### Literature Cited

BARNER, H. and CHRISTIANSEN, H.: The formation of pollen, the pollination mechanism and the determination of the most favourable time for controlled pollination in Larix. Silvae Genetica 9-1 p 1–41 (1960). — DOGRA, P. D.: Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae. Studia Forestalia Suecica No 45 p 1–97 (1967). — DOYIE, J. and O'LEARY, M.: Pollination in Tsuga, Cedrus, Pseudotsuga and Larix. Sc. Proc. Royal Dublin Soc., Vol 21 (N.S.), p 191 (1935). — HAGMANN, M. and MIKKOIA, L: Observations on cross-, self- and intraspecific pollinations in Pinus *peuce* Griseb. Silvae Genetica 12: p 73–79 (1963). — HAKANSSON, A.: Seed development in Larix. Botaniska Notiser Vol 113, Fasc. 1 p 29–40 (1960). — HALL, J. P.: Yield of seed and hybridization in the genus Larix Miller PhD thesis, University of Aberdeen pp 1–215 (1976). — HALL, J. P. and BROWN, I. R.: Microsporogenesis, pollination and potential yield of seed of Larix in North-east Scotland. Silvae Genetica 25, 3-4, 132–137 (1976). — KOSKI, V.: On self-pollination, genetic load, and subsequent inbreeding in some conifers. Comm. Inst. For. Fenn. 78.10 p 1–42 (1973). — KRIEBEL, H. B.: Embryo development and hybridity barriers in the white pines (section Strobus) Jour. Paper No 32–70 Ohio Agric. Res. and Dev. Centre p 1–20 (1970). — JOHANSEN, D. A.: Plant microtechnique. McGraw-Hill Book Co. Inc., New York. 523 pp (1940). — SCHOPF, J. M.: The embryology of Larix. III. Biol. Mon., **19**:4 p 1–97 (1943). — SMOLSKA, A.: Die Entwicklung des Archegoniums und der Befruchtungsprozeß bei *Larix europea* D. C. Bull. Acad. Pol. Sci et Lettres, Ser. B., Sci. Nat., 993–1028 (1927).


# Increased Flowering of Sitka spruce (Picea sitchensis (Bong.) Carr.) in a Polythene House


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## Introduction

Sitka spruce (Picea sitchensis (BONG.) CARR.) is the economically most important forest tree species in Great Britain, comprising more than 40% of the total number of plants produced by the Forestry Commission in the last decade. There is considerable scope for improving the productivity of Sitka spruce by breeding, but the absence of rmethods for controlling flowering has been a fundamental obstacle to progress. Flowering does not occur readily in Sitka spruce and in nature there is little before trees are 25–30 years old. In the related species Norway spruce (Picea abies) plentiful flowering often follows high temperatures in the previous summer (TIREN 1935, EKLUND 1957) and similar findings were reported for Fagus *sylvatica* (HOLMSGAARD and OLSEN 1960), Larix leptolepis (YANAGIHARA et al. 1960) and Pinus ponderosa (DAUBENMIRE 1960). However, very few studies have been made to assess the effect on flowering of artificially altering the environment around the tree.

Experiments have been carried out at the Forestry Commission Northern Research Station to determine the effect of growth in a polythene tunnel house on the flowering of 4-years-old grafted plants of Sitka spruce.

## Materials and Methods

Grafted plants of Sitka spruce (Picea sitchensis (BONG.) CAHR.) comprising young seedling rootstocks and scions from the tops of mature trees are known to be capable of producing strobili from about 8 years after grafting and were consequently chosen for use in this study. Scions from 19 clones were grafted on to rootstocks of 3-years-old seedlings in 1970 and transferred to 33-cm-diameter polythene buckets in 1972. Twelve uniform plants, about 0.6 m in height, were selected from each clone in the spring of 1973; nine of these plants were transferred to a polythene-skinned house while three remained outside as controls. The polythene skin, made from a mixture of polyethylene and ethyl vinyl acetate, covers a framework 3.1 X 15 X 10 m.

The buckets were weighed each week and sufficient water was supplied to keep the mean weight of those in the polythene house approximately equal to the mean weight of those containing the control plants. The polythene house was ventilated by opening the doors at both ends during the day. Air ternperatures were recorded at seven different heights between 0.1 m and 1.07 m above ground level inside the polythene house and at two heights outside the polythene house; overall mean temperatures are presented in Table 1.

Table 1. — Mean daily maximum and minimum air temperatures (°C) in the polythene house (PH) and among the control plants outside (CON) in the spring and summer of 1976.

| | May | | June | | July | | August | |
|---|---|---|---|---|---|---|---|---|
| | PH | CON | PH | CON | PH | CON | PH | CON |
| Min | 7.9 | 6.1 | 11.6 | 9.7 | 13.1 | 10.0 | 11.0 | 9.3 |
| Max | 34.5 | 16.8 | 39.3 | 22.3 | 39.3 | 23.1 | 38.2 | 23.7 |

## Results and Discussion

Grafted plants were retained in the polythene house for periods during three growth cycles. Following an initial treatment for 3.5 months in the spring and summer of 1973 a striking increase in both the proportion of plants flowering and the numbers of male and female strobili per plant was observed in 1974 (Table 2). The same group of plants was returned to the polythene house in April 1974 and remained there for a further 12 months. This second and longer treatment led to a still greater increase in flowering in 1975, and a response also occurred following the third year of treatment (Table 2). It should be noted, however, that individual plants within the group did not flower strongly in two successive years.

Buds of Sitka spruce are initiated in April and become distinguishable as reproductive or vegetative by September in Scotland (TOMPSETT unpubl.); the processes leading to flower differentiation must therefore take place within the intervening period of four months. Detailed observations made during these months in 1976 indicate that there are large differences in the mean daily temperature maxima between positions inside and outside the polythene house (Table *1),* confirming preliminary observations made


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*Table 2.* — Increased flowering of grafted plants in a polythene house. Plants were kept in the polythene house between early April and mid-July in 1973 and between early April in 1974 and May in 1976.

| Year of assessment | Polythene house | | | Control | | |
|---|---|---|---|---|---|---|
| | Mean number of strobili per plant | | Number of plants flowering/ Total number | Mean number of strobili per plant | | Number of plants flowering/ Total number |
| | Male | Female | | Male | Female | |
| 1974 | 1.53 | 0.81 | 36/171 (21.0%) | 0.09 | 0.05 | 4/57 (7.2%) |
| 1975 | 16.2 | 7.22 | 153/171 (89.5%) | 0.09 | 0.17 | 4/57 (7.2%) |
| 1976 | 4.01 | 0.11 | 63/162 (38.9%) | 1.15 | None | 9/54 (16.7%) |

in 1973—75. Mean daily maximum temperatures for 1976 did not differ greatly from those for 1973—75 *(Table 3)*. The results from this study agree with reports that retention under cold-room conditions during the spring can reduce flowering of *Pinus banksiana* (Larson 1961) and that an increase in male flowering was induced in grafted plants of *Picea abies* by covering with polythene tents in June and July (Brondbo 1969).

*Table 3.* — Mean daily maximum air temperatures (°C) at the Bush Meteorological Station, Roslin, for four months in the spring and summer periods of 1973—76.

| | May | June | July | August |
|---|---|---|---|---|
| 1973 | 12.4 | 17.3 | 17.6 | 17.8 |
| 1974 | 13.8 | 15.6 | 16.2 | 17.0 |
| 1975 | 11.7 | 17.2 | 18.7 | 20.5 |
| 1976 | 13.5 | 18.2 | 20.3 | 19.5 |

It is the authors' opinion that higher temperatures within the polythene house were directly responsible for the observed increase in flowering; an influence of other factors cannot, however, be ruled out. Drought has been shown to induce flowering in *Fagus sylvatica* (Holmsgaard and Olsen 1966); in the present study the mean weight of the buckets containing the polythene house grafts was maintained approximately equal to that of the control grafts by frequent watering so there was little difference in water stress between the two groups. Light quality is known to affect the flowering of plants; transmittance of radiation through the polythene film used in the present experiments was approximately 85% at all wavelengths between 400 and 800 nm so that the quality of light entering the polythene house was little changed within the part of the spectrum expected to influence plant growth. A reduction in the total amount of light reaching the plants would be expected to reduce flowering (Jackson and Sweet 1972) and therefore is unlikely to have caused the observed effect. Mean daily relative humidity readings were a little lower inside than outside the polythene house in the spring and summer of 1976, but small differences in humidity levels *per se* have never been shown to influence flower induction significantly. Finally, application of ethylene-inducing agents has been found to cause flowering in seedlings of some trees *(e.g. Mangifera indica* — Chacko *et al.* 1974), raising the question whether naturally-produced ethylene could have influenced flowering inside the polythene house. However, natural ethylene production by plant tissues is extremely small (Burg 1962) and the polythene house used was both large and well ventilated; it is unlikely that sufficient gas would have accumulated to affect plant growth.

Brondbo (1969) observed a relatively small increase in flowering after grafted plants of *Picea abies* had been covered by polythene tents for periods of two weeks; the stronger flowering of Sitka spruce in the present study may have been due to the longer period of treatment.

*Table 2* shows only small differences in minimum (night) temperatures between positions inside and outside the polythene house (overall mean 2.1° C). These observations suggest that the day temperatures, here represented by the maximum temperatures, are especially important in controlling flowering.

Heavy seed crops occur at irregular intervals on Sitka spruce and are difficult to harvest under natural conditions. The plentiful female flowering observed on plants after polythene house treatment shows that large quantities of seed might be conveniently obtained for forest plantings in this way. The greater degree of control over flowering which is possible using this method should enable the tree breeder to produce improved types more efficiently. In addition, the ability to enhance flowering strongly will be important for future studies of the internal mechanisms regulating the flowering process.


## Summary

The effect on flowering of keeping 4-years-old grafted plants of mature Sitka spruce *(Picea sitchensis* (Bong.) Carr.) in a polythene house for long periods was assessed in three successive years; overall mean increases of 15-fold in numbers of male strobili and 43-fold in numbers of female strobili over untreated controls were observed. This response was associated with large increase in day temperatures inside the polythene house during the months preceding the differentiation of distinct reproductive and vegetative buds. The implications of these findings for tree breeding and seed production are discussed.



*Key words:* Sitka spruce, flowering, temperature, polythene house, tree breeding, seed production.



## Zusammenfassung

Pfropflinge von *Picea sitchensis* (Bong.) Carr. wurden 2 Jahre nach der Pfropfung in Kübel gepflanzt und 1 Jahr später in ein Polyäthylen-Gewächshaus gebracht, in dem sie danach während bestimmter Perioden während der Vegetationszeit 3 Jahre lang verblieben. Gegenüber Vergleichspflanzen im Freien erhöhte sich die Fruktifikationsrate auf das 15fache bei den männlichen bzw. auf das 43-fache bei den weiblichen Blüten.


## References


Brondbo, P.: Blomstringsinduksjon ved varmebehandling hos granpodninger. Medd. Nor. Skogforsoksves. 27, 295—311 (1969). — Burg, S. P.: The physiology of ethylene formation. Annu. Rev. Plant Physiol. 13, 265—302 (1962). — Chacko, E. K., Kohli, R. R., Dore Swamy, R. and Randhawa, G. S.: Effect of (2-chloroethyl) phosphonic acid on flower induction in juvenile mango *(Mangifera indica* L.) *seedlings.* Physiol. Plant. 32, 188—190 (1974). — Daubenmire, R.: A seven-year study of cone production as related to xylem layers and temperature in *Pinus ponderosa.* Am. Midl. Nat. 64, 187—193 (1960).

— Eklund, B.: Om granens årsringsvariationer inom mellersta Norrland och deras samband med klimatet. Medd. Statens Skogsforskningsinst. 47, 1—63 (1957). — Holmsgaard, E. and Olsen, H. C.: The influence of weather on the production of beech mast. Forstl. Forsøgsvaes. Dan. 26, 345—370 (1960). — Holmsgaard, E. and Olsen, H. C.: Experimental induction of flowering in beech. Ibid. 30, 1—17 (1966). — Jackson, D. J. and Sweet, G. B.: Flower initiation in temperate woody plants. Hortic. Abstr. 42, 9—24 (1972). — Larson, P. R.: Influence of date of flushing on flowering of *Pinus banksiana*. Nature (Lond.) 192, 82—83 (1961). — Tiren, L.: Om granens kottsättning, dess periodicitet och samband med temperatur och nederbörd. Medd. Statens Skogförsöksanst. 28, 413—524 (1935). — Yanagihara, T., Tochiaki, K. and Arai, K.: On the relation between the harvest of Japanese Larch seed and meteorological conditions. (In Japanese). J. Jap. For. Soc. 42, 347—351 (1960).

# Zum Problem der Umstimmung von vegetativem zu generativem Wachstum bei Picea abies (L.) Karst.

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## 1. Einleitung

Es gibt eine Vielzahl von Arbeiten, die sich mit dem Phänomen der Blühstimulation bei verschiedensten Pflanzenarten befassen, wobei bei biochemischen Untersuchungen besonders der Funktion von pflanzlichen Wirkstoffen, z. B. Hormonen, Aufmerksamkeit geschenkt wurde.

Blütenbildung scheint das Ergebnis eines aufeinander folgenden Zusammenwirkens verschiedener Phytohormone zu sein (Bleymüller 1973, 1976). Sinngemäß dürften auch bei der Hemmung des vegetativen Wachstums, wie sie bei der Unterdrückung der Apikaldominanz zu Fruktifikationssteigerungen verwandt wird, bestimmte Wirkstoffe wie Hormone beteiligt sein. Zusammenhängend mit dem Anliegen der forstlichen Praxis, die Fruktifikationsvorgänge in Waldbaumarten wirksam beeinflussen zu können, wird die Gewinnung eines physiologisch aktiven Extraktes aus männlichen Fichtenblüten beschrieben und anhand der Ergebnisse von Biotests mit diesem Extrakt ein Arbeitskonzept zur Umstimmung von vegetativem zu generativem Wachstum (Blüteninitiierung) diskutiert.

## 2. Methoden

2.1 Herstellung eines sauren Extraktes aus männlichen Blüten

Blütenmaterial: Herkunft aus Siegsdorf (Adlgaß) bei Traunstein (1300 m). Die Ernte der noch nicht abgeblühten Blüten erfolgte am 14. 5. 76 an frisch gefällten Mutterbäumen. Die Zweige mit Blüten wurden nach ca. 5 h Transport (10—20°) in einer Tiefkühltruhe einige Tage eingefroren. Die Blüten wurden dann von den Zweigen abgenommen und bis zur späteren Aufarbeitung am 11. 10. 76 bei —20° aufbewahrt.

2.11 Ätherphase

531 g gefrorene Blüten wurden mit insgesamt 1,5 Liter ungekühltem dest. Wasser versetzt und in 2 Portionen bei Raumtemperatur 15 Min. auf Stufe III (Star-Mix) aufgeschlossen. Das Aufschlußgut wurde anschließend 15 Min. bei 0° und ca. 20.000 g in Stahlbechern zentrifugiert. Zur Erleichterung der Aufarbeitung wurde der Extrakt vor der Zentrifugation mit einem Seihtuch von groben Gewebetrümmern durch Abpressen befreit. 1.6 Liter klarer, gelber Überstand (pH ca. 4.5) wurden weiter verarbeitet. Es wurden 130 g NaCl im Überstand gelöst. Je 500 ml Überstand wurden 2× mit je 100 ml p. a. Äther ausgeschüttelt. Die fast farblose Ätherphase enthält lipoide neutrale und z. T. auch saure Extraktkomponenten. Die vereinten Ätherphasen wurden über wasserfreiem $Na_2SO_4$ 24 h getrocknet und bei 40° vom Äther befreit (Rotationsverdampfer). Nach 24 h Trocknen i. Vak. über KOH-Plätzchen unter Lichtausschluß und bei Raumtemperatur wurden 82,9 mg eines harzigen heterogenen Rückstandes (pH ca. 2—3) erhalten.

2.12 Essigesterphase

Die gelbe Wasserphase der Ätherextraktion wurde nunmehr mit konz. HCl unter Rühren ad pH ca. 3 gebracht, wobei eine Orangefärbung der Lösung eintrat. Je 500 ml der angesäuerten Phase wurden mit 2× je 100 ml p. a. Essigester ausgeschüttelt. Die gelbe Esterphase enthält saure Bestandteile der generativen Zellen. Die vereinten Esterphasen wurden 24 h über $Na_2SO_4$ getrocknet und bei 40° vom Solvens befreit. Nach 24 h Trocknen i. Vak. wie unter Kap. 2.11 wurden 113 mg ölig-harziger, heterogener Rückstand vom pH 2—3 erhalten.

Die Aufarbeitung des Aufschlußgutes wurde bis zur $Na_2SO_4$-Trocknung der organischen Phasen an einem Tag durchgeführt. Die verbliebene orangefarbene, saure Wasserphase der Essigesterextraktion enthält basische Zellkomponenten und wurde nicht weiter untersucht. Die erhaltenen Rückstände wurden zu weiteren Untersuchungen unter Lichtausschluß bei —20° verschlossen aufbewahrt.

Bei Bedarf wurde der Essigesterrückstand mit Essigester vollständig gelöst (Vibrationsmischer) und Aliquote für die Biotests bzw. Diazomethanbehandlung entnommen. Von den Aliquoten kann das Solvens leicht abgedampft werden. Nach Substanzentnahme wurde stets wieder vom Solvens befreit und wie beschrieben aufbewahrt.

2.2 Methylierung des sauren Extraktes mit Diazomethan

Das saure Extraktgemisch (2.12) wurde nach den Reaktionen a) und b) mit Diazomethan verestert bzw. verätherт.

a) $RCOOH + CH_2N_2 = RCOOCH_3 + N_2$

b) Phenole $+ CH_2N_2 =$ Methyläther $+ N_2$

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