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Cost-Effective Promotion of Flowering in a Douglas-Fir Seed Orchard by Girdling and Pulsed Stem Injection of Gibberellin $A_{4/7}$

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Abstract

Douglas-fir (*Pseudotsuga menziesii*) grafts of 5 cm to 14 cm diameter received different combinations of partial saw-cut stem girdles and ethanolic solutions of $GA_{4/7}$ injected into shallow holes drilled around the main stem. Grafts averaged 79 seed-cone buds and 4500 pollen-cone buds each without treatment. Girdling alone increased the tree production of seed- and pollen-cone buds to 325 and 9300, respectively. $GA_{4/7}$ alone was nearly as effective as girdling alone, the response being marginally greater at the high than low dosage of $GA_{4/7}$ (3.82 vs. 1.27 mg cm^{-2} of stem cross sectional area), but independent of whether the total dose was applied all at once or over two or three injections at 2-week intervals. Together, girdling and $GA_{4/7}$ had an additive effect on flowering, increasing the tree production of seed- and pollen-cone buds to 585 and 18,250. The combined treatment was particularly effective on smaller trees that flowered poorly or not at all without treatment, while also enhancing production significantly on larger trees. The combined treatment was safe and highly cost effective. It cost \$ 63.75 per year to maintain each tree in the orchard, so that without any treatment the cost per seed-cone bud initiated was \$ 0.91. Girdling (at \$ 2.07 per tree) reduced this cost to \$ 0.20, and girdling + $GA_{4/7}$ (at \$ 7.87 per tree) to only \$ 0.13.

Key words: Cone induction, Economics, Gibberellins, Girdling, Seed orchard, *Pseudotsuga menziesii*.

Introduction

Numerous studies have established the efficacy of exogenously applied gibberellin A_4 and A_7 mixture ($GA_{4/7}$) for promoting flowering in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (see Pharis *et al.*, 1987). The treatment is particularly effective in conjunction with such cultural practices as girdling and rootpruning (Ross *et al.*, 1985; Pharis and Ross, 1986; Pharis *et al.*, 1987). However, owing to practical problems of applying the growth regulator, $GA_{4/7}$ may be occassionally used to accelerate breeding but seldom operationally to enhance cone production in seed

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orchards. For this species the most effective mode of $GA_{4/7}$ application has been continuous stem injection in dilute aqueous solution. A „hanging bottle" (reservoir) is affixed to the tree or a supporting stake, with a connecting tube that is either inserted into a hole drilled partway through the stem (PHARIS and ROSS, 1976) or over the end of a freshly cut branch (MCMILLAN, 1980). Both techniques are labor intensive and impractical for treating large seed orchard trees.

Unlike some other conifers (see BONNET-MASIMBERT, 1987), Douglas-fir does not respond well, if at all, to aqueous (water-surfactant) foliar sprays of $GA_{4/7}$ (PHARIS and ROSS, 1976; BOWER and ROSS, 1986). MASTERS (1982) reported otherwise, but from our experience his success is a notable exception. Ultra-low volume spraying of an oil-water formulation of $GA_{4/7}$ gave promising results in one recent trial with Douglas-fir (BOWER and ROSS, 1986). However, this technique also has practical limitations. Spraying cannot be done on windy or rainy days that can prevail throughout much of the spring treatment period.

PHILIPSON (1985) and LONGMAN *et al.* (1986) recently described a pulsed stem-injection technique for $GA_{4/7}$ treatment of pole-size grafts of Sitka spruce (*Picea sitchensis* (BONG) CARR). A concentrated ethanolic solution of the growth regulator is injected into shallow drill holes on

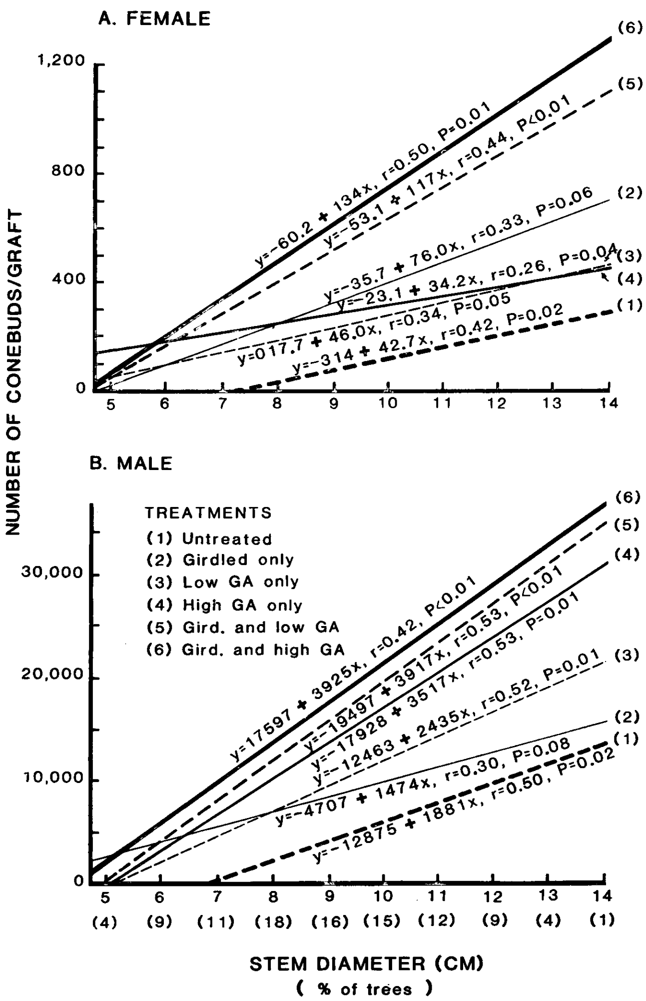

Figure 1. — Size-flowering relationships compared for different treatment groupings (on x-axis, values in parentheses refer to the average number of grafts per treatment in each diameter class). Means are based on 35 grafts, except for the girdling + $GA_{4/7}$ treatments where injection schedules are pooled and n = 105.

opposite sides of the stem, with a second injection 2 weeks later. The treatment appears to be operationally practical and, in these studies on Sitka spruce, was especially effective in conjunction with girdling.

Our study assessed the cost effectiveness of pulsed $GA_{4/7}$ stem injections, with and without girdling, for enhancing flowering in a Douglas-fir seed orchard. Not considered in the original studies on spruce, but of operational importance and thus investigated, were the effects of $GA_{4/7}$ dosage in relation to tree size and number of injections.

Materials and Methods

The study was conducted in the MacMillan Bloedel Harmac Seed Orchard near Nanaimo, B. C., using Douglas-fir grafts (mature parent tree selections on seedling rootstocks) 10 to 15 years old when treated in 1985. A total of 350 grafts, 5 cm to 14 cm in diameter at breast height (DBH, 140 cm above ground), were selected for good vigor and straight stems. These were partitioned among 10 treatment groups such that each treatment contained a different group of 35 clones (1 ramet each) but had the same distribution of tree diameter classes (*Fig. 1*). Grafts either received no treatment, girdling alone, $GA_{4/7}$ injections alone or both treatments. For the combined treatment, trees received a low or high dosage of $GA_{4/7}$ applied all at once or spread over two or three injections. The same two $GA_{4/7}$ dosages, but two injections only, were compared for nongirdled trees (see *Table 1*).

Double overlapping, half-circumferential girdles were made with a pruning saw (WHEELER *et al.*, 1985) at the vegetative bud swelling stage in late April, and the wound sealed with pruning compound. The lower girdle was positioned above the major whorl of branches nearest to breast height and separated from the upper girdle by a distance equal to the stem diameter.

The $GA_{4/7}$ dosage was proportional to the stem cross sectional area at breast height, the low and high dosages being 1.27 and 3.82 mg cm^{-2}, respectively (i.e. 100 mg and 300 mg per tree of DBH 10 cm). The total dosage was applied all at once when the majority of grafts had flushed (24 May), or divided equally among a second or third injection at subsequent 2-week intervals.

Two, three or four injection holes evenly spaced around the stem were used for grafts of DBH 5 cm to 6 cm, 7 cm to 10 cm and 11 cm to 14 cm, respectively. These were 12-mm diameter, approximately 30-mm deep, drilled slightly downward 5 cm to 10 cm above the uppermost girdle, or at a comparable height in nongirdled grafts. For each injection, a new set of holes was dilled above and bisecting the previous ones. Depending on the tree size, dosage and number of applications, 0.7 mL to 4.1 mL of a 95% ethanol solution containing 6 mg or 36 mg mL^{-1} $GA_{4/7}$ (ICI Ltd., Plant Protection Division, Haslemere, Surrey, UK) were injected per hole using an adjustable autopipette.

Seed- and pollen-cone buds were counted the following February and March for one north- and one south-facing branch in each major whorl. These counts were multiplied by a standard factor of 4.4, the mean number of branches per average whorl, and the products summed to provide an estimate of total tree production. Selected treatment groupings were compared by the method of planned contrasts (SNEDECOR, 1976), using a general linear model with DBH as a covariant. Analyses were performed on the square root-transformed conebud counts.

Table 1. — Average flowering responses by Douglas-fir grafts to partial stem girdles and different gibberellin $A_{4/7}$ injection regimes.

Treatment			Conebuds/tree		Trees producing	
	$GA_{4/7}$ injections		seed	pollen	females	males
Girdled	times applied	total dose (mg cm^{-2})	(no. ± se)	(no. ± se)	(%)	(%)
-	---- 0 ----		79 ± 36	4470 ± 1350	20	43
-	2	1.27	230 ± 45	10000 ± 1700	69	83
-	2	3.82	302 ± 56	13390 ± 2360	74	94
+	---- 0 ----		325 ± 68	9310 ± 1660	69	80
+	1	1.27	479 ± 96	14720 ± 2400	74	96
+	2	1.27	513 ± 85	17380 ± 2680	91	97
+	3	1.27	522 ± 77	15280 ± 2350	94	94
+	1	3.82	586 ± 88	18260 ± 3464	85	94
+	2	3.82	573 ± 79	17310 ± 3180	94	100
+	3	3.82	689 ± 103	21080 ± 3490	97	100

Note: Values are based on 35 grafts per treatment balanced with respect to diameter distribution.

Results

Tree condition

Tree condition was assessed at the end of the 1985 growing season following an unusually severe late summer drought. Eleven percent of grafts that received $GA_{4/7}$ alone, and 30% of those receiving the growth regulator plus girdling, exhibited a slight yellowing of foliage and loss of older needles above the injection point. This is a typical response to $GA_{4/7}$, and one from which the trees rapidly recover (Ross, 1983).

Nine (4%) of the girdled + $GA_{4/7}$-treated grafts exhibited more severe defoliation and some shoot dieback in the upper crown; another four similarly treated grafts died. However, all but one of these was located in an area of shallow soil overlaying bedrock where trees would have been more severely drought stressed than elsewhere in the orchard. Several nonstudy grafts in the same area became similarly affected, but none of these died.

Girdles and/or injection holes on 4% of the treated grafts were infected with *Diorectria* spp., but the injury was not severe.

Flowering Response

Table 1 summarizes treatment responses for proportion of grafts flowering and the mean number of conebuds per tree. Results of the analysis of covariance with planned contrasts are given in *Table 2*.

Without treatment, 20% of the grafts produced seed-cone buds and 43% produced pollen-cone buds, indicative of a light to medium natural cone crop year. Girdling alone increased the proportion of female- and male-flowering grafts to 69% and 83%, respectively. The mean production of seed-cone buds was increased three fold ($P < 0.001$), and that of pollen-cone buds was more than doubled ($P < 0.001$), by this treatment.

Two injections of $GA_{4/7}$ at the high dosage were as effective as girdling alone in increasing female flowering, and slightly more promotive ($P = 0.06$) of pollen-cone buds. Girdling and $GA_{4/7}$ together had an additive effect both on female and male flowering. Ninety-four percent of the grafts produced seed-cone buds and 100% produced pollen-cone buds, an average of 573 and 17 310, respectively, per treated tree in response to girdling plus two injections of $GA_{4/7}$ at the high dosage.

For girdled as well as nongirdled grafts, the response to $GA_{4/7}$ was only slightly better at the high than low dosage ($P = 0.10$). The per-tree production of seed- and pollen-cone buds was independent (respectively, $P > 0.14$ and $P > 0.5$) of whether the $GA_{4/7}$ was applied all at once or spread over two or three injections. Multiple injections resulted in a higher proportion of grafts producing seed-cone buds but not pollen-cone buds.

Size-flowering relationships

Tree production of seed- and pollen-cone buds was positively correlated ($P < 0.05$) with DBH for all but the girdled-only treatment (*Fig. 1*). The linear correlation coefficients were not high ($r = 0.26$ to 0.53), but site and genotype were highly variable. Furthermore, the largest and smallest DBH classes were represented by relatively few individuals (see *Fig. 1*).

Covariant analysis (*Table 2*) forced the regression slopes to be equal and used the intercepts to test treatment differences. The assumption of slope homogeneity was verified in a separate analysis of covariance using the treatment by DBH interaction as the error term. However, it appeared that the more effective the treatment in promot-

Table 2. — Analysis of covariance with planned contrasts on square-root transformed counts of seed- and pollen-cone buds per tree.

		Seed-cone buds		Pollen-cone buds	
Source of variation	d.f.	SS	PR>F	SS	PF>F
Treatment	9	11318.3	.001	210662	.001
Stem diameter	1	6319.5	.001	282722	.001
Contrasts:					
gird alone vs. cont	1	1679.0	.001	28051	.001
low GA vs. control	1	1327.1	.001	34867	.001
high GA vs. control	1	1924.4	.001	66529	.001
low GA vs. girdling	1	16.2	.70	481	.67
high GA vs. gird.	1	14.7	.71	9102	.065
low GA+gird vs. cont	1	993.0	.003	30137	.001
high GA+gird vs. cont	1	1932.2	.001	39044	.001
high vs low GA+gird	1	288.9	.10	1045	.53
2 vs. 1 GA injection	1	62.1	.45	53	.88
3 vs. 1 GA injection	1	236.7	.14	1132	.51
Error	318	33995.6		843013	
Total	328	51633.4		1336397	

Note: SS = sums of squares; PR > F is the calculated probability level.

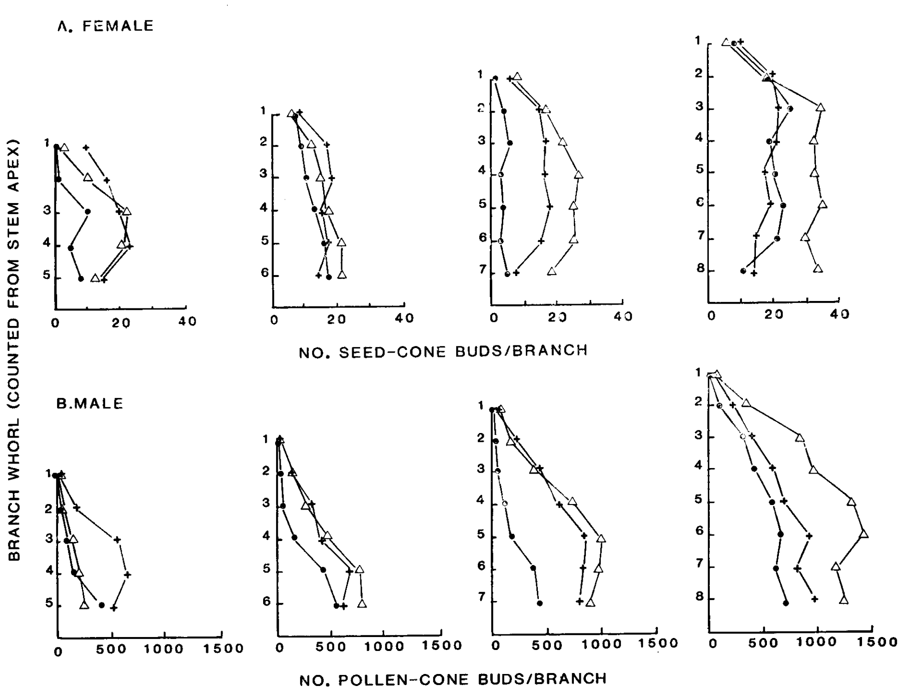

Figure 2. — Distribution of seed and pollen cones by branch whorl as a function of tree size and number of whorls compared for girdled grafts without $GA_{4/7}$ (●) and with $GA_{4/7}$ at the low (+) and high (△) dosages (injection times pooled).

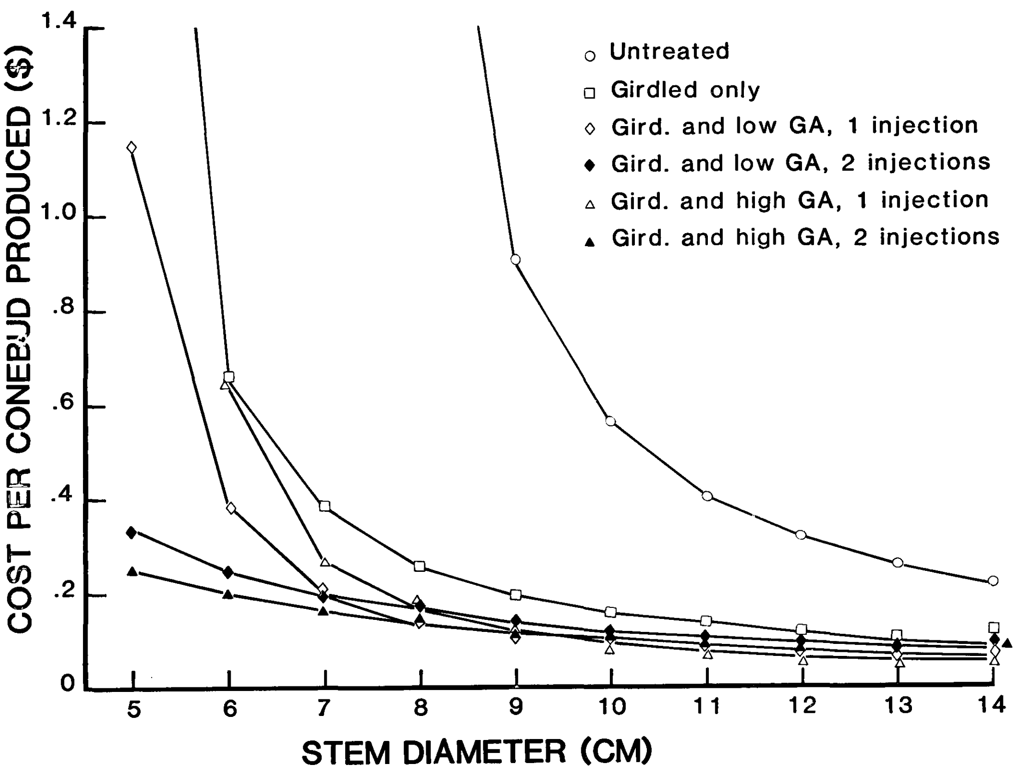

Figure 3. — The estimated cost per seed-cone bud produced compared for selected treatments as a function of tree size (cost derivations are detailed in the text, and conebud production is extrapolated from *Fig. 1*).

ing flowering the steeper the regression slope and larger its intercept value (*Fig. 1*). Without treatment, only 1 of 25 trees of < 10-cm DBH produced seed-cone buds, and the minimum tree size for male flowering was 8 cm. By comparison, grafts of only 5-cm DBH flowered (both sexes) in response to girdling + high $GA_{4/7}$.

Within-crown distribution of conebuds

Branches in whorls below the girdle and(or) $GA_{4/7}$ injection produced few, if any, conebuds of either sex. *Figure 2* compares the distribution of conebuds by branch whorl above the point of treatment for girdled grafts with and without $GA_{4/7}$ at the low and high dosages (application times pooled). Too few untreated controls flowered to warrant inclusion. Number of branch whorls above the treatment internode ranged from four to nine, but the 4- and 9-whorl classes contained few representatives and were therefore also excluded for this comparison.

The effectiveness of $GA_{4/7}$ in promoting female flowering in the uppermost crown decreased with increasing tree size and distance from the point of injection (*Fig. 2*). Increasing the $GA_{4/7}$ dosage three fold did not alter this relationship, but the female response by branches in the middle and lower crown was affected. Here the low dosage was as effective as the high dosage for grafts with five and six whorls, but only half as effective for those with seven whorls, For grafts with eight whorls, only the high $GA_{4/7}$ dosage elicited a female response for middle- and lower-crown branches.

Relative to girdling alone, $GA_{4/7}$ treatment extended the crown zone of female production downward and that of male production upward (*Fig. 2*). Otherwise, the effectiveness of $GA_{4/7}$ in promoting pollen-cone buds diminished similarly with distance from point of injection.

Treatment cost effectiveness

Figure 3 compares the cost effectiveness (in Canadian dollars) of selected treatments as a function of tree size. Cost effectiveness is calculated as the fixed plus treatment cost divided by the number of seed-cone buds produced extrapolated from Figure 1. Independent of treatment, it cost \$ 63.75 annually to maintain a graft in this seed orchard. This considers direct operating and amortized establishment costs plus overhead.

To girdle a graft required about 5 min. (at \$ 23.75 h^{-1}), including the time for selection of study trees and travel between trees. Adding another \$ 0.10 for materials (pruning compound and saw), brought the total to \$ 2.07 per tree. On average it required 6 min. per tree (\$ 2.38) for each injection of $GA_{4/7}$, to which must be added \$ 0.47 per tree for the portable electric drill and automatic syringe (3-y depreciation on each assumed). The cost of $GA_{4/7}$ (at \$ 11.00 g^{-1}) was a function of tree size (5 cm to 14 cm); at the low dosage being \$ 0.28 to \$ 1.58 per tree, and at the high dosage \$ 0.82 to \$ 4.74 per tree.

Calculated on this basis, the girdling and especially girdling + $GA_{4/7}$ treatments were highly cost effective (*Fig. 3*). Without treatment, the per-conebud production cost for the average tree of 9 cm DBH was \$ 0.91 (\$ 63.75/70 cones). Girdling alone reduced this cost to \$ 0.20 (\$ 65.82/325 cones), and girdling + $GA_{4/7}$ (high dosage, 1 injection) to \$ 0.13 (\$ 71.34/549 cones). Although $GA_{4/7}$ alone was nearly as effective in promoting female flowering as girdling alone, a higher application cost made it less cost effective (\$ 0.25 per conebud).

Treatments were relatively more cost effective for smaller (5 cm to 7 cm) grafts that otherwise would not have flowered. However, even for grafts of 12-cm DBH, girdling alone and girdling + $GA_{4/7}$ injections reduced the per-conebud production cost by 62% and 68%, respectively, relative to no treatment. For grafts larger than 7-cm DBH, the different $GA_{4/7}$ injection regimes were similarly cost effective, but for smaller grafts, the low $GA_{4/7}$ dosage was marginally more economical than the high dosage, as were two injections relative to one or three (least cost effective).

Discussion

Other studies have demonstrated the effectiveness of girdling and $GA_{4/7}$ treatments, especially together, for promoting flowering in field-grown Douglas-fir trees EBELL, 1971; MASTERS, 1982; ROSS, 1983; ROSS *et al.*, 1985; WHEELER *et al.*, 1985; BOWER and ROSS, 1986; PHARIS *et al.*, 1987). Partial saw-cut stem girdles are known to be a safe and practical treatment for use in Douglas-fir seed orchards (WHEELER *et al.*, 1985). $GA_{4/7}$ treatments, on the other hand, have heretofore been considered either too costly or inconvenient for treating larger seed orchard trees (see Introduction). However, that assessment must now be revised given the success of the pulsed-stem injection technique reported here for Douglas-fir (*Table 1*), and previously for Sitka spruce (PHILIPSON, 1985; LONGMAN *et al.*, 1986).

The 5- to 14-cm DBH Douglas-fir grafts were selected as being representative of a young Douglas-fir seed orchard approaching production, as this is where cone-enhancement treatment might be most profitably employed. For this seed orchard, in the year tested, the girdling + $GA_{4/7}$ treatment was safe, provided that grafts were not also severely water stressed. It was also cost effective (*Fig. 3*). The annual cost to maintain a graft in the orchard was nearly \$ 64[1]), and without treatment the graft of average DBH (9 cm) produced only 70 seed-cone buds. Girdling alone increased female flowering three fold while reducing the per-conebud production cost from \$ 0.91 to \$ 0.20. Where girdled grafts also received a single injection of $GA_{4/7}$ at high dosage, flowering was increased an additional two fold and the per-conebud production cost reduced to \$ 0.13. Of course, the treatment costs incurred in this small study were much higher than would occur in operational practice.

The relative cost effectiveness of treatments diminished with increasing tree size (*Fig. 3*). For grafts larger than 10-cm DBH, girdling + $GA_{4/7}$ was only marginally more economical than girdling alone, and thus one might assume that for such grafts the additional effort and cost of stem injection was not warranted. However, this fails to take into account the increased flowering achieved (*Fig. 1*), and value of the additional wood that the genetically improved seed represent. Internal resistances to $GA_{4/7}$ transport (*Fig. 2*) may limit the size of tree that can be profitably injected, but our study did not determine what that size might be.

We assessed treatment cost effectiveness on the basis of seed-cone buds initiated, whereas viable seed is the product of interest. LONGMAN *et al.* (1986) reported nonsignificantly lower cone survival and filled seed per cone for girdled + $GA_{4/7}$-treated Sitka spruce grafts. However, their girdling treatment was more severe than our's, involving the removal of a complete ring of bark 8-mm wide from around the stem. WHEELER *et al.* (1985) noted a similar deleterious effect for Douglas-fir where the partial girdles were similarly wide, but not for narrow (ca. 3-mm wide) saw-cut girdles as used in the present study.

Used at a proper concentration, and in the absence of phytotoxic adjuvants (ROSS, 1983), there is no evidence that exogenously applied $GA_{4/7}$ has adverse carry-over effects on development of the induced seed and pollen (PURITCH *et al.*, 1979; ROSS *et al.*, 1980). Of course, any treatment or condition that causes profuse flowering may result in increased cone and ovule abortion due to competition effects for nutrients. However, this could be offset by improved seed set s a result of increased pollen production (WHEELER *et al.*, 1985).

In their studies on Sitka spruce, PHILIPSON (1985) and LONGMAN *et al.* (1986) used two injections of $GA_{4/7}$, spaced 2 weeks apart, timed to bracket the period when conebud differentiation was presumed to occur. For the Douglas-fir grafts in this study, a single injection of $GA_{4/7}$ at the proper dosage and time [i.e., when ca. 50% of the grafts had flushed in spring (ROSS, 1983)] was nearly as effective as the same dosage divided among a subsequent second or third application (*Table 1*), and it was more cost effective (*Fig. 3*).

The requirement for only a single injection makes the $GA_{4/7}$ treatment operationally even more attractive by easing the burden on seed orchard staff at a normally busy time of the year. Fewer injection holes also means less potential for insect attack and other wounding effects. The particular design of our study necessitated relatively large (12-mm diameter) holes to accomodate the range of injection volumes used (0.7 mL to 4.1 mL), whereas operationally a 6-mm hole would suffice. Adjusting $GA_{4/7}$ dosage for grafts of different sizes on the basis of stem cross-sectional area seemed to work well. However, as the flowering response was not highly dependent on dosage (*Fig. 1*), it might be more convenient to make the dosage proportional to stem circumference. One hole per 7 cm of circumference, and 30 mg $GA_{4/7}$ injected in 0.5 mL 95% ethanol per hole would have provided a near optimal dosage for trees of the size used in this study.

Although our results are confined to a single year and orchard, the conclusion that girdling + $GA_{4/7}$ injection is a practical and cost-effective technique for enhancing flowering in Douglas-fir seed orchards is supported by other work on Douglas-fir (N. C. WHEELER, Weyerhaeuser Co., Centralia, WA, personal communications), and Sitka Spruce (PHILIPSON, 1985; LONGMAN *et al.*, 1986). The treatment was successfully used by the B. C. Forest Service in its older coastal Douglas-fir orchards to bring otherwise recalcitrant families and clones into production to improve parental balance and, hence, hopefully the genetic quality of seed produced (J. PARKINSON, personal communication). There are other potential advantages of integrating cone enhancement as a routine part of orchard management. Existing orchards can be rogued more severely to improve genetic gains and still meet planting requirements. Shortening the long nonproductive phase of orchard development means a quicker and more substantial payoff from tree improvement. And, in the future, planting requirements will be met through a smaller, less costly seed orchard program. Research is continuing to ensure that these benefits are fully realized for Douglas-fir and extended to other important tree improvement species.

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[1]) This is about average for similar age Douglas-fir seed orchards of coastal British Columbia (M. CROWN, B. C. Ministry of Forests, personal communication).

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Genetic Control of Isozyme Systems and Heterogeneity of Pollen Contribution in Beech (Fagus sylvatica L.)

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Summary

The study of the alloenzymatic polymorphism and the genetic structure of beechwoods began some years ago, but until now, only few markers have principally been used. For common beech, several new loci, mostly polymorphous, are described in the present paper. The genetic control is carried out analysing the offspring of heterozygous mothers (in case of diallelic loci), assuming the codominance of the alleles for loci coding the MDH, 6PGD, PGI, and SOD systems and testing the segregation ratio 1:1. For the triallelic loci that control IDH and ACP isozyme variants, we only verified that the different genotypes observed in each offspring corresponded to the genotypes expected in the case of three codominant alleles. Additional some presumptions of the genetic control of PX3 and SKDH1 are given.

Some markers were used for studying the variations of the allelic frequencies of the paternal contribution within three populations. In all cases, the paternal contribution is heterogeneous. This heterogeneity presents an inter-group and an intra-group components with allelic frequencies different as well for neighbouring trees as for isolated trees several hundred meters apart.

Key words: allozymes, inheritance, pollen pool variations, beech.

Résumé

L'étude du polymorphisme enzymatique et de la structuration génétique des hêtraies a débuté il y a quelques années, mais jusqu'à présent, seuls quelques marqueurs ont été utilisés. Dans cet article, de nouveaux loci sont décrits chez le Hêtre, la plupart d'entre eux étant polymorphes. Le contrôle du déterminisme génétique est assuré par l'étude de la descendance de mères hétérozygotes (dans le cas de loci dialléliques) en postulant la codominance des allèles pour les loci codant les systèmes MDH, 6PGD, PGI et SOD et en testant le rapport de ségrégation 1:1. Dans le cas des loci trialléliques qui contrôlent les allozymes d'IDH et d'ACP, nous avons seulement pu vérifier que les différents génotypes observés dans chaque descendance correspondaient aux génotypes attendus sous l'hypothèse de trois allèles codominants. Des hypothèses sur le contrôle génétique de PX3 et SKDH1 ont également été avancées.

Plusieurs marqueurs ont été appliqués à l'étude de la variation des fréquences alléliques du pool génique paternel dans trois populations. Dans tous les cas, la contribution allélique paternelle est hétérogène. Cette hétérogénéité présente une composante inter-groupes et une composante intra-groupe avec des fréquences alléliques différentes aussi bien pour des arbres voisins que pour des arbres isolés, séparés les uns des autres de plusieurs centaines de mètres.

Mots-clés: allozymes, contribution paternelle, Hêtre.

Introduction

The isozyme technique offers a practical way of examining genetic variation in natural populations. At the present time, the number of biochemical studies on beech tree is very small: Paganelli *et al.* (1973) examined the variation of dehydrogenases in winter buds; Kim (1979) studied the genetics of two enzymatic markers in young leaves, one

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