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Regulation of flowering in Scots pine (Pinus sylvestris L.) grafts by gibberellins

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Summary

Spraying shoots of Scots pine grafts with gibberellins from the end of May to mid August caused significant changes in flowering. The $GA_{4/7}$ mixture tripled the intensity of female flowering and GA_3 had a negative effect on male flowering. The gibberellins used did not affect significantly the length and girth increments of shoots. There were no interactions between the less polar $GA_{4/7}$ and the more polar GA_3. It is suggested that earlier treatment with $GA_{4/7}$ favours the formation of male flowers and later treatment stimulates female flowers.

Key words: *Pinus sylvestris* L., flowering, gibberellins, sexual differentiation.

Zusammenfassung

Besprühen von Zweigen in der Krone von Kiefernpfropflingen (*Pinus sylvestris* L.) mit Gibberellinlösungen in der Zeit von Ende Mai bis Mitte August hatte eine signifikante Änderung der Blütenbildung zur Folge. Durch $GA_{4/7}$ wurde die Zahl der weiblichen Blüten verdreifacht, GA_3 wirkte sich auf die Bildung männlicher Blüten negativ aus. Die genannten Gibberelline haben den Durchmesser- und Längenzuwachs der behandelten Zweige nicht signifikant beeinflußt. Eine Interaktion der Wirkung der schwach polaren $GA_{4/7}$ mit der stärker polaren GA_3 wurde nicht festgestellt.

Auf Grund der bisherigen Ergebnisse darf man annehmen, daß die Behandlung mit $GA_{4/7}$ am Anfang der Vegetationsperiode die Bildung der männlichen Blüten fördert, während eine wiederholte Behandlung während der gesamten Periode die Bildung der weiblichen Blüten stimuliert.

Introduction

Since the last literature review on the physiology of flowering in conifers (PHARIS and KUO, 1977) several papers have been published on the effect of gibberellins on flowering in Pinaceae. These reports confirmed the positive effect of mixed $GA_{4/7}$ either alone or in combination with other factors on the stimulation of male flowering (CHALUPKA, 1978; LUUKKANEN and JOHANSSON, 1980), female flowering (GREENWOOD, 1979; ROSS and GREENWOOD, 1979) or both (TOMPSETT, 1977; TOMPSETT and FLETCHER, 1979; HARE *et al.*, 1979; PURITCH *et al.*, 1979; ROSS 1979). It turned out also that GA_3 may under certain conditions stimulate flowering in *Picea abies* (BLEYMÜLLER, 1976, 1978; CHALUPKA, 1979).

The paper presented here reports further data on the hormonal stimulation of flowering in Scots pine from the Institute of Dendrology in Kórnik.

Materials and Methods

The experiment was set up in the spring of 1978 on 17 year old Scots pine grafts growing in a seed orchard in Kórnik. Each of the selected clones was represented by 12 grafts on which 3 branches were chosen from successive whorls. These branches were designated as upper, middle and lower and shoots of the previous (1977) year, and new shoots developing in 1978 were treated. The treatments used gibberellins in the following combinations and concentrations: $GA_{4/7}$ at 200 mg/l, GA_3 at 200 mg/l, $GA_{4/7}$ + GA_3 at 200 mg/l + 200 mg/l, and a water control. The gibberellins were applied as a water solution with 0.05 % of surfactant AROMOX — C12 added. The sprays were applied five times on 29-30 May, 28-29 June, 10-11 July, 28-31 July and 15-17 August 1978. The whole experiment was replicated three times.

In spring 1979 a scoring was made of the number of shoots with male flowers, number of shoots with female flowers and total number of female flowers. Also the current annual increment in diameter and length of the treated shoots was measured. The orthogonal design of the experiment (5 clones × 2 × 2 gibberellin treatments × 3 types of branches) in three replicates permitted evaluation of results by analysis of variance *(Table 1)*.

Results

A. Male flowering

Supplying GA_3 from the end of May to mid-August has caused a significant reduction in the proportion of shoots with male flowers from 14.0 % to 8.9 % *(Table 1)*. This negative effect of GA_3 was manifested primarily in the middle (M) and lower (L) branches *(Fig. 1)*. On the upper (U) branches a slightly positive effect of GA_3 was observed but the interaction between GA_3 and branches was not significant ($F_{emp.} = 2.53 < F_{0.05} = 3.07$).

Male flowering was also affected by position of the branch in the crown and clones and there was an interaction between these two sources of variation *(Table 1)*.

B. Female flowering

The treated branches responded to $GA_{4/7}$ with a significant increase in female flowering *(Table 1)*. The proportion of shoots with female flowers increased on average from 2.7 % to 9.2 %, that is, almost threefold. The total number of female flowers per 100 shoots increased on average from 6.4 to 15.7. The success of $GA_{4/7}$ application in stimulating both the proportion of shoots with female flowers and the total number of female flowers was much greater in the middle and lower branches than in the upper ones *(Figs. 2 and 3)*.

As in male flowering, female flowering was strongly affected by the variability between branches and clones *(Table 1)*. However there was no interaction between gibberellin treatment and branches or clones since the positive effect of $GA_{4/7}$ occured in all the variants of the experiment though with varying intensity.

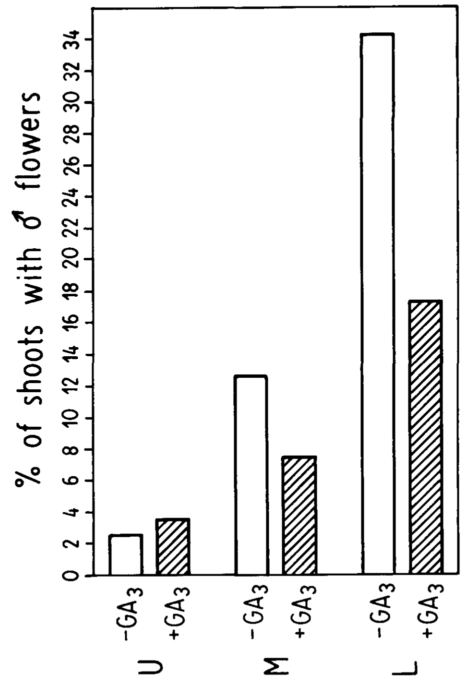

Figure 1. — Effect of Ga_3 on male flowering in upper, middle and low branches of Scots pine grafts.

In both male and female flowering there was no synergism (interaction) between the effects of the two gibberellins, GA_3 and $GA_{4/7}$, applied *(Table 1)*.

C. Growth increment

In comparison with the untreated controls each of the gibberellin combinations increased the length of the treated 1978 shoots by about 7% and their girths by 10%. These effects however were not significant, which is consistent with the data quoted earlier by Pharis and Kuo (1977).

Discussion

The negative effect of GA_3 on male flowering observed this year and the earlier experiments with *Larix lepto-*

Table 1. — Results of analysis of variance.

Source of variance	DF	% of shoots with male flowers (After arc - sine transformation) MS	F	% of shoots with female flowers (After arc - sine transformation) MS	F	Number of female flowers per 100 shoots MS	F
Total	179	469,05		120,24		186,53	
Growth regulators /G/	3	318,08	1,44	1057,72	$12,21^{++}$	1389,57	$9,75^{++}$
GA_3	1	929,80	$4,20^{+}$	158,63	1,83	199,08	1,40
$GA_{4/7}$	1	0,90	0,00	3011,06	$34,75^{++}$	3898,56	$27,36^{++}$
GA_3 x $GA_{4/7}$	1	23,54	0,11	3,46	0,04	71,07	0,50
Branches /B/	2	6329,04	$28,60^{++}$	1130,01	$13,04^{++}$	1383,10	$9,71^{++}$
Clones /C/	4	6402,11	$28,93^{++}$	239,46	$2,76^{+}$	543,38	$3,81^{++}$
G x B	6	317,73	1,44	73,57	0,85	190,22	1,34
G x C	12	397,42	1,80	128,08	1,48	134,67	0,94
B x C	8	829,82	$3,75^{++}$	180,82	$2,09^{+}$	297,20	$2,09^{+}$
G x B x C	24	220,71	1,00	39,23	0,45	48,52	0,34
Replications	2	7,70	0,03	271,16	$3,13^{+}$	583,45	$4,10^{+}$
Residual	118	221,30		86,64		142,47	

* significant at 0,05 level

** significant at 0,01 level

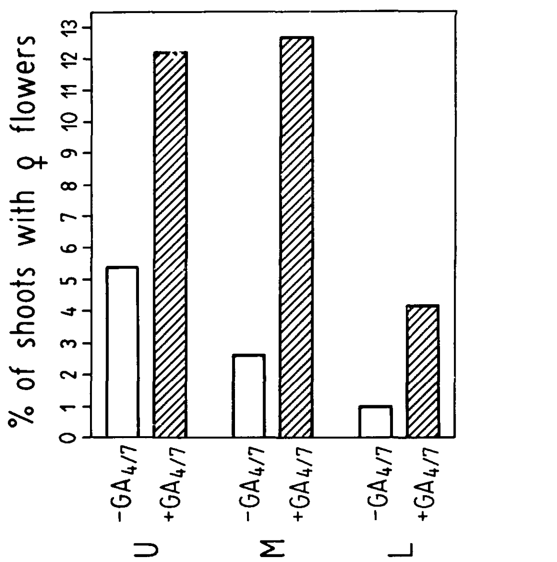

Figure 2. — Effect of $GA_{4/7}$ on the percentage of shoots flowering female in upper, middle and low branches of Scots pine grafts.

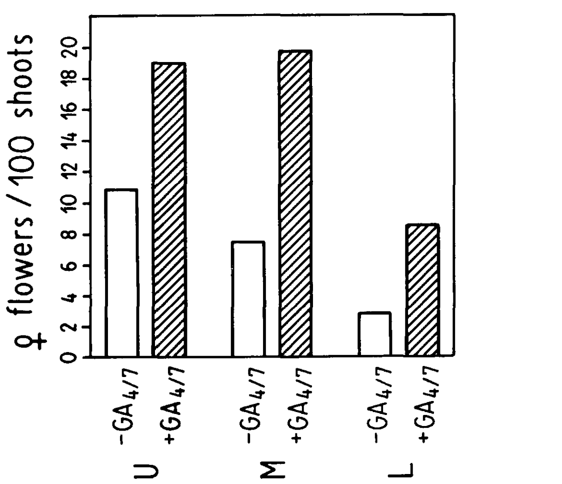

Figure 3. — Effect of $GA_{4/7}$ on the number of female flowers in upper, middle and low branches of Scots pine grafts.

lepis (HASHIZUME, 1967, after PHARIS and KUO, 1977), *Picea abies* (BLEYMÜLLER, 1976 and 1978; CHALUPKA, 1979) and *Pinus taeda* (ROSS and GREENWOOD, 1979; GREENWOOD, 1979) indicate that this gibberellin may actively participate in the regulation of flowering in *Pinaceae*. According to ROSS and GREENWOOD (1979) the effect of GA_3 on flowering in *Pinaceae* is small compared to that of $GA_{4/7}$; however on the basis of results reported so far its effect cannot be ignored.

The decidedly positive effect of $GA_{4/7}$ on female flowering in the 1978 experiment differed from that obtained in 1977, when the same mixture of hormones significantly stimulated male flowering (CHALUPKA, 1978).

The differential action of $GA_{4/7}$ on stimulation of flowers of different sex is well known, but no good explanation for this has been proposed. The listing of data available so far for *Pinus sp.* (*Table 2*) suggests a certain regularity. It appears that $GA_{4/7}$ given in the first part of the vegetative season, that is in May and June, causes a significant stimulation of male flowering. Extension of the time of $GA_{4/7}$ application to the latter part of the vegetative season, that is to July. August and September, results in a significant stimulation of female flowering. This regularity was observed only for grafts. In cases where $GA_{4/7}$ was applied to young seedlings the effect on the sex of the induced flowers was different and did not appear to be related to time of treatment. None the less the data presented in *Table 2* suggest that the effect of the $GA_{4/7}$ mixture was dependent on time of treatment, and therefore on the stage of development within the stem apex. This agrees with the results of HASHIZUME (1973) where later gibberellin application to *Cryptomeria japonica* and *Chamaecyparis obtusa* favoured female, and earlier application male flowers. In Scots pine male flowers form at the base and female flowers at the top of the developing shoot and, it is not surprising that the maximum sensitivity of lateral primordia to gibberellin or other external influences promoting flowering occurs at a different time for the two sexes.

There are suggestions in the literature about the relation between growth activity of apical meristems and the eventual course of development of the buds formed (ROMBERGER

Table 2. — Effect of time of $GA_{4/7}$ application on flowering in *Pinus sp.*

Reference	Plant material	Time of $GA_{4/7}$ application	Singnificant increase of flowering
CHALUPKA (1978)	*P. sylvestris* grafts	6 V — 20 V 1976	♂
LUUKKANEN and JOHANSSON (1980)	*P. sylvestris* grafts	24 V — 14 VII 1976	♂ and ♀
ROSS and GREENWOOD (1979)	*P. taeda* grafts	a. 20 V — mid-Sept. 1973 b. 20 V — mid-Sept. 1975	♀ ♀
GREENWOOD (1979)	*P. taeda* grafts	mid-June — end Sept. 1975	♀
CHALUPKA (1980)	*P. sylvestris* grafts	29 V — 21 VIII 1978	♀
PHARIS, WAMPLE and KAMINSKA (1975)	*P. contorta* seedlings	a. VI — VII 1972 b. VIII — IX 1972	♀ ♀
HARE, SNYDER and SCHMIDTLING (1979)	*P. palustris* seedlings	1 VI — 10 VIII 1976	♀ and ♂

and GREGORY, 1974; TOMPSETT 1978). It is also believed that gibberellins affect growth in the apical meristems of the *Pinaceae* (ROMBERGER and GREGORY, 1974) but this does not mean that they are directly involved in the process of sexual differentiation. It is known that the active zones within the apical meristems at the critical moments for the differentiation of primordia are sites for major changes in the activity of various enzymes (VANDEN BORN, 1963) and in the content of DNA, RNA and proteins (CECICH, LERSTEN and MIKSCHE, 1972).

It appears therefore that during the processes leading to sexual differentiation of primordia in the *Pinaceae* gibberellins are triggering agents that first induce changes in the rate of mitotic activity of the apical domes, as happens in other plants (SACHS, BRETZ and LANG, 1959). As a result of this, gibberellin action, depending on various factors (position in the crown, availability of light, time and method of application etc.), may lead to the formation of various types of growth in the meristems that end in the formation of vegetative, male or female primordia.

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