FGC

extension note 09

# Increasing Quality Seed Production in Western Redcedar Orchards:

## *A synthesis of a multi-year foliar-applied gibberellin $A_3$ study*

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Authors: O. Hak
Hak Consulting
Victoria, BC
Oldrich@shaw.ca

And: J.H. Russell
Cowichan Lake Research Station
BC Ministry of Forests and Range
Mesachie Lake, BC
John.Russell@gov.bc.ca

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## About the Forest Genetics Council of British Columbia

The Forest Genetics Council of BC (FGC) is a multi-stakeholder group representing the forest industry, Ministry of Forests and Range, Canadian Forest Service, and universities. Council's mandate is to champion forest genetic resource management in British Columbia, to oversee strategic and business planning for a cooperative provincial forest gene resource management program, and to advise the Chief Forester on forest gene resource management policies.

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## Introduction

At sexual maturity, western redcedar is a prolific seed producer. Even though each cone contains only a few seeds, trees are capable of producing abundant seed cones (Owens and Molder 1980). As well, western redcedar can be artificially induced with gibberellin $A_3$ ($GA_3$) to enhance female and male strobili and seed cone production in orchards (Pharis et al. 1969), and it responds well to induction treatments at a very young age (Owens and Molder 1984). Therefore, why would we need any more studies on inducing cones in western redcedar?

Most conifer species carry a high genetic load such that inbreeding can result in the expression of lethal and semi-lethal genes. This translates to poor seed viability and germination, seedling mortality and dramatically reduced vigour. As a result, inbred seedlings do not normally represent a very high percentage of an outplanted seedlot. In addition, physical separation in the crown of male and female strobili, asynchronous receptivity and pollen competition aid in reducing inbred seed in most conifer species.

Western redcedar is a notable exception to the above given that it has a mixed-mating system. Both natural and orchard seedlots exhibit on average 30% selfing, with reported selfing rates varying from 0 to 100% (El Kassaby et al. 1994, O'Connell et al. 2001, Ritland et al. 2004). There is no evidence of early life cycle inbreeding depression; selfed seed germinate on average the same as outcrossed seed, and subsequent seedlings have similar survival and growth rates in a greenhouse environment (Owens et al. 1990, Cherry 1995, Russell et al. 2003). However, selfed trees have been shown to grow 10% slower than outcrossed trees at age 9 in the field (Russell et al. 2003). This translates to 8% on average volume reduction at rotation (Wang and Russell 2006). In addition, both male and female strobili occur throughout the crown and receptivity can be synchronized within a very short time period.

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In natural conditions, western redcedar male strobili are initiated under long and increasing day lengths, and female strobili under long but decreasing day lengths (Owens and Pharis 1971). Consequently, it has been suggested that treatments with $GA_3$ applied during increasing day lengths enhance the proportion of male strobili induced, while treatments applied during decreasing day lengths increase the proportion of female strobili induced (Pharis at al.1969; Owens and Pharis 1971; Owens and Molder 1984). Owens and Molder (1984) suggested that foliar application of $GA_3$ at a concentration of 100 mg/L twice weekly for about six weeks results in the maximum promotion of male and female flowering. Cones develop normally if the treatments occur early enough for them to differentiate fully before dormancy (Owens and Molder 1984). Stress treatments such as heat, girdling, and root pruning are ineffective with western redcedar when used in conjunction with $GA_3$ (Owens and Molder 1984).

We know then, that inducing cones on western redcedar is quite easy to accomplish. However, it is not enough to just spray foliar $GA_3$ to enhance the number of cones, but to judicially apply the hormones at the right time and concentration, and to utilize appropriate management techniques to maximize the production of quality seed. To this end, a study was initiated to investigate foliar-applied $GA_3$ induction techniques for male and female strobili to improve the operational efficiency of producing western redcedar seed while potentially minimizing selfing. Under natural conditions, western redcedar tends to produce prolific pollen buds on the less vigorous, pendulous lateral branches (Owens and Molder 1984). Pollen from these male strobili has the potential to pollinate the female strobili that are produced at the tips of these branches. Fewer male strobili occur on the vigorous, upright outer shoots on the primary and secondary branches. Thus, particular attention was paid to induction treatments on these vigorous branches in relation to female and male stobili production to investigate the impact on potential selfing rates in seed orchards (Ritland et al. 2004).

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This report summarizes four separate foliar-applied $GA_3$ cone induction trials done over a span of four years. The objective of the first trial was to investigate the effects of timing, concentration and frequency of $GA_3$ induction treatments on male and female stobili production in mature seed orchards. The objective of the second trial was to examine the effects of induction timing treatments in young seed orchards. The third trial involved retesting the second trial at a different location and in a different year. Finally, the objective of the fourth trial was to determine the time period during late summer when stobili induction treatments become ineffective.

This report synthesis results from the above trials. Major conclusions from the research are listed and orchard management techniques that have the prospect of enhancing seed quality through the potential reduction of selfing are suggested. Details of the individual study designs, statistical analyses and results can be found in Russell and Hak (2007).

## Major Study Results and Orchard Management Suggestions

### 1. In general, foliar spray $GA_3$ anytime from late spring to late summer to initiate male and female strobili on western redcedar trees.

The entire period, and also the peak period of male and female strobili initiation, are less precise in western redcedar than in the Pinaceae species. Consequently, the timing of strobili induction treatments is also less exact. Western redcedar has indeterminate shoot growth, lacks vegetative buds and does not display a fixed cycle of bud development such as species from the Pinaceae family (Owens and Molder 1984). It is more important that gibberellin applied for strobili induction and/or differentiation in Pinaceae species be tied to bud development and early shoot elongation (e.g., Owens and Colangeli 1988, Ho and Hak 1994, Webber 2000) than for species with indeterminate shoot growth as in the Cupressaceae. For example, male and female strobili were induced over the whole span of shoot elongation in western redcedar, from April to September, without any apparent specific day length requirements (Figure 1). Male strobili production on low-vigour lateral branches was maximized over a four month time span from May to August, and female strobili production was the highest between mid-July and mid-August, a one month time span.

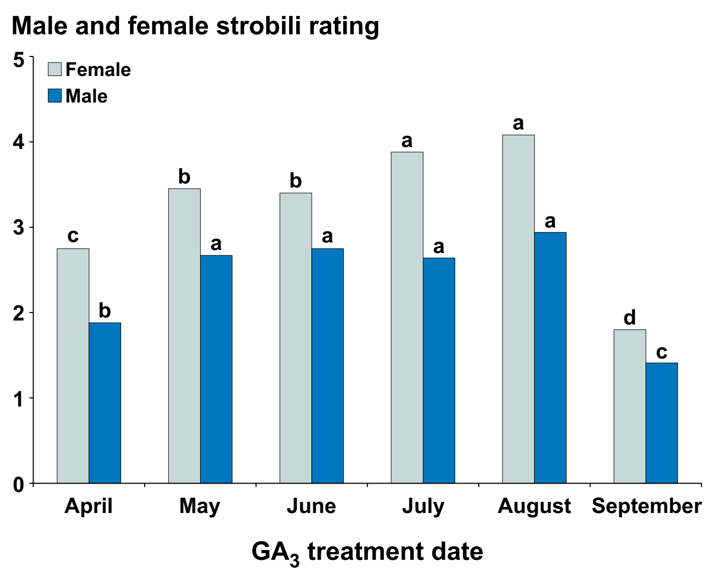


**Figure 1.** *Effect of $GA_3$ timing on female and male strobili production. Different letters denote significant differences ($p<0.05$) between treatment periods for male and female strobili rating according to Fisher's LSD mean separation test. Strobili rating of 0 = no strobili and a rating of 5 = very heavy.*

## 2. To maximize seed production, foliar spray $GA_3$ once around mid-July at a concentration of 200 mg/l.

If the main objective of seed production is operational efficiency, a one-time treatment with $GA_3$ concentration of 200 mg/l during the last two weeks in July appears to be the most promising (Figures 2 and 3). Even though the same treatment in mid-August produced a similar number of cones, this and subsequent treatment periods had a negative effect on final cone and seed qualities such as cone length, number of filled seed per cone, seed weight, and seed germination (Figures 4 and 5). Similarly, treatments with 400 mg/l $GA_3$ in July were significantly more effective, but excessive cone production dramatically decreased the number of sites for subsequent vegetative growth. Consequently, trees treated with these higher $GA_3$ concentrations may require more than a one-year resting period to replenish the number of potential sites for sufficient male and female strobili production. Spraying twice over a one-week interval had minimal effect on female stobili production (Figure 3).

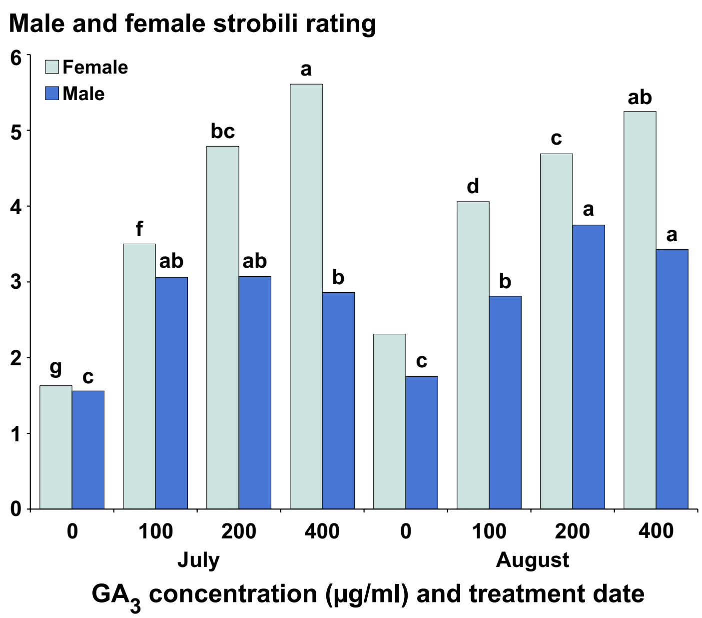


**Figure 2.** *Effect of $GA_3$ timing and concentration during July and August on male and female strobili production. Different letters denote significant differences ($p<0.05$) between concentration and treatment periods for male and female strobili rating according to Fisher's LSD mean separation test. Strobili rating of 0 = no strobili and a rating of 6 = very heavy.*

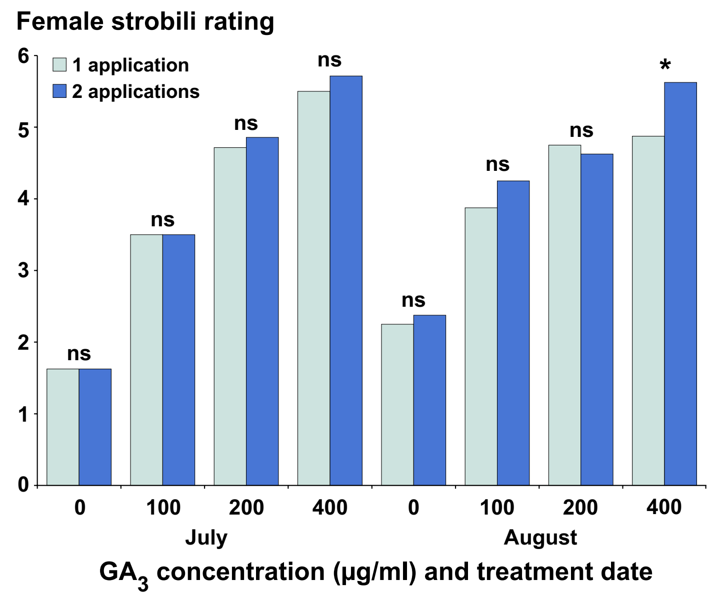


**Figure 3.** *Effect of $GA_3$ application frequency and concentration during July and August on female strobili. *= significant differences ($p<0.05$) between frequency treatments within concentration and treatment periods according to Fisher's LSD mean separation test, ns=not significant. Strobili rating of 0 = no strobili and a rating of 6 = very heavy.*

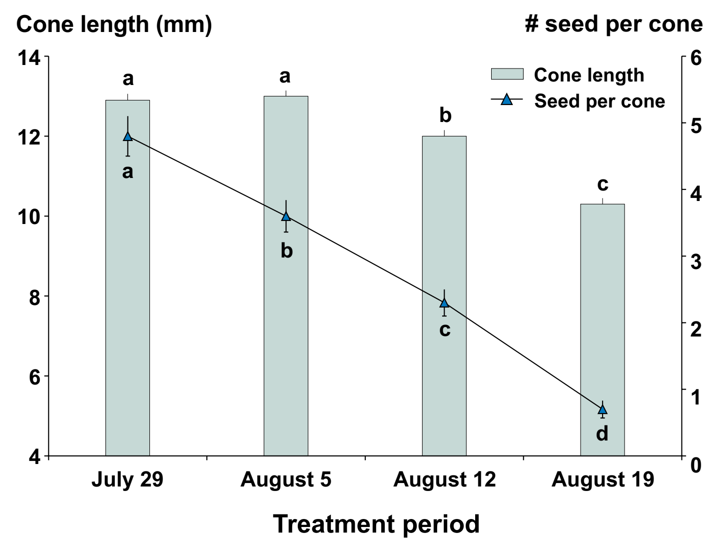


**Figure 4.** *Effect of late summer $GA_3$ treatments on cone length and the number of filled seeds per cone (+/- s.e.). Different letters denote significant differences ($p<0.05$) between treatment periods for cone length and seed per cone according to Fisher's LSD mean separation test.*

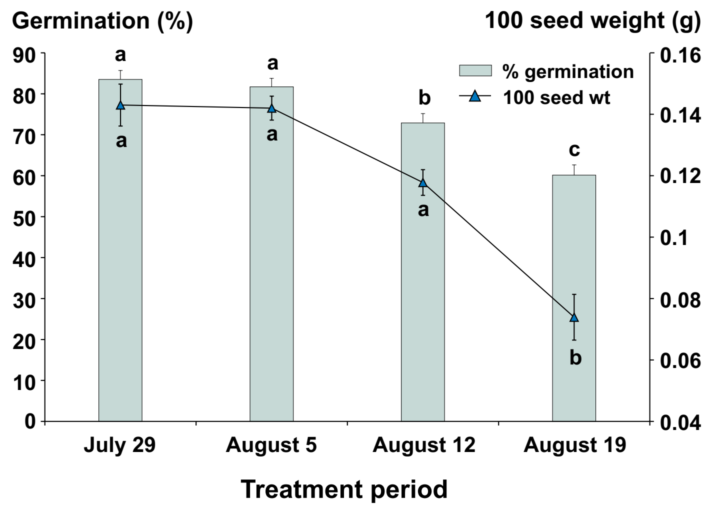


**Figure 5.** *Effect of late summer $GA_3$ treatments on seed germination and on seed weight (+/- s.e.). Different letters denote significant differences ($p<0.05$) between treatment periods for % germination and 100 seed weight according to Fisher's LSD mean separation test.*

### 3. Alternatively, time $GA_3$ induction treatments with the greatest shoot increment to maximize seed production.

Female stobili production in response to induction treatments was related to the rate of shoot increment. The highest level of female stobili production was associated with the peak of shoot increments (Figure 6). It appears that the significant increase in female strobili around mid-summer resulted from a greater expansion of the vigorous lateral branches than of the non-vigorous ones during the growing season. Since branch growth is influenced to a great degree by the prevailing weather, more attention should be given to shoot increment rates than to dates in order to establish the best treatment period for a specific seed orchard location.

### 4. Initiating strobili on vigorous as opposed to non-vigorous shoots can potentially result in less selfed seed through the manipulation of female to male strobili ratios.

One of the objectives of western redcedar seed orchard management is to minimize selfing (Ritland et al. 2004). This may be possible through manipulation of the female to male strobili ratio within a branch where selfing has a higher potential to occur. Western redcedar has a short, compact, and plastic response to receptivity, such that male and female strobili within a tree tend to mature at similar times (El Kassaby 1999). Pollen from the above neighbouring branches of the same tree can shed directly onto receptive females on the short, vigorous tips below, potentially resulting in selfed seed (Ritland et al. 2004). In this study, it was difficult to control the production of males in favour of females in low-vigour pendulant lateral branches in the proximal region of especially large/older western redcedar trees where an overwhelming amount of male pollen is produced. There were, however,

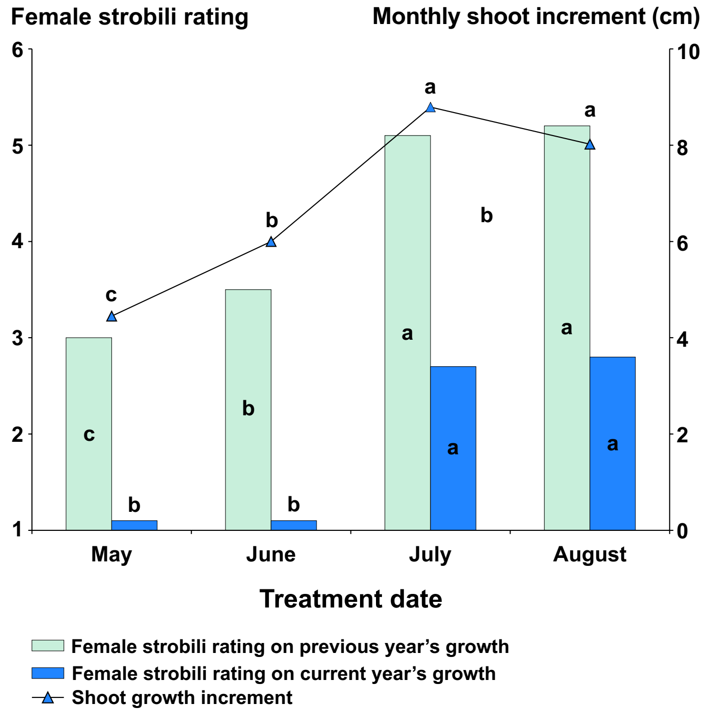


**Figure 6.** *Relationship between $GA_3$ treatment date, female stobili production and monthly shoot growth increments for western redcedar. Different letters denote significant differences ($p<0.05$) between treatment periods for the three traits according to Fisher's LSD mean separation test. Strobili rating of 0 = no strobili and a rating of 6 = very heavy.*

significantly fewer male strobili on the vigorous, distal branches resulting in significantly more female than male strobili. Thus, branch vigour seems to be associated with higher female and lower male strobili production as suggested by Owen and Molder (1984).

### 5. To maximize female to male strobili ratios, induce twice: first during the early season at the start of growth initiation (May) and then mid-summer when the highest shoot increments occur (mid-July).

Timing of strobili induction treatments can further enhance the female to male strobili ratio, especially on young vigorous trees. The highest female to male strobili ratio was achieved with a combination of earlier (May) and later (mid-August) induction treatments (Figure 7). However, late summer induction treatments, starting in mid-August have a negative effect on seed quality (see Figures 4 and 5 above). Substituting the mid-August induction with treatments that correspond to maximum shoot increment (around mid-July), in the above treatment combination, may prove to be a viable alternative since mid-July inductions produce good quality seed and female stobili production is similar to mid-August inductions.

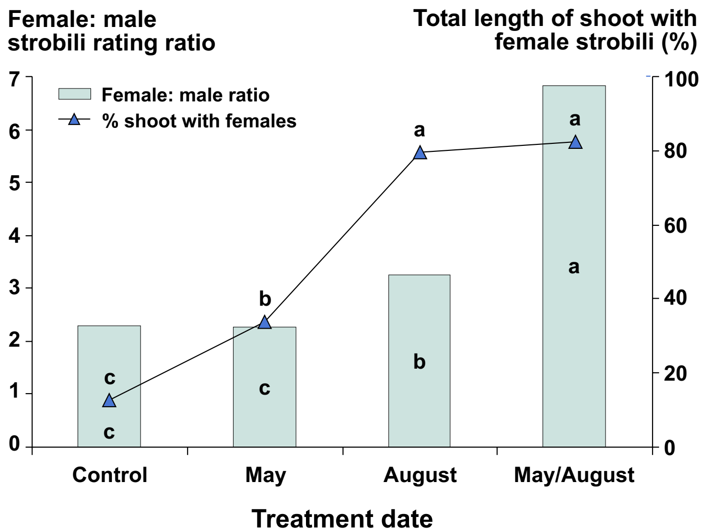


**Figure 7.** *Relationship between treatment date and female:male strobili rating ratio and percent of the total shoot length occupied by female strobili for western redcedar. Different letters denote significant differences ($p<0.05$) between treatment periods for female:male ratio and presence of females according to Fisher's LSD mean separation test.*

## 6. Develop separate male and female trees, and prune female trees to promote the establishment of vigorous branches at the expense of non-vigorous laterals.

Large, unpruned trees used for pollen production only, scattered among smaller, unrelated pruned trees for cone production, has the potential to promote outcrossing. The "female" trees should be pruned in such a way as to promote vigorous upright shoots and minimize pendulous non-vigorous branches. In addition to the female trees having less pollen, O'Connell et al. (2004) have shown that in natural western redcedar, smaller trees exhibit less selfing when surrounded by larger trees. Spraying only vigorous shoots on trees designated for cone production will potentially increase the female to male strobili ratio.

## Conclusions

Results from this study have implications for the management of western redcedar seed orchards in the Pacific Northwest. Timing of foliar induction treatments does not have to be as precise as with associated conifer species that develop vegetative buds and display determinate growth patterns. In these trials, male and female strobili were induced over the complete span of shoot elongation from April to September. However, to maximize quality cone production and management efficiency, a one-time treatment of foliar-applied $GA_3$ at a concentration of 200 mg/l during the last two weeks in July, or in combination with a mid-May treatment, appears to be the most promising. These treatments balance the appropriate timing for female stobili production without overproduction, which would result in a decreased number of sites for future vegetative growth and, at the same time, minimizing seed quality issues associated with late season induction treatments. However, actual spraying times to increase the female to male ratio, and potentially reduce selfing, should be keyed to shoot increment as opposed to a calendar date, since branch growth is influenced to a great degree by the prevailing weather. Thus, the best treatment period for a specific seed orchard location may vary.

*…actual spraying times to increase the female to male ratio, and potentially reduce selfing, should be keyed to shoot increment as opposed to a calendar date*

The negative impact of planting western redcedar selfed seedlings on growth and yield (Russell et al. 2003, Wang and Russell 2006) can be minimized through appropriate seed orchard management techniques that potentially reduce selfing and subsequently increase seedling quality. These include crown management, development of male and female trees, and utilization of vigorous shoots for cone production. The potential of these techniques to reduce selfing is influenced by 1) the overwhelming amount of pollen produced by large trees dominated by lower vigour branches, and 2) the predominance of females on vigorous current year's growth. The proposed management techniques include focusing female stobili production on vigorous shoots, minimizing cone collections from less vigorous shoots which produce an overwhelming number of male stobili, and timing foliar inductions to shoot growth. Tools for estimating selfing in western redcedar seed orchards have been developed (Ritland et al. 2004, O'Connell et al. 2004) and can be utilized to gauge the effectiveness of these new management techniques in reducing selfed seed.

## References

Cherry, M.L. 1995. Genetic Variation In Western Red Cedar (*Thuja plicata* Donn) Seedlings. PhD Thesis, Univ. of BC, Vancouver, BC. 200 pp.

El Kassaby, Y. 1999. Phenotypic plasticity in western redcedar. Forest Genetics 6(4):235–240.

El Kassaby, Y., J.H. Russell, and K. Ritland. 1994. Mixed mating in an experimental population of western redcedar. J.Hered. 85(3):227–231.

Ho, R.H. and O. Hak. 1994. Optimum timing of gibberellin $A_{4/7}$ sprays to promote cone production in jack pine seedlings. New For. 8:61–69.

O'Connell, L.M., F. Viard, J. Russell, and K. Ritland. 2001. The mating system in natural populations of western redcedar (*Thuja plicata*). Can. J. Bot. 79: 753–756.

O'Connell, L.M, J.H. Russell, K. and Ritland. 2004. Fine-scale estimation of outcrossing in western redcedar with microsatellite assay of bulked DNA. Heredity 93: 443–449.

Owens, J.N. and A.M. Colangeli. 1988. Promotion of flowering in western hemlock by gibberellin $A_{4/7}$ applied at different stages of bud and shoot development. Can. J. For. Res. 19:1050–1058.

Owens, J.N., A.M. Colangeli, and S.J. Morris. 1990. The effect of self-, cross-, and no pollination on ovule, embryo, seed, and cone development in western red cedar (*Thuja plicata*). Can. J. For. Res. 20: 66–75.

Owens, J.N. and M. Molder. 1980. Sexual reproduction in western red cedar (*Thuja plicata*). Can. J. Bot. 58: 1376–1393.

Owens, J.N. and M. Molder. 1984. The reproductive cycles of western redcedar and yellow-cedar. BC Min. For., Info. Serv. Br., Victoria, BC. 28 pp.

Owens, J.N., and R.P. Pharis. 1971. Initiation and development of western red cedar cones in response to gibberellin induction and under natural conditions. Can. J. Bot. 49: 1165–1175.

Pharis, R.P., W. Morf, and J.N. Owens. 1969. Development of the gibberellin induced ovulate stobili of western redcedar: quantitative requirement for long day -> short day -> long day. Can. J. Bot. 47: 415–420.

Ritland, K., P. Brown, B. Wigmore, and J. Russell. 2004. Controlling selfing rates in seed orchard populations of western redcedar. *In* Forest Genetic Council of BC Tree Improvement Program Proj. Rep. 2003/2004. 53 pp.

Russell, J.H., R.B. Burdon, and A.D. Yanchuk. 2003. Inbreeding depression and variance structures for height and adaptation in self- and outcross *Thuja plicata* families in varying environments. For. Gen. 10(3):171–184.

Russell, J.H. and O. Hak. 2007. Effect of foliar-applied gibberellin $A_3$ on male and female stobili production and cone and seed quality in western redcedar (*Thuja plicata* Donn). Western J. Appl. For. Vol. 22(3). In press.

Wang, T. and Russell, J.H. 2006. Evaluation of selfing effects on western redcedar growth in operational plantations using Tree and Stand Simulator (TASS). For. Sci. 52(3):281–289.

Webber, J.E. 2000. Western hemlock: a manual for tree improvement seed production. BC Min. For., Resear. Br., Victoria, BC. Work Pap. No. 44. 61 p.