Influences of crown size and maturation on flower production and sex expression in *Picea glauca* treated with gibberellin A$_4$/7

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**Summary** We evaluated the effects of exogenous gibberellin A$_4$/7 (GA$_4$/7) on the production of seed and pollen cones in four stock types of *Picea glauca* (Moench) Voss. From 1987 to 1991, a combined treatment that included GA$_4$/7, root pruning and heat stress was applied to 5- to 10-year-old container-grown grafts, representing over 90 clones and originating from 17- to 27-year-old ortets. Hormonal treatments were applied to pruned field-grown grafts in 1991 and 1992, and to unpruned field-grown grafts from the same set of clones in 1990, 1991 and 1992. In 1992, 26-year-old mature trees in a progeny test were treated with GA$_4$/7. The GA$_4$/7 treatment was effective in promoting seed-cone production in all four stock types, whereas it promoted pollen-cone production only in unpruned field-grown grafts and mature trees. The older and taller a tree, the less the response to the GA$_4$/7 treatment was influenced by the climatic conditions prevailing during the differentiation phase.

**Keywords:** pollen cones, seed cones, stock type, white spruce.

**Introduction**

Over the last 10 years, flower induction techniques have been designed to control flowering under artificial as well as natural growing conditions. The most significant advance has been achieved through the judicious use of plant hormones such as gibberellin A$_4$/7 (GA$_4$/7) in combination with appropriate cultural treatments (Bonnet-Masimbert 1987, Pharis et al. 1987, Owens 1991).

In white spruce (*Picea glauca* (Moench) Voss), GA$_4$/7 used alone or in combination with cultural treatments (heat stress and root pruning) promotes flowering of containerized grafts (Corriveau et al. 1989, Greenwood et al. 1991, Ross 1991), field-grown grafts (Cecich 1985, Daoust et al. 1993a) and mature trees (Pharis et al. 1986, Daoust et al. 1993b). However, there is no evidence that the timing of GA treatment preferentially promotes male or female flowering in different stock types or that the concentration of GA applied affects the sex ratio (Owens and Molder 1979, Marquard and Hanover 1984a, 1984b, Cecich 1985, Pharis et al. 1986).

We studied the effects of GA$_4$/7 treatment on seed- and pollen-cone production of four stock types of white spruce: containerized grafts, pruned and unpruned field-grown grafts and mature trees. Containerized grafts and pruned field-grown grafts were chosen because they are used in intensively managed control-pollinated seed orchards (Carson et al. 1992, Webber et al. 1993), and unpruned field-grown grafts and mature trees were selected because they are commonly used as either female or male parents in classical open-pollinated seed orchards.

**Materials and methods**

**Container-grown grafts**

Each year from 1987 to 1991, about 100 container-grown ramets, representing as many clones, originating from 17- to 27-year-old ortets received a combined treatment that included root pruning, GA$_4$/7 (sprayed or injected) and heat stress. The grafts were 1 to 2 m tall and 5 to 10 years old when first treated. A similar group of untreated container-grown grafts served as controls. Before the vegetative buds swelled in the spring, the treated grafts were root pruned, repotted, fertilized and placed in an unheated greenhouse ventilated at 20 °C. Once weekly for 4 weeks during the shoot elongation phase of years 1987 to 1990, treated grafts were sprayed to wetness with an aqueous solution of 400 mg l$^{-1}$ GA$_4$/7 (ICI, Plant Protection Division, U.K.) and 0.05% (w/v) Aromox C-12/W surfactant (Armax Chemical Ltd., Saskatoon). When the terminal shoots had nearly reached full elongation (90–100%), a 4-week heat treatment was applied at day/night temperatures of 30 ± 2/20 ± 2 °C.

In the fifth year, i.e., in 1991, at the end of the shoot elongation phase (over 80% elongation completed), 50 mg of GA$_4$/7 in 5 ml of ethanol (95%) was injected into a drilled hole (4 mm in diameter and 20 mm deep) in the trunk at the base of each treated graft. The grafts were not sprayed with GA$_4$/7 in 1991.

**Unpruned field-grown grafts**

In 1984, a set of 10 ramets per clone from the same clones used for the containerized trial was established in a field (5 ramets per block). The grafts averaged 12 years old and 3.5 m in height at the start of the treatment.

In 1990, 20 clones (four ramets per clone) were treated, and two ramets from the same clones were used as controls. In the
middle of the shoot elongation phase, 5 ml of ethanol (95%) containing 100 mg of GA\textsubscript{4/7} + 10 mg of naphthaleneacetic acid (NAA) was injected into the trunk of two of the four grafts, and the other two grafts were treated with GA\textsubscript{4/7} only. The number of grafts in the treatment and control groups was increased to 77 and 97 grafts per group in 1991 and 1992, respectively, and each graft represented a clone. For grafts with a diameter of less than 8 cm, 50 mg of GA\textsubscript{4/7} was used, and grafts 9–11 cm in diameter were treated with 75 mg of GA\textsubscript{4/7}. In all cases, the NAA concentration was adjusted to 10% of the GA\textsubscript{4/7} concentration.

**Pruned field-grown grafts**

In 1987, a set of three ramets per clone from the same clones used for the containerized trial was established in a field. The ramets averaged 12 years old. Grafts were top-pruned and side-trimmed on a 2-year basis to maintain an oblong tree shape not taller than 2 m.

In 1991 and 1992, 104 and 110 clones, respectively, (one ramet per clone) were treated, and one ramet per clone was used as a control. In 1991, each graft was injected in the trunk with 50 mg of GA\textsubscript{4/7} + 5 mg of NAA diluted in 5 ml of ethanol (95%). In 1992, the GA\textsubscript{4/7} concentration was adjusted to the diameter of the trunk; trees with a diameter more than 5 cm received 40 mg, whereas trunks with a diameter less than 5 cm received 20 mg. The NAA concentration was 10% of the GA concentration. Treatments were applied when shoot elongation had reached 50% of its final length.

**Mature trees**

In 1992, mature trees, averaging 10 m in height, were selected from a 26-year-old progeny test. The control and treated trees were progeny paired, and the treated trees were injected with 100 or 150 mg GA\textsubscript{4/7} + NAA (10% of the GA concentration) dissolved in 95% ethanol (50 mg GA\textsubscript{4/7} per 5 ml ethanol). The higher concentration was used when tree diameter exceeded 12 cm at the injection site. Hormone solution was injected at the bottom of the living crown during the shoot elongation phase.

The experiments were conducted at the Valcartier forest station (46°57’ N, 71°30’ W) except for the unpruned grafts experiments, which were conducted at Cap Tourmente National Wildlife Area (47°04’ N, 70°50’ W). The meteorological data in Figure 1 were collected at Quebec City Airport (46°47’ N, 71°23’ W) (Environment Canada).

For each experiment, seed and pollen cones were counted in the spring following each treatment. Data were subjected to ANOVA using the PROC GLM procedure (SAS Institute Inc., Cary, NC).

**Results**

**Seed-cone production**

In 4 out of 5 years, the treatments significantly increased seed-cone production in the container-grown grafts. Over the 5-year study, 69.8% of the clones responded to the combined treatments and produced an average of 84.1 seed cones per clone compared with 4.7 seed cones per clone in the control material (Table 1). The lack of a treatment response in 1990 was probably due to excessive irrigation that resulted in the death of many grafts.

Treatments applied to unpruned field-grown grafts in 1990 and 1992 significantly increased seed-cone production. The high annual variation in seed-cone production was possibly related to differences among years in the climatic conditions prevailing during the differentiation period in July and August (Figure 1). In 1990, when weather conditions closely approximated the long-term average for the July–August period, the treatments induced an average of 393.5 seed cones per clone, whereas in 1991, the treatments induced an average of about 3600 seed cones per clone. Climatic conditions during the summer of 1991 were especially favorable for flower bud

Figure 1. Rainfall, hours of bright sunshine and degree days above 5°C for July and August 1990, 1991, 1992 and for 1951–1980 (normal).
initiation as indicated by abundant flower production on the control material. During July–August 1991, total rainfall, mainly in July, was below normal, and hours of sunshine and accumulated degree days were slightly above or equal to normal. Compared with 1991, 1992 was less favorable for flower bud initiation: precipitation in July was more abundant, and hours of sunshine and accumulated degree days were below normal. Perhaps as a result of the prevailing climatic conditions during July–August 1992, the effect of the hormonal treatment was weakened because the treated clones produced, on average, only 19.1 seed cones per clone. Flower production on the control material was also less in 1992 than in 1991. The percentages of treated clones flowering were 100, 100 and 54.6%, in 1990, 1991 and 1992, respectively, compared with 55, 100 and 2.1% for the control stock.

In both 1991 and 1992, the treatments significantly increased seed-cone production of pruned field-grown grafts. However, a large annual variation was observed with 257 and 1.9 seed cones produced per clone following the 1991 and 1992 treatments, respectively. The larger treatment response in 1991 than in 1992 was attributed to the favorable climatic conditions in 1991.

Treatments applied to mature trees in 1992 did not promote seed-cone production significantly, although in preliminary experiments in 1991 a treatment-induced increase in seed-cone production was observed (unpublished data).

Table 1. Summary of the results of flower induction experiments conducted between 1987 and 1992 on four stock types of *Picea glauca* (Moench) Voss.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of clones</th>
<th>Average seed cones/clone</th>
<th>Average pollen cones/clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Container-grown grafts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td>99</td>
<td>99</td>
<td>55.2 a (64.7)</td>
</tr>
<tr>
<td>1988</td>
<td>100</td>
<td>83</td>
<td>114.8 a (79.0)</td>
</tr>
<tr>
<td>1989</td>
<td>83</td>
<td>96</td>
<td>101.1 a (81.9)</td>
</tr>
<tr>
<td>1990</td>
<td>96</td>
<td>10</td>
<td>27.1 a (45.8)</td>
</tr>
<tr>
<td>1991</td>
<td>86</td>
<td>12</td>
<td>129.0 a (80.2)</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>84.1 (69.8)</td>
</tr>
<tr>
<td></td>
<td>Unpruned field-grown grafts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>20²</td>
<td>20³</td>
<td>393.5 a (100.0)</td>
</tr>
<tr>
<td>1991</td>
<td>77</td>
<td>77</td>
<td>3600.0⁴ (100.0)</td>
</tr>
<tr>
<td>1992</td>
<td>97</td>
<td>97</td>
<td>19.1 a (54.6)</td>
</tr>
<tr>
<td></td>
<td>Pruned field-grown grafts</td>
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<td></td>
</tr>
<tr>
<td>1991</td>
<td>104</td>
<td>102</td>
<td>257.0 a (99.0)</td>
</tr>
<tr>
<td>1992</td>
<td>110</td>
<td>110</td>
<td>1.9 a (7.3)</td>
</tr>
<tr>
<td></td>
<td>Mature trees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>19⁵</td>
<td></td>
<td>4.2 a (15.8)</td>
</tr>
</tbody>
</table>

¹ Means within a type of stock and year of treatment not followed by the same letter are significantly different (P < 0.05). The percentage of clones with cones is shown in parentheses.
² Four ramets per clone.
³ Two ramets per clone.
⁴ Average numbers of seed and pollen cones were estimated following the total harvest of seed cone crop; field observations and data from 1990.

Pollen-cone production

The treatments did not significantly promote pollen-cone production in the container-grown grafts. Over the 5-year study, the average production was 6.1 pollen cones per treated clone compared with 0.9 pollen cones for the controls. In 1988, the treatments significantly promoted pollen-cone production although, in all cases, there were fewer than 10 pollen cones per treated clone. On average, 15.5% of the treated clones responded to the treatments.

Pollen-cone production of unpruned field-grown grafts was significantly stimulated by the treatments. In 1990, the average pollen-cone production of the treated grafts was 16 times the production of the control grafts. Although the treatments in 1992 were effective, pollen-cone production was only 34.3 pollen cones per treated clone. In 1990 and 1992, 95 and 63.9% of the treated clones and 35 and 0% of the control clones, respectively, produced pollen cones. The annual variation in pollen-cone production was closely linked to the climatic conditions during the differentiation period (Figure 1).

Although pruned field-grown grafts that received the hormonal treatments in 1991 and 1992 had significantly more pollen cones per clone than the control grafts, pollen-cone production was low in both groups of grafts. The highest average production, 20 pollen cones per clone, was obtained in 1991, a year with climatic conditions favorable for seed-cone differentiation. About 62% of the treated clones produced pollen cones compared with 9% of the control material.
The hormonal treatment increased pollen-cone production of mature trees in 1992, and an average of 190.7 pollen cones were produced per treated clone, whereas the control material did not produce any pollen cones. About 84% of the treated trees produced pollen cones even though the summer of 1992 appeared to be unfavorable for differentiation of male flowers.

Discussion

Under normal climatic conditions, the GA_4/7 treatment promoted seed-cone production on the four stock types of *P. glauca* tested. Although the GA_4/7 treatment was not effective in promoting pollen-cone production on stock used in intensively managed controlled-pollinated seed orchards (container-grown grafts and pruned field-grown grafts), it was effective on stock used in classical open-pollinated seed orchards (unpruned field-grown grafts and mature trees that are tall and have a well-developed crown).

Greenwood et al. (1991) and Ross (1991) reported that a combined GA_4/7 + heat treatment applied under controlled conditions enhances both seed- and pollen-cone production in container-grown grafts. In our study, however, the combined treatment enhanced seed-cone production of the container-grown grafts but had no effect on pollen-cone production (cf. Adams and Greenwood 1992). One possible explanation for this difference is that our container-grown grafts were small and in a phase of seed-cone production (Marquard and Hanover 1984a, Longman 1987). As a tree becomes older and taller, a zone that initially produced female flowers becomes a zone of male-flower production as its position in the crown changes (Marquard and Hanover 1984a). A second possible explanation for the observed differences is that our ortets were in an early stage of maturation at the time of grafting. Thus the crowns of the grafts did not present branch characteristics allowing the establishment of two zones of flower production, i.e., female and male, as is observed on grafts obtained from older trees that exhibit weak vigor, a compact form and plagiotropic growth. Vegetatively propagated shoots taken from the crowns of older trees often behave differently from seedlings, even when both are of similar size and are grown in the same environment (Longman 1987).

Despite a significant hormone-induced increase in pollen-cone production on pruned field-grown grafts, production was low (20 pollen cones per clone) and similar to that observed on container-grown grafts. Our results differ from those reported by Greenwood et al. (1991) who obtained an average of 159 pollen cones per 4- to 6-year-old field-grown graft (1.5 to 2.5 m tall) in a good flowering year. However, we obtained an abundant pollen-cone production (2300 pollen cones per clone) on unpruned field-grown grafts originating from the same ortets as the pruned grafts, indicating that unfavorable climatic conditions cannot account for the low pollen-cone production of the pruned field-grown grafts. Even in 1990 when climatic conditions approximated the long-term average, flower induction treatments were effective in increasing pollen-cone production on unpruned grafts but not on pruned grafts. Thus, it appears that limiting the growth of the grafted trees to a maximum of 2 m did not allow the natural establishment of sexual zonation as seen on the unpruned grafts, particularly when the grafts came from ortets in the early stage of sexual maturation.

The low production of seed cones on both pruned and unpruned field-grown grafts and on mature trees following the 1992 induction treatments was not due to the influence of endogenous cycles or to a required period of recovery following a heavy crop (Owens and Blake 1985) because the trees bore either no flowers or a below-average number of flowers in 1992, a year of naturally abundant flowering. We believe that the small response to the flower-induction treatments was related to the occurrence of climatic conditions in the summer of 1992 that were less favorable for cone-bud differentiation than in the summers of 1990 and 1991. July and August 1992 had above normal precipitation, and below normal sunshine and accumulated degree days (Figure 1). We conclude that climatic conditions modify the effectiveness of hormonal treatments and should be near normal for the treatment to have a significant positive effect.

We succeeded in significantly increasing pollen-cone production on two types of stock (unpruned grafts in 1990 and 1992, and mature trees in 1992) despite the unfavorable climatic conditions of 1992, whereas Pharis et al. (1986) did not significantly increase pollen-cone production or male flowering frequency on several different stock types including mature 55-year-old trees. Our failure to promote seed-cone production on mature trees in 1992 was unexpected because GA_4/7 generally promotes flower production of both sexes on mature material (Longman 1989). It is possible that the GA_4/7 concentration used was not high enough to raise the endogenous GA_4/7 concentration (which may have been lower than normal as a result of unfavorable climatic conditions) to the threshold required to induce differentiation of the vegetative buds into female flower buds.

We conclude that female flowers can be obtained on different stock types following GA_4/7 treatment; however, pollen-cone production is delayed, and flower induction treatments are not effective when grafts are pruned or grown in containers.

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References


