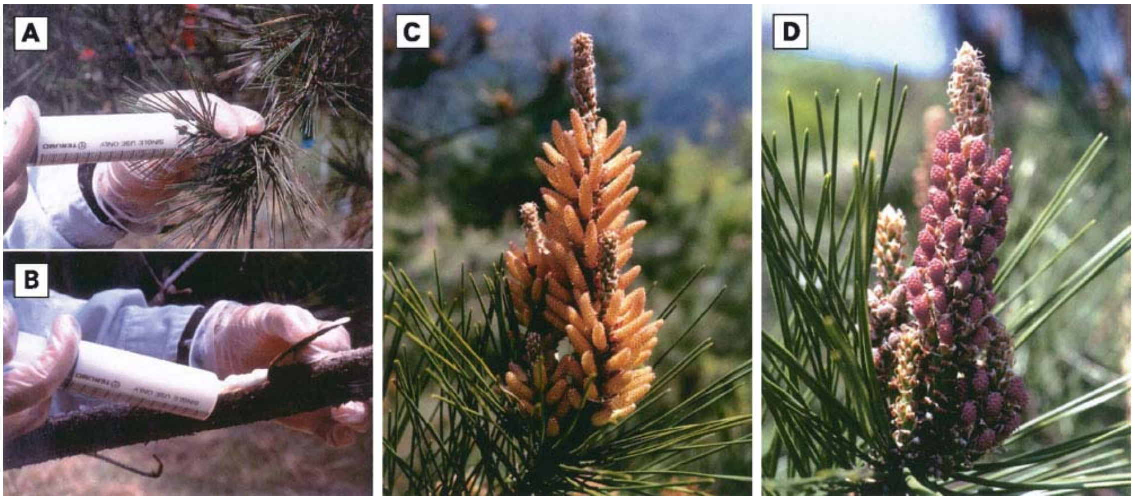
Plant growth regulators and cone induction in Pinaceae

Lisheng Kong and Patrick von Aderkas

Centre for Forest Biology, Department of Biology, University of Victoria,
3800 Finnerty Rd., Victoria, BC V8W 3N5 Canada



Inducing gender conversion in Japanese pines (Wakushima, 2004)

Contents

Summary

Many methods have been used for inducing or enhancing cone flowering in Pinaceae including girdling, pruning, application of nitrogen-containing fertilizers or drought treatments, and hormone induction. The most notable method for cone induction is to apply plant growth regulators (PGRs) to target trees. Research on application of PGRs for cone induction started in the middle of 50s and researched its high peak in 80s in the last century. Gibberellins (GAs), a group of plant hormones, have been used for cone induction in Pinaceae. GAs were the most intensively researched hormones. The most effective gibberellins in cone induction are those with less polar molecular structures. In practice, a mixture of GA $_{4/7}$ was commonly used for cone induction due to its active effects and relatively low cost. Combining GA treatments with other methods such as girdling or pruning proved more effective than GA treatment alone. Gibberellins are thought to stimulate cell and bud activity resulting in more male and/or female flowers. Other PGRs, such as cytokinins and auxin were also tested in cone induction. Cytokinins showed negative effects on cone flower induction until successful results became available recently. When synthetic cytokinin, benzylamino purine (BAP) was applied at specific floral developmental stages, male primordia were converted into female ones in two pine species. Since female cone flower induction by BAP was much more effective than that by Gas, this study showed that cytokinins had great potential in cone induction. Seeds produced from “transgendered” cones demonstrated similar germinability to ordinary seeds. Auxins, such as NAA and IAA, have often been used in combination with Gas. Auxins appeared to enhance GA-effects in cone induction. Application of auxin alone or in combination with GAs enhanced only male cone flowering. Abscisic acid (ABA) increased during effective cone induction treatments, such as GA application, pruning and drought

treatments. Little research has been carried out on application of exogenous ABA to stimulate flowering: no positive result is available at present. Another relatively neglected hormone class is ethylene: in spite of large amount of work on angiosperm trees, ethylene induction of cones in Pinaceae has been very limited.

Effects of PGRs on cone induction depend on many factors including PGR types, concentrations, application time, method and frequency, etc. Different responses to PGRs may also result from various genotypes and/or different physiological conditions.

Efforts were made to explore the mechanism behind cone flower induction by PGRs in the last a few decades. Although more research results become available and improved analysis methods have been developed, convincing answers remain illusive. A few hypotheses concerning cone induction are either discussed or proposed in this review.

Introduction

There are two possible goals in inducing trees to form floral buds, in particular female buds. The first is to achieve precocity. Precocious flowering in normally immature trees can provide advantages in breeding and in seed production because it overcomes a substantial seed production delay intrinsic to late-maturing conifers. The second reason has nothing to do with this phase change from juvenile growth to mature growth. Induction in most situations targets the creation of increased number of buds in sexually mature trees in order to increase yield of elite seeds. Induction of either precocity or increased male flower and female cone formation are generally the result of applied treatments. A few studies have focused on the intrinsic physiological differences between high and low producing clones (Moritz et al., 1990; Oden et al., 1987; 1995). Most studies involve treatments of either a physical, nutritional or hormonal nature (reviewed by Bonnet-Masimbert and Zaerr, 1987).

The traditional methods for improving seed yield involve treatments to manipulate physiological conditions of the parent trees, which in turn enhances flowering. Flower enhancement is achieved by either physically stressing the trees, altering the tree's nutritional status by applying various inorganic fertilizers (Sweet and Hong, 1978), or applying growth regulating substances such as gibberellins (GAs) (Bonnet-Masimbert, 1987; Owens 1991). Physical treatments are either applied to the shoot (top working, shoot training, girdling, wire-girdling, scoring) or to the root (pruning, trickle irrigation, fertigation, confinement). Some treatments are combinations of these general categories of tree management to enhance the effects of each treatment (Ross and Bower, 1991). The most notable method for cone induction is application of plant growth regulators due to its efficiency and practice in application.

The five major types of plant hormones include gibberellins, cytokinins, auxins, abscisic acid, and ethylene. This review focuses on plant hormones or growth regulators and their application in flower enhancement in Pinaceae, in particular, Douglas fir and lodgepole pine.

History of application of PGRs in cone induction

Plants are unable to escape from unfavorable living conditions or stresses. A frequent response of plants is to hasten reproduction by generating both more flowers and thus more seeds. There is a long history of studies whose goal was to enhance flowering. For decades, the search for a universal floral induction mechanism and its universal compound – florigen - consumed many careers. With the failure to find such a substance, it has been accepted that the flowering process is complex and possibly under control of multiple factors. Plant hormones play an important role in creating or maintaining a balance of the factors to initiate flowering process.

The history of exogenous plant hormones or plant growth regulators to induce flowers in coniferous species began in the middle of 1950s when gibberellins (GAs) were successfully used in induce cones of Cupressaceae and Taxodiaceae (Kato et al., 1958). Examples of the high efficacy of GAs within these families was that a 12-month-old plant sprayed with GA_3 produced 8,007 strobili out of 8,090 meristems, most of which were transformed quickly from vegetative to reproductive state (see review by Pharis and Kuo, 1977). Thereafter, GAs have been tested and proven effective in flower induction in many coniferous species. Some effort, albeit limited, was also made to use other PGRs alone or in combination for cone enhancement in Pinaceae.

Gibberellins

Gibberellins are a group of plant hormones with a common basic molecular structure derived from four isoprenoid units forming a system of four rings. Different GAs are distinguished by 19 and 20 C-atoms. To date, more than 110 GAs have been discovered, which differ in their biological effects. The less polar GAs, such as gibberellins GA_4, GA_7, and GA_9, were the most effective ones in flower induction in Pinaceae (Pharis, 1991). Preference of different GAs in different plant families depends not only on their stability, but on the turnover rate of GA metabolism in plant tissue. Application of such GAs alone or in combinations with other methods stimulated cone induction in Douglas fir (Pharis et al., 1980; Pharis, 1991, Ross and Bower, 1991), lodgepole pine (Longman, 1982; Wheeler et al., 1980), eastern white pine (Pijut, 2002), black spruce (Smith and Greenwood, 1997; Smith, 1998), western larch (Shearer, 1999); jack pine (Rong and Oldrich, 1994), Scots pine (Eriksson et al., 1998; Almqvist, 2003), Sitka spruce (Philipson, 1985; Longman et al., 1986), and western hemlock (Ross, 1989). More details could be found in Table 1. Effective stimulation of cone initiation and differentiation by GAs has been largely successful in conifers with one notable exception: application of $GA_{4/7}$ on white spruce was ineffective (Beaulieu et al. 1998). This is possibly due to the improper application and/or application at an inappropriate developmental stage, since positive effects of $GA_{4/7}$ were observed in the same species by other researchers (Marquard and Hanover, 1985; Pharis, 1991).

Table 1. Effects of GAs on flower induction in Pinaceae*

Species	Best PGRs	Application	Effects	Reference
Black spruce	$GA_{4/7}$	stem injection	Increased CFB	Smith and Greenwood, 1997; Smith, 1998
Caribbean Pine	$GA_{4/7}$	bud base injection	Enhanced flowering	Harrison and Slee, 1991
Chinese red pine	$GA_{4/7}$	foliar spray	Increased CFB	Sheng and Wang, 1990
Douglas fir	$GA_{4/7} + GA_9$	foliar spr/stem inj + GDL, PRN	Increased CFB	Pharis et al, 1980; Pharis, 1991
Eastern white pine	$GA_{4/7}$	foliar spray	Increased CFB and MFB	Ho and Schnekenburger 1992; Ho and Eng, 1995; Pijut et al., 2002
European larch	$GA_{4/7}$	stem injection	Increased CFB and MFB	Bonnet-Masimbert, 1982
Jack pine	$GA_{4/7}$	stem injection	More MFB, no effects on CFB	Fogal, et al, 1996
		foliar spay	Enhanced CFB	Cecich, 1981, 1983
Japanese larch	$GA_{4/7}$	stem injection	Increased CFB and MFB	Bonnet-Masimbert, 1982
Loblolly pine	$GA_{4/7}$, GA_3	foliar spr/stem inj	More CFB; $GA_{4/7} > GA_3$	Greenwood, 1982; Hare, 1984
Lodgepole pine	$GA_{4/7}$ + NAA	foliar spray	Increase CFB	Wheeler et al., 1980;
Longleaf pine	$GA_{4/7}$	foliar spray	Increase MFB	Hare, 1984
Norway spruce	$GA_{4/7}$	stem injection	More CFB; no effects on MFB	Fogal, et al, 1996;
Radiata pine	$GA_{4/7}$, GA_3	stem injection	More CFB; $GA_{4/7} > GA_3$	Ross et al., 1984
Sitka spruce	$GA_{4/7}$	*foliar spray*	More CFB; no effects on MFB	Almqvist, 2003 Philipson, 1985; Longman et al., 1986
	A_3, A_5, A_7	drops on buds	Enhanced CFB and MFB	Tompsett, 1977
Scots pine	$GA_{4/7}$	stem injection	More CFB+ MFB, smaller seeds	Eriksson et al., 1998
Slash pine	$GA_{4/7}$	foliar spray	Increase MFB	Hare, 1984
White spruce	$GA_{4/7}$	stem injection	More CFB, no effects on MFB	Fogal, et al, 1996
	$GA_{4/7+\text{ NAA}}$	stem injection	No effect on CFB	Beaulieu et al., 1998
Western hemlock	$GA_{4/7}$, GA_3	foliar spr/stem inj	More CFB and MFB	Ross et al., 1989; Harrison and Owens, 1992
Western larch	$GA_{4/7}$	stem injection	Enhanced CFB and MFB	Shearer et al., 1999

* Only the PGRs with the best effects were listed. CFN, cone flower bud; MFB, male flower bud; VB, vegetative bud; FLB, floral bud.

Cytokinins

Cytokinins are compounds with a structure resembling adenine. They promote cell division. Cytokinins have been shown to play a significant role in tree development in a wide variety of pinaceous trees. Exogenously applied cytokinins increase bud initiation and also control bud release. Exogenous applications of gibberellins that induce bud initiation have been accompanied by an increase in endogenous cytokinin levels (Imbault et al, 1988; Pilate et al., 1991). It is known that spring cytokinin levels increase in the root and that these compounds are transported via the xylem to shoots where they influence development. Endogenous cytokinin levels are known to increase in the xylem immediately prior to bud burst. Levels are also correlated with bud size and architecture in some conifers. Morris et al. (1990) reported that all buds in Douglas fir have three major cytokinins. In female buds and vegetative buds the zeatin-type cytokinin was relatively higher while in male flower buds concentration of isopentenyladenosine was higher with low level of zeatin-type cytokinin. Buds of radiata pine contained different cytokinins and varying patterns were observed in developing and mature buds. The implication is that bud development is in some way regulated by cytokinins (Zhang et al., 2003).

Cytokinins have been used alone or in combinations with other PGRs to induce cones (Table 2). Benzlaminopurine (BAP), a synthetic cytokinin, enhanced GA-induced flowering in Douglas fir (Ross and Pharis, 1976) and Sitka spruce (Tompsett, 1977). In contrast, application of benzyladenine (BA) alone not only enhanced vegetative growth but inhibited both male and female flower formation in Chinese red pine. BA also reduced GA effects on flowering when BA was applied in combinations of GAs in the same species (Sheng and Wang, 1990). Smith and Greenwood (1995) reported similar negative results when black spruce was treated with BAP, zeatin or zeatin riboside at early stages of bud development.

Recently, significant progress was made cytokinin-linked cone induction. When BAP was applied to Japanese black pine and Japanese red pine at specific developmental stages, female flower formation was greatly enhanced through conversion of male flower buds into female ones (Figs. 1 and 2). The most effective treatment was a combination of BAP with girdling (Wakushima et al., 1996, 1997; Wakushima, 2004). This method shows great potential since cone induction rates by BAP were 3- to 10-fold higher than cone induction by GAs in the same species. Cytokinin-induced gender conversion may involve an altered balance between cytokinin and other plant hormone(s). Seeds produced in these gender-shifted or transgendered cones demonstrated similar germinability as ordinary seed (Wakushima, 2004).

Table 2. Effects of cytokinins on flower induction in Pinaceae

Species	**PGRs**	**Application**	**Effects**	**References**
Black pruce	BAP, zeatin or zeatin riboside	Stem injection	Reduced flowering	Smith and Greenwood, 1995
	GA + Cks	Stem injection	Reduced flowering	Smith and Greenwood, 1995
Chinese red pine	BA	Stem injection	Increased VB; decreased FLB	Sheng and Wang, 1990
	GA + BA		Decreased CFB	Sheng and Wang, 1990
Douglas fir	GA + BAP		Enhance flowering	Ross and Pharis, 1976
	iPA		Enhance CFB	Imbault et al., 1988
Japanese red pine	BAP	Paste on branch/ stem injection	Increased CFB by sex switch	Wakushima et al., 1996, 1997; Wakushima, 2004
Japanese black pine	BAP	Paste on branch stem injection	Increased CFB by sex switch	Wakushima et al., 1996, 1997; Wakushima, 2004
Sitka spruce	GA + BAP	Bud application	Enhance flowering	Tompsett, 1977
	kinetin		No effect	

CFB, cone flower bud; MFB, male flower bud; VB, vegetative bud; FLB, floral bud.

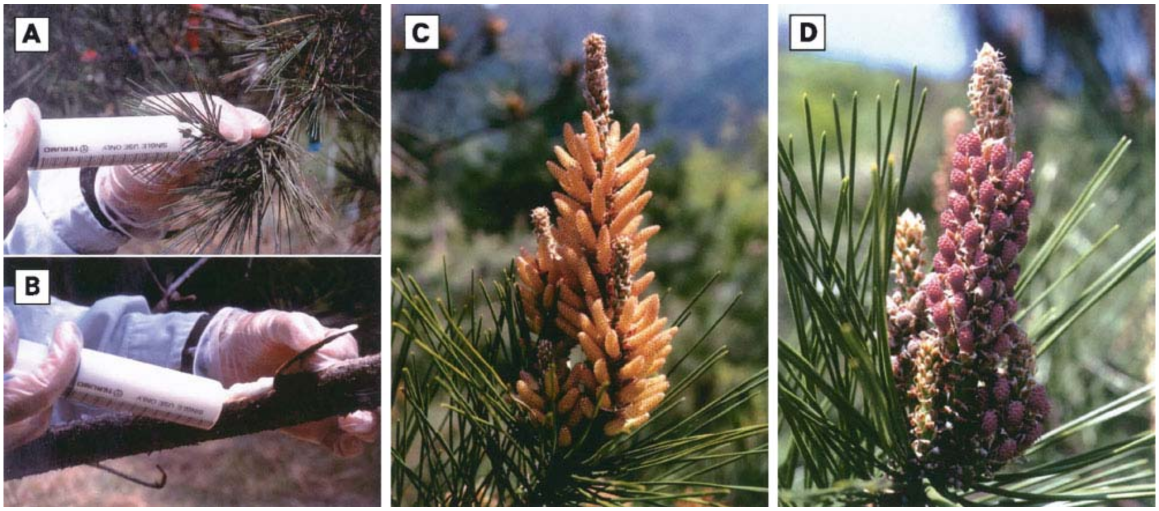


Figure 1 Application of BAP for cone induction (**A**) Terminal bud treatment. Terminal buds were covered with 2 ml volume of BAP paste. (**B**) Girdling treatment. Selected branches (4-year or 5-year-old portions) were girdled with a craft knife. About 1–2 cm (width) × 5 cm (length) of bark was stripped off. Five ml volume of BAP paste from a syringe was applied to the stripped part. (**C**) Normal male strobili developed at the lower part of new shoots (Japanese black pine). (**D**) Numerous lateral female strobili developed on the lower part of new shoots treated with BAP (Japanese black pine) – by Wakushima (2004).

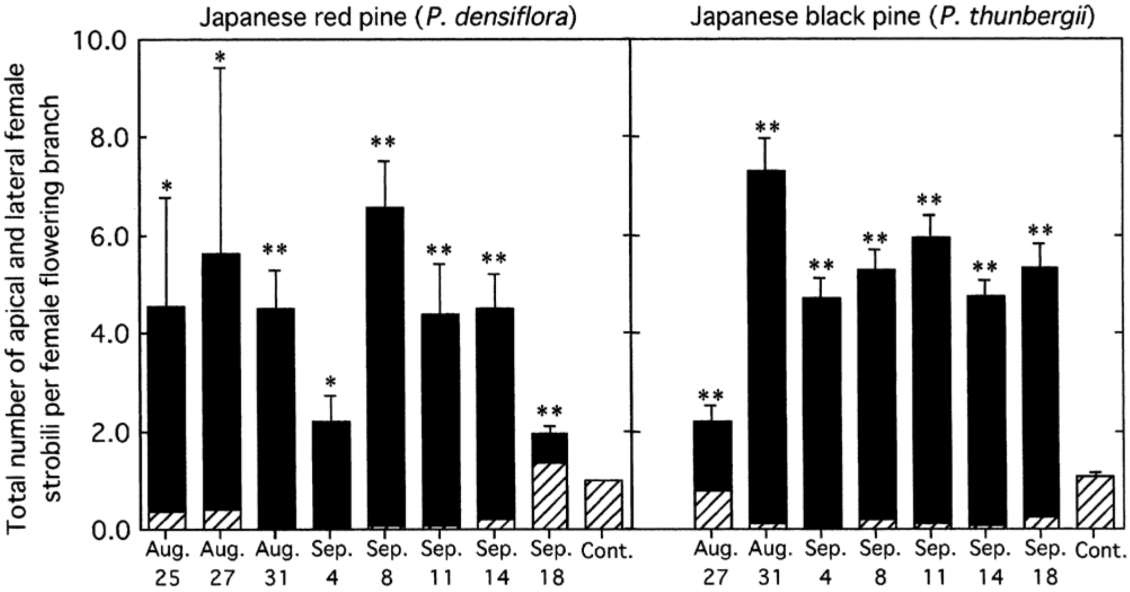


Figure 2 Total number of apical and lateral female strobili per female flowering branch by terminal bud treatment in Japanese red pine and black pine. Means of apical and lateral female strobili are shown by hatched and solid columns, respectively. Vertical bars indicate standard error – by Wakushima (2004).

Auxin

The term auxin is a Greek word meaning to grow. Compounds are generally considered auxins if they have functions similar to indoleacetic acid (IAA), the first auxin isolated from plants. The typical function is to induce cell elongation in stems.

Responses by coniferous trees to auxin induction vary by species. Auxins, such as naphthaleneacetic acid (NAA), are usually applied with GAs in order to enhance effects of GA effects (Pharis et al., 1980). In these combinations, low NAA concentration favored female flower development in Douglas fir, whereas high NAA concentrations stimulated male flower formation. Application of NAA alone decreased female flower formation in lodgepole pine (Wheeler et al., 1980) and in Chinese red pine (Sheng and Wang, 1990). However, application of NAA alone enhanced male flowering in Chinese red pine (Sheng and Wang, 1990). In combined applications, addition of NAA enhanced the $GA_{4/7}$ effect in loblolly pine but diminished it in slash pine and longleaf pine during male flower bud induction (Hare, 1984). No enhancement by GA was observed in either Douglas fir (Puritch et al, 1979) or white spruce (Beaulieu et al., 1998).

Abscisic acid

Abscisic acid (ABA) was initially discovered to play a role in leaf abscission in the autumn. ABA has been found to be a key factor regulating many physiological responses, including transpiration, stress response, germination of seeds and embryogenesis. ABA influences plant growth and development, but often by interactions that involve other plant hormone pathways. For optimal growth, plants require an optimal level of ABA. Plant growth suffers when ABA levels are too low or too high.

ABA is the major player in mediating the adaptation of the plant to stress. Drought treatments can enhance flowering and seed yields in both conifers and angiosperm trees, especially if they are applied during the period of shoot elongation. It has been known since the early 1980s that drought-treated conifers send ABA from their roots into their shoots within 10 h of such a treatment. Some studies show that bud differentiation may well involve, in part, a response to increased ABA metabolism. Other studies imply an interaction between cytokinins and ABA in these processes (Gille et al., 1990). Girdling and pruning are commonly used methods for enhancing flowering in conifers. These physical treatments may influence endogenous ABA concentrations in the stressed trees. It was found that flower induction by GAs also involved increased endogenous ABA concentrations. In Douglas fir, exogenous $GA_{4/7}$ enhanced flowering, while doubling ABA concentrations in GA-treated trees compared to controls (Pilate et al., 1990). ABA's role during flowering process has never been clarified to any degree. In a few limited experiments, exogenously applied ABA reversed the promotion of flowering by GAs in Sitka spruce (Tompsett, 1977).

Ethylene and polyamines

Ethylene is a gaseous plant hormone. It is well known for its role in fruit maturation. Ethylene is also produced during stress responses, such as water stress, tissue wounding, and high temperature, all of which are used in the conventional methods for cone induction. Enhanced flowering by ethylene in angiosperms is well documented and ethylene-releasing compounds have been applied to enhance flowering in agriculture plants (Abeles et al., 1992; Marie-Louise et al., 1998). Behaviour of flowering genes can be mediated by ethylene, such as ACS10 (Fig. 3) in *Arabidopsis* (Devlin and Kay, 2000). Historically, little effort has been made in application of ethylene directly for cone induction in Pinaceae. Initial tests, mostly in the 1970s, showed mixed results (reviewed by Bonnet-Masimbert and Zaerr, 1987). Ethrel, an ethylene enhancer, demonstrated a synergistic effect on GA_3 induction in *Cryptomeria japonica* and loblolly pine. Female cone was doubled by Ethyl in Norway spruce. However, Ethyl had no effect in Douglas fir.

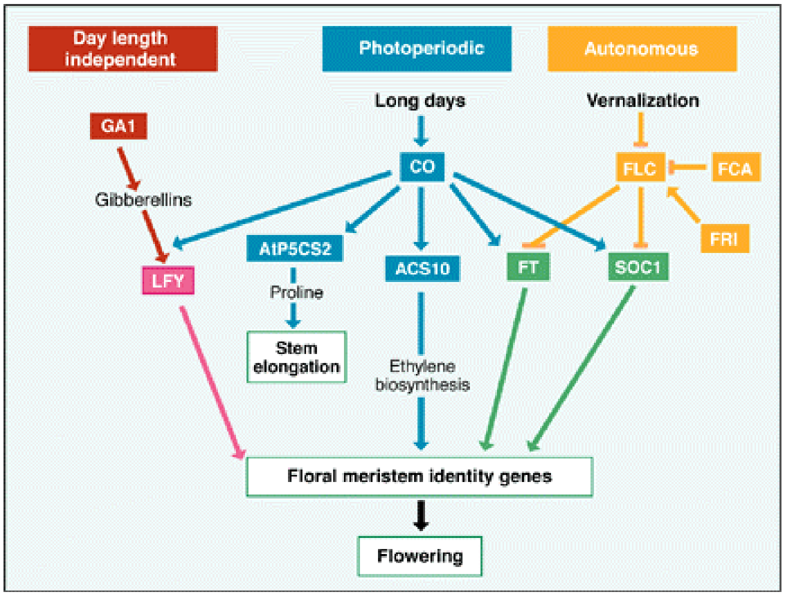


Fig. 3 Flower arranging in *Arabidopsis* by Devlin and Kay (2000).

Polyamines, mainly putrescine, spermine and spermidine are commonly found in higher plants. Both ethylene and polyamines share steps in their metabolic pathways and are important in flowering of angiosperms (Liu et al., 2006). Polyamines are known to influence floral induction and gender. Methylglyoxalbisguanylhydrazone (MGBG), a polyamine synthesis inhibitor, inhibited flowering, whereas application of exogenous spermidine reversed this effect (reviewed by Liu et al., 2006). Daoudi et al. (1994) reported that application of a combination of nitrogen fertilizer and GAs brought about marked changes in polyamines and amino acids.   At present, no information is available on effects of polyamines on flowering in Pinaceae.

Understanding hormonal mechanisms of cone flowering

Plant metabolomics

Practical manipulation of sex expression may be possible through different PGR combinations, selective crown pruning, treatment timing and/or photoperiod control, but the development of such techniques for operational use will require a better basic understanding of the endogenous regulatory mechanisms involved (Ross and Pharis, 1987). Unfortunately, endogenous hormone measurement was mainly limited to bioassay experiments and enzyme-linked immunosorbent assays and radioimmunoassays carried out in the 80s and early 90s. This work suffered from limitations in sensitivity. These have been overcome with the use of mass spectrometry. Few research groups have sufficient expertise to use these methods (Oden et al., 1987, 1994; Wang et al., 1992; 1995;1996; Zhang et al., 2001; 2003). Consequently, most hormone classes, including newer additions such as jasmonate, have not been studied in this respect.

This shortfall in research has been compounded by the singular physiological nature of conifers. The inductive role of GA is unique to conifers. By comparison, angiosperm trees, such as poplars and birches, are inhibited by GA; in a complete reversal of the situation in conifers, induction is possible with *anti*-gibberellins. As a result, expanding our knowledge of conifer tree physiology depends on direct experimentation rather than drawing on lessons learned from studying other trees.

Neutral gibberellins such as $GA_{4/7}$ have been used extensively in the Pinaceae whereas the Taxodiaceae and Cupressaceae have been more responsive to GA_3. Where GAs have been more closely studied using GC-SIM (gas chromatography single ion monitoring) differences between poor flowering clones and good flowering clones have been correlated to differences in GA metabolism: good clones lack GA_1 and GA_3 found abundantly in poor clones. The ratio of GA 9 to GA1 was ca. 10 fold higher in good clones than that in poor ones (Oden et al., 1994). Reproductive development appears to be regulated by GA_9 and its conjugated form, which both readily form GA_4, the form thought to play an important role in regulation. Limits in interpretation were encountered because of lack of sensitivity in the methods. Compounds that affect gibberellin metabolism, such as the inhibitors paclo-butrazol and prohexidione have also been used to study the gibberellin biosynthesis within elongating and differentiating shoots (Wang et al., 1995).

Hypotheses of cone induction

A variety of hypotheses exist (reviewed by Bonnet-Masimbert and Zaerr, 1987), including ones that claim an endogenous threshold must be met, others that propose interactions between different gibberellins, as well as interactions between gibberellins and each of the different classes of hormones (cytokinin, auxin, abscisic acid and ethylene). A limitation in all these hypotheses is that most hormones are too poorly studied for conclusions to be drawn concerning their importance in bud differentiation. A good example is the effects of BAP on cone induction. It was a long period of time before positive results were reported for this plant growth regulator; when it was finally applied at the right developmental stage it's potential became evident. This breakthrough provided a potential powerful method to enhance female cone flowering in Pinaceae. Effects of BAP on gender-conversion bring us a new hypothesis that the ratio of cytokinin to auxin during floral bud differentiation may affect flower gender, i.e. higher ratio for the female and lower for the male. This is supported by the facts that exogenous auxin alone reduced female flowering in lodgepole pine (Wheeler et al., 1980) and enhanced male flower formation in Chinese red pine (Sheng and Wang, 1990). Furthermore, the number of female strobili was negatively correlated with vegetative shoot length in Sitka spruce (Tompsett, 1977), Caribbean pine (Harrison and Slee, 1990) radiata pine (Sweet, 1979) and loblolly pine (Schmidtling 1981). Little and MacDonald (2003) found that exogenous IAA and NAA neither affect nor inhibit neoformed growth in terminal bud stem unit number I of Scots pine and white spruce. The authors suggested that the endogenous auxin concentrations were likely optimal or supra-optimal. Longer shoot length may result from higher endogenous auxin concentrations. If so, it lowers the ratio of cytokinin to auxin and suppresses female flower formation. This hypothesis needs further experiments for verification.

Fig. 5 A productive tree of lodgepole pine showing very short vegetative shoots. Note the similar apical position of female and male cones. Photo provided by Jack Woods.

Applications of PGRs

Timing PGR application

Stimulation in female or male flower bud differentiation not only relies on types of treatments but also on developmental stages of buds at time of treatments. Different results may result from the same treatment applied at different times (Greenwood, 1982; Ho and Hak, 1994). To determine the correct application time it is first necessary to establish which developmental stages are most sensitive to PGR treatments. Responses of plants to induction treatments vary by species and by PGRs. GAs were used before or during initiation stage of floral buds in order to enhance flowering, whereas cytokinins, such as BAP, were used at floral differentiation stage for converting sexual expression of male buds to female buds. Application of BAP to plants before or during the initiation stage did not influence sexuality, but only enhanced vegetative growth. If BAP was applied too late, i.e. after differentiation stage (Fig. 4), there was no effect at all. Thus, timing is more important than PGR application frequency (Wakushima et al., 1996, 1997; Wakushima, 2004). Once the targeted developmental stages are determined, the next important step is to be able to predict when these stages are reached. This is not an obvious process, as seasonal variations in temperature and rainfall can hasten or delay bud development. Improved methods usually include histological exam, the percent of shoot or needle elongation in relation to final lengths of these plant parts, and, finally, heat sum accumulation. Heat sum accumulation models were more accurate than percent of shoot or needle elongation and it is more practical than histological exam. The recommended heat sum accumulation model is the heat sum degree-days (Almqvist, 2003).

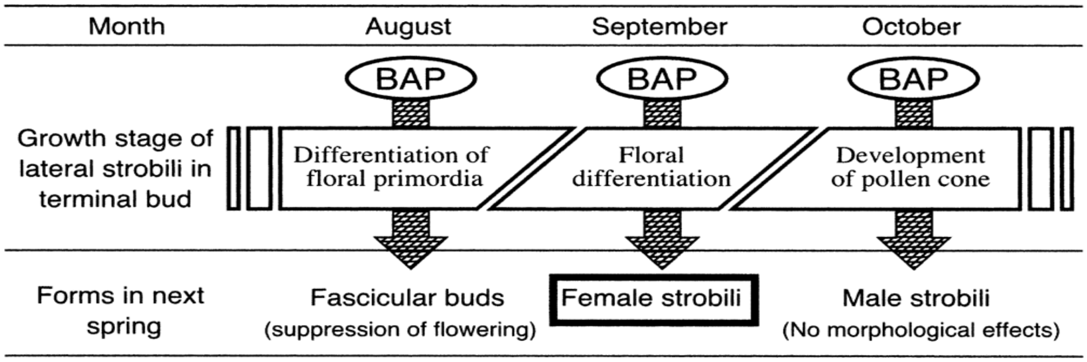


Figure 4. Relationships between the growth stage of lateral strobili and the morphological effects of BAP application. BAP treatment at the stage of floral primordia prior to the floral differentiation stage of two Japanese pine species transforms the lateral floral primordia into fascicular bud primordia. When the BAP treatment is done in October or thereafter, no floral sex transformation occurs because all lateral floral primordia are already differentiated into male strobili. Only BAP treatment during the floral differentiation stage induces sex transformation of lateral floral primordia into female strobili in the next spring. – by Wakushima (2004)

PGR quality and quantity in applications

The quality of the chemicals can make a difference. Of all the synthetically produced chemicals (e.g. NAA, BAP and various GAs), it is gibberellins that are the most notoriously variable. A mixture of GA_4 to GA_7 is commonly used for cone induction. The ratios of GA_4 to GA_7 vary on different supplements sources. Trace amounts of other GAs such as GA_1, GA_3 and GA_9 may exist in the mixture. Generally, GA_7 is more effective than GA_4. GA_4 was inactive in floral induction in Sitka spruce (Tompsett, 1977). Total amounts of applied PGRs must be determined in relation to the size of the trees (i.e. larger tree, more PGR), after which concentrations must be calculated in relation to the application method, e.g. stem injection or foliar spray.

PGR application methodology

Application methods of PGRs include foliar spay (water, ethanol and etc. as solvents), topping, paste, bud injection, and stem injection etc. Applications of PGRs are usually combined with other methods such as girdling, pruning, drought, and/or fertilization.

Application frequency and duration

Bud initiation and development is generally not synchronized. In order to maximize induction effects genotype variation must be taken into account. Exogenous PGR applications are expected to last for a period of time. The duration can be augmented either by multiple applications of PGRs or by using slow-release methods.

Current situation of cone induction in Douglas fir and lodgepole pine

Douglas fir has been the object of many papers published by reputable physiologists. Greater advances have been made on floral induction in Douglas fir than most other conifers (Puritch et al., 1979; Pharis et al, 1980; Ross et al., 1980; Ross and Bower, 1989; 1991). $GA_{4/7}$ plus GA_9 was reported the best hormonal treatment (Pharis et al, 1980; Pharis, 1991). However, a single injection of $GA_{4/7}$ plus stem girdling was the method that proved to be the most economically effective. This treatment increased floral induction by 1.9- to 5.6-fold over controls in different experimental years (Ross and Bower, 1991). Although this produced many more cones, a noted effect was that after GA and girdling treatment in Douglas fir a slight decline was found in cone size, seed per cone and percentage of filled seeds. Percentage of filled seeds ranged from 44% to 53% in all the treatments and control (Ross and Bower, 1991).

Major drawbacks of seed production in lodgepole pine included poor cone yield and cone drop. Cone drop occurred after pollination and during early embryo development. This was possibly caused by unsuccessful pollination or self-pollination (Owens and Molder, 1984). Two decades ago, a few reports were published on floral induction in lodgepole pine (Pharis et al., 1975; Wheeler et al. 1980; Longman 1982). Flower buds were increased in lodgepole pine by foliar spraying $GA_{4/7}$ alone or $GA_{4/7}$ plus NAA (Wheeler et al., 1980). Highly significant clonal variation occurred in response of lodgepole pine to exogenous GAs (Longman 1982). Since 1982, no further research has been published.

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