Flowering on long and short shoots of *Larix laricina* in response to differential timing of GA$_{4/7}$ applications

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**Summary** In *Larix*, reproductive buds most often occur terminally on short shoots, but they can also differentiate in lateral positions on long shoots. The phenology of long and short shoots differs considerably, with short shoots breaking bud and expanding about 5 weeks before the start of long shoot extension. Foliar sprays of GA$_{4/7}$ were applied to 160 branches on 10 greenhouse-grown *Larix laricina* (Du Roi) K. Koch grafts either before (early) or after (late) the start of long shoot extension, or during both periods, to test whether the timing of GA$_{4/7}$ application affects flowering on short and long shoots. All three treatments induced flowering on both long and short shoots. The early GA$_{4/7}$ treatment led to slightly, but not significantly, more flowering on short shoots than on long shoots, whereas the late GA$_{4/7}$ treatment resulted in increased flowering on both types of shoots, but primarily on long shoots. Application of GA$_{4/7}$ during both periods did not result in increased flowering over the early or late treatment alone. Based on the fact that gibberellins are metabolized rapidly in conifers and our finding that GA$_{4/7}$ applied before shoot elongation, when the bud primordia were at a very early stage of development (detectable bud differentiation only occurred several weeks later), induced flowering on long shoots, we conclude that the early GA$_{4/7}$ treatment did not affect differentiation as it was occurring, but somehow predisposed the bud primordia to differentiate reproducitively.

*Keywords: bud primordia, differentiation, flower induction.*

**Introduction**

Gibberellin A$_{4/7}$ is commonly used to promote flowering in trees of the Pinaceae for research purposes and less commonly for commercial seed production. The mechanism by which GA$_{4/7}$ promotes flowering is not known. However, the importance of the timing of GA$_{4/7}$ application, as well as the timing of adjunct treatments, has been demonstrated (Greenwood 1982, Ross 1985, 1988, Owens and Colangeli 1989, Eysteinsson and Greenwood 1990, Ho 1991). Pharis et al. (1987) proposed that flowering only occurs when endogenous concentrations of the less polar gibberellins (including GA$_3$ and GA$_7$) exceed vegetative demand during bud differentiation. However, in some cases, application of GA$_{4/7}$ before detectable differentiation of bud primordia is more effective in promoting flowering than application close to or during the period of differentiation (Owens and Colangeli 1989, Eysteinsson and Greenwood 1990, Ho 1991).

In the genus *Larix*, reproductive buds most often occur in the terminal position on short (dwarf) shoots, but they can also occur laterally on long shoots. The phenology of the two types of shoots is very different, with short shoots breaking bud about 5 weeks before the start of long shoot extension and also setting bud much earlier than long shoots. Powell et al. (1984) reported flowering on long shoots of tamarack (*Larix laricina* (Du Roi) K. Koch), and we have observed female flowering on long shoots of Japanese larch (*Larix leptolepis* Gord.) and European larch (*Larix decidua* Mill.). Flowering on young, indoor-grown tamarack grafts in response to GA$_{4/7}$ occurred primarily on long shoots when GA$_{4/7}$ was applied after the start of long-shoot extension (Eysteinsson and Greenwood 1993), suggesting that timing of flower induction treatment influences the pattern and frequency of flowering.

The experiment described here was designed to test whether early applications of GA$_{4/7}$, i.e., before the start of long shoot extension, would result in increased flowering by short shoots. This question is of practical importance to seed production in larch because the timing of flower induction treatments could target different types of shoots. Thus, optimum cone production on both types of shoots might require induction treatments at different times.

**Materials and methods**

Sixteen first-order branches on each of 10 indoor-grown tamarack grafts were selected for uniformity of size from throughout the crown. The grafts, which represented five clones and two ramets per clone, were beginning their fourth growing season from grafting. Ortet age ranged from 28 to 56 years.

The branches were randomly divided into four treatment groups each of 40 branches, with four branches per treatment per graft. One group received weekly GA$_{4/7}$ foliar spray applications for 5 weeks starting March 13, 1990, about 1 week after short shoot bud burst, and ending April 10 (early treatment). Another group received weekly GA$_{4/7}$ foliar spray applications for 5 weeks starting April 17, at about the start of long shoot...
extension, and ending May 10 (late treatment). The third group received 10 weekly GA$_{47}$ foliar sprays starting March 13 and ending May 10 (spanning both periods). The fourth group received control sprays weekly spanning both periods.

The GA$_{47}$ was applied as an aqueous foliar spray of 200 mg $1^{-1}$ in 5% ethanol with 0.2 ml $1^{-1}$ Aromox C/12w as a surfactant. Control branches were sprayed with the same solution minus the GA$_{47}$. The foliar sprays were applied to the entire branch at each application, which means that only short shoot foliage was sprayed during the early period, whereas both short shoot- and expanding long-shoot foliage were sprayed during the late period.

Buds were counted in spring 1991, when pollen cone, seed cone and vegetative buds could easily be distinguished. Bud counts were analyzed by ANOVA and Duncan’s multiple range test to separate means.

**Results**

Both the early and late GA$_{47}$ applications resulted in increased female flowering on short shoots ($P < 0.001$). Male flowering on short shoots was only significantly increased by the GA$_{47}$ treatment that spanned both periods ($P < 0.027$). The early GA$_{47}$ applications (before the start of long shoot extension), led to slightly, but not significantly, more male and female flowering on short shoots than the late applications (after the start of long shoot extension) (Table 1).

Male flowering on long shoots was significantly increased by the late GA$_{47}$ treatment and the treatment spanning both periods ($P < 0.001$), but not by the early treatment. Female flowering on long shoots was increased 6-fold by the early GA$_{47}$ treatment and 14-fold by the late treatment ($P < 0.001$).

Differences between the late GA$_{47}$ treatment and the GA$_{47}$ treatment spanning both periods were nonsignificant for both sexes at both shoot positions.

At the end of the 1990 growing season, the current-year long shoots comprised an average 63% of the length of each branch, and the short shoots comprised 37%. Shoot length was a good indicator of the number of potential flowering sites because the number of buds per unit of branch length (1.4 ± 0.1 buds cm$^{-1}$) was strongly conserved in the greenhouse-grown trees. Across all treatments, long shoots bore 60 and 73% of the total number of pollen and seed cones, respectively. Roughly the same proportion of buds differentiated reproductively on the two types of shoots, about 11% of short shoot buds and 14% of long shoot buds.

Average shoot length (including laterals) at the end of the 1990 growing season was 92 cm. Application of GA$_{47}$ did not affect shoot length.

**Discussion**

The effects of differential timing of GA$_{47}$ applications on flowering by short shoots were inconclusive. The optimum time of GA$_{47}$ application for stimulating flowering on long shoots was during early shoot elongation. The late treatment may have been better than the early treatment because of the increasing amount of expanding foliage in proximity to the primordia affected, leading to greater uptake of GA$_{47}$.

The treatment spanning both time periods (lasting 10 weeks) did not result in significantly more flowering than the early or late treatments, indicating that five weekly GA$_{47}$ applications were sufficient to maximize flowering. Previous work has indicated that as few as two weekly GA$_{47}$ foliar spray applications can significantly induce flowering (Eysteinsson et al. 1993). We conclude, therefore, that repeated applications are not required to induce individual primordia to differentiate reproductively. However, repeated applications are more effective than a single application because of clonal differences and phenological differences between branches within a tree, and because of the increased chance that sufficient GA$_{47}$ will reach the maximum number of target buds.

Early GA$_{47}$ applications enhanced female flowering on both long and short shoots. Lateral bud primordia are visible soon after a long shoot starts to elongate (Frampton 1960), but may be detectable before elongation (John N. Owens, personal communication). Detectable differentiation of reproductive buds occurs in short shoots of western larch ( *Larix occidentalis* Nutt.) and, we can assume, in other larch species as well at about the end of long shoot elongation (Owens and Molder 1979). We can also assume that lateral buds on long shoots do not differentiate earlier than short-shoot buds. Thus, GA$_{47}$ was effective at a very early stage of bud development, as has been found for western hemlock ( *Tsuga heterophylla* (Raf.) Sarg.) where the best flowering results were obtained when GA$_{47}$ was applied before bud burst (Owens and Colangeli 1989).

For many species, GA$_{47}$ appears to promote flowering only when applied before visible bud differentiation (Ross 1985, 1988, Owens and Colangeli 1989, Eysteinsson and Greenwood 1990, Ho 1991). There is also evidence that GA$_{47}$ is rapidly metabolized in conifers (Duberg et al. 1983, Moritz et al. 1989, 1990). Taken together, these observations suggest that the signal prompting buds to differentiate reproductively can occur when bud primordia consist of relatively few cells and is maintained through many cell divisions until bud differentiation takes place. The nature of this signal is not known, but one possibility is that GA$_{47}$ affects methylation or demethylation of genes that control cone bud development.

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