

the highest proportion of variation in the subject tree measurements. This is also the most complicated index to calculate, requiring measurements of crown radius from all competitors and the subject tree. The Weighted Tally is one of the simplest indices to calculate, requiring only the diameter of adjacent competitors. And it is also significantly correlated to subject tree measurements. The choice of which index to use will vary; the differences in correlations may well be offset by the time required to obtain measurements necessary to calculate the index. In either case once the index has been selected, simple linear regression models of the index predicting subject tree diameter and height should be generated for each progeny test. Based upon the results of these regression models, individuals within families should be selected based upon 1) the subject tree's measurements alone prior to the onset of competition and 2) those subject trees whose measurements, after the onset of competition, exceed measurements predicted by the derived regression equations.

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## Propagation of Norway Spruce Cuttings Free of Topophysis and Cyclophysis Effects

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#### Abstract

A method is described with which within 14 months from an ortet of *Picea abies* (L.) KARST. about 64 very evenly growing, topophysis-free ramets can be vegetatively propagated at an early developmental phase. This number increases exponentially with a longer propagation period. Starting from a seedling, first stock plants were propagated and repropagated by cuttings and from these the cuttings were taken. At the age of two years, a comparison of clones propagated by this method and clones propagated from 12-year old trees showed that topophysis effects influence many growth characters. Cuttings propagated by this method showed better height growth, more free growth as well as orthotropic growth and a radial arrangement of needles and branches. The growth characters of the clones propagated from the 12-year old trees showed that a complete change of the developmental phase caused by topophysis and cyclophysis back to a seedlings phase is unlikely. Implications for sustained growth potential of repeatedly repropagated clones are discussed.

Key words: *Picea abies*, cyclophysis, topophysis, repropagation, developmental phase.

#### Zusammenfassung

Eine Methode wird beschrieben, mit deren Hilfe bei Fichte (*Picea abies* (L.) KARST.) in einer Zeit von 14 Mona-

ten ca. 64 sehr einheitlich gewachsene topophysisfreie Stecklinge pro Klon vegetativ vermehrt werden können. Sie befinden sich in einem frühen Entwicklungsstadium. Die Anzahl der gewonnenen Stecklinge steigt exponentiell mit der Dauer der Vermehrung. Beginnend mit einem Sämling wurden zunächst Ausgangspflanzen in mehreren Vermehrungszyklen hergestellt und von diesen die Stecklinge gewonnen. Ein Vergleich dieser Stecklinge im Alter von zwei Jahren mit gleichalten Stecklingen, die von zwölfjährigen Mutterbäumen gewonnen wurden, zeigt, daß Topophysiseffekte verschiedene Wuchsmerkmale deutlich beeinflussen. Solche topophysisfreien Stecklinge zeigen ein größeres Höhenwachstum, mehr freies Wachstum sowie orthotropes Wachstum und radiärsymmetrisch angeordnete Nadeln und Zweige. Wachstumsmerkmale von Stecklingen zwölfjähriger Mutterbäume lassen ein vollständiges Verschwinden der Topo- bzw. Zyklophysiseffekte durch das von den Mutterbäumen erreichte Entwicklungsstadium nicht erwarten. Diese Stecklinge beginnen also ihre Entwicklung in einem späteren als das Sämlingstadium. Die Auswirkungen einer wiederholten Vermehrung von Klonen auf ein anhaltendes Wuchsvermögen wird diskutiert.

#### Résumé

Nous décrivons une méthode par laquelle environ 64 ramets de *Picea abies* (L.) KARST. poussés de nature uniforme et sans topophysis, peuvent être propagés végéta-

tivement à partir d'un clone en 14 mois. Les ramets se trouvent alors dans une première phase de développement. Le nombre de ramets obtenus augmente exponentiellement durant la période de reproduction. Partant d'un semis, des ortets ont été propagés en plusieurs cycles de reproduction dont on reçoit les boutures. Une comparaison entre ces clones de deux ans et ceux du même âge propagés des arbres-mères de douze ans montre que les effets de topophysis agissent nettement sur les différentes caractéristiques de croissance. Les boutures propagées selon la méthode décrite montrent une meilleure croissance de hauteur, plus de croissance libre ainsi qu'une croissance orthotrope et un arrangement radiaire des aiguilles et des branches. Les caractéristiques de croissance des clones provenant des arbres de douze ans et avant subi l'influence de la topophysis et de la cyclophysis ont peu de chance de disparaître au cours du développement jusqu'au stade d'un semis.

Sont discutées, les conséquences d'une propagation à plusieurs reprises portant sur le potentiel de croissance permanent.

### Introduction

The shoot growth characteristics of young ramets of Norway spruce clones were to be studied in a parallel investigation, however, the experiments were seriously handicapped by plagiotropic growth, lateral symmetry of needles and branches, within-clone differences in the growth of ramets and differences in the morphology of needles and branches. These effects are due to topophysis and cyclophysis (SEELIGER 1924, SCHAFFALITZKY DE MUCKADELL 1959, MOORBY *et al.* 1963, ROBBINS 1964, ROULUND 1973, OLESEN 1978). Degree of differentiation which increases with increasing age of the ortet seems to be responsible for these effects. Least topophysis and cyclophysis effects can therefore be expected on cuttings taken from seedlings. However, only few cuttings can be taken from seedlings. To be

able to propagate a relatively large number of ramets per clone but still have the advantages of a seedlings' developmental phase, in this study a method suggested by LIBBY (1969) and used for *Picea mariana* (RAUTER 1979, ARMSON *et al.* 1980) was applied with modifications. Until the age of two, different growth characters of these clones were compared to those of a second group of clones which were propagated from 12-year old trees. An abstract of this study has already been given (MUHS *et al.* 1983).

### Material and Methods

For the first group of clones (S), seeds from five sources, two Roumanian, two Czechoslovakian and one from West-Germany (Westerhof) were germinated in February in a greenhouse. Five seedlings of each source (altogether 25 clones) were potted in a standard greenhouse soil and kept in a glasshouse at 20 hours light per day to induce continuous shoot growth. Natural daylight was supplemented by fluorescent lamps giving a light intensity at plant level of about 10 000 lux and higher.

In May, when the seedlings were about 10–15 cm high, the first cuttings were taken. The seedlings started new shoot growth from lateral buds. About every 3–5 weeks further cuttings were made, usually one to two, sometimes three cuttings per plant. Care had to be taken to leave at least one lateral bud on the seedlings for further shoot growth.

The propagules were cut to a length of about 4 cm. Before planting, they were dipped into a fungicide solution (0,05% Benomyl) with which the rooting medium was also treated. For rooting the relative humidity was kept above 95% by covering the cuttings with clear plastic sheets. The temperature was held between 18–24°C and the photoperiod and light intensity about the same as for the seedlings. The cuttings were not treated with rooting hor-

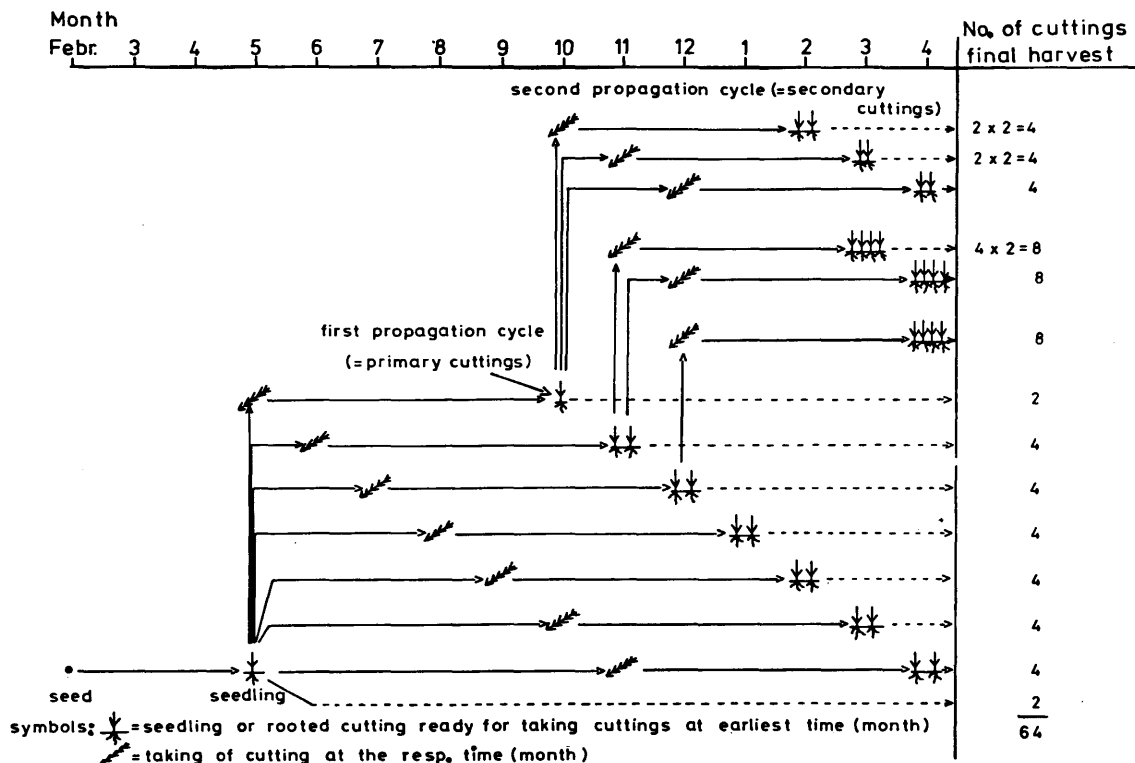


Fig. 1. — Propagation schedule going from sowing to the propagation of stock plants to the final harvest of cuttings of *Picea abies*. This schematic schedule assumes that always two cuttings were taken from the seedling and the rooted cuttings except at the very first time, when only one cutting was taken from the seedling.

mones. As a rooting medium a mixture of sand and perlite (50/50%, vol/vol) was used until November. Later than November a mixture of peat/perlite/sand (50/40/10%, vol/vol) was used. Shortly after the cuttings had rooted they started shoot growth. They were then potted, kept together with the seedlings and used as stock plants for further propagation of cuttings. After sufficient shoot growth had taken place cuttings were taken from these plants also. By April of the following year (Fig. 1) the seedling and all the stock plants which had been propagated so far were harvested. These harvested cuttings were rooted as described, potted and kept in the nursery for further investigations.

In the same year a second group of clones (T) was propagated. The cuttings were not taken from seedlings, but from seed-grown 12-year old trees of Norway spruce of similar seed sources in Roumania and Czechoslovakia. The rooting procedure was similar to that for the first group of clones, using also no rooting hormones, a peat/perlite/sand rooting medium and clear plastic sheets to keep the relative humidity high. As distinct from the rooting procedure of the first group of clones the cuttings of the second group were woody, from first order branches and had a length of about 10 cm. No cuttings were repropagated, that is all cuttings were primary cuttings. After rooting, the cuttings were potted and put in the nursery together with the clones of the first group. The rooting percentage of the second group of clones was only roughly estimated, whereas on the first group of clones rooting time and percentages were exactly recorded.

After one year of growth total heights and lengths of shoots formed by free growth (JABLANCZY 1971) were measured on four clones of each group and orthotropic growth and radial symmetry of needles and branches were scored. To classify orthotropic growth and radial symmetry five types were distinguished for each trait, 1 representing a seedlings' habit and 5 representing strong plagiotropic growth with lateral symmetry of the needles and branches. Except free growth these characters were recorded again after the second year of growth (Table 1).

## Results

### Rooting

All of the cuttings of the first group of clones rooted, but there was a large variation in rooting time between the clones. Peat in the rooting medium accelerated rooting

markedly. In the sand/perlite rooting medium during eight weeks only 38% of the cuttings rooted, whereas in the peat/perlite/sand medium used later than November over the same time already 100% rooting had occurred.

The second group of cuttings from 12-year old trees also showed differences in rooting time which was altogether longer. However the difference between the groups of clones in rootability was much larger. In none of the clones of the second group there was a rooting percentage anywhere near 100%. Rootabilities of both groups are difficult to compare because the cuttings of the first group of clones were succulent and only 4 cm long, whereas the cuttings of the second group were overwintered first order branches of about 10 cm length.

After five months the cuttings of the first group of clones had rooted and grown enough to be able to take from them the first cuttings of the second propagation cycle. From these in turn already after four months the first cuttings of the third propagation cycle could be repropagated (Fig. 1), presumably due to the better rooting substrate with peat.

By the middle of April, 14.5 months after sowing, altogether 867 cuttings could be harvested from the 25 clones of the first group. The average was 35 cuttings per clone and the range varied between 18 and 65 ramets per clone. However, further repropagation of stock plants would increase exponentially the number of cuttings which can be harvested.

### Topophysis and Cyclophysis Effects

Comparing the two groups of clones marked differences were found. Cuttings of the first group of clones rooted 100%, whereas cuttings of the second group gave rooting percentages roughly estimated to lie between 50 and 75%.

In both years height growth was on the whole much better in the first group of clones (S) than in clones of the second group (T), although in the second group clone C2/3/5 also shows good height growth (Table 1).

Free growth length as well as its incidence in the first year are not uniform in the second group of clones. Clones of the first group (S) are highly uniform (about 20–30% of total increment is due to free growth, standard deviation is low, incidence of free growth is 100%). Two clones of the second group (T) show a similar free growth pattern while the other two clones had a reduced length and incidence of free growth (Table 1).

Table 1. — Means and Standard Deviation Respective Ranges of Different Characters of Two-Year Old Clones Propagated From Seedlings (S) and From 12-Year Old Trees (T).

| Clone      | Rooting % | Number of ramets studied | Height    | Height    | Increment 2-year | Free Growth 1-year |           | Orthotropism of Growth |           | Symmetry of Needles |           |
|------------|-----------|--------------------------|-----------|-----------|------------------|--------------------|-----------|------------------------|-----------|---------------------|-----------|
|            |           |                          | 1-year    | 2-year    |                  | Length             | Incidence | 1-year                 | 2-year    | 1-year              | 2-year    |
|            |           |                          | cm        | cm        | cm               | cm                 | %         |                        |           |                     |           |
| 6777-5 (S) | 100       | 13                       | 23,7 ±1,7 | 42,6 ±2,0 | 18,9 ±1,1        | 6,0 ±1,1           | 100       | 1,0                    | 1,0       | 1,0                 | 1,0       |
| 6777-3 (S) | 100       | 13                       | 22,9 ±2,4 | 42,5 ±3,7 | 19,5 ±2,3        | 4,4 ±1,3           | 100       | 1,0                    | 1,0       | 1,0                 | 1,0       |
| 6782-3 (S) | 100       | 14                       | 20,7 ±1,6 | 38,7 ±3,7 | 18,0 ±2,9        | 6,0 ±1,3           | 100       | 1,0                    | 1,0       | 1,0                 | 1,0       |
| 6787-3 (S) | 100       | 14                       | 24,3 ±2,2 | 39,8 ±3,3 | 15,5 ±2,2        | 4,8 ±1,6           | 100       | 1,0                    | 1,0       | 1,0                 | 1,0       |
| C1/3/3 (T) | 50-75     | 11                       | 12,5 ±3,2 | 22,4 ±4,5 | 9,9 ±1,5         | 3,8 ±2,2           | 90        | 3,8 (2-5)              | 3,4 (2-5) | 4,8 (4-5)           | 4,5 (4-5) |
| C2/3/5 (T) | 50-75     | 11                       | 20,9 ±3,8 | 37,2 ±6,8 | 18,3 ±4,7        | 6,5 ±2,2           | 100       | 1,6 (1-3)              | 1,4 (1-2) | 3,6 (3-5)           | 1,8 (1-4) |
| C3/1/9 (T) | 50-75     | 10                       | 15,8 ±2,5 | 31,5 ±5,5 | 15,7 ±4,5        | 5,2 ±2,1           | 100       | 1,9 (1-3)              | 1,5 (1-2) | 4,4 (3-5)           | 2,1 (1-3) |
| R7/5/16(T) | 50-75     | 10                       | 15,3 ±2,3 | 29,3 ±5,7 | 14,0 ±4,2        | 2,8 ±1,8           | 80        | 2,4 (1-3)              | 1,7 (1-3) | 4,7 (4-5)           | 2,5 (1-4) |

Classification of orthotropism of growth and symmetry of needle arrangement ranging from 1 to 5, 1 representing a seedling's habit and 5 representing plagiotropic growth and lateral symmetry of needle and branch growth.

Orthotropic growth and radial arrangement of needles and branches are different between the groups. While the first group (S) shows in both characters seedling growth patterns, clones of the second group (T) maintain more or less their plagiotropic growth and a lateral arrangement of needles and branches even after two years, although a trend towards orthotropic growth and radial arrangement can be observed when comparing the values of the first and second year. Low height as well as free growth are associated with plagiotropic growth and lateral symmetry (Table 1).

### Discussion

In this experiment within 14,5 months between 18 and 65 ramets per clone have been propagated. This is not a very high number of ramets, but it is comparable to the propagation efficiency of other methods (BENTZER 1980) and there are possibilities of raising this number. To achieve this, the shoot growth of the stock plants should be kept high, there should be many lateral branches and buds and the time for rooting and subsequent growth should be short. Differences between clones in the number of lateral buds and branches explain the large variation in the number of ramets per clone propagated. Shoot growth can be accelerated by extending the photoperiod, applying sufficient nutrients (DORMLING *et al.* 1968) and possibly by raising the CO<sub>2</sub> level (ALDÉN 1971), which was not done in the study here. Rooting can be accelerated by using rooting hormones, keeping humidity above 95% and using an adequate rooting medium (KLEINSCHMIT *et al.* 1973). In this study the addition of peat to the medium reduced rooting time by more than a half. When these growth factors are put close to the optimum the production of cuttings could be raised to about 100 per clone in the first year. Furthermore, the number of cuttings increases exponentially when stock plants are repropagated for a longer period. This seems to be possible for several years (LIBBY 1983) but is probably connected with an increase of topo- and cyclophysis effects the longer the repropagation of stock plants goes on.

The differences between the two groups of clones are very significant, whereas within group differences are comparatively small in most characters. Strong differences between groups become apparent in rooting, height increment, free growth, orthotropic growth, arrangement of needles and branches either radially or laterally and also in the morphology of needles and branches which have been observed but not measured here. These results are in accordance with those of ROULUND (1973, 1975, 1979), ROSS (1975), ALDÉN *et al.* (1977), KLEINSCHMIT *et al.* (1977), OLESEN (1978), JESTAEDT (1980) and WEISGERBER (1980) who showed that with increasing age of the ortet rootability decreases and plagiotropism increases. HERRMANN (1961) also found that height growth of Norway spruce cuttings is effected strongly by the age of the ortet.

After two years of growth a slow trend toward orthotropic growth and radial arrangement of needles and branches on the leader was observed on the second group of clones. This agrees with the results of KLEINSCHMIT (1958, 1961), ROULUND (1975, 1979), BLACK (1973) and JESTAEDT (1980) who have found, that the older an ortet is, the longer time it takes before the ramets start to grow upright. It is to be expected that a rejuvenation takes place more easily the younger the ortet is because not as much of the ontogenetic development has to be reversed.

The change of some characters influenced by topophysis as documented in an upright position and the general

appearance of a normally growing tree can lead to the deceptive conclusion that cyclophysis effects have also disappeared and also the cutting has completely rejuvenated. But ROBINSON and WAREING (1969) could show on repeatedly repropagated *Ribes nigrum*, that the plants started flowering after a few rooting cycles. This means that cyclophysis effects have increased, although the plants have never reached any large size or high degree of complexity. Also KRUSCHE *et al.* (1976) showed that grafts of Norway Spruce did not return to a juvenile stage after growing upright but were flowering. Contrary to that KLEINSCHMIT *et al.* (1977) found that with repeated repropagation of Norway spruce cuttings rootability increased with every propagation cycle. Also FRÖHLICH (1961) could show that cuttings from very old Norway spruce trees that would not root could be rejuvenated by way of grafting and then cuttings taken from the grafts could be rooted. It can be concluded, that for Norway spruce some characters can show some degree of rejuvenation e.g. rootability, orthotropic growth and radial arrangement of branches and needles (KRUSCHE *et al.* 1976, KLEINSCHMIT *et al.* 1977) and some characters show no rejuvenation at all or even a further development according to their ontogeny, e.g. flowering (KRUSCHE *et al.* 1976, ROBINSON and WAREING 1969, HACKETT 1980). These changes in the ontogeny of a cutting with some characters rejuvenating while others continue developing probably influences the whole growth performance of a cutting. It can at least be expected to differ from that of an ortet and is probably also different between cuttings of different propagation cycles. This could explain the weak correlations of growth performance between Norway spruce cuttings of different propagation cycles as well as between them and their ortet found by DIETRICHSON and KIERULF (1980). Similar results were also found by KLEINSCHMIT (1982).

There is no guarantee for sustained high mass production in forestry, when the ramets of a clone only assume upright growth and the general habit of a normally growing tree but have reached a phase past the grand period of growth. The developmental phase of a cutting should be tested with the aid of other characters to be sure of sustained high growth potential lasting into the harvesting age. Characters which might give hints about the developmental phase of a clone are bark and tracheid structure as well as needle and branch morphology and the way a clone reacts to specific environmental influences (SCHAFFALITZKY DE MUCKADELL, 1959, BORCHERT 1976, OLESEN 1978).

The method employed here is suitable for the propagation of clonal material free of topophysis and cyclophysis effects. This is due to the circumstance that very young seedlings have been used as basic material in which differentiation and ageing processes have not advanced far.

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## Rapid Multiplication of Bamboo by Tissue Culture\*)

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### Abstract

Multiple shoots of *Dendrocalamus strictus* from seedlings were obtained in shake flasks on liquid medium containing Murashige & Skoog's medium supplemented with BAP and coconut milk. Rooting of these shoots was obtained on Murashige & Skoog's half strength liquid medium after treatment with IBA for 48 hours in dark. The rooted plantlets were transferred to field. Multiple shoots from nodal segments of mature trees of *Dendrocalamus strictus*, *Bambusa arundinacea* and *Bambusa vulgaris* were obtained on Murashige & Skoog's medium supplemented with coconut milk, kinetin and BAP. Rooting of the shoots of *Dendrocalamus strictus* was obtained on Murashige & Skoog's half strength medium with activated charcoal after treatment with IBA for 96 hours in dark. Fifteen subcultures have been carried out from seedling explants without any diminution in the capacity for shoot or root formation. By this method of subculture it can be estimated that 10,000 plantlets can be obtained from one single seedling in a year.

**Key words:** Tissue culture, plantlets, seedling, mature nodal segments, *Dendrocalamus strictus*, *Bambusa arundinacea*, *Bambusa vulgaris*.

### Zusammenfassung

Die Vermehrung von Sproßtrieben aus Sämlingen von *Dendrocalamus strictus* wurde im Schüttelkolben mit einem flüssigen Medium erreicht, welches aus Murashige & Skoog's Medium, angereichert mit BAP und Kokosmilch, bestand. Die Bewurzelung solcher Triebe wurde mit Murashige & Skoog's halbkonzentriertem Flüssigmedium nach 48stündiger Behandlung mit IBA im Dunkeln erzielt. Die bewurzelten Plantlets wurden danach auf die Freifläche verpflanzt. Die Vermehrung von Sproßteilen aus nodalen Segmenten von älteren Mutterbäumen von *Dendrocalamus strictus*, *Bambusa arundinacea* und *Bambusa vulgaris* werden mit dem Medium von Murashige & Skoog erzielt, ergänzt durch Kokosmilch, Kinetin und BAP. Die Bewurzelung von *Dendrocalamus strictus* Trieben wurde mit dem halbkonzentrierten Murashige & Skoog's Medium mit Aktivkohle nach einer 96stündigen Behandlung mit IBA im Dunkeln erzielt. Es wurden 15 Subkulturen mit Sämlings-explantaten angelegt, ohne Verringerung der Kapazität der Sproß- oder Wurzelbildung. Mit dieser Methode der Subkulturen können in einem Jahr schätzungsweise 10.000 Plantlets von einem einzelnen Sämling erzielt werden.

### Introduction

Bamboo is a versatile multipurpose forest tree which plays a vital role in the world's industrial and domestic

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