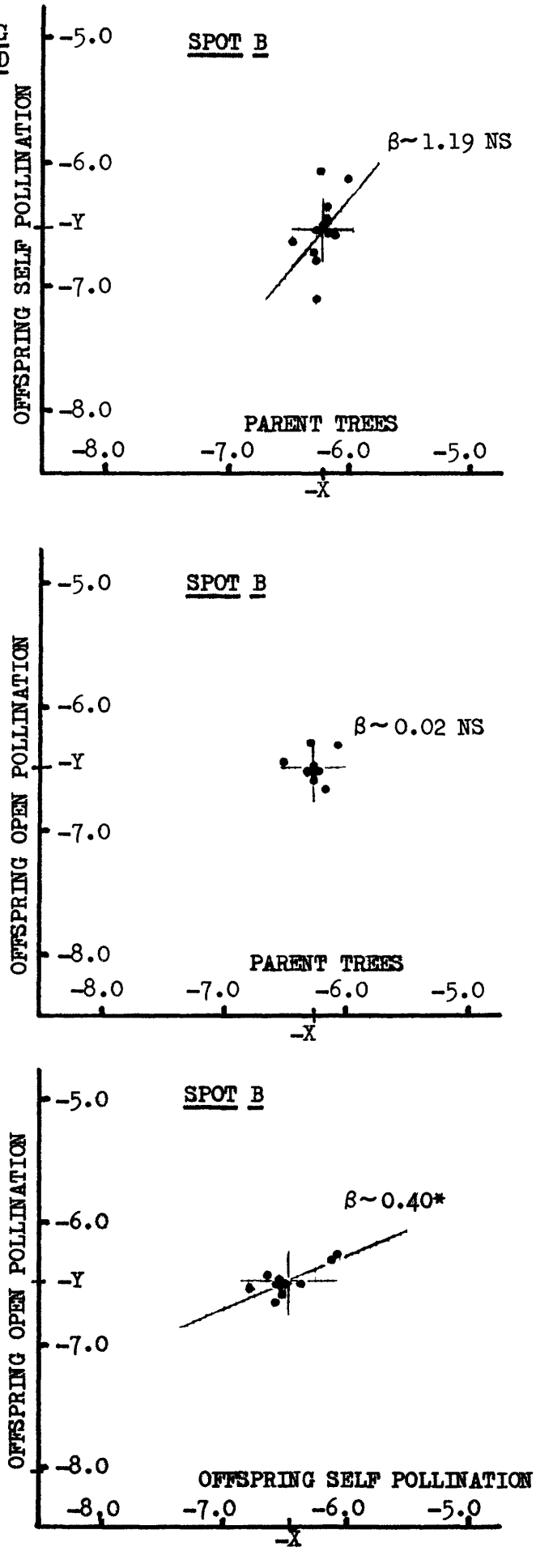
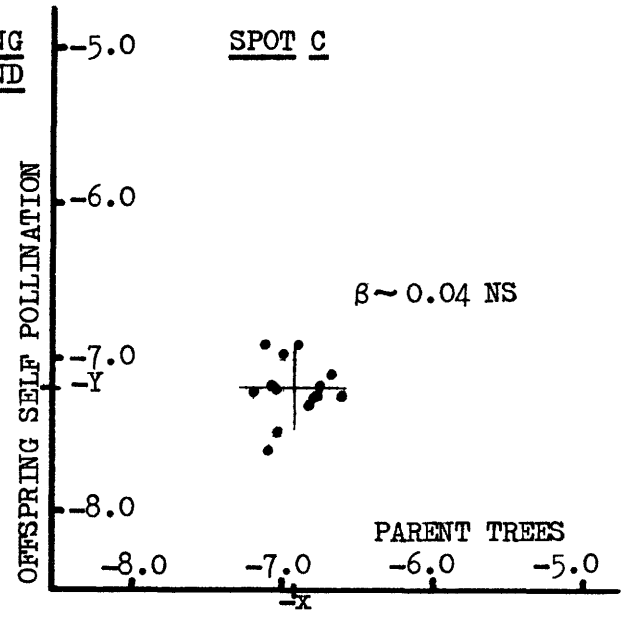
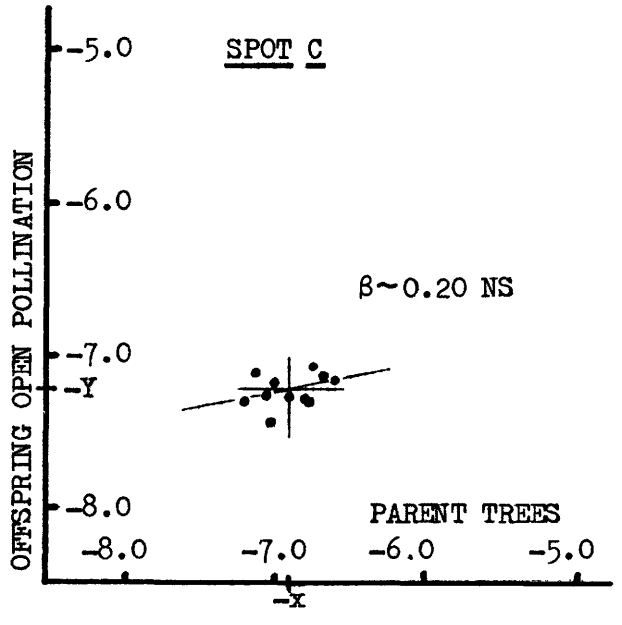


Fig. 9. PARENT TREES AND OFFSPRING AFTER SELF-POLLINATION AND AFTER OPEN POLLINATION.

SPOT C. MATURE PARENT TREES AND 6-YEAR OLD OFFSPRING OF AUTOCHTONE SOUTHERN FINNISH SPRUCE POPULATION.





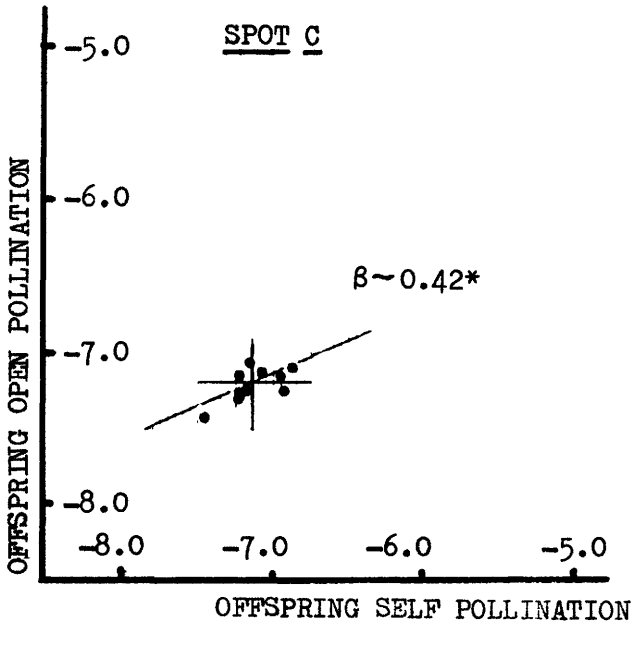


Fig. 10. DISTRIBUTION OF MATURE PARENT TREES AND THEIR 6-YEAR OLD OFFSPRING AFTER OPEN POLLINATION IN THE SOUTHERN FINNISH AUTOCHTONE SPRUCE POPULATION AT TUUSULA.

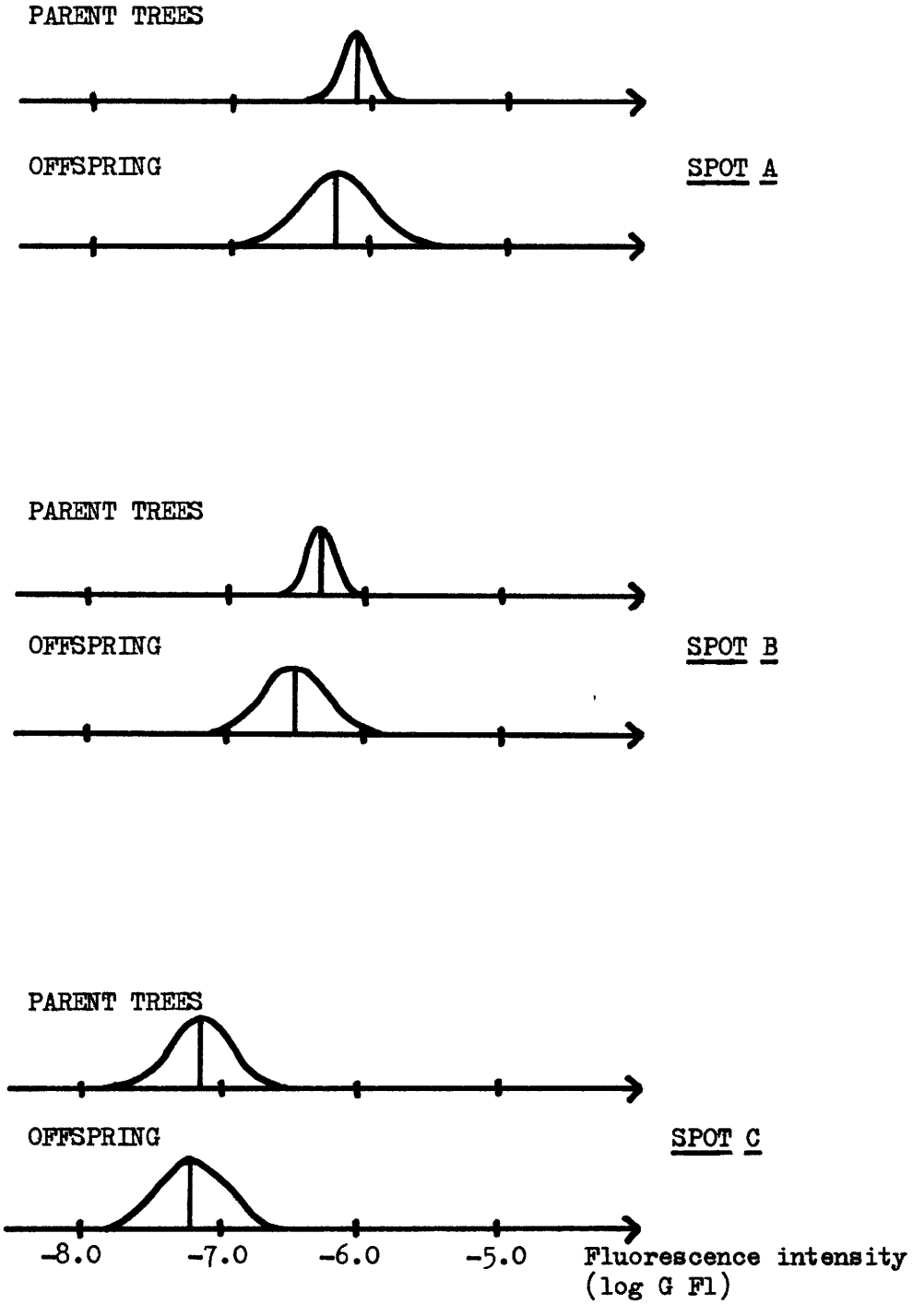


TABLE 1. SUMMARY OF QUANTITATIVE GENETIC ANALYSES.

MATERIAL	PARENTS			OFFSPRING					PARENT-OFFSPRING REGRESSIONS	
					VARIANCES					
	spot	mean	variance	mean	between-family	within-family	total	var.-homog within fam. Bartl. χ^2	b	$h^2_{N.S.}$
16 parent clones and offspring after open pollination of ortets. 20 year old material of German origin (FUSSINGØ)	A	- 7.05	0.1865	- 7.08	0.04373	0.1282	0.1719	21.33 NS	0.42**	(~0.8)
	B	- 7.42	0.2680	- 7.54	0.04672	0.1475	0.1942	33.45 **	0.35**	(~0.7)
	C	- 7.88	0.07473	- 7.92	0.01142	0.06335	0.07477	27.36 *	0.34***	(~0.7)
	D	- 7.96	0.04893	- 7.96	0.01248	0.04620	0.05868	28.53 *	0.45***	(~0.9)
	E	- 7.97	0.07061	- 8.03	0.01501	0.05322	0.06823	11.00 NS	0.45***	(~0.9)
13 parent clones and 12 offspring families after controlled crosses. 20 year old material of German origin (EKEBO)	A	- 7.10	0.1902	- 7.17	0.08067	0.06486	0.1455	26.96 ***	0.61*	~0.6
	B	- 7.53	0.1267	- 7.67	0.05831	0.05544	0.1138	55.88 ***	0.98***	~1.0
	C	- 8.01	0.07563	- 8.16	0.07685	0.02066	0.09751	23.49 *	0.46 NS	(~0.5)
	D	- 8.04	0.04982	- 8.14	0.005077	0.01742	0.02250	12.40 NS	0.89***	~0.9
14 parent trees and their offspring after open pollination. Old parent trees and 6-year old progeny from autochton S-Finnish stand (TUUSULA)	A	- 6.09	0.009448	- 6.22	0.01317	0.04786	0.06103	11.0 NS	0.33 NS	
	B	- 6.32	0.009269	- 6.49	0.00881	0.04248	0.05129	22.5 *	0.02 NS	
	C	- 7.12	0.03451	- 7.20	0.00800	0.05484	0.06285	11.0 NS	0.18 NS	
Same 14 parent trees and their offspring after self pollination	A	- 6.07	0.009448	- 6.22	0.03924	0.05184	0.09108	13.6 NS	0.58 NS	
	B	- 6.26	0.009269	- 6.55	0.05344	0.06883	0.12226	27.4 *	1.19 NS	
	C	- 6.92	0.03451	- 7.17	0.02535	0.06053	0.08588	24.0 *	0.04 NS	

TABLE 2: REGISTERED CORRELATIONS BETWEEN SPOTS AND BREEDING GOALS

MATERIAL	BREEDING GOAL	SIGNIFICANCE FOR THE MULTIPLE REGRESSION. F-VALUE	PARTIAL CORRELATION COEFFICIENTS BETWEEN BREEDING GOAL AND SPOT				
			A	B	C	D	E
13 clones. 20 year old material of German origin (FUSSINGØ)	Height growth :						
	- total height	2.39 NS	0.32	- 0.02	0.09	- 0.61	0.38
	- Δ H (4 years)	0.96 NS	- 0.10	0.02	0.11	0.07	0.21
	- mean height of progenies	0.08 NS	- 0.14	0.11	- 0.11	0.04	0.12
	Flushing	0.31 NS	0.02	- 0.26	0.15	0.27	- 0.18
11 clones. 20 year old material of Polish origin (IUFRO 1939)	Height growth :						
	- total height	0.19 NS	- 0.12	0.12	0.20	- 0.21	0.13
	Wood density :						
	- uncorrected	0.87 NS	0.01	0.00	- 0.44	0.12	- 0.33
	- corrected 1)	1.37 NS	0.04	- 0.23	- 0.51	0.21	- 0.37
	Flushing	5.05 *	- 0.08	- 0.49	- 0.47	- 0.65	0.11
19 plus-tree candidates selected in a 35 year old stand of KARPATO-UKRAINE origin	Wood density :						
	- uncorrected	2.85 NS	- 0.31	- 0.31	0.56	0.59	- 0.69
	- corrected 1)	0.95 NS	- 0.15	- 0.22	0.31	0.45	- 0.47

1) Corrected for the influence of ring width:

SESSION PAPER
SEANCE IV DOCUMENT 10
SITZUNG DOKUMENT

THE USE OF SEROLOGICAL METHODS FOR THE IDENTIFICATION OF SPECIES, PROVENANCES AND CLONES OF FOREST TREES.

L'EMPLOI DES METHODES SEROLOGIQUES POUR L'IDENTIFICATION DES ESPECES, DES PROVENANCES ET DES CLONES DES ARBRES FORESTIERS.

DIE ANWENDUNG SEROLOGISCHER METHODEN BEI DER IDENTIFIZIERUNG VON ARTEN, PROVENIENZEN UND KLONEN DER WALDBÄUME.

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SUMMARY

With the expansion of the trade in forest reproductive material, the need for identification of species, provenances and single clones is increasing, and with the development of cultivars of forest trees, the unequivocal identification of such cultivars will assume great importance.

Morphological descriptions are often difficult at the subspecies level and even at the species level; the material at hand does not always permit a morphological identification in the usual way of classical taxonomy.

Biochemical techniques must therefore be developed to aid in the identification and they already offer considerable help for this purpose. With different biochemical methods data can now be obtained that can be treated together with other information using numerical taxonomic procedures.

Serological methods, well known for their highly specific use in the identification of micro-organisms, have also been applied to forest trees and the results are promising. Differences at the subgenus level have been found in experiments with pine pollen and intraspecific differences between geographic sources as well as between individuals has been found.

Substances capable of cross reacting with antisera against known chemotypes of Salmonella bacteria have been found in pine pollen and the reaction patterns differ for different pine species as well as for the different serotypes of the bacteria.

Experiments using the same technique for endosperms from seeds are in progress.

It is suggested that specific bacterial antisera which are easily obtainable might be a good tool for the identification work of forest trees.

RESUMÉ

A cause de l'expansion du commerce du matériel reproductif forestier il y a un besoin croissant pour l'identification des espèces, des provenances et

des clones. En outre, l'identification sans équivoque des cultivars des arbres forestiers aura une grande importance à cause du développement des mêmes.

La description morphologique est souvent difficile sur le niveau des sous-espèces, même sur le niveau des espèces, le matériel obtenible ne permettant pas toujours l'identification morphologique de la façon habituelle de la taxonomie classique.

A cause de cela on a besoin de développer des méthodes biochimiques pour faciliter l'identification et ils offrent déjà une aide considérable pour ce but.

Maintenant on peut obtenir des données par les méthodes biochimiques diverses et on peut les traiter ensemble avec d'autres données employant des procédés numériques taxonomiques.

Les méthodes sérologiques, connues pour leur emploi spécifique dans l'identification des micro-organismes ont été appliquées aux arbres forestiers avec résultats promettants. Dans les essais avec le pollen du pin on a trouvé des différences sur le niveau des sous-genres et des différences intraspecifiques entre les sources géographiques de même que entre les individus.

Substances capables de réaction croisière avec les antisérums contre les chemotypes connus du bactère Salmonella ont été trouvées dans le pollen du pin. Les modèles de réaction varient selon les espèces différentes de même que selon les sérotypes différents des bactères.

On est en train de faire des essais avec les endospermes des graines employant la même méthode.

On a proposé que les antisérums bactériels spécifiques facilement obtenibles pourraient servir d'un bon outil dans les travaux de l'identification des arbres forestiers.

ZUSAMMENFASSUNG

Mit der Entwicklung der Handel mit Forstpflanzen und Forstsaatgut ist schon

vorhanden ein immer grösserer Bedarf für Bestimmungsmethoden für Arten, Herkünfte und Einzelklonen.

Die Züchtung von Sorten der Waldbäume fordert auch das solche zweifellos identifiziert werden können.

Morphologische Beschreibungen sind oft am Unter-Arten-Niveau schwierig und eben mit Arten ist es nicht immer möglich mit vorhandenes Material eine Identifizierung mit klassischen taxonomischen Methoden zu machen.

Biochemische Methoden müssen für solche Zwecke auch entwickelt werden, und es gibt schon viele Methoden die dafür geeignet sind. In dieser Weise kann man nunmehr eine grosse Menge von Daten bekommen die zusammen mit anderen Informationen mit der Methode der numerischen Taxonomie behandelt werden können.

Serologische Methoden die ja bekannt sind für ihre hochspezifische Anwendungsmöglichkeiten in der Identifizierung von Mikroorganismen, sind auf Waldbäumen probiert und die Resultate sind versprechend. Unterschiede zwischen Sub-Genera sind in Experimente mit Kiefernpollen gefunden und dazu intraspezifische Unterschiede zwischen geographischenHerkünften und auch zwischen Individuen.

Substanzen die mit Antisera gegen Salmonella-Bakterien reagieren sind in Kiefernpollen gefunden und die Reaktionsmuster sind für viele Arten sowohl als für die verschiedenen Serotypen der Bakterien verschieden.

Experimente mit derselben Technik mit Endospermen aus Samen sind im Anfang.

Es wird gedacht, das spezifische Antisera gegen Bakterien, die ja leicht produzierbar sind, in der Identifizierung von Waldbäumen sehr brauchbar sein könnten.

THE USE OF SEROLOGICAL METHODS FOR THE IDENTIFICATION OF SPECIES, PROVENANCES AND CLONES OF FOREST TREES

INTRODUCTION

The international trade in forest reproductive material is expanding and with this development, the need for identification of species, provenances and clonal material is increasing.

The breeding of forest cultivars makes the unequivocal identification of such cultivars a necessity, but this will often meet with difficulties since, as pointed out by Grant (1973), morphological descriptions of cultivars present problems in identification, as clearcut distinguishable differences between cultivars are often lacking.

Biochemical techniques however, offer additional ways for identification and discrimination and the application of biochemical taxonomy is therefore gaining more and more importance especially now that improved techniques such as chromatography, electrophoresis and serology are available. Alston and Turner (1963) have reviewed the situation and they have also given a very comprehensive discussion about the earlier literature on plant serology.

As a matter of fact, one group of forest trees, the genus Pinus is one of the best chemotaxonomically investigated plant groups that hitherto has been studied (Erdtman, 1963, 1968a, 1968b, Mirov, 1967).

In order to get a"fingerprint picture" as true as possible it is necessary to apply many different methods and finally to try to combine the results. But to identify complex substances from tissues is difficult and the separation of them in their final parts might not disclose their nature and their activity in vivo. If the substances are surface components they may, however, in many cases be identified by immunochemical methods.

The specificity of cell surface substances has been sufficiently documentated in bacteriology, histo-incompatibility and allergenic reactions. In plants it has been possible to trace serologically detectable differences between geno-types to single pollen grains (Lewis, Burrage & Walls, 1967).

Already Heidelberger has pointed out the possibilities for using serological methods in the study of complex polysaccharides (Heidelberger, 1959) and later he showed the usefulness of specific antisera for Pneumococci-bacteria as a tool for detecting specific sugars (Heidelberger, 1960).

With this technique he found e.g. that an arabogalactan from Jeffrey pine reacted specifically with antisera to Pneumococcus strains type IV and type XIV. Reactions were also obtained with Salmonella-bacteria. This method could furnish powerful criteria of inhomogenity in polysaccharides and often rapidly yield clues as to the sugars present and even the chemical linkages (Heidelberger, 1964).

Conifers have a constitution containing a great number of complex polysaccharidic substances, but most of the studies have hitherto been made of the woody parts of the trees. It has, however, been found that pollen also might contain many complex sugars. For further references see e.g. Hagman (1975).

A serological approach towards tree species seems therefore justified. The method might, however, also have its drawbacks. Plants have been found to contain several substances with the ability to precipitate antisera and react with red blood cells. Many of them, including a compound from Taxus cuspidata, have been found to be specific to the same substances that determine the serological blood group types (Springer, 1966). Lectins have mainly been found in the Leguminosae (Mäkelä, 1957) and although the list by Boyd, Waszezenko-Zacherczenko and Goldwasser, (1961) stated that seeds of Pinus cembroides monophylla were negative or non-specific for human erythrocytes A, B and O and seeds of P.griffithii were negative with A_1 A_2, B,O and several other types, no pollen tests were reported nor tests on further pine species.

Serological methods have been applied to forest trees for various purposes. Clarkson and Fairbrothers (1970) used serology for taxonomical work with Abies. Hagman applied the method in incompatibility studies of Betula

(Hagman, 1971) and Pinus (Hagman, 1975). Knox, Willing and Ashford (1972) studied the pollen of Populus. Recently Borzan and Widakovic have used the same technique for the identification of those Pinus nigra clones having high crossability with Pinus silvestris (Proceed. I. Yugoslav. Congress of Genetics, in the press).

One of the most comprehensive serological studies on seed proteins of Pinus has been made by Saito (1968) who in addition to species investigations also compared provenances.

It therefore seemed worthwhile to investigate, as a kind of pilot project, if serological differences could be detected between species, provenances and individuals using pollen from pines—a genus with a high degree of variation and a world-wide distribution.

MATERIALS AND METHODS

The plant material used consisted almost exclusively of pollen of pines. This pollen was obtained from many different sources and if not especially mentioned, the pollen sample comes always from a single tree. Several of the pollen lots used in the serological studies were also used in hybridization work to be reported elsewhere. These pollen sources are given in table 1. The pollen samples used in the serological part only are listed in table 1a. It should be noted that the Placerville pollen samples were coded at Placerville at the time of shipment in order to exclude any possible influence of the knowledge of the species when evaluating the results.

With a few exceptions, the exotic pollen samples are limited to one source per species, which, of course, is a limitation for the applicability of the results.

Only with Pinus sylvestris is it possible to get some view of the intraspecific variation.

The need for an additional supply of the same pollen source restricted the material for the antisera production to Pinus cembra, P.peuce, P.balfouriana, P.sylvestris and P.contorta. Thus the antisera are restricted to the sections Strobus, Parrya and Pinus.

The nomenclature in this study follows Chritchfield and Little (1966).

The Salmonella antisera used were provided by the State Serological Institute, Helsinki. Sera of five types were obtained:

	T_1	Serum K 829 and 838
	C_1	K 170
S.Paratyphi	B	K 990
S.typhi	D	K 370
	G_{22}	K 010

Extraction of antigens from pollen and the production of antisera followed standard procedures as earlier described by Hagman (1971).

Double diffusion in agar plates was carried out as described by Ouchterlony (1965) using punched patterns of three, four or several wells (Feinberg, 1957).

The general technique of immunoelectrophoresis followed Nerenberg (1966).

RESULTS

Double diffusion plates

The double diffusion method disclosed that good serological reactions are obtainable with pine pollen antigens.

The double diffusion plates showed patterns both of similarity and dissimilarity. If the two antigen reactants are present in equivalent amounts the arcs formed will be sharp and symmetrical, but if the systems are unbalanced asymmetric arcs, double lines and false spurs may develop. Since it was not possible to keep the concentrations of the pollen antigens exactly equal in all samples, some of the differences seen in the patterns may

be due to this fact.

On the other hand, it is obvious that the different pines show distinct differences from each other. The total number of precipitates found in the different experiments is summarized in table 2. Since this does not give an idea about the identities or non-identities, the kind of precipitates in each combination is summarized in tables 3-6.

If one looks at these tables and to the corresponding figures, a general impression is that there are not very many serologically active substances in pine pollen. The highest number of precipitates mentioned in table 2 is four although occasionally as much as six precipitates could be observed. This low figure indicates that a very detailed system of differences is probably not to be found among the relatively numerous species of the pines.

First inclinations are therefore to look for patterns common to higher taxonomical groups and one is immediately struck by the obvious differences between the two sub-genera Haploxylon and Diploxylon. There has, of course, been ample biochemical evidence earlier for the differences between these two groups, but it is interesting to note that this difference, so well founded also on morphological studies, can be observed also in the serological reaction of the pollen grains.

From the serological results one gets the impression that the Haploxylon pines consist of a fairly homogeneous and rather easily classifiable group.

This is somewhat surprising when one thinks of the very remote location of the North American and the Asiatic pines of this group. Nevertheless, the reactions of for example P.balfouriana and P.bungeana are in many cases identical. The serologically detectable similarities,respectively differences, may thus have been founded during a very early period of the evolution of the pines. One wonders, if in the subgenus Diploxylon, which also has a very discontinuous distribution of species, there could also be found examples of similarity, as in the Haploxylon pines.

One case which offers a particularly interesting object for comparison involves the species P. canariensis and P. roxburghii. When the pollen of these species was tested with antiserum for P. sylvestris, both pollen antigens formed 4 precipitates of which 3 were identical. Thus tested with P. sylvestris, P. canariensis and P. roxburghii show great similarity. It would be highly interesting if antisera against antigens of both these species could be produced and then the homologous and heterologous reactions performed.

One might also note the very small reactivity shown by the pollen antigen of P. pringlei, one of the very few representatives of the Mexican pines in this study.

One of the species showing several clear reactions of non identity is P. taeda. P. rigida also differs from the others in many cases. More of these interesting members of the subsection Australes should be included in further studies since it is well known that they cross easily with each other within the southern pines of this group but that there are great difficulties with these pines in hybridization with species from other parts of the distribution area of the Australes.

Observations on the amount of intraspecific difference in serological behaviour are limited. Some facts could be obtained from the tests with Finnish sources and the material of P. sylvestris could be expanded to include pines of Scottish origin.

The trees available of P. cembra and P. peuce showed a high degree of similarity within the species. But the sample trees are from cultivated stands where the provenance used probably originated in a rather limited area. The P. peuce has a limited distribution also in nature and seems to be fairly homogeneous morphologically. A similarity in serology to a certain degree could therefore be expected.

In P. sylvestris the tree E 144 appeared to be more similar to the other individuals than E. 111. Many trees showed different reactions with serum for E 111.

Several trees were tested against antiserum for the tree E 2. Many of them showed differences from the E2 pattern. Most similar were the trees E 302, E 35 and E 67. At least one clearly different precipitate was shown by

the trees E 144, E 90, E 360, E 110, E 610, E 630, E 635, E 1003 and La 37. It could not be noted that the northern trees La 37 and P 2504 should have been particularly more different from E 2 than the southern trees.

When tested against antiserum for P.sylvestris E 2 of Southern Finland, several of the Scottish pines showed clear differences. Among these were 30 Grant, 223 Tanar, 46 Maree and 41 Maree. Similarity, on the other hand was shown by 45 Maree, 39 Grant, 229 Tanar and 54 Maree. When Scottish trees were tested in pairs against antiserum for the Finnish tree E 2, the Scottish patterns were very similar. When Finnish trees were tested in comparison with Scottish trees, the differences were very pronounced. These experiments would indicate that the Scottish and the Finnish populations of P.sylvestris contain individuals which are, at least in some cases, more serologically different than the trees from each country. This result suggests that further investigations into the biochemical differences of races and provenances might open interesting perspectives on the evolution of geographical varieties and species formation.

There is also some evidence for intraspecific variation in the samples of P.contorta and P.muricata. More samples of both species are needed in order to clarify the degree of difference.

In our samples of P.muricata the tree N18 represents the Mendocino County provenenance which has shown differences in crossability with other pro-provenances (Critchfield, 1967). The sample USA 2246 comes from south of Mendocino and is perhaps of the same type. The sample V 28 represents the area around Monterey whereas the mix 16-48 from San Vicente represents the Baja California area.

Immunoelectrophoresis

The immunoelectrophoretic method offers another way of comparing the antigenic composition of two samples. As could be expected from the double diffusion plates, pollen antigens and pollen antisera formed nice arcs of precipitate in the immunoelectrophoretic plates. The number of arcs was, however, often less than the number observed in the Ouchterlony plates.

In these experiments the own antigen was always run as a standard on one side of the plates whereas the other side contained different antigens. In tables 7-11 the results of these experiments are summarized and in the upper right corner of the table the standard "normal" pattern for the homologous reaction is given.

There are similarities as well as differences but it is difficult to find patterns consistent with the detailed taxonomy of the pines.

One pattern is, however, evident from the immunoelectrophoresis plates and this is the total absence of the anodic fraction (precipitate arc No 1) from all the members of the subgenus Haploxylon that has been tested.

The substance responsible for the formation of this anodic precipitate seems on the other hand to be present in most of the Diploxylon pines.

There seems also to be at least one substance which is common to almost all of the pines, but it has not yet been tested to see if this compound is not perhaps a common feature also of pollen of other plants and so cannot be said to be Pinus-specific.

The similarity or dissimilarity of precipitating arcs in the immunoelectrophoresis plates offers possibilities for further chemotaxonomical investigations. When enough pollen is available from the same individual source of each species it would be possible through combined "Sephadex" separation and electrophoresis to separate the substances responsible for each one of the serologically detectable precipitates. Separated fractions could then be collected and used in the production of very specific antisera and as specific antigens as well.

Cross reactions between pine pollen and bacterial antigens of Salmonella.

Encouraged by the studies by Heidelberger mentioned in the introduction, I decided to see if the use of specific Salmonella-sera could help in the classification of the pines. The reactions between the five types of Salmonella sera available and with the pine pollen as antigens were carried out in double diffusion plates. The reactions were in these cases very distinct and in most cases only one band of precipitate was formed. Thus

the classification could be limited to a positive or a negative reaction. The results of these studies are summarized in table 12.

The pattern of reaction is complex but if one looks for group differences the presence of a positive reaction with the Salmonella type C_1 in many of the Haploxylon pines, particularly in the section Strobus and the absence of the same reaction in all the Diploxylon pines tested except P.sabiniana is notable. Generally speaking the reaction pattern in the Haploxylon pines seems to be more homogeneous than in the Diploxylon pines. The similarity of the isolated P.bungeana compared with P.edulis and also partly with P.balfouriana is evident.

Some information about the intraspecific variation in P.sylvestris of Finnish and Scottish origin is collected in table 13. There seems, indeed, to be differences also at the individual level, and although the absence of a reaction sometimes can be due to low level of the antigen, studies with concentrated antigens did not change the patterns shown. Differences similar to those found in the Finnish pines were also found in the Scottish samples. Notable is the absence of any positive reaction with the type G_{22} in the Scottish material.

The fraction containing the anodic compound specific for the Diploxylon pines gave a positive reaction with all the Salmonella antisera. Thus it was not possible to relate the anodic specificity to any of the type-specific Salmonella antibodies. On the other hand, the first cathodic fraction gave a negative reaction with the Salmonella antisera.

As mentioned earlier the Salmonella types available for this study were D_1, B, C_1, T_1 and G_{22}. The specific sugars in the Salmonella types used are according to Lüderitz, Staub and Westphal (1966) and Straub (1960)

for S.typhi	D_1	tyvelose but contains also glucose, galactose and rhamnose.
S.paratyphi	B	abequose, mannose and rhamnose but contains also glucose and galactose
S.paratyphi	C_1	mannose
	G_{22}	galactosamine and fucose
	T_1	rhamnose

The positive reaction with pine pollen substances would therefore indicate that these sugars were present in the pollen antigen compounds. It is, however, to be observed, that the specificity of an antigen is defined by the presence of multiple and discrete determinant groups. A given determinant group produces a family of antibodies. Some of these antibodies can cross-react with other similar not identical determinant groups. The method is nevertheless interesting and more samples of the pines as well as of the very numerous Salmonella types should be tried.

Discussion

To make any far-reaching conclusions from the results here presented would be too early since one has to be aware of the rather small basis of this study. Although serological differences have been detected at many levels the detection is based on a very small number of antisera. These sera represent only a few of the pine species.

The variation found between individuals of the same species points towards the necessity of using several antigens in the study of the immunology for a particular species. If the intention is to detect genetically bound differences between trees it is clear that individual samples must be used, but also in this case samples should be taken in different years, to take account of any environmental effects.

If individual differences are found, which are consistent even after the production of antisera in several animals these identities offer great possibilities for identification, for use as genetical markers in crossing experiments and also for the detection of partners later suitable for hybridization.

A detailed study, which from a systematic point of view would be very interesting, would, judging from the case of P. pringlei, be the investigation of the Mexican pines and some other subtropical or tropical pine complexes.

The grouping obtained with the Salmonella antisera suggest that further experiments should be made with this technique using more types of this well known bacterial group. The advantages for using bacterial antigens

and antisera for mapping are obvious. They are easily obtainable and are often produced commercially in standard batches by bacteriological and serological institutes. The antigens are highly specific and the substances and/or structural compositions responsible for the type of serological reaction are in many cases already known (See e.g. Jann and Westphal, 1975). This would make them a very sensitive tool for classification work of many kinds.

Cronquist (1973) said that the great virtue of serological data is that they are objectively measurable and absolutely independent of all other characters. The usefulness of serological reactions is limited, however, by uncertainty as to what is actually being measured, by the occurrence of "antisystematic" reactions (commonly associated with lectins), and especially by the fact that they exist only as one to one comparisons rather than concrete data. The number of tests required to check a large number of items reciprocally against each other is astronomical.

This is, of course, true if we try to produce antisera against every tree to be tested. However, as we have seen the grouping using commonly available bacterial sera can to a certain extent be applied with a smaller number of identificators.

Even if the number of the tests grows larger there are techniques now available for the rapid handling of a great number of analytical data for taxonomical purposes. There is also rapid progress in the development of such techniques (See e.g. Willcox and Lapage, 1975; Hedén, 1974).

Once the basic investigations have been made there seems to be good possibilities for applying similar methods also in standard routines for forest trees.

Although the few experiments made with pine endosperms have not yet given consistent results (there seems to be endospermal substances upsetting the reactions in the agar gels) other techniques such as absorption of blood cells might lead to success.

More studies should be made of the effect of storage and preparation of antigens since differences have been found also among tree species (Fairbrothers, 1975).

The door to further specification is also open.
Recently techniques in the analysing of complex plant polysaccharides have made great progress (See e.g. Dea and Morrison, 1975) and it would be very desirable that seeds and also other organs of the more important forest trees should be further investigated for these matters.

The combination of serology with biochemical methods may give carbohydrate chemistry new tools for the structural analysis of polysaccharides (Glaudemans, 1975).

Since many of the polysaccharides might be connected with the surface of the plant cells they may also be of importance in recognition and in immune response to infections. This could bring us further into other aspects, both theoretical and practical, of the biochemistry of forest trees.

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Table 1. List of foreign trees used as male parents in crossability studies 1964-1967.

Trees with underlined tree nrs were also used for serological studies.

Our tree No.	Foreign tree number	Species	Location collected from: Natural stand	Name of arboretum or planting	Geographic origin: Place	Elev. in ft	Crop year	Pollen quality germin % in year: 64	65	66	67	Used in year: 64	65	66	67
USA 3020		P.albicaulis	x	Coll.by Inst.For.Gen.,Placerville, Calif. from	Alpine Co.,Calif.Lat. 38°40' N. Long. 115°58' W.	8400	1963	16				x			
USA 3024	Eld-2-7	P.monticola	x	Coll.by Inst.For.Gen.,Placerville, Calif. from	El Dorado Co.,Calif., Lat.32° 51.2'N.,Long. 120°14.5'W.	6800	1963	16				x			
USA 3022	Nr. 69	P.lambertiana		Eddy Arboretum, Placerville, Calif.	El Dorado Co..Calif.,local cross		1963	16				x	x		
USA 3333	K-5	P.lambertiana		Badger Hill Seed Orchard	Near Seiad Valley, Calif., Lat.41°48'N.,Long. 123°14'W.	3900	1966								x
USA 3023		P.monophylla	x	Coll.by Inst.For.Gen.,Placerville, Calif. from	Diamond Valley Alpine Co.Calif. Lat.35°46'N.,Long. 119°47'W.	5600	1963	27				x			
USA 3021	Inyo-3-6	P.aristata	x	Coll.by Inst.For.Gen.,Placerville, Calif. from	White Mount.,Inyo Co.,Calif. Lat.37°23.6'N.,Long.118°10.6'W.	10300	1963	31				x			
YO 3036	1	P.pinea		Coll.by Dept.For.Gen.Zagreb Univ.Yugoslavia	Yugoslavia				0				x		
YO 3037	2	P.pinea		Coll.by Dept.For.Gen.Zagreb Univ.Yugoslavia	Yugoslavia								x		
USA 3028	V 26	P.resinosa		Eddy Arboretum, Placerville, Calif.			1963	6				x			
YO 3035	45	P.nigra		Coll.by Dept.For.Gen.Zagreb Univ.Yugoslavia	Yugoslavia				5				x		
YO 3032	234	P.nigra		Coll.by Dept.For.Gen.Zagreb Univ.Yugoslavia	Yugoslavia								x		
YO 3041	47	P.nigra		Coll.by Dept.For.Gen.Zagreb Univ.Yugoslavia	Yugoslavia								x		
J 3016	Mix 1	P.densiflora		Coll.by Yamanashi For.Exp.St. Japan, from	Mt. Fuji, Japan				66				x		
J 3017	Mix 2	P.densiflora		Coll.by Yamanashi For.Exp.St. Japan, from	Mt. Fuji, Japan				69				x		
J 3018	Mix 3	P.densiflora		Coll.by Yamanashi For.Exp.St. Japan, from	Mt. Fuji, Japan				48				x		
J 3019	Mix 1	P.thunbergii		Coll.by Yamanashi For.Exp.St. Japan, from	Mt. Fuji, Japan				64				x		
USA 3044	T-V-62	P.taeda		Eddy Arboretum, Placerville, Calif.						37				x	
USA 3026	V 13	P.echinata		Eddy Arboretum, Placerville, Calif.	Probably Lousiana		1963	2				x			
USA 3043	Ri-61	P.rigida		Eddy Arboretum, Placerville, Calif.						33				x	
USA 3027	Eld-131-1	P.ponderosa	x	Coll.by Inst.For.Gen.,Placerville, Calif.	Near Forebay,ElDorado Co.,Calif.	3680	1963	3		12		x		x	
USA 3042	P-Eld-4A-61	P.ponderosa	x	Coll.by Inst.For.Gen.,Placerville, Calif.	El Dorado Co., Calif.					11				x	
USA 2245	J-V8	P.jeffreyi		Coll.by Inst.For.Gen.,Placerville, Calif.	Unknown		1964		6				x		
USA 3029	Eld-11-2	P.sabiniana	x	Coll.by Inst.For.Gen.,Placerville, Calif.	M.S.Dixon Ranch,near Folsom Lake		1963	15		44		x		x	
USA 3045	V-V-10	P.virginiana		Eddy Arboretum, Placerville, Calif.						23				x	
USA 2247	R-Mix	P.radiata		Mix.6 trees,P.Volz place Eldorado Co. Calif.	Unknown		1965		24				x		
USA 3025	Mix (V26, V29 & V31)	P.attenuata		Eddy Arboretum, Placerville, Calif.			1963	67				x			
USA 2246	MC-V22	P.muricata		Coll.by Inst.For.Gen.,Placerville, Calif.	South of Mendicino,Calif.		1964		3				x		

Table 1 a. List of trees used in the serological studies

Our tree no.	Foreign tree no.	Species	Location collected from Name of arboretum or plantation	Geographic origin Place
E 1938		P.cembra	Ruotsinkylä Exp.For., Cult.No 79/30	Finland, Turku (Unknown)
E 2421		P.cembra	Ruotsinkylä Exp.For., Cult.No 157	Finland, Turku (Unknown)
	St - N7	P.strobus	Inst. For.Gen., Placerville, Calif.	"Canada"
	F-Inyo-15	P.flexilis	Coll.by Inst.For.Gen.,Placerville	Inyo County, Calif..Plot 1 tree
E 2402		P.peuce	Ruotsinkylä Exp. For., Cult. No 50	Bulgaria, Rilo Planino
E 2403		P.peuce	Ruotsinkylä Exp. For., Cult. No 50	Bulgaria, Rilo Planino
E 2404		P.peuce	Ruotsinkylä Exp. For., Cult. No 50	Bulgaria, Rilo Planino
E-7	E-V7	P.griffithii	Inst.For.Gen., Placerville, Calif.	NW India, Chakrata Forest, Lat. 30° 55'N, Long.77°47'E,6509
	V7	P.edulis	Inst.For.Gen., Placerville, Calif.	No information available
	N1	P.bungeana	Inst.For.Gen., Placerville, Calif.	No information available
	Invo-1-5	P.balfouriana	Coll.by Inst. For. Gen., Placerville from a natural stand	Inyo County, Calif.Onion Valley Lat.36°46'N,Long.118°21'W, 920
	90	P.canariensis	Coll.by Inst. For. Gen., Placerville from a planted tree	Alameda County, Calif.
Ind 3563		P.roxburghii	Coll.by Forest Res. Inst., Dehra Dun	India, Dehra Dun, U.P.
Ind 3564		P.roxburghii	Coll.by Forest Res. Inst., Dehra Dun	India, Lucknow, Botanical gard.
	V 20	P.nigra	Inst. For. Gen., Placerville, Calif.	Received from Austria
E2		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Punkaharju
E35		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Pyhäjärvi U1.
E62		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Lammi
E67		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Hyvinkää
E90		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sysmä

cont.

Table 1 a. Cont.

Our tree nr.	Foreign tree nr.	Species	Location collected from Name of arboretum or plantation	Geographic origin Place
E 94		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela
E 102		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela
E 104		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela
E 107		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela
E 108		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela
E 109		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Kalvola
E 110		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Kalvola
E 111		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Kalvola
E 144		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Loppi
E 360		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Keuruu
E 362		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Keuruu
E 610		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Suomenniemi
E 620 D		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Suomenniemi
E 630		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sulkava
E 631		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sulkava
E 635		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sulkava
E 637		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sulkava
E 641		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Sulkava
E 1003		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tammela, Swamp-pine
E 1101		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Punkaharju
K 608		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Tohmajärvi
P 2504		P.sylvestris	Ruotsinkylä Tree Bank	Finland, Kolari
La 37		P.sylvestris	Laanila Exp.Forest, natural stand	Finland, Ivalo, Laanila, tree 37
La 38		P.sylvestris	Laanila Exp.Forest, Natural stand	Finland, Ivalo, Laanila, tree 38

cont.

Table 1 a. Cont.

Our tree nr.	Foreign tree nr.	Species	Location collected from Name of arboretum or plantation	Geographic origin Place
	41	P.sylvestris	Coll. by Forestry Comm. Res. Branch, Edinburgh, Scotland	Maree, Scotland, culture Lat. 57°38' N, Long.5°25'W
	45	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Maree, Scotland, culture
	46	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Maree, Scotland, culture
	54	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Maree, Scotland, culture
	223	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Tanar, Scotland, culture Lat. 57°1'N, Long 2°53' W.
	229	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Tanar, Scotland, culture
	30	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Grant, Scotland, culture (Spey side
	39	P.sylvestris	Coll. by Forestry Comm. Res. Branch	Grant, Scotland, culture (Spey side Lat.57°28'N, Long.3°37'W.
	SiY-V4	P.yunnanensis	Inst. For. Gen., Placerville, Calif.	Likiang Snow Range,Yunnan, China 9500 ft.
	V 7	P.engelmannii	Inst. For. Gen., Placerville, Calif.	Chiricahua Mts., Coronado N.F. S. Arizona.
	V-26	P.coulteri	Inst. For. Gen., Placerville, Calif.	Julian, San Diego County, Calif.
4		P.banksiana	Ruotsinkylä Exp. Forest, Cult. No 359/37	Canada, Br. Col., Eagle River, Lat.50°56'N, Long. 118°50' W.
E513		P.contorta v. latifolia	Elimäki, Mustila Arboretum	Canada, Br. Col., M:t Ida.
	CT-54	P.contorta	Coll. by Inst. For. Gen., Placerville	Near Reedsport, Oregon, Lat.43°40'N, Long.124°10'W, 50
E 1934		P.contorta v. latifolia	Ruotsinkylä Exp. Forest, Cult. No 116	Canada, Br.Col.,Upper Hat Creek
	N 18	P.muricata	Inst. For. Gen., Placerville, Calif.	Fort Bragg, California, coast Lat. 39°27'N, Long. 123°48'W.

Table 1 a. Cont.

Our tree nr.	Foreign tree nr.	Species	Location collected from Name of arboretum or plantation	Geographic origin Place
	V 28	P.muricata	Inst. For.Gen.,Placerville, California	Del Monte estate,near Monterey Lat. 36°36'N, Long.121°52'W.
	N 40	P.muricata	Inst. For.Gen.,Placerville, California	
	Mix V46-V-48	P.muricata	Inst. For.Gen.,Placerville, California mixed pollen from 2 clones.	San Vicente, California Lat. 31°18'N, Long.116°19'W.
	38	P.patula	Inst. For. Gen.,Placerville, California	"Mexico" Received at Placerville as P.teocote
	Lot E	P.pringlei	Coll. by Inst. For.Gen., Placerville, Calif. from native forest	Oaxaca state, Mexico, Lat.16°29'N, Long.97°01'W, 205 0

Trees with underlined tree nos. in this table were also used for the production of antisera.

Total number of precipitates

Table 2

Antigen \ Antisera		cembra		peuce		bal-four.	sylvestris			contort.	
		1938	2421	2402	2404	In-1 - 5	2	111	144	513	CT-54
Subgen.Strobus (Haploxylon)											
Sect.Strobus											
Subsect.Cembrae											
P.cembra	E1938									3	
	E2421	3	3	4	2	2	4	2	1		3
Subsect.Strobi											
P.strobus	N7		3		1			3	1		
P.lambertiana	USA 3333	2		3		2	2			4	4
P.flexilis	Inyo 1-5		2		1			2	1		
P.peuce	E2402			3	2		2	-		3	
	E2403				2				1		
	E2404	3	3		2	3		2	1		2
P.griffithii	E-7		2		1			1	1		
Sect.Parrya											
Subsect.Cembroides											
P.edulis	V7		2		2			2	2		
Subsect.Gerardianeae											
P.bungeana	N1		2		1			1	-		
Subsect.Balfourianae											
P.balfouriana	Inyo 1-5	2	2	2	1	3	1	2	-	2	2
Subgen.Pinus (Diploxylon)											
Sect.Ternatae											
Subsect.Canariensis											
P.canariensis	90		3		1		4	2	2		
P.roxburghii	Ind.3563			5			4				
	Ind.3564			4			4				
Sect.Pinus											
Subsect.Sylvestres											
P.nigra	V20	1	1	2	-	1	2	2	1		2
	YU3041		2	2	2		2	2	2	2	
P.sylvestris	E2						4			4	3
	E111	1	1	2	-			3	1		
	E144		2		(1)	2			2		
P.yunnanensis	V4		3		1			2	1		
Subsect.Australes											
P.taeda	USA 3044	2	2	3	1	1	3	2	2	4	3
P.rigida	USA 3043	2	3	2	2	1	3	3	3	3	3
Subsect.Ponderosae											
P.ponderosa	USA 3042	1	3	3	1	2	4	2	2	4	3
P.jeffrey	USA 2445		2		1		1	1	2		
P.engelmannii	V7		2		2	3	3	2	2		
Subsect.Sabinianeae											
P.sabiniana	USA 3029	3	4	4	2	2	3	2	2	3	4
P.coulterii	V26		2		(1)		3		1	1	
Subsect.Contortae											
P.banksiana	4	2	2	3	2	1	2	1	-	4	3
P.contorta	E513	1	1	3	1	1	3	1	-	4	2
	E1934		2		1		1	1	1		3
	CT-54	4	2	4	1	3	2	3	2	2	4
P.virginiana	USA 3045	2	2	3	1	1	4	2	2	4	4
Subsect.Oocarpae											
P.muricata	USA 2246		3		1			1	1		
	N18		3		1			3	2		
	V28		3		2			3	2		
	N40		3		1			3	3		
	Mix 46-48		1		-			2	1		
P.patula	38		3		2			3	2		
P.pringlei	Lot E		(1)		-				-		

Number and nature of precipitates.

Table 3

Antigen \ Antisera		cembra E1938 id.	cembra E1938 part	cembra E1938 non	cembra E2421 id.	cembra E2421 part	cembra E2421 non			
Subgen.Strobus (Haploxylon)										
Sect.Strobus										
Subsect.Cembrae										
P.cembra	E1938	3			3					
	E2421	3			3					
Subsect.Strobi										
P.strobus	N7				2	1				
P.lambertiana	USA 3333	1	1							
P.flexilis	Inyo 1-5				2					
P.peuce	E2402									
	E2403									
	E2404	2	1		2	1				
P.griffithii	E-7				1		1			
Sect.Parrya										
Subsect.Cembroides										
P.edulis	V7				2					
Subsect.Gerardianeae										
P.bungeana	N1				2					
Subsect.Balfourianae										
P.balfouriana	Inyo 1-5	1	1		2					
Subgen.Pinus (Diploxylon)										
Sect.Ternatae										
Subsect.Canariensis										
P.canariensis	90				2		1			
P.roxburghii	Ind. 3563									
	Ind. 3564									
Sect.Pinus										
Subsect.Sylvestres										
P.nigra	V20			1	1					
	YU3041				1		1			
P.sylvestris	E2									
	E111	1			1					
	E144				1	1				
P.yunnanensis	V4				1	2?				
Subsect.Australes										
P.taeda	USA 3044			2	1		1			
P.rigida	USA 3043		1	1	1	1	1			
Subsect.Ponderosae										
P.ponderosa	USA 3042	1			1	1	1			
P.jeffrey	USA 2445				1	1				
P.engelmannii	V7				1		1			
Subsect.Sabinianeae										
P.sabiniana	USA 3029	1		2	1	3				
P.coulterii	V26				1	1				
Subsect.Contortae										
P.banksiana	4	1	1		1	1				
P.contorta	E513	1			1					
	E1934					1	1			
	CT-54	1	1	2	1		1			
P.virginiana	USA 3045	1	1		1	1				
Subsect.Oocarpae										
P.muricata	USA 2246						3?			
	N18				2	1				
	V28				1	1	1			
	N40				1		2			
	Mix 46-48				1					
P.patula	38				1	2?				
P.pringlei	Lot E				(1)					

Number and nature of precipitates.

Table 4

Antigen \ Antisera		peuce E2402			peuce E2404			balfouriana In - 1 - 5		
		id.	part	non	id.	part	non	id.	part	non
Subgen.Strobus (Haploxylon)										
Sect.Strobus										
Subsect.Cembrae										
P.cembra	E1938									
	E2421	3	1		2			1	1	
Subsect.Strobi										
P.strobus	N7				1					
P.lambertiana	USA 3333	2	1					1	1	
P.flexilis	Inyo 1-5				1					
P.peuce	E2402	3			2					
	E2403				2					
	E2404				2			2	1	
P.griffithii	E-7				1					
Sect.Parrya										
Subsect.Cembroides										
P.edulis	V7				2					
Subsect.Gerardianeae										
P.bungeana	N1				1					
Subsect.Balfourianae										
P.balfouriana	Inyo 1-5	1	1		1			3		
Subgen.Pinus (Diploxylon)										
Sect.Ternatae										
Subsect.Canariensis										
P.canariensis	90				1					
P.roxburghii	Ind.3563	2	1	2						
	Ind.3564	1	2	1						
Sect.Pinus										
Subsect.Sylvestres										
P.nigra	V20	1		1	-	-	-		1	
	YU3041			2			2			
P.sylvestris	E2									
	E111	1	1		-	-	-			
	E144						1		2	
P.yunnanensis	V4				1					
Subsect.Australes										
P.taeda	USA 3044	1	1	1			1			1
P.rigida	USA 3043	1	2	1			2		1	
Subsect.Ponderosae										
P.ponderosa	USA 3042	1	1	1			1	1		1
P.jeffrey	USA 2445			1						
P.engelmannii	V7				1		1			
Subsect.Sabinianeae										
P.sabiniana	USA 3029	1	2	1			2		1	1
P.coulterii	V26					(1)				
Subsect.Contortae										
P.banksiana	4	1		2		1	1		1	
P.contorta	E513	1		2	1			1		
	E1934						1			
	CT-54	1		3	1				3	
P.virginiana	USA 3045	1	1	1			1			1
Subsect.Oocarpae										
P.muricata	USA 2246						1			
	N18						1			
	V28						2			
	N40						1			
	Mix 46-48				-	-	-			
P.patula	38				1	1				
P.pringlei	Lot E									

Number and nature of precipitates.

Table 5

Antigen \ Antisera		sylvestris E2			sylvestris E111			sylvestris E144		
		id.	part	non	id.	part	non	id.	part	non
Subgen.Strobus (Haploxylon)										
Sect.Strobus										
Subsect.Cembrae										
P.cembra	E1938									
	E2421	1		3	2					1
Subsect.Strobi										
P.strobus	N7				1	2?			1	
P.lambertiana	USA 3333		2							
P.flexilis	Inyo 1-5				1		1		1	
P.peuce	E2402		1	1	-	-	-			
	E2403							1		
	E2404				1		1			1
P.griffithii	E-7						1		1	
Sect.Parrya										
Subsect.Cembroides										
P.edulis	V7				1	1				2
Subsect.Gerardianeae										
P.bungeana	N1				1			-	-	-
Subsect.Balfourianae										
P.balfouriana	Inyo 1-5			1	1		1	-	-	-
Subgen.Pinus (Diploxylon)										
Sect.Ternatae										
Subsect.Canariensis		1	2	1	1		1	1		1
P.canariensis	90									
P.roxburghii	Ind. 3563	1	2	1						
	Ind. 3564	1	2	1						
Sect.Pinus										
Subsect.Sylvestres										
P.nigra	V20	1	1		1	1			1	
	YU3041	1	1		1	1		1	1	
P.sylvestris	E2	4								
	E111				3			1		
	E144				3			2		
P.yunnanensis	V4				2			1		
Subsect.Australes										
P.taeda	USA 3044	1		2	1	1		1	1	
P.rigida	USA 3043	1	1	1	1		2	1		2
Subsect.Ponderosae										
P.ponderosa	USA 3042	1	2	1	1		1	1		1
P.jeffrey	USA 2445	1			1			1	1	
P.engelmannii	V7	1	1	1	1		1	1		1
Subsect.Sabinianeae										
P.sabiniana	USA 3029	1	2		1	1		1	1	
P.coulterii	V26	1	2					1		
Subsect.Contortae										
P.banksiana	4		2		1					(1)
P.contorta	E513	1	1	1	1					1
	E1934	1			1			1?		
	CT-54		1	1	1	1?	1	1	1?	
P.virginiana	USA 3045	1	2	1	1	1		1	1	
Subsect.Oocarpae										
P.muricata	USA 2246						1			1
	N18				1	1?	1	1	1	
	V28				1	1?	1	1		1
	N40				1	1?	1	1	1	1
	Mix 46-48				2			1		
P.patula	38				1	2?		1	1	
P.pringlei	Lot E				(1)					

Number and nature of precipitates.

Table 6

Antigen \ Antisera		contorta E513			contorta CT-54					
		id.	part	non	id.	part	non			
Subgen.Strobus (Haploxylon)										
Sect.Strobus										
Subsect.Cembrae										
P.cembra	E1938	1		2						
	E2421					2	1			
Subsect.Strobi										
P.strobus	N7									
P.lambertiana	USA 3333	1		3	2	1	1			
P.flexilis	Inyo 1-5									
P.peuce	E2402	1		2						
	E2403									
	E2404						2			
P.griffithii	E-7									
Sect.Parrya										
Subsect.Cembroides										
P.edulis	V7									
Subsect.Gerardianeae										
P.bungeana	N1									
Subsect.Balfourianae										
P.balfouriana	Inyo 1-5			2	1	1				
Subgen.Pinus (Diploxylon)										
Sect.Ternatae										
Subsect.Canariensis										
P.canariensis	90									
P.roxburghii	Ind.3563									
	Ind.3564									
Sect.Pinus										
Subsect.Sylvestres										
P.nigra	V20				2					
	YU3041		2							
P.sylvestris	E2	1	2	1	1	1	1			
	E111									
	E144									
P.yunnanensis	V4									
Subsect.Australes										
P.taeda	USA 3044	2	2			2	1			
P.rigida	USA 3043	2		1	1	1	1			
Subsect.Ponderosae										
P.ponderosa	USA 3042	2	2			2	1			
P.jeffrey	USA 2445									
P.engelmannii	V7									
Subsect.Sabinianeae										
P.sabiniana	USA 3029	1	2		1	3				
P.coulterii	V26	1								
Subsect.Contortae										
P.banksiana	4	4			3		1			
P.contorta	E513	4			1	1				
	E1934				3					
	CT-54	2			4					
P.virginiana	USA 3045	1	3		2	1	1			
Subsect.Oocarpae										
P.muricata	USA 2246									
	N18									
	V28									
	N40									
	Mix 46-48									
P.patula	38									
P.pringlei	Lot E									

Table 7

Antigen \ Antisera		AG cembra 2421 / AS 2421	1	2	3	4	Differ.
Subgen.Strobus (Haploxylon)							
Sect.Strobus							
Subsect.Cembrae							
P.cembra	E1938			1	1		
	E2421			1	1	1	
Subsect.Strobi							
P.strobus	N7			1	-	1	
P.lambertiana	USA 3333			-	1	1	
P.flexilis	Inyo 1-5			1	1	1	
P.peuce	E2402			1	1	1	
	E2403						
	E2404						
P.griffithii	E-7			1	-	1	
Sect.Parrya							
Subsect.Cembroides							
P.edulis	V7			1	1	1	
Subsect.Gerardianeae							
P.bungeana	N1			1	-	1	
Subsect.Balfourianae							
P.balfouriana	Inyo 1-5			1	1	1	
Subgen.Pinus (Diploxylon)							
Sect.Ternatae							
Subsect.Canariensis				1	1	1	
P.canariensis	90						
P.roxburghii	Ind.3563						
	Ind.3564						
Sect.Pinus							
Subsect.Sylvestres							
P.nigra	V20			1	-	1	
	YU3041			1	1	1	
P.sylvestris	E2			1	-	1	
	E111			1	-	1	
	E144			1	-	1	
P.yunnanensis	V4			1	-	1	
Subsect.Australes							
P.taeda	USA 3044			1	-	1	
P.rigida	USA 3043			1	-	1	
Subsect.Ponderosae							
P.ponderosa	USA 3042			1	-	-	
P.jeffrey	USA 2445			1	1	1	
P.engelmannii	V7			1	-	1	
Subsect.Sabinianeae							
P.sabiniana	USA 3029			1	1	1	
P.coulterii	V26			1	-	1	
Subsect.Contortae							
P.banksiana	4			1	1	1	
P.contorta	E513			1	-	1	
	E1934			1	-	-	
	Ct-54			1	1	1	
P.virginiana	USA 3045			1	1	1	
Subsect.Oocarpae							
P.muricata	USA 2246			1	-	1	
	N18			1	1	1	
	V28			1	1	1	
	N40			-	-	1	
	Mix 46-48			1	-	1	
P.patula	38			1	1	1	
P.pringlei	Lot E			-	-	-	

Table 8

Antigen \ Antisera		AG P.peuce 2404				
		AS 2404	+	2 3		-
		1	2	3	4	Differ.
Subgen.Strobus (Haploxylon)						
Sect.Strobus						
Subsect.Cembrae						
P.cembra	E1938					
	E2421		1	-		
Subsect.Strobi						
P.strobus	N7		1	-		
P.lambertiana	USA 3333		1	1		
P.flexilis	Inyo 1-5		1	-		
P.peuce	E2402		1	1		
	E2403					
	E2404		1	1		
P.griffithii	E-7		1	-		
Sect.Parrya						
Subsect.Cembroides						
P.edulis	V7		1	-		
Subsect.Gerardianeae						
P.bungeana	N1		1	-		
Subsect.Balfourianae						
P.balfouriana	Inyo 1-5		1	-		
Subgen.Pinus (Diploxylon)						
Sect.Ternatae						
Subsect.Canariensis						
P.canariensis	90		1	-		
P.roxburghii	Ind.3563					
	Ind.3564					
Sect.Pinus						
Subsect.Sylvestres						
P.nigra	V20		1	-		
	YU3041		1	-		
P.sylvestris	E2					
	E111		-	-		
	E144		-	-		
P.yunnanensis	V4		1	-		
Subsect.Australes						
P.taeda	USA 3044		1			
P.rigida	USA 3043		-	-		
Subsect.Ponderosae						
P.ponderosa	USA 3042		-	-		1
P.jeffrey	USA 2445		1	-		
P.engelmannii	V7		1	-		
Subsect.Sabinianeae						
P.sabiniana	USA 3029		1	-		
P.coulterii	V26		1	-		
Subsect.Contortae						
P.banksiana	4		1	-		
P.contorta	E513		-	-		1
	E1934		-	-		
	CT-54		1	-		
P.virginiana	USA 3045		(1)	-		
Subsect.Oocarpae						
P.muricata	USA 2246		-	-		
	N18		1	-		
	V28		1	-		
	N40		-	-		
	Mix 46-48		1	-		
P.patula	38		1	-		
P.pringlei	Lot E		-	-		

Table 9

Antigen \ Antisera	AG P. sylvestris 111 AS 111	1	2	3	4	Differ.
Subgen.Strobus (Haploxylon)						
Sect.Strobus						
Subsect.Cembrae						
P.cembra	E1938					
	E2421	-	1	1	1	
Subsect.Strobi						
P.strobus	N7	-	-	-	1	
P.lambertiana	USA 3333					
P.flexilis	Inyo 1-5	-	1			
P.peuce	E2402	-	1	1	1	
	E2403					
	E2404					
P.griffithii	E-7	-	-	-	1	
Sect.Parrya						
Subsect.Cembroides						
P.edulis	V7	-	1	-	1	
Subsect.Gerardianeae						
P.bungeana	N1	-	-	-	1	
Subsect.Balfourianae						
P.balfouriana	Inyo 1-5	-	1	-	1	
Subgen.Pinus (Diploxylon)						
Sect.Ternatae						
Subsect.Canariensis						
P.canariensis	90	-	1		1	
P,roxburghii	Ind.3563					
	Ind.3564					
Sect.Pinus						
Subsect.Sylvestres						
P.nigra	V20	-	1	-	1	
	YU3041	1	1	1	1	
P.sylvestris	E2					
	E111	?	1	1	1	
	E144		1	1	1	
P.yunnanensis	V4	-	1		1	
Subsect.Australes						
P.taeda	USA 3044	-	1	1	1	
P.rigida	USA 3043	1	1	1	1	
Subsect.Ponderosae						
P.ponderosa	USA 3042		1	1	1	
P.jeffrey	USA 2445	-	1	1	1	
P.engelmannii	V7	-	1	-	1	
Subsect.Sabinianeae						
P.sabiniana	USA 3029	-	1	1	1	
P.coulterii	V26	-	-	-	1	
Subsect.Contortae						
P.banksiana	4		1	-	1	
P.contorta	E513				1	
	E1934		1	-	-	
	CT-54	-	-	-	1	
P.virginiana	USA 3045	-	1	1	1	
Subsect.Oocarpae						
P.muricata	USA 2246	-	1	1	1	
	N18	-	1	-	1	
	V28		1	-	1	
	N40		1	-	1	
	Mix 46-48		1	-	-	
P.patula	38				1	
P.pringlei	Lot E	-	-	-	-	

Table 10

Antigen \ Antisera		AG sylvestris 144 AS 144 + 1 0 2 3 -				
		1	2	3	4	Differ.
Subgen.Strobus (Haploxylon)						
Sect.Strobus						
Subsect.Cembrae						
P.cembra	E1938					
	E2421	-	-	-		
Subsect.Strobi						
P.strobus	N7	-	-	-		
P.lambertiana	USA 3333					
P.flexilis	Inyo 1-5			1		
P.peuce	E2402	-	-	-		
	E2403					
	E2404					
P.griffithii	E-7	-	1	1		
Sect.Parrya						
Subsect.Cembroides						
P.edulis	V7	-	1	1		
Subsect.Gerardianeae						
P.bungeana	N1	-	-	-		
Subsect.Balfourianae						
P.balfouriana	Inyo 1-5	-	-	-		
Subgen.Pinus (Diploxylon)						
Sect.Ternatae						
Subsect.Canariensis						
P.canariensis	90	-	-	1		
P.roxburghii	Ind.3563					
	Ind.3564					
Sect.Pinus						
Subsect.Sylvestres						
P.nigra	V20	-	1	-		
	YU3041	1	1	(1)		
P.sylvestris	E2					
	E111	1	1			
	E144	1	1	1		
P.yunnanensis	V4	-	1	-		
Subsect.Australes						
P.taeda	USA 3044	1 (+1)	-	-		
P.rigida	USA 3043	1 (+1)	1	1		
Subsect.Ponderosae						
P.ponderosa	USA 3042	1	1			
P.jeffrey	USA 2445	-	1	-		
P.engelmannii	V7	-	-	-		
Subsect.Sabinianeae						
P.sabiniana	USA 3029	1	1	(1)		
P.coulterii	V26	1	1	1		
Subsect.Contortae						
P.banksiana	4	1	1			
P.contorta	E513	1	1			
	E1934	-	-	-		
	CT-54	1	1	-		
P.virginiana	USA 3045	1	1	(1)		
Subsect.Oocarpae						
P.muricata	USA 2246	1 (+1)	1	1		
	N18		1	1		
	V28	1	1	1		
	N40	-	1	1		
	Mix 46-48	1	-	1		
P.patula	38	-	1	-		
P.pringlei	Lot E	-	1	-		

Table 11

Antigen \ Antisera	AG Cont.513 / AS 513	1	2	3	4	Differ.
Subgen.Strobus (Haploxylon)						
Sect.Strobus						
Subsect.Cembrae						
P.cembra	E1938					
	E2421	-	1	1	-	
Subsect.Strobi						
P.strobus	N7					
P.lambertiana	USA 3333	-	1	1	-	
P.flexilis	Inyo 1-5					
P.peuce	E2402	-	1	1	-	
	E2403					
	E2404					
P.griffithii	E-7					
Sect.Parrya						
Subsect.Cembroides						
P.edulis	V7					
Subsect.Gerardianeae						
P.bungeana	N1					
Subsect.Balfourianae						
P.balfouriana	Inyo 1-5	-	1	-	-	
Subgen.Pinus (Diploxylon)						
Sect.Ternatae						
Subsect.Canariensis						
P.canariensis	90					
P.roxburghii	Ind.3563					
	Ind.3564					
Sect.Pinus						
Subsect.Sylvestres						
P.nigra	V20					
	YU3041	-	-	1	1	
P.sylvestris	E2	1	1	1	1	
	E111					
	E144					
P.yunnanensis	V4					
Subsect.Australes						
P.taeda	USA 3044	1	1	1		1
P.rigida	USA 3043	1	1	1		1
Subsect.Ponderosae						
P.ponderosa	USA 3042	1	1	1	1	
P.jeffrey	USA 2445					
P.engelmannii	V7					
Subsect.Sabinianeae						
P.sabiniana	USA 3029	1	1			2
P.coulterii	V26					
Subsect.Contortae						
P.banksiana	4	1	1			
P.contorta	E513					
	E1934					
	CT-54	1	1	1	-	
P.virginiana	USA 3045	1	1			
Subsect.Oocarpae						
P.muricata	USA 2246					
	N18					
	V28					
	N40					
	Mix 46-48					
P.patula	38					
P.pringlei	Lot E					

Pine pollen antigens and Salmonella seratypes — Table 12

Antigen \ Antisera		Salmonella				
		C_1 K 170	T_1 K829	D typh. K 370	B parat. K 990	G_{22} K 010
Subgen.Strobus (Haploxylon)						
Sect.Strobus						
Subsect.Cembrae						
P.cembra	E1938	+	+	+ 2	+ 2	+
	E2421	+	+	+	+	+
Subsect.Strobi						
P.strobus	N7	+	-	+	-	+
P.lambertiana	USA 3333	-	+	+	+	-
P.flexilis	Inyo 1-5	-	+	+	-	+
P.peuce	E2402	+	+	+ 2	+ 2	+
	E2403	+	+	+	+	+
	E2404	+	+	+	+	+
P.griffithii	E-7	-	+	-	-	+
Sect.Parrya						
Subsect.Cembroides						
P.edulis	V7	-	+	+	+	+
Subsect.Gerardianeae						
P.bungeana	N1	-	+	+	+	+
Subsect.Balfourianae						
P.balfouriana	Inyo 1-5	-	+	+	+	-
Subgen.Pinus (Diploxylon)						
Sect.Ternatae						
Subsect.Canariensis						
P.canariensis	90	-	+	+	+	-
P.roxburghii	Ind.3563	(-)	(-)	(-)	(-)	(-)
	Ind.3564	(-)	(-)	(-)	(-)	(-)
Sect.Pinus						
Subsect.Sylvestres						
P.nigra	V20	-	+	-	-	+
	YU3041	-	+	+	-	-
P.sylvestris	E2		+	-		-
	E111	-	+	+	+	+
	E144		-	+		+
P.yunnanensis	V4	-	+	-	-	-
Subsect.Australes						
P.taeda	USA 3044	-	+	-	-	+
P.rigida	USA 3043	-	+	+	-	+
Subsect.Ponderosae						
P.ponderosa	USA 3042	-	+	-	-	+
P.jeffrey	USA 2445	-	-	+	+	-
P.engelmannii	V7	-	+	+	-	+
Subsect.Sabinianeae						
P.sabiniana	USA 3029	+	+	+	+	+
P.coulterii	V26	-	+ 2	+	+	+
Subsect.Contortae						
P.banksiana	4					
P.contorta	E513	-	+ 2	+	+	+
	E1934	-	+	+	+	-
	CT-54	-	+	+	+	+
P.virginiana	USA 3045					
Subsect.Oocarpae						
P.muricata	USA 2246	-	+	+	+	-
	N18	-	+	+	+	+
	V28	-	+	+	+	+
	N40	-	+	+	+	+
	Mix 46-48	-	+	-	-	+
P.patula	38	-	+	+	+	+
P.pringlei	Lot E	-	-	-	-	-

Table 13.

Pinus sylvestris pollen antigens from different sources and *Salmonella* antisera.

P.sylvestris pollen Origin and tree Nr.	*Salmonella* T_1 K838	*Salmonella* D *typhi* K370	*Salmonella* G_{22} K010
Finland			
E2	+	-	-
E90	+	-	-
E620 D	+	+	-
E144	-	+	+
E110	-	+	+
E62	-	-	+
E35	+	+	+
E630	-	-	-
E610	+	+	+
La37	+	+	-
Scotland			
30 Grant	-	-	-
39 Grant	+	+	-
223 Tanar	+	+	-
229 Tanar	+	+	-
41 Maree	-	-	-
45 Maree	+	+	-
46 Maree	-	-	-
54 Maree	+	+	-

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MÖGLICHKEITEN UND PROBLEME BEI DER ANWENDUNG BIOCHEMISCHER METHODEN ZUR IDENTIFIKATION VON WALDBÄUMEN.

POSSIBILITIES AND PROBLEMS IN THE APPLICATION OF BIOCHEMICAL METHODS TO THE IDENTIFICATION OF FOREST TREES.

POSSIBILITES ET PROBLEMES EN APPLICATION DES METHODES BIOCHIMIQUES A L'IDENTIFICATION D'ARBRES FORESTIERS.

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ZUSAMMENFASSUNG

Die Problematik der Anwendung biochemischer Methoden zur Identifikation von Klonen, Sorten, Populationen bei Waldbäumen wird diskutiert. Grundsätzlich soll ein biochemisches Merkmal nur zusammen mit anderen morphologischen oder physiologischen Merkmalen für Identifikationszwecke gebraucht werden. Nur biochemische Merkmale, die einfach, schnell und kostengünstig zu erfassen sind und die einen hohen Anteil genetischer Varianz an der Gesamtstreuung besitzen, kommen infrage.

Es werden Vor- und Nachteile der Verwendung von biochemischen Polymorphismen einerseits und quantitativen Merkmalen andererseits abgewogen. Polymorphismen bedingen Einzelanalysen, finden Verwendung bei kleinen Individuenzahlen, haben jedoch eine hohe Aussagekraft. Quantitative Merkmale lassen Mischanalysen zu und sind deshalb auch bei Untersuchungen an hohen Stückzahlen interessant.

Die Methodik muss die genetische Struktur des zu prüfenden Materials berücksichtigen. Klone, Sorten, Populationen und Arten werden getrennt behandelt. Für Klone sind biochemische Polymorphismen besonders bei hohen Stückzahlen eine grosse Hilfe für die Identifikation. Populationsanalysen von Waldbäumen sind durch weitgehende Zerstörung natürlicher Verteilungsmuster in Mitteleuropa am problematischsten. Hier wird beispielhaft die unterschiedliche Situation bei Fichte und Douglasie gegenübergestellt.

Neben der genetischen Struktur werden die verschiedenen ontogenetischen Phasen behandelt. Als Haupteinsatzbereiche für biochemische Analysen werden Saatgut und Altbäume im Vordergrund stehen. Bei Jungpflanzen sind biochemische Methoden für hohe Stückzahlen oder für Frühselektion sinnvoll anwendbar.

SUMMARY

The problems involved in the application of biochemical methods to the identification of clones, varieties, and populations of forest trees are discussed.

Generally speaking, biochemical characteristics should only be used together with morphological and physiological traits for identification purposes. Only those biochemical characteristics which can be evaluated simply, quickly and cheaply and which show a high proportion of genetic variation are of especial interest.

The advantages and disadvantages of using biochemical polymorphisms on the one hand and quantitative genetic characteristics on the other hand are discussed. Polymorphisms can be used in single analyses for small samples of individuals and are valuable for discrimination. Quantitative characteristics can be analysed in mixed samples and thus allow the analysis of large numbers of individuals.

The methods have to reflect the genetic structure of the tested material. Clones, varieties, populations and species are treated separately. In identifying large numbers of clones, biochemical polymorphisms are very helpful. The analysis of population structure and ecological patterns of forest trees in Central Europe is difficult because of the destruction of the natural pattern. As an example of this the different situations of Norway spruce and Douglas fir are demonstrated.

In addition to the genetic structure of the material, the ontogenetic phases are also dealt with. Biochemical analyses are mainly useful in seeds and adult trees. In young plants biochemical methods are especially helpful for discrimination of large numbers of plants or for early selection.

RESUME

Les problèmes en application des méthodes biochimiques à l'identification de clones, de sortes, et de populations chez des arbres forestiers sont discutée.

En principe aux fins d'identification une caractéristique biochimique ne doit être utilisée qu'en commun avec d'autres caractéristiques morphologiques ou physiologiques. Il est seulement question de caractéristiques biochimiques qui peuvent être évaluées d'une manière simple, rapide, et pas chère, et qui ont une bonne part de variation génétique de la totalité de la variation.

Les avantages et les désavantages de l'utilisation des polymorphismes biochimiques d'une part et des caractéristiques quantatives d'autre part sont évalués. Les polymorphismes exigent des analyses individuelles, et sont utilisés s'il s'agit d'un petit nombre d'individues, mais ils servent beaucoup à la discrimination. Des caractéristiques quantitatives permettent des analyses mélangées et c'est pourquoi qu'elles sont aussi intéressantes pour des analyses de grand nombre.

La méthodologie doit tenir compte de la structure génétique du matériel qui est à examiner. Les clones, les sortes, les populations, et les espèces sont traités séparément. Pour les clones les polymorphismes biochimiques aident beaucoup à l'identification surtout s'il s'agit d'un grand nombre. Les analyses de populations d'arbres forestiers sont les plus difficiles à cause de la vaste destruction de la distribution naturelle en Europe centrale. Ici, la situation différente de l'épicéa et du Douglas vert sert d'exemple contraire.

En plus de la structure génétique les différentes phases ontogénétiques sont traitées. Les analyses biochimiques seront surtout utilisées en cas de semences et de vieux bois. En cas des jeunes arbres les méthodes biochimiques sont très utilisables pour un grand nombre ou pour une sélection précoce.

MÖGLICHKEITEN UND PROBLEME BEI DER ANWENDUNG BIOCHEMISCHER METHODEN ZUR IDENTIFIKATION VON WALDBÄUMEN

EINLEITUNG

Die Anwendung biochemischer Methoden in der Forstpflanzenzüchtung und das Interesse an der Verwendung solcher Methoden zur Identifikation von Material hat in den letzten Jahren stark zugenommen. Die Erfolge, die durch Anwendung biochemischer Methoden z.B. in der Rapszüchtung erzielt wurden (Appelqvist et al. 1970, Lein 1970, Thies 1971, Rakow 1973, Jönsson 1973 u.a.) sind augenfällig.
In der Forstpflanzenzüchtung finden biochemische Merkmale für die Selektion z.B. bei korrelierten Resistenzeigenschaften (Santamour et al. 1958, Cvrkal 1959, Scholz und Reck 1975, Boyer 1964, Hanover 1964, Smith 1966, Smeljanez 1973, Lunderstädt et al. 1975, Lunderstädt 1976 u.a.), für die Analyse von Populationsstrukturen (Park 1972, Squillace 1966, 1971, Sakai et al. 1971, Matsuura et al. 1972, Sakai et al. 1972, Feret 1974 a, b, Tigerstedt 1974, Rudin et al. 1974, Bergmann 1975, Lundkvist 1976 a, b, Lang 1976) und für biosystematische Untersuchungen (Hillis 1967 a, b, c, Rudloff 1967, Erdtman et al. 1966, Wellendorf et al. 1971, Thielges 1972, Hunt et al. 1974 u.a.) Verwendung. Sowohl im Rahmen von Züchtungsprogrammen als auch für Handelskontrollen sollen biochemische Merkmale als Identifikationshilfen verwendet werden (Bergmann 1975, Bartels 1971).

Hier werden vorrangig die Probleme, die bei der Identifikation von Waldbäumen auftreten, besprochen.

EINFLUSS DER METHODE

Auch wenn geeignete biochemische Identifikationsmerkmale zur Verfügung stehen, sollten morphologische und physiologische Eigenschaften, die zum Teil mit vergleichsweise geringem experimentellen Aufwand zu erfassen sind, zur Identifikation herangezogen werden.

Oft ermöglichen biochemische Methoden allerdings schnelle Entscheidungen ohne langwierige Beobachtung der Pflanzen.
Ein Identifikationsmerkmal ist nur dann gut, wenn es einfach, schnell und kostengünstig zu erfassen ist und wenn der Anteil genetischer Varianz an der auftretenden Gesamtstreuung hoch ist. Dies trifft z.B. bei unseren Waldbaumarten für phänologische Merkmale ebenso wie für Polymorphismen (Bartels 1971, Sauer et al. 1973, Simonsen et al. 1975) weitgehend zu.

Qualitativ unt erscheidbare Merkmale bzw. Substanzen (Polymorphismen) werden überwiegend oder vollständig genetisch kontrolliert. Sie eignen sich als Identifikationshilfe sehr gut. Der methodische Aufwand ist meist geringer als bei der quantitativen Analyse. Der Nachteil von qualitativen biochemischen Merkmalen liegt darin, dass grundsätzlich keine Mischproben, sondern nur Einzelproben analysiert werden können. Eine Mischprobe würde das Fehlen einer Substanz bei einigen Individuen verschleiern und nur eine quantitative Verschiebung bewirken, das qualitative Merkmal wäre dann wie ein quantitatives Merkmal zu behandeln. Die qualitativen Merkmale werden in erster Linie für die Identifizierung bzw. Unterscheidung mit Hilfe von Einzelanalysen, also bei vergleichsweise kleinen Individuenzahlen (Lundkvist 1975, Rudin et al. 1973, Feret 1971, Rasmuson et al. 1971) geeignet sein. Durch Analysenungenauigkeit, Allel-Interaktionen, Einfluss der Umweltänderung u.a. können biochemische Polymorphismen so verändert werden, dass sie wie quantitativ variierende Merkmale erscheinen (Feret 1971). Umgekehrt können durch Artefakte qualitative Merkmale vorgetäuscht werden.

Quantitativ unterscheidbare Merkmale werden genereller anwendbar sein. Besonders Phenole und Terpene sind bei Koniferen untersucht worden (Hanover 1964, Squillace 1966, Lunderstädt 1976 u.a.).
Doch der methodische Aufwand ist hier z.T. erheblich grösser. Die Analysenzahl wird durch die auftretende Streuung bestimmt, die durch Versuchsfehler und Umwelteinflüsse verursacht wird.

Häufiger werden ontogenetische Veränderungen zu berücksichtigen sein. Der erhebliche Kostenaufwand bei hoher Analysenzahl kann nur gesenkt werden, wenn die Möglichkeit besteht, Mischproben zu untersuchen.

Für die Analyse von Saatgut oder auch sehr kleinen Stecklingen oder Sämlingen wird die Verwendung von Mischproben oft schon wegen der Substanzmenge notwendig sein.

EINFLUSS DES MATERIALS

Wenn man über die Identifikation von Waldbäumen mit Hilfe biochemischer Methoden spricht, so muss zunächst einmal unterschieden werden, welches Material man mit welcher Zielsetzung identifizieren will. Die Möglichkeiten und Verfahren werden in Abhängigkeit davon, ob man Klone, Sorten, Populationen, Hybriden oder Arten identifizieren will, unterschiedlich aussehen müssen (Adams 1974, Crawford und Dorn 1974). Es wird weiterhin danach zu unterscheiden sein, ob man Saatgut, Jungpflanzen oder alte Bäume zu bearbeiten hat. Nur in seltenen Fällen wird eine eindeutige Identifizierung von Arten, Sorten, Klonen u.a. allein durch eine biochemische Analyse möglich sein. Die biochemische Analyse ergibt neben morphologischen und physiologischen Eigenschaften nur weitere Informationen (Packer und Denford 1974, Challice und Westwood 1973). Oft kann die biochemische Eigenschaft nur dazu dienen, die Nichtidentität zweier Proben zu beweisen.
Eine eindeutige Identifikation kann möglich werden, wenn die biochemische Analyse eine Vielzahl von Eigenschaften erfasst (möglich z.B. bei Terpenen, Flavonolen u.a.) (Spethmann 1975, Belzer und Ownbey 1971, Bate-Smith 1973). In diesen Fällen kann die Variationsmöglichkeit so gross sein, dass die Analyse dann sozusagen als "Fingerabdruck" anzusehen ist.

Klone

Klone können nur als junge Pflanzen bzw. ältere Bäume identifiziert werden. Hier ist von der genetischen Seite die Grundlage insofern am einfachsten, als es sich immer um ganz bestimmte

Genotypen und damit um identische Reaktionsnormen handelt. Andererseits werden hier biochemische Methoden erst beim Vorliegen grösserer Klonzahlen notwendig werden, weil bei Pflanzen oft sehr viel einfacher erfassbare, genetisch stark kontrollierte Merkmale verfügbar sind, wie z.B. Austrieb, Vegetationsabschluss, Johannistriebbildung, Länge der Vegetationszeit, Höhenwachstum, Verzweigungsform, Blatt-bzw. Nadellänge und Form, Zahl und Anordnung der Stomata u.a..

Bei Verwendung geeigneter biochemischer Merkmale wie z.B. der Esterasen oder der Terpene und Phenole sind eindeutige Klonunterschiede feststellbar (Squillace et al. 1966, Sauer et al. 1973, Kaufmann et al. 1974). Hier kommt es vorrangig darauf an, geeignete Merkmale zu finden und die Analysenmethoden so zu vereinfachen, dass grosse Serien schnell und billig durchgezogen werden können. Für die Klone muss eine Merkmalsinventur und Katalogisierung erfolgen. Bei der Identifikationsarbeit kann dann im Abfrageverfahren oder bei quantitativ variierenden Merkmalen mit Hilfe von Diskriminanzanalysen die Klonidentität überprüft werden, wobei Fehlentscheidungen weitgehend ausgeschlossen werden können, wenn eine ausreichende Zahl anderer Eigenschaften einbezogen wird.
Da in der Praxis bei Waldbäumen ganz erhebliche Klonzahlen verwendet werden, wird man hier im allgemeinen auch biochemische Methoden zur Hilfe nehmen müssen, um den Merkmalskatalog zu erweitern und die Identifikationsmöglichkeiten zu verbessern. Dies insbesondere deswegen, weil viele der biochemischen Merkmale dichter an der primären Genwirkung liegen als die meisten der morphologischen und physiologischen Merkmale. Biochemische Polymorphismen sind zur Identifikation von Klonen gut geeignet.

Bei Fichte z.B. ist die Trennung von Klonen allein mit morphologischen und physiologischen Hilfsmitteln dann schwer oder sogar unmöglich, wenn die Klonzahl sehr gross wird. Für den praktischen Anbau sollen aber Klongemische verwendet werden, die mehrere tausend Klone umfassen. Hier ist eine Identifikation der Einzelklone nur noch möglich, wenn auch biochemische Merkmale zu Hilfe genommen werden. Besonders Phenole und Terpene scheinen sich hier gut zu eignen (Sauer et al. 1973).

Sorten

Zuchtsorten werden bei Waldbäumen derzeit noch in vergleichsweise begrenztem Umfang in der Praxis verwendet. Dieses können Plantagenabsaaten, Hybridsorten aus Plantagen oder anderen Versuchsanlagen, Vollgeschwisterfamilien oder Halbgeschwisterfamilien aus kontrollierten Kreuzungen und Klongemische sein.

Je stärker eine Zuchtsorte genetisch eingeengt ist (z.B. Vollgeschwisterfamilien), um so leichter wird ihre Identifikation und ihre eindeutige Zuordnung möglich sein. Je genetisch heterogener das Material und je grösser die Anzahl der Alternativen ist, z.B. Plantagenabsaaten aus klonzahlreichen Plantagen, um so schwieriger wird eine Identifikation und um so kostenaufwendiger muss sie zwangsläufig sein. Hierbei ist der Übergang zu Populationen fliessend.

Die vergleichsweise geringe Zahl der verschiedenen Sorten erleichtert grundsätzlich deren Identifikation, wenn geeignete Unterscheidungsmerkmale gefunden werden können. Die Unterscheidung müsste hier sowohl am Saatgut als auch an den Jungpflanzen möglich sein, wobei das grössere praktische Interesse beim Saatgut liegt. Eine Erschwernis ist die Tatsache, dass bei Plantagensorten die Fruktifikationshäufigkeit der einzelnen Klone von Jahr zu Jahr sehr schwankt und damit die genetische Zusammensetzung des Plantagensaatgutes und der Nachkommen aus den Plantagen über die Jahre nicht konstant ist. Dies hat zur Folge, dass bei der Identifikation ein mehr oder minder weiter Toleranzrahmen vorgesehen sein muss, der diesen biologischen Gegebenheiten Rechnung trägt, und dass die Einzelkomponenten der Gemische vorher bekannt sein müssen. Die Einzelanalyse findet ihre Grenze in dem notwendigen Kosten- und Arbeitsaufwand. Die Verwendung von Mischproben andererseits, die von der Kosten- und Arbeitsseite her sehr viel einfacher ist, verschleiert wiederum Sachverhalte, die möglicherweise für die Identifikation solcher Sorten von ausschlaggebender Bedeutung sind. Dies gilt insbesondere dann, wenn sich solche Sorten aus einer grossen Zahl verschiedener Individuen zusammensetzen, die ihrerseits unterschiedliche

biochemische Muster aufweisen. Bei Jungpflanzen bietet sich genau wie bei den Klonen eine Kombination morphologischer, physiologischer und biochemischer Betrachtung an.

Populationen

Die Identifikation von Populationen, die aus einer grossen Zahl unterschiedlicher Genotypen in weitgehend heterozygotem Zustand zusammengesetzt sind und die noch unter dem Einfluss der natürlichen Auslese an ihrem jeweiligen Anbauort stehen, ist sicher am problematischsten (Townsend et al. 1972). Für Mitteleuropa kommt zudem erschwerend hinzu, dass die natürlichen Verteilungsmuster der Waldbaumpopulationen der meisten Arten durch den Menschen weitestgehend in Unordnung gebracht sind und daher heute keine klinalen Variationsmuster mehr feststellbar sind. Andererseits sind hier Methoden der Saatgutidentifizierung für die Praxis am interessantesten (Bergmann 1971, 1972).

Das theoretisch optimale Vorgehen für die Identifikation von Populationen wäre :

- dass zunächst aufgrund der genetischen Struktur der Populationen die Mindestzahl der zu analysierenden Individuen festgelegt wird (Bergmann 1973)
- dass sodann eine Karte der Häufigkeiten bestimmter Merkmale bzw. der Verteilungsmuster innerhalb der Populationen in Abhängigkeit von deren geographischer Verbreitung und der Höhenlage erstellt wird (Weimarck 1974) und
- dass schliesslich durch einen Vergleich der Analysenergebnisse mit diesen Karten die Populationen lokalisiert bzw. identifiziert werden.

Eine Erleichterung wäre auch hier vom Arbeits- und Kostenaufwand zu erreichen, wenn die Einzelanalysen durch eine Pauschalanalyse ersetzt werden könnten.
Die forstgeschichtliche Entwicklung in Europa erlaubt für die einheimischen Baumarten eine Identifikation von Populationen

praktisch nicht. Ausnahmen hiervon könnten ggf. die weitgehend natürlich verjüngten Arten wie Buche und Birke sein.
Das Problem der Populationsidentifikation muss daher differenziert nach den einzelnen Arten betrachtet werden. Hier sollen beispielhaft zwei nach ihrer Situation sehr unterschiedlich zu beurteilende Arten besprochen werden :

1. Fichte

Der Fichtenanbau in Mitteleuropa wird seit mindestens 200 Jahren in so erheblichem Umfang künstlich vorgenommen, wobei fast alle natürlichen Verteilungsmuster aufgehoben wurden, dass heute in Mitteleuropa ein kaum noch rekonstruierbares Gemisch von Populationen verschiedensten Ursprungs vorliegt. Dabei wurden sowohl Hochlagen mit Tieflagenherkünften und umgekehrt vermischt als auch geographisch weit voneinander entfernt liegende Quellen. Diese künstlichen Anbauten ihrerseits sind durch Pollenflug von Nachbarbeständen in der nächsten Generation ebenso genetisch verändert worden wie durch die natürliche Auslese am neuen Anbauort. Beides hat mit Sicherheit erhebliche Auswirkungen auf die Zusammensetzung der Genfrequenzen in den einzelnen Populationen.
Eine Inventur dieses oft sehr kleinräumig variierenden künstlichen Verteilungsmusters ist aus Arbeitsgründen ausgeschlossen. Für ein praktisch anwendbares Vorgehen bleiben eigentlich nur zwei Möglichkeiten :

a) Von extremen Populationen ausgehend, die mit grosser Wahrscheinlichkeit noch an ihrem Ursprungsort stocken, kann versucht werden, eine Zuordnungstabelle zu erstellen, die einen gewissen Wahrscheinlichkeitsgehalt hat. Hierbei kann man sowohl von Hochlagen zu Tieflagen als auch von geographisch entfernten Orten, z.B. Nord-Süd, Ost-West-Querschnitt vorgehen. Die Erstellung solcher Kreuztabellen wird bei der geschilderten Situation in Mitteleuropa nur mit ausserordentlich grosser Mühe und zudem mit wenig Sicherheit möglich sein.

b) In der Praxis wird man mit einem Ausschlussverfahren, das die Nichtidentität zweier Populationen nachweist, weiterkommen.

Die Verteilungsmuster müssen in jedem Fall über mehrere Jahre geprüft werden. Für Fichte ist bekannt, dass von Jahr zu Jahr sehr unterschiedliche Bestandesglieder fruktifizieren und damit die genetische Zusammensetzung der Bestandesbeerntung von Jahr zu Jahr unterschiedlich sein kann. Diese Unterschiede verlieren sich zwar in Vollmastjahren, bei Fichte wird aber auch in Sprengmastjahren geerntet. Dieses Vorgehen mag zwar einen Perfektionisten nicht befriedigen, es ist aber bei der vorliegenden Situation wahrscheinlich die einzige praktikable Möglichkeit.

2. Douglasie

Bei der Douglasie ist, zumindest in ihrem Heimatgebiet, noch auf grossen Flächen das natürliche Verteilungsmuster vorhanden, so dass man hier nach morphologischen, physiologischen und biochemischen Merkmalen eine Kartierung vornehmen kann, wie dies auch versucht worden ist (Rudloff 1972, 1973 a, b, 1975, Sziklai 1973, Kleinschmit et al. 1974). Eine Saatgutidentifikation von Importsaatgut über biochemische Methoden scheint deswegen für Douglasie durchaus sinnvoll.

Ganz anders ist die Möglichkeit der Identifikation von in Europa angebauten Altbeständen dieser Baumart und deren nachträglicher Zuordnung zu einem Herkunftsgebiet zu beurteilen. Wir wissen aus geschichtlichen Unterlagen, dass für die Begründung unserer heutigen Douglasien-Altbestände oft lange Jahre erforderlich gewesen sind (Kleinschmit 1973). In aufeinanderfolgenden Jahren ist oft Saatgut aus unterschiedlichen Quellen verwendet worden, so dass die Bestände keine reinen Populationen sind, genau wie wir heute bei der Begründung von Douglasienbeständen und Nachbesserungen von Ausfällen immer wieder Material anderer Herkünfte verwenden.

Hinzu kommt, dass für die Begründung von Beständen oft enorme Mengen von Saatgut notwendig gewesen sind, d.h. dass eine sehr starke natürliche Auslese am Anbauort stattgefunden hat. Diese Tatsache erschwert eine nachträgliche Zuordnung durch Verschiebung der Genfrequenzen so, dass sie u.E. kaum sinnvoll sein kann. Hier ist es für die Praxis sehr viel entscheidender, ob dieser Bestand unter den gegebenen Anbauverhältnissen wirtschaftlich eine gute Leistung gezeigt hat. Ist dies der Fall, wird man durch Beschreibung des Merkmalsmusters dieses Bestandes sicherstellen, dass tatsächlich dessen spezielle Nachkommen weiter verwendet werden. Zuordnungs- oder Identifikationsmöglichkeiten sind bei diesen Beständen etwa gleich zu beurteilen wie bei Fichte.

Arten

Zwischen Arten sind neben morphologischen und physiologischen Unterschieden auch grosse biochemische Verschiedenheiten gefunden worden (Harborne & Williams 1973, Spethmann 1975, Hegnauer 1962, Heywood 1971). Diese können nicht nur für systematische Fragen von Bedeutung sein, sondern auch die oft schwierige Identifikation von Arthybriden ermöglichen (Hanover et al. 1970, Mirov 1956, Hoff 1968, Thielges 1972).

Neben der Gliederung nach der genetischen Struktur des Untersuchungsmaterials kann eine Gliederung nach dem ontogenetischen Entwicklungszustand für die Methodenwahl ausschlaggebend sein.

Saatgut

Bei Nadelbaumarten ist der Einfluss von Fremdpollen durch Analyse des haploiden Endosperms auszuschliessen. Deshalb können exaktere Aussagen über die Mutterpflanzen bzw. Mutterpopulationen gewonnen werden als bei Laubbaumarten. Hier dürfte ein Haupteinsatzbereich für biochemische Analysen liegen, denn die Untersuchungsmöglichkeiten morphologischer und physiologischer Merkmale sind beim Saatgut sehr begrenzt.

Bei der Methodik können Schwierigkeiten auftreten, da für Einzelanalysen oft sehr aufwendige Mikromethoden notwendig sind. Bei der Untersuchung des Endosperms bei kleinsamigen Gymnospermen können Präparationsschwierigkeiten auftreten. Hinzu kommt, dass die Alternativen der zu untersuchenden Substanzen bei Samen vergleichsweise gering sind. Insbesondere beim Saatgut sollte die Eignung von Misch- oder Gruppenanalysen geprüft werden.

Jungpflanzen

Hier werden zunächst einmal morphologische und physiologische Merkmale zur Identifikation hilfreich sein. Solche Merkmale sind für die verschiedensten Arten bereits erarbeitet. Zusätzlich müssen hier ggf. bei grösseren Pflanzenzahlen oder differenzierten Fragestellungen biochemische Merkmale herangezogen werden. Dies kann besonders sinnvoll sein, wenn Stoffe vorhanden sind, die nur qualitativ erfasst werden müssen und ontogenetisch stabil sind, oder solche, die Frühselektion ermöglichen.

Bei Jung- und Altpflanzen steht für biochemische Zwecke ein erheblich grösserer Untersuchungsspielraum zur Verfügung. Abgesehen von der grösseren verfügbaren Masse können hier Substanzen aus Blatt, Nadel, Holz, Rinde, Blüte u.a. ausgewählt werden. Für Einzelanalysen können hier einfacher zu handhabende Makromethoden benutzt werden.

Altbäume

Bei Altbäumen werden zahlreiche der morphologischen Merkmale durch die Bestandeserziehung und den Standort so stark verwischt, dass hier wieder biochemische Merkmale nützlicher sein können (Sakai et al. 1972). Auch die Beobachtung der Phänologie ist bei Altbäumen nicht immer ganz einfach. Hierdurch wird nach dem Saatgut für die Altbäume die Anwendung biochemischer Methoden vorrangig interessant sein. Allerdings sind die Jugend-Alters-Korrelationen nicht für alle biochemischen Merkmale gut, so dass

Vergleiche nur zwischen entsprechenden Entwicklungsstadien zulässig sind (Baradat et al. 1972). Die Entscheidung, ob man Nadel, Blatt, Holz, Blüten oder Samenproben verwendet, muss nach der Zielsetzung vom Versuchsansteller entschieden werden.

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European Communities - Commission

EEC symposium on forest tree biochemistry

by D.T. Seals, G.I. Forest, J.J. Philipson, H. Parkes

Directorate-General for Agriculture

Luxembourg: Office des publications officielles des Communautés européennes

1977 - 290 p. - 21×29,7

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