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Effect of growth regulators on the flowering of Scots pine (*Pinus sylvestris* L.) grafts

By W. CHALUPKA

Institute of Dendrology, 63-120 Kórnik, Poland

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Summary

Application of $GA_{4/7}$ made thrice in May 1976 at weekly intervals has significantly increased the percentage of Scots pine shoots that flowered male in 1977. This result was independent of method application and over and above substantial clonal variation. Application of CCC had no measurable effect. The girdling of branches has significantly increased both male and female flowering. There was no interaction between the $GA_{4/7}$ and the girdling treatments indicating that the responses are additive rather than synergistic. There are indications that the applied gibberellins also migrate to adjacent untreated twigs causing similar flowering responses there.

Key words: *Pinus sylvestris* L., flowering, gibberellins, girdling.

Zusammenfassung

Durch die Anwendung von Gibberellinsäure im Mai 1976 konnte bei *Pinus sylvestris* im folgenden Frühjahr 1977 eine signifikant verstärkte Ausbildung männlicher Blüten beobachtet werden. Das Ergebnis war unabhängig von der Anwendungsmethode und unabhängig von den einzelnen Klonen. Die Anwendung von Chlorcolinchlorid hatte keinen meßbaren Effekt. Das Ringeln der Zweige verstärkte die Ausbildung sowohl männlicher Blüten als zugleich weiblicher Blüten.

Introduction

Interest in the role of growth regulators in the regulation of flowering in coniferous trees is on the increase. In the fifties and sixties most investigators concentrated their work on the study of species from the families Cupressaceae and Taxodiaceae in view of the positive responses that were being obtained following treatments, particularly with gibberellic acid (GA_3). This same gibberellin however proved completely inadequate or only minimally so for the induction of flowering in the representatives of the Pinaceae family. In recent years other gibberellins were being investigated and it turned out that some of them could also stimulate flowering in the Pinaceae. A detailed review of achievements in this field has been recently published by PHARIS and KUO (1977). In addition to the results quoted

there one should also mention those of BLEYMÜLLER (1976) which were not mentioned in that review. He has applied gibberellic acid (GA_3) as sprays on 16-year old grafts of *Picea abies* and then followed the treatment 4 and 8 weeks later with a dose of CCG [(2-chloroethyl) trimethylammonium chloride]. This treatment favoured the induction of female flowers, and reduced male flowering. The idea behind BLEYMÜLLER's method was to give the promoter in the early phase of vegetation and then to inhibit the growth processes with a retardant during the final stage of extension growth, thus to divert the built up potential towards reproductive functions.

In 1976 studies were begun in the Institute of Dendrology of the Polish Academy of Sciences in Kórnik on the effect of growth regulators on flowering of Norway spruce and Scots pine. In the case of spruce GA_3 was used at a concentration of 100 ppm and CCC at a concentration of 2000 ppm in order to attempt a confirmation of the result BLEYMÜLLER obtained. Unfortunately this experiment has not provided any meaningful results. A similar method was tried on Scots pine, but in this case instead of GA_3 , the $GA_{4/7}$ mixture was used. The results presented below concern this latter experiment.

Materials and Methods

In the spring of 1976 on a 15-year old seed orchard of Scots pine in Kórnik 5 clones were selected each represented by 12 ramets. Individual grafts within a clone were treated either with $GA_{4/7}$, CCC, $GA_{4/7} + CCC$ or not at all (control). Thus there were 3 grafts (replicates) per treatment. For the treatments in the central part of the crown two branches were selected from adjacent whorls, one of which was given a full girdle 5 mm wide close to the base of the branch. On both the branches three more or less uniform 2-year old twigs were selected from the same whorl, and these were given the same hormonal treatment but the method of application to each twig was different. Three methods of application were tried: 1^o in lanoline onto a longitudinal 4-5 cm slit in the stem, 2^o by spraying aqueous solution and 3^o by dipping in an aqueous solution. The

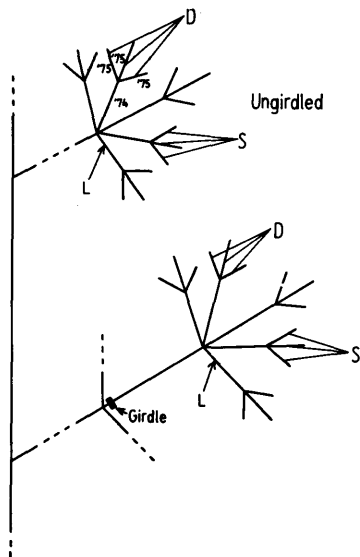


Figure 1. — Distribution of treatments on experimental grafts.
D — dipping, S — spraying, L — lanoline.

design of the experiment on a graft is shown in fig. 1. It consisted of a $(2 \times 2 \times 2 \times 3 \times 5)$ factorial with 3 replicates suited for variance analysis.

The $GA_{4/7}$ reagent was used at a 200 ppm concentration and CCC at 2000 ppm. For the spraying and dipping an aqueous solution with 0,1% Tween 20 was used while into the slit on the stems a lanoline preparation was given. The control variant received water with Tween 20 or lanoline only. The dipping and spraying were made three times on the the 6th, 13th and 20th of May while the lanoline treatment was given only once on May the 12th. The treatment with CCC was performed through dipping and spraying on the 18 th and 25 th of June and the 2nd of July, and application in lanoline to the slits on the stems on July the 3rd.

In the spring of 1977 all the shoots were counted on the experimental branches, shoots with male flowers, shoots with female flowers and all the female flowers. The observations were made separately for the twigs treated with the reagents and for the rest of the branch above the girdle (or the place where girdle would have been on the ungirdled branches).

In view of the differences in the number of replicates due to the breaking of some of the girdled branches under the influence of heavy snow in April 1977 when making the

Table 1. — The effect of $GA_{4/7}$ and CCC on the flowering of Scots pine grafts. Significant results of variance analyses. GA — $GA_{4/7}$ treatment, C — CCC treatment, B — branches girdled or not girdled, M — method of applying growth regulators, Cl — clones.

Source of variance	Treated twigs			Twigs adjacent to treated ones			
	No of 1977 shoots	% shoots with male fl.	% shoots with female fl.	No of female fl. per 100 shoots	% shoots with male fl.	% shoots with female fl.	No of female fl. per 100 shoots
GA	—	++	n.s.	—	n.s.	—	n.s.
C	n.s.	—	—	—	—	—	—
B	++	++	++	++	++	++	++
M	++	—	+	+	—	—	—
Cl	n.s.	++	n.s.	n.s.	++	++	+
GA × C	—	—	n.s.	—	—	n.s.	—
GA × B	—	n.s.	—	—	n.s.	—	—
GA × M	—	—	—	—	—	—	—
GA × Cl	—	n.s.	+	+	+	n.s.	n.s.
C × B	—	—	n.s.	—	—	—	—
C × M	—	—	+	—	—	—	—
C × Cl	—	+	n.s.	—	+	+	n.s.
B × M	—	—	—	—	—	—	—
B × Cl	n.s.	+	++	++	++	+	n.s.
M × Cl	—	—	n.s.	—	—	—	—
GA × C × B	—	—	n.s.	n.s.	—	n.s.	n.s.
GA × C × M	—	—	—	n.s.	—	—	—
GA × C × Cl	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	+
GA × B × M	—	—	—	—	—	—	—
GA × B × Cl	n.s.	n.s.	++	++	—	—	—
GA × M × Cl	—	—	—	—	—	—	—
C × B × M	—	—	—	—	—	—	—
C × B × Cl	—	n.s.	++	+	—	+	+
C × M × Cl	—	—	—	—	—	—	—
B × M × Cl	—	—	—	—	—	—	—
GA × C × B × M	—	—	—	n.s.	—	—	—
GA × C × B × Cl	n.s.	n.s.	++	++	n.s.	++	++
GA × C × M × Cl	—	—	—	—	—	—	—
GA × B × M × Cl	—	—	—	—	—	—	—
C × B × M × Cl	—	—	—	—	—	—	—
GA × C × B × M × Cl	—	++	—	—	—	—	—

+ significant at 0.05 level
++ significant at 0.01 level
n.s. not significant
— $F < 1$.

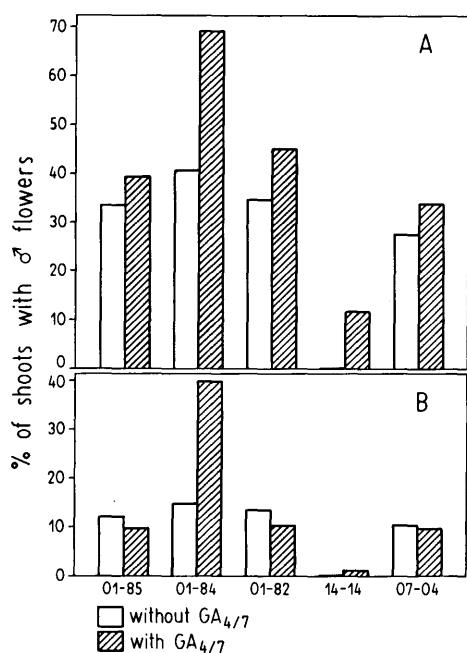


Figure 2. — The effect of GA_{4/7} on the percentage of shoots with male flowers in individual clones on treated parts of the branch (A) and on parts that were not treated directly (B).

variance analysis use was made of the modification for a full factorial design with unequal subclass numbers (SNEDECOR 1956).

Results

Table 1 shows all the significant results of the variance analysis. Below the most important ones are discussed.

A. Total number of shoots

The total number of shoots expanded in 1977 was significantly reduced from 14,5 to 11,8 following girdling of the branch. This reduction occurred on all clones. On the other hand the introduction of lanoline to a slit on the stem, with or without the growth regulators has had a positive effect on this character, which resulted in a significant difference between application methods.

B. Male flowering

The triple application of GA_{4/7} at weekly intervals from 6th to 20th of May has caused a significant increase in the proportion of shoots with male flowers, from 22,7% to 38,1% (Fig. 2 A). Girdling has also had a very significant effect on this feature, increasing the proportion of shoots with male flowers from 11,1% to 53,5%. A slight synergism was observed in the joint action of GA_{4/7} and girdling, however the interaction between gibberellins and branches was not significant (Fig. 3).

Analysis of male flowering on the untreated parts of the branches did not of course include the methods of hormone application. Similarly as the treated twigs the rest of the shoots on the branch reacted positively to the girdling by an increase in the percentage of flowering shoots, from 2,0% to 23,1%. Both in the case of the treated and untreated twigs there occurred very obvious natural differences between clones which have also had a differential response to the treatments evidenced in many interactions involving clones and other factors including GA_{4/7} and CCC. In fig. 2B for example the significant GA_{4/7} interaction with clones is shown for the male flowering on the untreated part of the branch. The GA_{4/7} treatment is detectable in that part of the branch only in one of the studied clones.

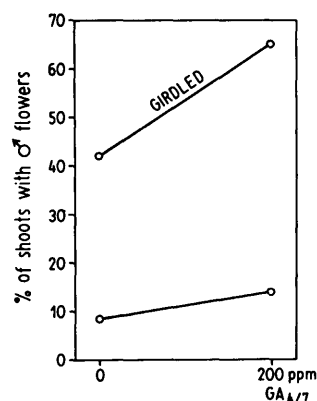


Figure 3. — The effect of GA_{4/7} and girdling on the percentage of shoots with male flowers.

C. Female flowering

The treated branches responded favourably to girdling, which increased the proportion of shoots with female flowers from 1,0% to 2,2% and the number of female flowers per 100 shoots from 2,2 to 4,8. In both these characters of female flowering there appeared significant differences between the methods of applying reagents. The greatest response was observed following application of lanoline with or without the growth regulators to the slit on the stem. The proportion of shoots with female flowers on twigs with the lanoline treated slits was 2,4% compared to 1,2% on the twigs that were sprayed and 1,1% on those that were dipped. Thus the numerical effect of making the longitudinal slit on the twigs was the same as that following girdling.

Among the higher order interactions caused by the natural variability between clones there occurred also an interaction between clones and GA_{4/7} treatment (Fig. 4).

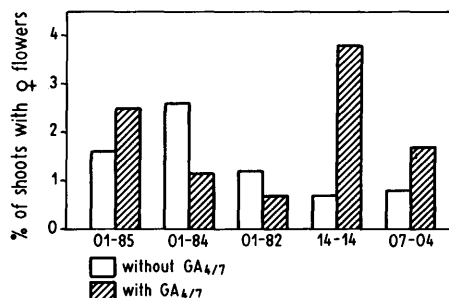


Figure 4. — The effect of GA_{4/7} on the percentage of shoots with female flowers in individual clones.

There was also an interaction between CCC and methods of application. The use of the spraying technique with CCC reduced the percentage of shoots with female flowers, while application of the retardant by other methods has had a slightly promotive effect on this character.

An analysis of female flowering on the parts of the branch untreated with reagents has shown that girdling has a significant positive effect and also the clonal differences in flowering intensity were confirmed.

Discussion

The most interesting result obtained in this investigation is the significant increase in the proportion of shoots with male flowers following GA_{4/7} application. This result was independent of the method of GA_{4/7} application and it was not obliterated by the very large and significant natural variation in flowering potential between clones. At the

same time there was no significant effect of GA_{4/7} on female flowering. The interaction of GA_{4/7} treatment with clones which appeared in the variance analysis was caused by the considerable variation between clones. Absence of GA_{4/7} effect on female flowering could have been caused by the treatment being too early. It is known that the initiation of stamens takes place before the initiation of ovuliferous scales (GIERTYCH, 1967 a, HEJNOWICZ, 1970). It is possible that the times of GA_{4/7} application were favourable for the initiation of male strobiles. The positive effect of GA_{4/7} on male flowering supports the data of KOPCEWICZ *et al.* (1977) who have found that in Scots pine the initiation of male floral initials is associated with a high level of gibberellins and low level of auxins, while the initiation of female strobiles is associated with a low level of gibberellins and a high level of auxins. The significant effect of GA_{4/7} on the male flowering in pine confirms also the suggestion of GIERTYCH (1967 b) about there being an analogy in reaction to gibberellin between photoperiodic plants and sex of flowers in pine. The data of HASHIZUME (1973) is also worth quoting. Three year old individuals (seedlings or grafts?) of *Pinus thunbergii* have had a more abundant male flowering following GA₇ application. At the same time two year old individuals of *P. densiflora* have had a reduced male flowering under influence of the same gibberellin.

In contrast to the result reported here PHARIS *et al.* (1975) have shown a significant positive effect of GA_{4/7} and other gibberellins on the female flowering of girdled *Pinus contorta* seedlings, also in the situation where the gibberellin application coincided with the time of male flower initiation. Differences in results could perhaps be explicable by differences in the material used. PHARIS and his co-workers have used juvenile material 4—7 years old. These individuals when attaining generative maturity usually begin by flowering female. In experiments with Scots pine in Kórnik the base material were generatively mature grafts with a distinctly stratified crown.

The positive effect of girdling that was obtained both on male and on female flowering has been known from the literature for a long time (GIERTYCH, 1967 a). On the other hand the negative effect of girdling on the total number of shoots is probably associated with the transport blockage through phloem that the girdle provided which could have resulted in abortion of some lateral shoot initials. On the other hand the positive effect of placing lanoline with or without growth regulators onto a longitudinal slit was probably an artefact associated with the fact that for this method of treatment out of the available 3 twigs probably the thickest one was selected in view of the need to make the cut and the greater danger of breakage. Such twigs were probably stronger, more viable and therefore producing more shoots.

As regards the effect of this cutting and lanoline treatment on flowering intensity it is possible that at least part of the explanation lies in the analogy with the girdling

treatment, both methods of injuring branches having a stimulative effect on the female flowering.

The significant interaction of the GA_{4/7} treatment with clones in the percentage of shoots with male flowers on the part of the branch that was not treated with the growth regulators (Table 1, Fig. 2B) indicates that there exists the possibility for the gibberellins to migrate to adjacent twigs, first basipetally and then acropetally. This is in agreement with the results of PHARIS *et al.* (1975) who after having injected labelled GA₄ to shoots of *P. contorta* have found that there was 24 times more label in needles above the injection point than in those below it.

Absence of significant interaction of GA_{4/7} treatment with the girdling treatment in the characters of both male and female flowering indicates that we are dealing here with additive rather than synergistic responses. Also absence of interaction between GA_{4/7} treatment and methods of application indicates that the method of gibberellin application is immaterial — spraying, dipping and applying in lanoline onto a cut being all equally efficient (or inefficient).

The many interactions found to be significant in this study are all associated with clones, and it is a well known fact that there are great differences in flowering response between individuals. Only results that are significant over and above individual variation are worth considering.

The results obtained so far (BLEYMÜLLER, 1976, PHARIS and KUO, 1977) indicate that with growth regulators one can associate considerable hopes of developing efficient and practical methods of stimulating flowering on forest seed orchards of species from the family Pinaceae.

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