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# Flower Induction in Greenhouse-grown Hybrid Larch Grafts and Field-grown European Larch Seedlings[1*]

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# 溫室에서 자라는 落葉松의 接木苗와 野外에서 자라는 實生苗로부터 開花의 誘導[1*]

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## ABSTRACT

GA4/7 spray, injection and several cultural treatments were applied to the greenhouse-grown potted hybrid larch(*Larix decidua × leptolepis*) grafts and field-grown European larch seedlings to induce early flowering. A treatment consisting of repeat-ed GA4/7 sprays, alone, was the most effective flower induction treatment for greenhouse-grown, potted larch grafts. Root pruning as a adjunct treatment did not show synergistic effects. Injection for potted grafts with GA4/7 was not useful approach in this study and it resulted in increased mortality. In the field experiment with 10-year-old larch seedlings, repeated GA4/7 sprays in combination with root pruning or with plastic mulching appears to be useful and practical means for inducing larch flowers:

*Key words: Flower induction, indoor seed orchard, GA4/7, cultural treatments,* larch.

## 요 약

Indoor seed orchard의 가능성을 시험하기 위해 온실의 화분에서 자라는 낙엽송 접목묘와 야외에서 자라는 실생묘에 Gibberellins(GA4/7) 살포, 주입 및 몇가지 보조 처리를 사용하여 조기개화를 유도하였다. 온실의 화분에 자라는 접목묘에 대해서는 GA4/7 반복 살포 단독처리가 가장 효과적이었으나 보조처리로서 사용된 root pruning은 상승효과를 보이지 못했다. GA4/7 주입은 접목묘의 치사를 초래하였기 때문에 유용한 방법이 아니었다. 10년생 실생묘를 이용한 야외실험에서는 GA4/7 살포와 보조처리로서 root pruning 또는 plastic mulching의 사용이 개화의 유도에 가장 효과적이었다.

## INTRODUCTION

Early, consistent and abundant flowering are necessary for tree breeding and for seed production from seed orchards. Unfortunately, however, adequate flowering is often a limiting factor to successful tree improvement programs, especially for those utilizing advanced generation crossings. Two common biological constraints for tree breeding are : (1)the long juvenile period needed for trees to reach flowering maturity and (2)irregular flowering once trees reach flower maturity age (Ross and Pharis, 1985). This irregular flowering is due to unfavorable climatic or other environmental factors, or due to the as yet unknown genetic control of flowering.

Attempts to overcome the long juvenile period

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in conifer trees have generally focused on the application of gibberellins(GAs) with or without adjunct cultural treatments. Successful flower induction using a gibberellin mixture A4/7(GA4/7) and/or adjunct cultural treatments has been reported for 19 members of Pinaceae, including 10 *Pinus* species, 5 *Picea* species, 3 *Larix* species, and *Tsuga heterophylla*(Ceich, 1985; Chalupka, 1981; Dunberg, 1980; Eynsteisson and Greenwood, 1990; Pharis *et al.*, 1987; Philipsson, 1985; Ross, 1985, 1988).

Seed production in traditional seed orchards is significantly affected by environmental conditions. Late spring frosts, for instance, or extremely cold winters can cause entire seed crops to be lost in a given year. Another problem with traditional seed orchards is that regardless of tree size or clonal arrangement, there remain practical difficulties in controlling pollen parentage in field-grown trees. Thus, the development and evaluation of indoor, potted seed orchards has been suggested as a practical alternative(Ross, 1985). This system has advantage of working with small, potted trees which are easily manipulated and allows for strict environmental control during all phases of flower induction and development.

Because of their rapid growth rate and high productivity, larch and hybrids are very promising conifers. However, seed production from seed orchards in larch has been limited by low yields of viable seeds resulting from a lack of abundant flowering, irregular flowering, and from cold weather during flower and seed production as larches flower early in the spring.

The objectives of this study were to find effec-tive methods for flower induction in greenhouse-grown hybrid larch grafts and field-grown European larch seedlings and evaluate the results with consideration towards the development of indoor-seed orchards for larch hybridization.

## MATERIALS AND METHODS

### Greenhouse experiments with grafts

Experiments were carried out in 1992, 1993, and 1994 at Mead Paper Company greenhouse located in Escanaba, Michigan.

Scions were taken from the upper one-third of the crown of field-grown mature European X Japanese hybrid larches growing in the USDA Forest Service Harshaw Experimental Area located at Oneida County, Wisconsin and were grafted onto the rootstocks of 2-year-old European larch seedlings on Febuary, 1992. They were grown in 15cm diameter pots containing peat : vermiculite (3 : 1) under greenhouse conditions. Surviving grafts were moved out of the greenhouse in late May and they remained until February, 1993 when the grafts were moved back into the greenhouse and forced to break dormancy.

Three different treatments, GA4/7 spray, GA4/7 injection, and GA4/7 spray with root pruning, were applied to the grafts when long shoot began to elongate on February 9, 1993. Twenty four grafts per each treatment were assigned randomly. GA4/7 spray was applied as an aqueous foliar spray(200mg/l; 88% active ingredient, in 5% ethanol with Aromox C-12/w 0.02% added as a surfactant) to the whole plant until it was completely wet (approximately 50ml per graft). Spraying was done 5 times at biweekly intervals using a hand-held sprayer.

For injections, a small hole(3mm wide by 5mm deep) was made at downward angle on about 5cm above the stem base of the grafts and GA4/7 solution (5mg/0.2ml of 20% ethanol) was injected through the hole using a pipet. After the solution was taken up, the stem was wrapped with parafilm. A second injection was made two weeks later through a new hole drilled about 5 cm above the first hole.

Root pruning was done by cutting the roots using a scissors that reached approximately three-quarters of the way to the side of the pot. Four 10-cm-long cuts, parallel to the side of the pots and about midway between the stem and the pot, were made. The treated grafts were watered daily and given liquid fertilizer(N : P : K=20 : 20 : 20) weekly. Control plants were treated with either 5% ethanol spray or 20% ethanol injection. Grafts were maintained in the greenhouse until late May when they were moved outdoor. Buds were counted in February, 1994 when male, female and vegetative buds could easily be distinguished.

**Field experiments with seedlings**

Experiments were carried out in 1993 and 1994 using 10-year-old European larch seedlings growing at the Michigan Technological University Tree Improvement Arboretum located near South Range, Michigan.

Seventy two trees with similar heights and DBH's(approximately 12 to 15cm) were chosen and assigned randomly for 8 replications of 9 different treatments including a control. Treatments consisted of : 1. five biweekly sprays with GA4/7(400mg/l in 5% ethanol with 0.02% Aromox); 2. sprays (as in 1) with plastic mulching (black plastic mulch was used to cover the soil in tree's dripline); 3. sprays (as in 1) with root pruning (root pruning consisted of a 15cm deep by 15cm wide trench being dug around the tree's dripline); 4. sprays (as in 1) with both mulching and root pruning; 5. three biweekly injections of GA4/7 (60mg/ml); 6. Injections (as in 5) with mulching; 7. Injections (as in 5) with root pruning; 8. Injections (as in 5) with both mulching and root pruning. Mulching and root pruning were made on November 14, 1992. Spray and injection treatments were made on June 6, 1993 when long shoots were starting to extend. For the GA4/7 sprays, three branches at similar height (1 to 1.5m) on each tree were chosen and were sprayed with the solution until wet using a hand held sprayer.

The 400mg/l concentration of GA4/7 was chosen because previous studies with field-grown larch trees suggested that this higher dose than in the potted plant experiment was necessary for flower induction. For the GA4/7 injections, 2cm deep by 0.5cm wide hole, drilled at a downward angle, was made on the stem 20cm above the stem base using a portable electric drill. After the GA4/7 solution was completely taken up, the hole was sealed with a piece of wood dowel. New holes were made 2 weeks and 4 weeks after the first treatment at 5cm above the previous hole and GA4/7 concentration for injection was determined based on the tree's DBH(Ross, 1990, unpublised data). Male and female buds were counted in mid April, 1994. The Student-Newman-Keuls test was used for separating of the means of the each treatment.

## RESULTS

**Flower induction on greenhouse-grown grafts**

The effects of GA4/7 sprays, GA4/7 injections, and GA4/7 sprays plus root pruning on the flowering of the greenhouse-grown larch grafts are summarized in Table 1. GA4/7 spray was most effective treatment. Root pruning did not enhance flowering and GA4/7 injection was not as effective as when it was applied as a spray.

There was high variation in the number of flowers among grafts applied with the same treatment. For example, in the grafts treated with

**Table 1.** Response of greenhouse-grown hybrid larch grafts to various flower induction treatments in the previous year including GA4/7 spray, GA4/7 spray with root pruning, and GA4/7 stem injection.

| Treatment | Male flowers per grafts $\bar{x}$ ± S.E | Female flowers per grafts $\bar{x}$ ± S.E | Total No. flower buds per graft $\bar{x}$ ± S.E | Grafts producing flowers (%) |
|---|---|---|---|---|
| Control* | 0 b | 0 b | 0 b | 0 |
| GA4/7 spray** | 5.6 ± 2.9 a | 4.3 ± 4.2 a | 9.9 ± 5.0 a | 38 |
| GA4/7 spray and root pruning*** | 2.2 ± 0.7 a | 0.5 ± 0.3 b | 2.6 ± 0.8 a | 42 |
| GA4/ 7injection**** | 0.3 ± 0.3 ab | 0 b | 0.3 ± 0.3 ab | 7 |

Treatments included.

Means followed by the same letter are not significantly different($p<0.05$), as determined by Student-Newman-Keuls test.

* Five biweekly sprays with 5% ethanol.
** Five biweekly GA4/7 sprays.
*** Five biweekly GA4/7 sprays with root pruning.
**** Two biweekly GA4/7 injections.

GA4/7 sprays, total flower buds per graft ranged from 0 to 80 ( $\bar{x}$=9.9; S.E=5.0), with 6 out of 16 grafts flowering.

Similarily, in the grafts treated with the GA4/7 spray plus root pruning, total buds per graft ranged from 0 to 10 ( $\bar{x}$=2.6; S.E=0.8), with 8 out of 19 grafts flowering. The patterns of male and female flowers were almost same in the three treatments. No flowers were developed on control plants. A high degree of mortality occured in all treatments including control treatment in this study (5 to 10 died out of 24 grafts for each treatment).

**Flower induction on field-grown seedlings**

In contrast to the results of the greenhouse trials with grafts, GA4/7 spray alone did not have a significant effect on flower induction in field conditions(Table 2).

However, GA4/7 sprays with mulching and/or root pruning showed significant promotion effects on flowering, especially on male flower induction. The combination of GA4/7 spray with both mulching and root pruning appeared to be the most effective teatment although it was not statistically different from the GA4/7 spray plus mulching or the GA4/7 spray plus root pruning.

As with the greenhouse study, large difference in the flowering response to treatments occurred. The number of total flower buds on the branches of trees with the best treatment ranged 0 to 353 ( $\bar{x}$=54.1; S.E=27.7) and 22% of treated branches did not show any flower buds. All trees given this treatment flowered, but variation between branches in a given tree was significant( $\bar{x}$=1.67 to 121.3; S.E=1.7 to 115.4). About 90% of branches treated with the GA4/7 spray plus mulching or GA4/7 spray plus root pruning treat-ments produced flower buds. Treatment with GA4/7 injection alone and with GA4/7 injection plus root pruning and/or root pruning had poor promotion effects as shown by both total number of flower buds and percentage of branches producing flowers.

Male flowers were much more easily induced in this field study than were female flowers. The best rate of female flower induction was 1.1 per branch.

## DISCUSSION

Flower induction responses in the greenhouse-grown grafts and field-grown seedlings were different. First, GA4/7 sprays were effective in

**Table 2.** Response of field-grown 10-year-old European larch trees to various flower induction treatments applied in the previous year.

| Treatment | Male flower per branch $\bar{x}$ ± S.E* | Female flowers per branch $\bar{x}$ ± S.E | Total cone bud per branch $\bar{x}$ ± S.E | Branches producing flowers (%) |
|---|---|---|---|---|
| control (1) | 0.1 ± 0.1 e | 0 b | 0.1 ± 0.1 e | 6 |
| spray (2) | 2.4 ± 0.8 cde | 0.1 ± 0.1 ab | 2.6 ± 0.8 de | 56 |
| s + mulch (3) | 22.3 ± 5.9 ab | 1.1 ± 0.6 ab | 23.4 ± 6.3 ab | 89 |
| s + r.p (4) | 37.3 ± 8.8 a | 0.8 ± 0.3 a | 39.5 ± 10.5 ab | 89 |
| s + m + r.p (5) | 53.2 ± 27.3 ab | 0.8 ± 0.3 a | 54.1 ± 27.7 ab | 78 |
| injection (6) | 1.9 ± 1.0 de | 0.1 ± 0.1 b | 1.9 ± 1.0 de | 28 |
| i + mulch (7) | 6.2 ± 1.8 bcd | 0.1 ± 0.1 b | 6.3 ± 1.7 bc | 72 |
| i + r.p (8) | 7.3 ± 2.6 bcd | 0.2 ± 0.2 ab | 7.5 ± 2.6 bcd | 61 |
| i + m + r.p (9) | 8.2 ± 1.9 bc | 0.1 ± 0.1 ab | 8.3 ± 1.9 bc | 72 |

Treatments included; (1) 5 biweekly sprays with 5% ethanol, (2) 5 biweekly sprays with 400mg/l GA4/7, (3) 5 biweekly GA4/7 sprays and plastic mulching, (4) 5 biweekly GA4/7 sprays and root pruning, (5) 5 biweekly GA4/7 sprays, mulching and root pruning, (6) 3 biweekly GA4/7 injections (60mg/ml), (7) 3 biweekly GA4/7 injections and mulching, (8) 3 biweekly GA4/7 injections and root pruning, (9) 3 biweekly GA4/7 injections, mulching and root pruning.

* Means followed by the same letter are not significantly different($p<0.05$), as determined by Student-Newman-Keuls test.

greenhouse-grown grafts but not in field-grown seedlings. Second, the best flower induction treatments were almost equally effective in stimulating both male and female flowers in the greenhouse-grown grafts but all the treatments applied onto field-grown seedlings produced more male than female flowers and only a few female flowers were produced even in the best field treatments.

GA4/7 injections in this study were not effective in inducing flowers on either greenhouse-grown grafts or field-grown seedlings. Injection through holes drilled into the stem of small (1 to 1.5cm in diameter) grafts seemed to damage these trees as indicated by higher mortality in injection treatments than any other treatments in greenhouse-grown grafts. The high mortality also may be due to phytotoxicity of GA4/7 or ethanol as reported in western larch(Ross; unpublished data). GA4/7 injections with the same concentration of GA4/7 used in this study has been previously shown to induce flowers on treated 2-year-old *Pinus contorta* cuttings(Longman, 1983) and 14-year-old *Picea sitchensis* grafts(Philipson, 1985). The lack of response in this study may have been caused by the prolific resins which are present in larch stems and which have been previously suspected to minimize the movement of injected GA4/7 into the stem(Greenwood, 1982).

Root pruning did not improve the effect of the GA4/7 spray for greenhouse-grown potted grafts, but it did appear to improve the efficiency of GA4/7 spray on field-grown seedlings. The effect of root pruning appears to vary depending on the species and ages of the trees and can be different between greenhouse-grown potted plants and field-grown seedlings of the same species. It has been reported that root pruning was more effective in inducing female flowers on both field-grown Douglas-fir seedlings and field-grown Dougla-fir grafts of the same age, and the effect for flowering induction was greater on seedlings than on grafts(Ross *et al.*, 1985).

The relationship between root activity and a tree's ability to respond to GA application has been investigated in Douglas-fir and white spruce. Bonnet-Masimbert and Zaerr(1989) reported that root growth was retarded by GA4/7 injection but that flower buds were induced on Douglas-fir grafts. Significant reduction in shoot water potential was reported in 6-year-old field-grown white spruce seedlings that had been root pruned (Marquard and Hanover, 1985). The authors speculated that the root pruning may have affected the internal GA levels in the treated trees.

The ineffectiveness of GA4/7 spray treatment on the field-grown seedlings in this study may have been due to the considerable loss of sprayed GA4/7 by wind or rain fall. This is a commonly encountered problem in the field situations(Bonnet-Masimbert, 1987).

Plastic mulching in combination with GA4/7 spray also increased flower bud formation in the field-grown seedlings in this study. Bonnet-Masimbert(1982) found that both plastic mulching alone and with GA4/7 were very effective in inducing male flowers of field-grown European larch. The effect of plastic mulching may be attributed to mild water stress, but soil warming is also important. Lavender *et al.*,(1973) noted an effect of increased soil temperature on increased endogenous GA's sent from Douglas-fir roots to the shoot. The effectiveness of water stress in combination with GA4/7 spray in flowering induction has been demonstrated in field experiments with 1 to 5-year old western hemlock seedlings (Brix and Portlock, 1982). Plastic mulching appears to be a safe and practical method for inducing mild water stress under field condition.

The highest flowering promotion effect on field-grown seedlings was observed in GA4/7 sprays in combination with both mulching and root pruning. However, this response was not significantly different statistically from GA4/7 sprays with mulching, and GA4/7 sprays with root pruning. This result indicated that a single cultural treatment may be enough to cause synergistic effect with GA4/7 and that additional treatments are not useful. Most flower induction studies have used only on cultural treatment in combination with GAs.

GA treatments have been known to induce more female flowers than male flowers (Marquard and Hanover, 1985; Ross *et al.*, 1985). However, GAs also often increased male flowering (Chalupka 1978, 1981; Tompsett and Fletcher, 1979). The fact that hermaphroditic strobili are sometimes observed on treated trees (Marquard and Hanover, 1984) indicates that GA is affecting a meristem that is gradually differentiating over a considerable length of time. Bonnet-Masimbert(1987) suggested that the key to sex differentiation by GA is probably more related to treatment timing, environmental factors and position in the crown where the treatments are applied than to the treatments themselves. The dominant production of male strobili in this field study could have been probably due to crown position effects because branches were chosen in the lower crown (Owens and Marje 1979). In normal conditions, male flowers are more abundant in the lower crown of larch trees. It is also can not be ruled out that treatment timing was unfavorable for female flowering induction in this study.

Finding the optimal level of GA for injection and determining the best timing for injection may improve the efficiency of flower induction. Treatment of branches in different parts of the crown may offer the possibility of differential development of male and female flowers. A definitive comparison of flower induction efficiency between greenhouse-grown grafts and field-grown seedlings is difficult because this experiments were carried with different genotypes, different aged materials, and in different environmental conditions. Because indoor seed orchards have great advantages compared with seed orchards in field, it would seem that more studies are needed for optimizing male and female flower induction from potted grafts.

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