vem Wachstum (erhöhte Blühwilligkeit) möglicherweise intensivieren oder prinzipiell einleiten zu können.

Untersuchungen über chemische Natur und Wirkungsweise der (vielleicht synergistisch) wirksamen Komponente(n) der beschriebenen aktiven Substanzgemische wären die nächsten Schritte um zu klären, ob die erhaltenen Ergebnisse für die Praxis mittelbar nützlich sein können.

4. Zusammenfassung

Es wird die Darstellung eines Gemisches unbekannter saurer Verbindungen aus männlichen Fichtenblüten beschrieben. Der Extrakt enthält mindestens eine wachstumsregulierende Komponente, da er sowohl in saurer als auch in methylierter Form im Biotest das vegetative Wachstum von Salat- und Fichtenkeimlingen hemmt.

Anhand dieser Ergebnisse wird folgendes Arbeitskonzept zur Beeinflussung der Blütenbildung bei Fichte vorgeschlagen:

Derldie gefundene(n) Hemmstoff(e) wird/werden nur bei generativem Wachstum (z. B. in Blüten) angehäuft, während des vegetativen Wachstums (Jugendphase) hingegen nur in geringerem Umfang oder gar nicht. Der/die Hemmstoff(e) werden nur unter definierten äußeren und inneren Bedingungen (Licht, Temperatur, Ernährung, Alter der Pflanze) gebildet.

Möglicherweise kann das generative Wachstum der Fichte durch den/die beschriebenen Hemmstoff(e) des vegetativen Wachstums stimuliert werden.

Schlagworte: Vegetatives Wachstum, Umstimmung, generatives Wachstum, Picea abies (L.) KARST.

Summary

A method is described to prepare a mixture of unknown acidic compounds from male flowers of Norway spruce, containing at least one growth-regulating component. According to the results of various bio-assays showing inhibition of the vegetative growth of lettuce and Norway spruce by the acidic or methylated mixture, a conception for influencing flower formation of Norway spruce is proposed.

The detected inhibitor(s) is/are thought to accumulate only when generative growth takes place, e. g. in flowers. During vegetative growth (juvenile phase) inhibitor(s) should be produced only at a low concentration or not at all. Synthesis of inhibitor(s) is thought to be possible only when well defined external and internal conditions (such as light, temperature, nutrition, age of plant) are provided.

Possibly the generative growth of Norway spruce could be stimulated by applying the described inhibitor(s) of the vegetative growth to the plant.

Key words: Vegetative growth, shift, generative growth, Picea abies (L.) KARST.

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The Application of DNA Reassociation Kinetics to evaluate Picea Crossability Patterns

By J. P. MIKSCHE and Y. HOTTA[1])

(Received October 1976 / March 1977)

Introduction

Interspecific hybridization is an important method of tree improvement. Hybrid plant development is often unsuccessful because of interfering factors such as pollen incompatibility, syngamic inhibition, and embryo inviability or subsequent abnormal ontogeny yielding embryo abortion (MIKKOLA, 1969; KRIEBEL, 1973).

Picea glauca (MOENCH) VOSS hybridizes successfully with *Picea sitchensis* (BONG.) CARR but not with *Picea abies* (L.) KARST. (NIENSTAEDT and TEICH, 1972; ROCHE and FOWLER, 1975). However, a putative hybrid between *Picea glauca* and *Picea abies* cv *acrocona* (FRIES) KRII has been reported (JEFFERS, 1971). MIKKOLA (1969) performed reciprocal crosses between *Picea abies* and *Picea glauca* and abnormal zygotes were formed that displayed inhibited development at various stages of pro-embryo ontogeny.

The *Picea glauca* X *Picea sitchensis* and *Picea glauca* X *Picea abies* hybridizations are yes and no crossing situations, respectively. This combination of interspecific genetic compatibilities offers a system that is amenable to the evaluation of the crosses at the molecular DNA level. The DNA-DNA homologies of the three species were, therefore, tested by means of liquid reassociation kinetics (BRITTEN and KOHNE, 1968) of repetitious, intermediate, and near unique copy DNA fractions to ascertain if reassociation kinetics are in agreement with the expected reannealing kinetic hypothesis in view of the demonstrated crossability patterns.

Materials and Methods

Extraction and purification of DNA from dormant seeds of *Picea abies, Picea glauca,* and *Picea sitchensis* are similar to the method of STERN (1968) and subsequently modified for conifers by MIKSCHE and HOTTA (1973) and HALL, MIKSCHE, and HANSEN (1976).

The seeds were washed in 10 volumes of 10% sodium hypochlorite ($NaClO\text{-}5H_2O$) in a beaker covered with cheesecloth. The seed and hypochlorite mixture was stirred vigorously for 20 minutes and the hypochlorite solution was decanted and the seeds were rinsed in running, cold, tap water for 45 minutes. The seeds were then placed on a flat surface and dried. The washed and dried seeds were ground to a fine powder with sand (acid washed and ignited) in a

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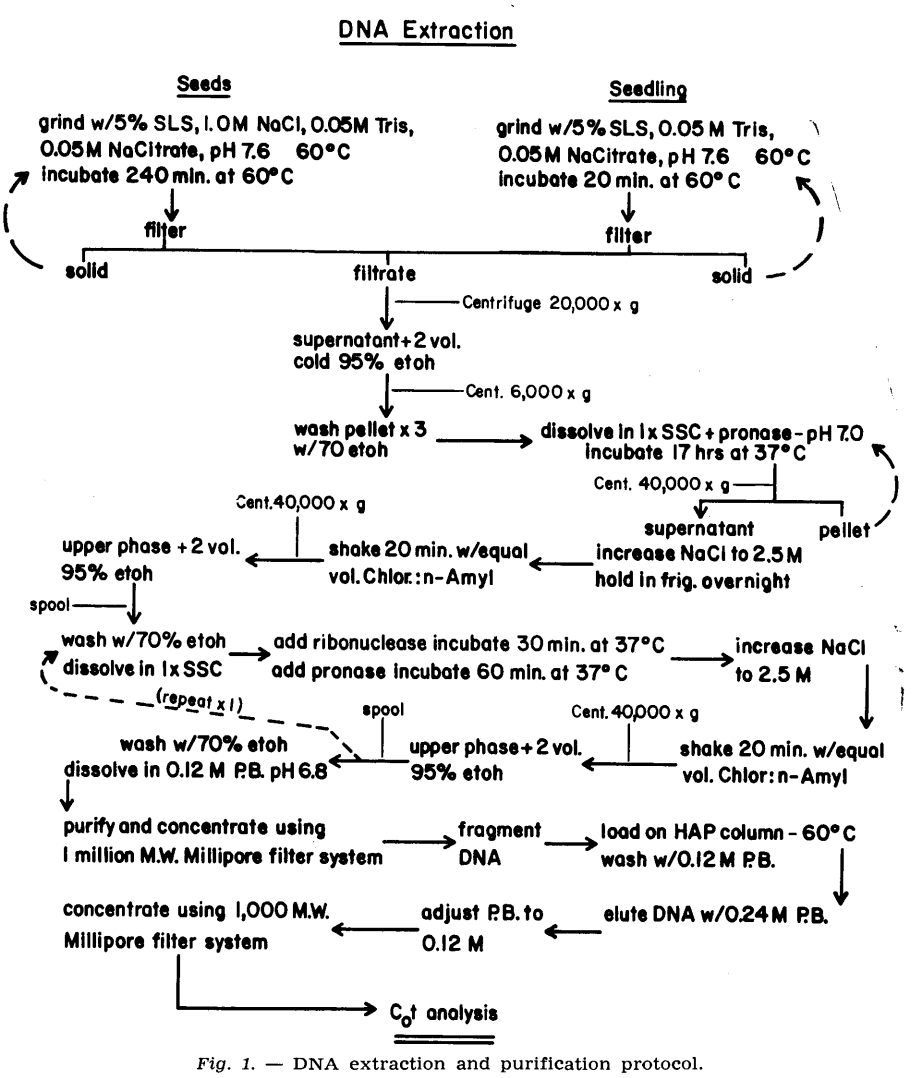

Fig. 1. — DNA extraction and purification protocol.

mortar and pestle and the DNA was extracted and purified according to the protocol illustrated in *Figure 1.*

The DNA precipitate was resuspended in 1 X SSC (0.15M NaCl, 0.015M Na citrate) and centrifuged at 25,000 x g at 4° C for 50 minutes. The DNA solution was further purified by passing it through a Pellicon (Millipore) 1 million molecular weight filter to remove small pieces of DNA and certain protein contaminants. Larger macro-molecules of DNA are retained on the filter. The effluent was monitored by a 230 through 300 nm spectral scan to ascertain passage of the small molecules. The DNA solution was centrifuged at 40,000 x g at 4° C for 60 minutes and the supernatant was decanted and precipitated with two volumes of 95% ethyl alcohol. The spooled DNA was held in cold 70% ethyl alcohol for subsequent use. The optical density, 260/280 mm ratio of a DNA-1X SSC solution was 1.90 at this stage of purification.

In DNA reassociation experiments using hydroxyapatite (HAP), the native or double strand DNA molecules are sheared, forming smaller pieces, and denatured into single strands. The single-strand fragments are incubated and molecular movement causes collisions between DNA strands. If the two colliding strands possess unlike nucleotide sequences, reassociation does not occur. However, if the strands are alike (complementary) a stable reassociated duplex is formed. The rate at which two complementary strands reanneal depends on their respective concentrations in solution. The experimental procedure employed in this study is described below.

The DNA samples were sonicated in 0.12M phosphate buffer (PB) (pH 6.8) with a Model W 185 Sonifer-Cell Disruptor (Heat systems — Ultrasonics, Inc., Plainview, N.Y.), microprobe at 60 watts in an ice water bath for 10 minutes using a 2 minute sonifer "on" and a 2 minute sonifer "off" cycle (Miksche and Hotta, 1973). The phosphate buffer was prepared by adding 51 ml of 0.24 M monobasic potassium phosphate to 49 ml of 0.24 M dibasic potassium phosphate and diluted to 200 ml. A mean molecular weight of 2×10^5 daltons was determined by sedimentation centrifugation (Studier, 1965) and intrinsic viscosity analysis (Crothers and Zimm, 1965). The DNA samples were purified additionally after sonication by loading the sheared DNA on a hydroxyapatite column (Hypatite, Clarkson Chemical Co., Williamsport, Pa.) at 60° C and washing with 0.12 M PB and the double strand DNA fragments were eluted with 0.24 M PB. A 1,000-molecular weight Pellicon Millipore fiter was used to reconcentrate the DNA samples and reduce the PB concentration to 0.12 M from 0.24 M.

For the generation of Cot (DNA concentration $\times$ time versus percent DNA reannealing), the *Picea* DNA samples were denatured by heating in boiling water for 20 minutes. The denatured samples were allowed to reanneal at 60° C in various concentrations of 5 to 3,000 ug/ml for different times. Single- and double-strand DNA were separated on

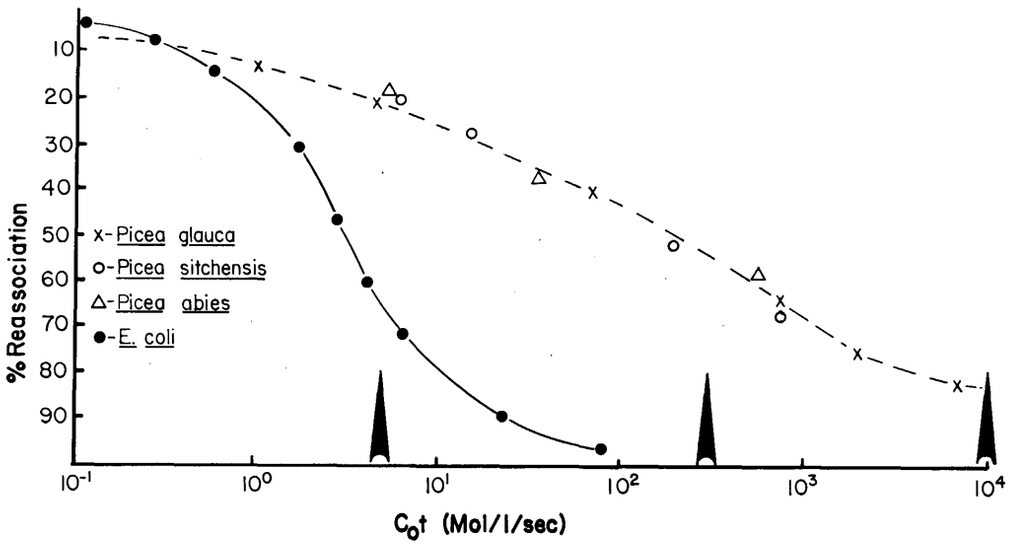

Fig. 2. — Reassociation curve of sheared total DNA of *Picea abies, Picea glauca* and *Picea sitchensis* in 0.12 M phosphate buffer at 60° C. Cot is the product of the initial DNA concentrations (in moles nucleotide per liter) loaded on the column and time (in seconds). The arrows delineate Cot fractions utilized in the molecular hybridization portions of the experiment.

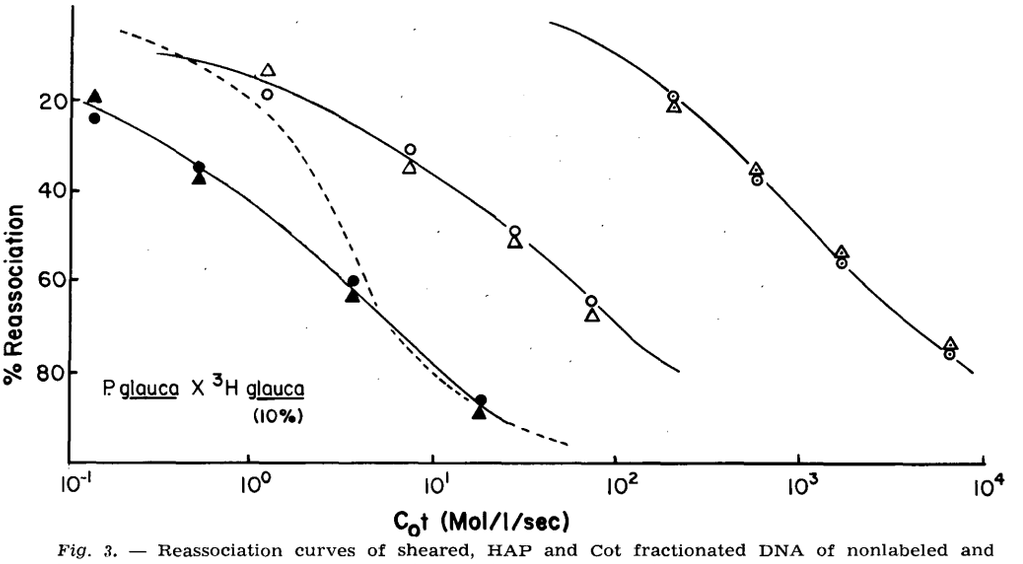

Fig. 3. — Reassociation curves of sheared, HAP and Cot fractionated DNA of nonlabeled and labeled *Picea glauca* in 0.12 M PB. The ^3H labeled *P. glauca* fractionated DNA was added to the respective Cot fractions at a quantity of 10% to the reaction mixture (1 ug ^3H *P. glauca;* 10 ug cold *P. glauca*). Optical densities (260 mm) are coded; 0-highly repetitive, 0-intermediate repetitive, and ⊙-near unique; and the cpm readings are represented by: Δ-highly repetitive, Δ-intermediate repetitive and Δ-near unique for the three Cot fractions, respectively. The reassociation curve of E. coli ^{14}C DNA is represented by a dashed line.

a 60° C water-jacked hydrohyapatite column and reassociation of the coniferous DNA and the internal standard E. coli DNA labeled with ^{14}C thymidine was determined by hyperchromicity and scintillation counting methods. The DNA samples were fractionated into three portions by termination of the reassociation reaction at Cot values of $1 \times 10^{-1} - 5 \times 10^{0}$ (highly repetitious), $5 \times 10^{0} - 3 \times 10^{2}$ (intermediate repetitious), and $3 \times 10^{2} - 1 \times 10^{4}$ (near unique) as demarcated by the arrows in *Figure 2.* The Cot fractions derived from the DNA of the three species were used for the DNA-DNA crosses to ascertain the homology potential of the reassociation of homologous and heterologous DNA nucleotide sequences using the method of liquid reannealing *(Figs. 3, 4,* and *5). Picea glauca* purified DNA was labeled with tritium *in vitro* with ^3H-dimethylsulfate substitution according to the method described by Smith, Armstrong and McCarthy (1967), and 740 cpm/ug DNA was obtained; E. Coli DNA was also labeled by the substitution method using ^{14}C dimethylsulfate and 6,000 cpm /ug DNA was obtained. The optical densities were read at 260 nm and the radioactivity was determined by a Beckman scintillation counter after 5% TCA precipitation on a glass filter (Reeve Angel, Clifton, N.J.) and drying.

Results and Discussion

The nuclear DNA of eukaryotes is composed of two or more fractions as was first demonstrated by the nucleic acid reassociation experiments of Britten and Kohne (1967, 1968). The DNA fractions consist of single and multiple copies, also denoted as unique and redundant or repetitious DNA, respectively. Repetitious DNA, implies the presence

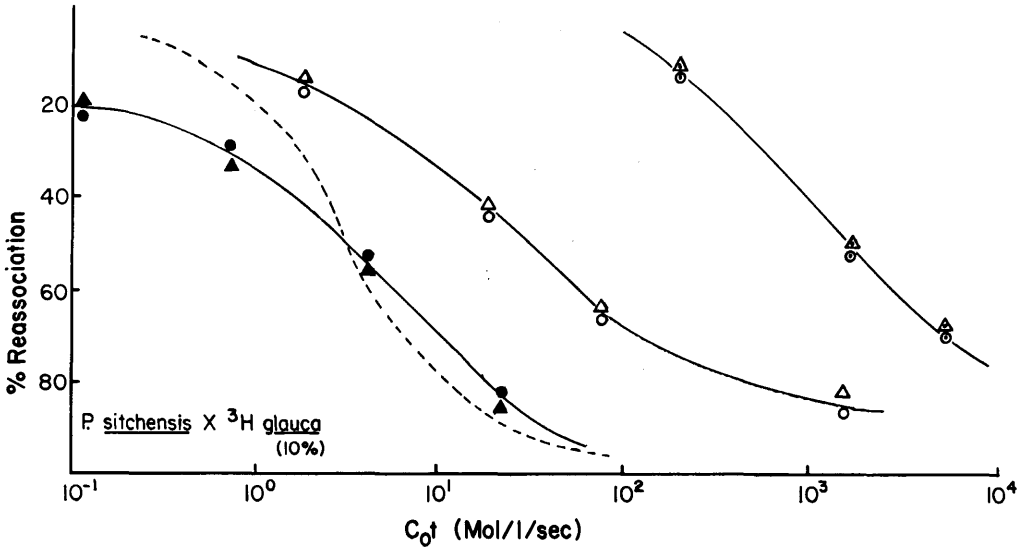

Fig. 4. — Reassociation curves between cold *P. sitchensis* and ³H labeled *P. glauca* highly repetitive, intermediate repetitive and near unique DNA. See *Figure 2* legend for coding of the labeled and non-labeled DNA fractions. The dashed line represents the reassociation curve of ¹⁴C E. coli DNA.

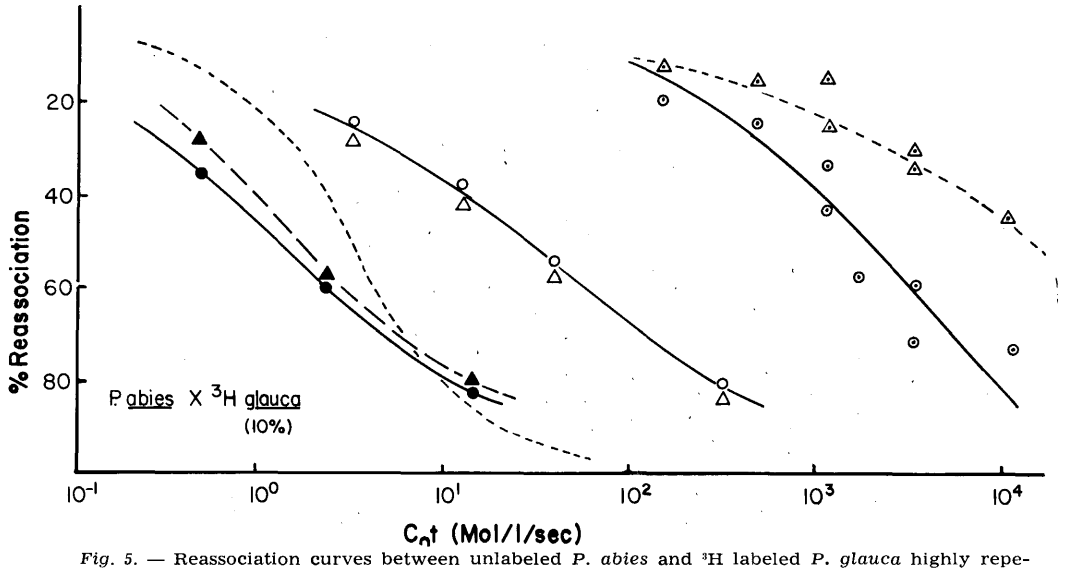

Fig. 5. — Reassociation curves between unlabeled *P. abies* and ³H labeled *P. glauca* highly repetitive, intermediate repetitive and near unique DNA. Notice the disparity of the curves in the high Cot fraction indicating a lack of homology. See he *Fgure 2* legend for coding. The dashed line represents the reassociation curve of ¹⁴C E. coli DNA.

of multiple copies of certain DNA sequences within a genome.

The studies listed below concerned with denaturing and reannealing manipulations and kinetics of DNA strands (and RNA molecular species) have been uitilized to study molecular taxonomic-genetic relatedness in viruses (Schildkraut, *et al.*, 1962) bacteria (McCarthy and Bolton, 1963), fungi (Dutta, 1975) higher plants (Bendich and McCarthy, 1970; Miksche and Hotta, 1973; Nze-Ekekang, *et al.*, 1974; Hall, *et al.*, 1975; Hotta and Miksche, 1975) and animals (Church and McCarthy, 1968, Laird and McCarthy, 1968; Walker, 1968; Entingh, 1970; Teshima, 1972; Rice, 1972; Schultz and Church, 1972; Hoyer, *et al.*, 1972; Santiago and Rake, 1973; Shields and Straus, 1975).

The present study incorporates DNA-DNA reassociation methodology in a manner somewhat different from the above studies. Although the general objective is similar, the result may offer a different type of interpretation of reassociation kinetics of DNA-DNA homology between species.

The purified whole genome DNA obtained from *Picea abies, Picea glauca,* and *Picea sitchensis* and subjected to Cot analysis procedures using hydroxyapatite columns yielded similar reassociation curves justifying the termination of the reassociation reactions of the *P. abies, P. glauca,* and *P. sitchensis* DNA to produce highly repetitive, intermediate repetitive, and near unique fractions *(Fig. 2).*

The *glauca-glauca DNA* hybridization portion of the experiment, *i.e.,* the reassociation of three fractions of labeled and unlabeled *P. glauca* DNA, demonstrates uniform second order kinetics, which is indicative of DNA homology among the highly repetitive, intermediate repetitive, and near unique DNA nucleotide copies *(Fig. 3).*

Examination of the reassociation curves of the three fractions of repeated nucleotide sequences from the *P. glauca* selfing and interspecific crosses of the three species by O.D. 260 hyperchomicity readings reveals similarities *(Figs. 3, 4, 5* and *Table 1).* The small deviations in the 1/2 Cot values of the highly repetitive fraction of *P. sitchensis* and in the near unique fraction of *P. abies* are probably

Table 1. — Cot 1/2 values of DNA from *Picea abies*, *Picea glauca* and *Picea sitchensis* taken from the O.D. 260 reannealing curves of the three fractions of DNA. The highly repetitive fraction of *P. sitchensis* and the near unique fraction of *P. abies* display some deviation.

Species	Cot Fractions		
	$1 \times 10^{-1} - 5 \times 10^{0}$	$5 \times 10^{0} - 3 \times 10^{2}$	$3 \times 10^{2} - 1 \times 10^{4}$
Picea abies	1.4	27	2000
Picea glauca	1.5	23	1300
Picea sitchensis	3	30	1500

not important enough to interfere with the reassociation kinetics.

The reassociation curves generated by scintillation counting of labeled and non-labeled DNA duplexes are also similar in shape and kinetics in all repeated nucleotide fractions with the critical exception of *Picea abies* against *Picea glauca* near unique fractions *(Fig. 5)*. In this case, reannealing curves measured by optical and radioactive counting methods are distinctly different as the isotope curve varies markedly from second order kinetics. A similar crossing result using meiotic DNA from different *Lilium* ultivars supports the findings of this report (Hotta and Stern, 1975).

The experiments reported here indicate, therefore, that to ascertain predictability of interspecific crosses, the near unique DNA should be used in the molecular DNA-DNA hybridizations. Of course, additional experiments with other interspecific crossing situations should be performed to further verify the practicability of the liquid reassociation technique as a prediction tool. If, indeed, the method is further verified much time and effort can be saved by the forest geneticist.

Acknowledgements

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Conclusion

Picea glauca hybridizes successfully with *Picea sitchensis* but does not with *Picea abies* using conventional breeding methods. DNA base complementarity among these species was evaluated by the DNA-DNA reassociation and Cot analysis as a possible indicator of field hybridization performance. Whole genome and reannealed Cot genome fractions from the three species were evaluated by reassociation kinetics. The DNA reassociation kinetics of the hydroxyapatite fractions — low (Cot 0—5), middle (Cot 5—300), and high (Cot 300—10,000) — of the three species were studied and the reannealing kinetics between the DNA segments from (*Picea glauca* and *Picea abies* yielded the greatest deviation from second order kinetics. These preliminary results indicate that reassociation kinetic analysis between near unique copies may be useful in indicating success of controlled as well as natural crossing of spruces and possible other plant species, but this technique does not measure or ascertain ontogenetic and physiological barriers inherent in certain crossing situations.

Key words: Cot analysis, DNA-DNA homology molecular crossing, reassociation kinetics. redundant DNA, repeated nucleotide sequences, repetitious DNA, unique DNA.

Zusammenfassung

Picea glauca läßt sich mit *Picea sitchensis* relativ leicht kreuzen, mit *Picea abies* bei Verwendung der herkömmlichen Methoden jedoch nicht. Als ein möglicher Indikator für das Kreuzungsverhalten wurde die Komplementarität der DNS-Basen dieser Arten durch DNS-DNS Hybridisierung und Cot-Analyse bestimmt. Die Kinetik der DNS-Renaturierung der Hydroxyapatitfraktionen — niedrig (Cot 300—10 000) — wurde bei den drei Arten untersucht. Die Hybridisierung der DNS von *Picea glauca* und *Picea abies* ergab die größte Abweichung von einer Reaktion zweiten Grades. Diese vorläufigen Ergebnisse weisen darauf hin, daß durch Analyse der Kinetik der DNS Renaturierung Hinweise auf Kreuzungsmöglichkeiten bei Fichten und möglicherweise auch anderen Pflanzenarten gewonnen werden können. Inkompatibilität aufgrund ontogenetischer oder physiologischer Unterschiede kann mit dieser Methode nicht ermittel werden.

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Ergebnisse einer Versuchsanlage mit europäischen Lärchen (Larix decidua Mill.) und Hybridlärchen (Larix eurolepis Henry).

Von S. RECK

(Eingegangen Februar 1977)

Im Frühjahr 1976 wurden bei einer Sturmkatastrophe auf einer 21 Jahre alten Lärchenversuchsfläche im Forstamt Farchau, Kreis Herzogtum Lauenburg in Schleswig-Holstein, 73% der Versuchsbäume geworfen, bzw. wurde der Wurzelteller so stark gehoben, daß die Bäume gefällt werden mußten. Wegen dieser hohen Ausfälle ist die Versuchsanlage nicht weiterzuführen. Mit der Aufnahme und Auswertung der Windwurfschäden erfolgte deshalb gleichzeitig eine Endauswertung, wobei auf Meßwerte und Boniturен aus vergangenen Jahren zurückgegriffen werden mußte. Mit Ausnahme der Stammformeigenschaften, die im Herbst 1975 erhoben worden waren, standen über Baummerkmale im Endzustand der Versuchsanlage vor der Zerstörung keine Daten zur Verfügung. Wegen der starken Schäden und der Unmöglichkeit, die zum Teil übereinander liegenden Bäume zweifelsfrei den einzelnen Versuchsnummern zuzuordnen, waren auch nachträgliche Merkmalserhebungen nicht möglich. Bei der Auswertung und Interpretation der Ergebnisse wurden deshalb Jugend-Alters-Korrelationen, besonders für Merkmale der Wüchsigkeit, unterstellt (RECK *u. a.* 1976) und Höhenmeßwerte der Versuchsbäume in einem jüngeren Alter für relative Vergleiche im Endalter verwendet. Hierdurch sind einige der mitgeteilten Ergebnisse mit zusätzlichen Unsicherheiten belastet, die auch durch Anwendung mathematisch-statistischer Tests nicht sicher zu quantifizieren waren. Insbesondere trifft das zu für Versuche, Abhängigkeiten der in den Kreuzungstypen und „Wiederholungen" unterschiedlich starken Windwurfschäden von Baumhöhe und Bestandesstruktur einzuschätzen. In der Absicht, Tendenzen für sortentypische und durch Umwelt modifizierte Wuchs- und Baummerkmale auch dann aufzuzeigen, wenn signifikante Unterschiede nicht nachzuweisen waren, wurde teilweise auf übliche mathematisch-statistische Auswertungsmethoden verzichtet, teilweise wurden sie in vereinfachter Form angewendet.

Aus der Bundesforschungsanstalt für Forst- und Holzwirtschaft Hamburg-Reinbek, Institut für Forstgenetik und Forstpflanzenzüchtung, Siekerlandstr. 2, 2070 Großhansdorf-Schmalenbeck.

[1]) Dem Forstamtsleiter sowie den Revierbeamten sei an dieser Stelle nochmals besonders gedankt für wertvolle Hilfeleistungen, beginnend mit der Begründung der Versuchsanlage bis zur abschließenden Untersuchung und Aufarbeitung nach der Sturmkatastrophe.

Material und Versuchsdurchführung

Die Versuchsanlage Lä 19 wurde im Herbst 1956 im Forstamt Farchau, Revier Salem[1]), mit 2jährigen verschulten Pflanzen begründet. Der Standort besteht aus humosem Sand auf einer stark lehmhaltigen Endmoräne, der im Untergrund schwach pseudovergleyt ist.

Bei den Versuchspflanzen handelt es sich um Nachkommenschaften aus einem Kreuzungsprogramm, in dem vier europäische Lärchen als Mutterbäume (Nr. 671, 672, 674, 1181) mit einer weiteren europäischen Lärche als Vaterbaum (Nr. 676) sowie mit einer Population der europäischen Lärche (Mischpollen Sudetenlärche) und einer japanischen Lärche als Vaterbaum (Nr. 1370) gekreuzt wurden *(Tab. 1)*.

Neben einer mißlungenen Kreuzung (1181 × 676) entstanden sieben Familien reine *Larix decidua* MILL. (Gruppe A und B) und vier Hybridfamilien *Larix decidua* MILL. × *Larix leptolepis* (SIEB. et ZUCC.) GORD. (Gruppe C). Zwischen den Familien innerhalb der Gruppen liegen ungleiche Verwandtschaftsverhältnisse vor: Gruppe A und Gruppe C enthalten jeweils Vollgeschwisterfamilien, die miteinander über denselben Vaterbaum (Nr. 676 bzw. 1370) verwandt sind. Gruppe B dagegen besteht unter der Annahme, daß der „Mischpollen" eine größere Anzahl von Bäumen repräsentiert, mit hoher Wahrscheinlichkeit aus vier Halbgeschwisterfamilien, die miteinander nicht verwandt sind. Diese Unterschiede sind für die genetische Interpretation, aber auch für die Anwendung von Auswertungsmethoden von Bedeutung.

Ziel der Untersuchungen war die Beurteilung der Anbaueignung von Hybridlärchen durch Vergleich mit europäischen Lärchen, wobei neben den Mittelwerten interessierender Merkmale auch — bei bestimmten Wirtschaftszielen sogar vorwiegend — die zu erwartende Variation innerhalb einer Hybridlärchen-Population zu berücksichtigen ist. Aus diesem Grunde erfolgte die Auswertung und die Schätzung der Variation getrennt für die drei Kreuzungsgruppen. Als Kriterium für diese Variation wurde die Variationsbreite (Spannweite) der Mittelwerte verwendet, die in Anlehnung an varianzanalytische Verfahren zur Heritabilitätsschätzung aufgegliedert wurde nach möglichen genetischen und Standortseffekten (vgl. RECK *u. a.* 1976).

Die Versuchsanlage wurde als Blockversuch mit 4 Wiederholungen mit je 4 Blocks zu 4 Parzellen und 49 Pflanzen