

### 3. CONTROL OF SEX EXPRESSION IN CONIFERS

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#### ABSTRACT

Despite considerable progress in recent years in the promotion of flowering in conifers, relatively little is known about the endogenous control of sex expression or its practical manipulation to aid pollen and crop management in seed orchards. Well defined patterns of sexual zonation exist within the tree crown and shoot, and these appear to be associated with hormonal and possibly nutritional gradients. No firm conclusions, however, are possible regarding the specific role(s) of different PGRs in sex expression, this being as much as a problem of lack of critical study as the complexity of the process itself. Whether a PGR such as gibberellins or auxins, or a cultural treatment that itself may influence endogenous hormone levels, primarily promotes female or male flowering often depends on the position within the crown of the shoot being treated, and its timing in relation to stage of primordia development. Photoperiod also may be involved-- short days favoring females and long days males, at least in some species-- although its role in controlling sex expression is by no means clear. Practical manipulation of sex expression may be possible through selective crown pruning, different PGR combinations, 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.

#### 1. INTRODUCTION

The control, hormonal and otherwise, of sex expression in conifers, and especially those commercially important species of the Pinaceae, has received little attention. This is no doubt due to the general, and heretofore more pressing problems, of overcoming a delayed and inadequate flowering. However, promotion of early and enhanced flowering is now possible for many, if not most conifers through the properly timed application of specific gibberellins (GAs), usually administered with adjunct cultural treatments (e.g. nitrogen fertilization, root-pruning, girdling, drought and heat) [see Chapter 2 by Bonnet-Masimbert and Zaerr; also reviews by Owens and Blake (85) and Ross and Pharis (115,116)]. Except in very young seed orchards, where pollen production naturally is sparse, the proper species-dependent GA<sub>4/7</sub> + cultural treatment will generally provide a good balance of female and male strobili.

However, with the growing interest in artificial pollination, both to improve seed yields and to maximize genetic gains through control of pollen parentage, an ability to regulate the sex of strobili produced becomes increasingly important. Sweet and Krugman (131) discussed the advantages of maintaining separate seed and pollen orchards, with the latter being situated

in warmer climates to ensure that pollen matures earlier, thereby allowing time for its collection and processing before the females become receptive. The ability to specifically promote female or male strobili on different individuals will be even more valuable in containerized seed orchards, which presently are being evaluated as a cost-effective alternative to conventional field orchards (9,117).

The literature on sex expression in conifers is presently confused and yields few firm conclusions about its regulation by the plant per se or by environmental factors. Nor is its possible practical control through plant growth regulator (PGR) application or other means clearly discussed in the literature. In this chapter we review the spatial and temporal patterns of sex expression common to representative conifer species, and consider their possible hormonal, nutritional and environmental bases. We also identify promising areas for future research.

## 2. PATTERNS OF SEX EXPRESSION

Nearly all conifers are predominantly monoecious, although within any species are to be found certain genotypes that produce only female or male strobili [see Owens and Hardev (86)]. And, even within monoecious individuals, there exist spatial and temporal patterns of sex expression that transcend most species and family boundaries (86). A better knowledge of these patterns can not only provide insight into the physiological basis of sex expression, but may also give us the practical means for its control. On the other hand, failure to take these general patterns into account when sampling or designing experimental treatments has been a confounding factor in many studies.

### 2.1. Spatial and Ontogenetical Patterns

It is true of most conifers that, following a so-called "juvenile" phase of strictly vegetative development lasting several years to several decades, depending on the species [see discussion in Zimmerman *et al.* (147)], regular female flowering precedes regular male flowering by several years (32,33,140,147). Occasionally, one finds examples of extremely precocious male flowering at ages of one year or less from seed. Although this appears to be an accidental phenomenon in response to some unique inductive condition, a better understanding of its control could be very useful in designing manipulative treatments. Such precocious male-flowering individuals will usually revert to the non-flowering condition and the normal sequence of regular female then male flowering (33).

As with all generalizations there are exceptions. For example, in the Pinaceae regular male flowering typically occurs first in *Pinus radiata* (G.B. Sweet, per. comm.), and we have observed a similar tendency for many Cupressaceae and Taxodiaceae conifers.

In an interesting experiment Giertych (32,33) compared the development on seedling rootstock of grafted scions representing 'strong' and 'weak' shoots (with regard to vegetative vigor) from, respectively, the female and male crown zones of a mature *Pinus sylvestris* tree. Contrary to expectation, the "male" scion did not begin producing male strobili before female strobili. Nor did they come into male flowering at an earlier age than the "female" scion. Both scion types exhibited the typical pattern of regular female followed by male flowering, but the transition occurred more rapidly in the female scion concomitant with their also more vigorous vegetative development. Giertych (34) concluded that factors other than ontogenetic aging were thus responsible for the within-crown pattern of sex expression, and principal among them was the shoot's vegetative vigor.

Development of patterns of sexual zonation within the tree crown is similar for most conifers (61,70,118,120,140). As the tree matures and increases in size, female strobili are initiated/differentiated first on strong leading shoot in the upper crown. In some species (e.g. *Pinus* and *Tsuga*), this may include the main-stem terminal, whereas in others (e.g. *Picea* and *Pseudotsuga*) female strobili are first produced on somewhat less vigorous lateral shoots of the current-year leader, and on the terminal shoots of subtending first-order branches. With increasing age of the tree, the zone of female flowering extends downward and initially inward to include still vigorous shoots at the base of the branch. However, with further increasing branch age and declining vigor of some higher-order shoots, the zone of female flowering again retreats outward to include usually only the most vigorous distal shoots. Female flowering, in fact, usually terminates in the lower third of the crown in older trees. Development of the male zone follows the reverse pattern, male strobili being produced first on less vigorous shoots in the distal region of lower branches. But, as the tree ages their production extends upward and inward, usually terminating in the middle crown. There is commonly a transition zone where both types of strobili occur on the same branch, and in some species even on the same internodal shoot, but with females always in the more distal position. Again there are exceptions. Nagao et al. (81) note the existence of some unusual *Chamaecyparis obtusa* clones which bear female strobili in the lower crown and male strobili in the upper crown.

Sexual zonation generally is most pronounced in poor flowering individuals and years. In good flowering years and in response to successful induction treatments, the female zone extends inward and downward, sometimes to the base of the crown, and the male zone outward and upward (67,81,82, 118).

## 2.2. Mode and timing of differentiation

Owens and Blake (85) describe the mode and timing of differentiation of female and male strobili for many north temperate conifers (see Fig. 1). In *Pinus*, female strobili differentiate from lateral long-shoot primordia (potential branch buds) and male strobili differentiate from short-shoot primordia (potential needle fascicles). There are exceptions, however, such as rare cluster-cone variants in which female strobili differentiate from previously undetermined short-shoot primordia, or by the transition of an existing male strobili [see Rudolph et al. (121)]. In *Tsuga*, female strobili generally result from transition of an existing terminal vegetative apice, whereas male strobili may also occur this way but more frequently from differentiation of previously undetermined axillary primordia. Both types of differentiation also occur in *Picea*, but with the terminal mode predominating for females and the axillary mode predominating for males. In *Pseudotsuga*, strobili of both sexes differentiate from previously undetermined axillary primordia-- female strobili from distal primordia that would normally become vegetative branch buds, and male strobili from proximal primordia that would otherwise abort or remain latent.

The mode of strobilus differentiation in *Abies* is similar to that for *Pseudotsuga*, with the exception that vertical as well as horizontal orientation of an axillary primordia on the shoot can determine its developmental fate. Thus, female strobili tend to differentiate from primordia on the dorsal (upper) side of the diageotropic shoot, and male strobili from primordia in the ventral (lower) position. A similar situation exists for some species of *Larix*, although here strobili of both sexes generally occur on dwarf (short) shoots as a result of the transition of a

previously vegetative terminal bud (61,77). Interestingly, gravimorphic treatments only appear to influence flowering and sex expression in these two genera. In *L. leptolepis* and *L. kaemferi*, tying horizontal branches in a vertical position promotes female flowering, whereas bending vertical branches downward promotes male flowering (61,62). Similarly, bending *A. sibirica* shoots so that the normally lower side faces upward causes axillary primordia in that position to differentiate as female rather than as male strobili (77).

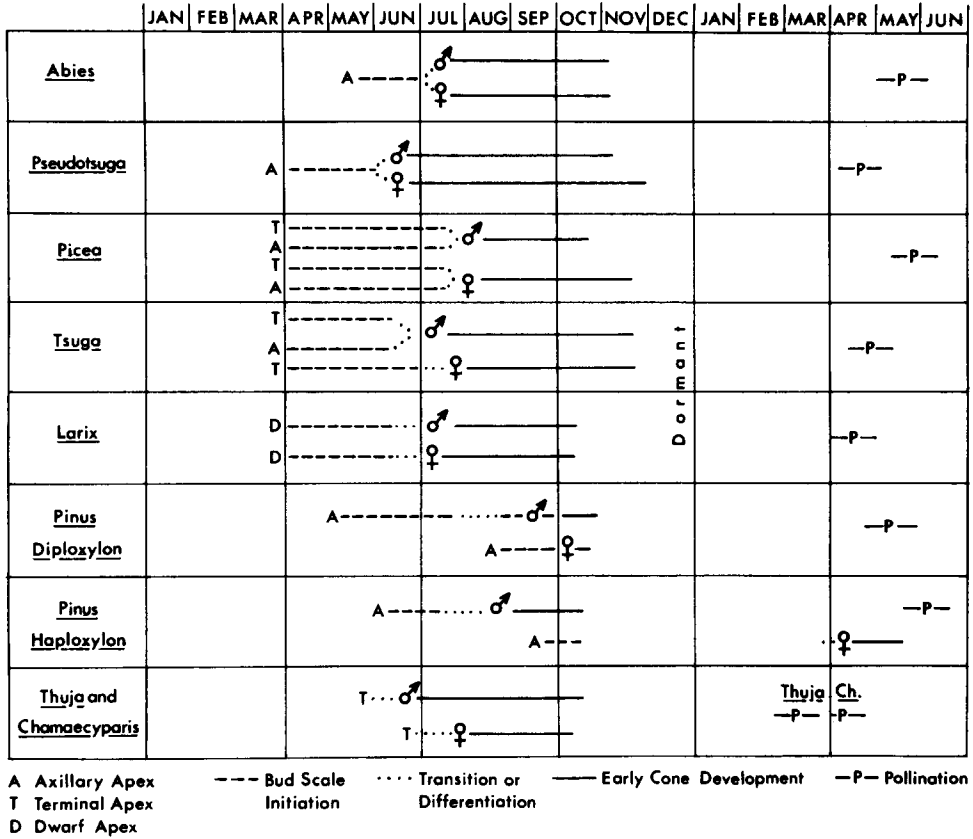


Figure 1. Times and modes of strobilus initiation in conifers [from Owens and Blake (85)].

Durand and Durand (21) concluded for angiosperms that "the male program is always expressed first, then the female one". Owens and Hardev (86) note that this applies to conifers as well if considered in a seasonal context (Fig. 1). This is very pronounced in *Pinus*, *Tsuga* and Cupressaceae and Taxodiaceae species, where on a given shoot male strobili are differentiated earlier in the season than female strobili, a fact confirmed by many timing trials using N fertilization and PGR treatments (16,48,81,82,127).

In *Larix*, *Picea* and *Pseudotsuga*, strobili of both sexes appear to differentiate at about the same stage of shoot development. Yet, even for these species the optimal time for promoting differentiation generally is

earlier for male than female strobili (6,17,115,116,136). Owens and Hardev (86) speculate that this is because male strobili usually differentiate on weak shoots in the lower crown which complete their seasonal development earlier than those more vigorous, upper-crown shoots on which female strobili are typically borne. The importance of positional effect is indicated by studies on *Tsuga heterophylla* (118) and *Larix* species (6), in which the optimal time for the  $GA_{4/7}$  promotion of female flowering was shown to be earlier for branches in the lower than upper crown.

### 2.3. Sex transformations

Although the reproductive structures of conifers are normally unisporangiate, bisporangiate and proliferated strobili occasionally occur in nearly all species (11,48,86,94,121,137). These range from mainly vegetative (Fig. 2A) to mainly female (Fig. 2B), and include all gradations of male>female transformations (Fig. 2C). Female>male and male>vegetative transformations also occur but more rarely. Such transformations may occur spontaneously as a more or less normal event in young trees entering sexual maturity (11, 137), but more commonly in response to promotive stresses (3,25), pruning (48) and PGR treatments (48,69,94; S.D. Ross and R.F. Piesch, unpubl.).

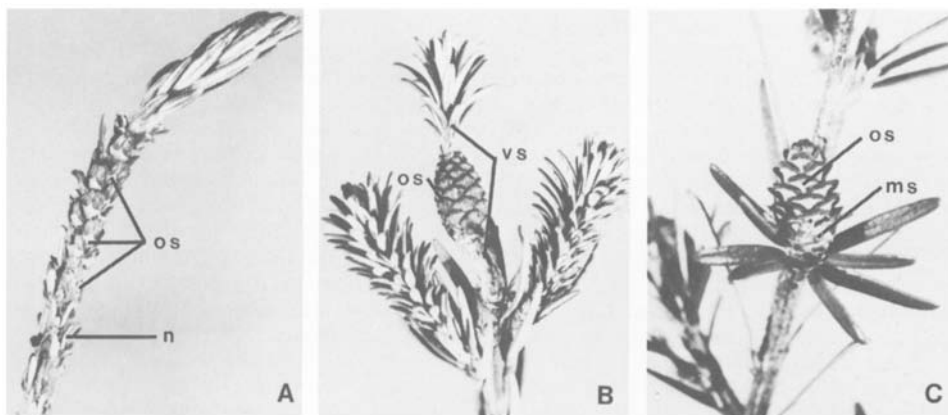


Figure 2. Examples of sex transformations produced by *Tsuga heterophylla* rooted cutting following treatment with  $GA_{4/7}$ . Fig. 2A. Rudimentary ovuliferous scales (os) interspersed among incompletely developed needles (n) along a vegetative shoot. Fig. 2B. Transformation of a vegetative shoot (vs) into a normal appearing female strobilus in which the apical meristem remained vegetative and produced a second growth flush. Fig. 2C. A bisporangiate cone in which megasporangiate sporophylla (ms) were initiated first followed by ovuliferous scales (os).

Sex transformations have long been a subject of anatomical interest, but their real significance is to illustrate the developmental plasticity of apical meristems during differentiation. Proliferated strobili are most likely to occur at the vegetative-female and vegetative-male transition zones in the tree crown, and bisporangiate cones in the male-female transition zone. Pharis and Morf (94) describe for *Thuja plicata* a bisporangiate strobili that started off female while on a vigorous shoot in the middle

crown, but then became male (female development was arrested) as the branch became part of the lower crown.

Figure 2A shows a proliferated shoot that developed on a GA<sub>4/7</sub>-treated *Tsuga heterophylla*. It began by initiating needle primordia (seen at the base), followed by megasporangiate primordia interspersed with needle primordia, and finally again only needle primordia. We attribute this unique development to a variable uptake of GA<sub>4/7</sub> throughout the prolonged differentiation period. Although GA<sub>4/7</sub> was applied by continuous stem injection, the injection hole rapidly became occluded and a new hole had to be drilled every 2 weeks over the 6-week treatment period [see Ross et al. (118)]. Thus the timing may have been such that the GA<sub>4/7</sub> dose was relatively low during the early and late stages of differentiation (resulting in vegetative development), but became high enough during the middle period to promote the formation (albeit sporadic) of ovuliferous scales (Fig. 2A). A similar situation may have existed for the shoot in Figure 2B, except that it was exposed to sufficient GA<sub>4/7</sub> for normal female strobilus development for a longer period midway through differentiation.

### 3. ROLE OF NUTRIENT STATUS AND VIGOR

#### 3.1. Carbohydrates and nitrogen

Female strobili generally occur on vigorous shoots in the exposed upper crown and male strobili on suppressed, usually lower shoots that often but not always grow in shade. This has led to the suggestion that a high photosynthetic rate favors female flowering, and conversely a low carbohydrate status favors male flowering (34,140). Although trees grown in dense shade may produce only male strobili (36,73,123), it is also well known that the production of both female and male strobili is maximized under conditions of wide tree spacing and associated high light intensities (34,70). The specific effect of light intensity on sex expression was investigated for *Chamaecyparis obtusa* under controlled-environment conditions (80). Nagao and Sasaki (80) confirmed that the threshold light intensity required for initiation and development of male strobili (12 klux) was significantly lower than that for female strobili (21 klux), but also demonstrated that production of strobili of both sexes was greatest under the highest light intensity.

Silen (129) observed that enclosing *Pseudotsuga menziesii* shoots in white cloth bags (from 6 weeks before until 8 weeks after vegetative bud burst in spring) inhibited female flowering while increasing the production of male strobili. Temperatures, however, were up to 6.6°C higher in the bags, and Ross (110; unpubl.) found that comparably timed heat treatment in a polyethylene-covered house can have a similar effect on sex expression in this species. Changes in light quality may also be involved in the response to bagging. Hashizume (48) found that placing *Pinus densiflora* shoots in brown paper bags caused potential female strobili to apparently revert to male, whereas the reverse transformation occurred within red paper bags.

Removal of the photosynthetic apparatus by defoliation has stimulated male and inhibited female flowering in *P. sylvestris* (32), but a similar approach caused feminization of potential male strobili in *P. thunbergii* (48). A solely nutritional hypothesis also is difficult to reconcile with the observation that girdling, a treatment that may be expected to cause an accumulation of carbohydrates above the phloem blockage, will often preferentially promote male flowering (64,75,96,143). Thus, as with floral initiation/differentiation in general [see Ross and Pharis (115)], a direct morphogenic role for carbohydrates in sex expression of conifers remains unproven.

Wareing (140) has proposed that a low N status (i.e. high C/N ratio) favors male flowering, whereas a higher N status is necessary for production of female strobili. Reports of profuse male flowering in young, potted seedlings of *Cryptomeria japonica* (66), *Cupressus arizonica* (59) and *Pinus radiata* (132) in response to induced N deficiency are consistent with such a hypothesis. However, for all three species male flowering is more precocious than female, and in each of these studies the plant material was relatively young. Hence, a non-specific response of N deficiency on flowering per se-- as opposed to sex expression-- cannot be ruled out. Interestingly, in another study where *C. japonica* seedlings also received GA<sub>3</sub>, NH<sub>4</sub><sup>+</sup>-N promoted only male flowering and NO<sub>3</sub><sup>-</sup>-N only female flowering (82).

There are reports that N fertilization has promoted only female flowering, with the sometimes inhibition of male flowering, in *Pinus* species (35,138) and *Cryptomeria japonica* (82). However, the treatment has promoted male flowering in other studies on *Pinus* (1,32,50,125), sometimes to a greater extent than female flowering (127). Further adding to the confusion is Mergan's (74) finding that application of liberal doses of NPK fertilizer increased the frequency of female to male transformations in *P. griffithii* x *P. strobus* seedlings. Schmittling (127) noted that the optimal time for promotion of male flowering in *P. taeda* by ammonium-nitrate was earlier than that for promotion of female flowering, and thus timing of N treatment may partly account for these conflicting results.

Barnes and Bengtson (1) also questioned that a low shoot N content might favor male flowering in *P. elliottii*. They note that even though male flowering was inhibited by ammonium-nitrate treatment, there was no relationship among non-fertilized trees between the shoot content of N and production of male strobili. Similarly, in the dioecious angiosperm papaya (*Carica papaya*), N fertilization may increase the percentage of female plants and also causes sex reversal in male plants; yet mature leaves from male plants can have higher N contents than those from female plants (30). It thus appears that if the nutritional status of shoots has a role in sex expression it may be indirect through effects on bud vigor and/or hormonal status [see Ross and Pharis (115)].

### 3.2 Bud vigor

Among the first to notice the involvement of vigor in sex expression was Meehan [cited by Mergen (74)], who proposed that the sex of a strobilus is determined by the vigor of the branch on which it is formed. Tompsett (134) also concluded that there exists a causal relationship between the early vigor or meristematic activity of a primordial shoot and its developmental fate in *Picea sitchensis*. In that species and in many other Pinaceae conifers (86,108), the probable development of a primordial shoot appears to change as follows with decreasing early vigor: strong vegetative > female strobilus > intermediate vegetative > male strobilus > weak vegetative.

Exogenous GAs are known to stimulate subapical meristem activity in many higher plants (54), and Tompsett and Fletcher (136) suggested that this may be the mechanism by which GA<sub>4/7</sub> treatment promotes flowering in conifers. Thus, whether exogenously applied GA<sub>4/7</sub> promotes female or male flowering, or continued vegetative development in a primordial shoot, depends on the new vigor class into which the treated shoot is elevated.

Girdling, rootpruning and drought are all "stress" treatments that retard, at least temporarily, shoot elongation and bud development (41,46,62,89,90,142). As was previously noted for girdling, rootpruning and

drought also tend to preferentially promote male flowering (10,23,117,120), except in young, vigorously growing trees where female flowering only may be increased (70,118,120). This is in agreement with Tompsett and Fletcher's (136) hypothesis, which predicts that stress treatments should preferentially promote female flowering in young tree where a majority of shoots fall into a "high vigor" category, but mainly male flowering in older trees where most of the shoots are of an intermediate vigor.

In Pseudotsuga menziesii, the promotion of flowering by calcium nitrate fertilization may be accompanied by either an enhanced (24) or a decreased (115) rate of shoot elongation, apparently depending on the tree's physiological status and dosage applied. Thus, given the variable influence of N fertilization on vegetative vigor, an inconsistent effect on strobilus sexuality is not surprising.

The variable response to top and branch pruning [see Ross and Pharis (115)] also seems amenable to explanation by a bud vigor hypothesis. Where only the main-stem terminal and leading shoots of vigorous upper-crown branches are pruned, production of female strobili on subtending branches is invariably depressed, even though these shoots are in the normal female region of the crown. However, the shoots are moderately vigorous, retaining the capacity to respond to the top pruning with greatly enhanced vegetative growth. And, when released from apical control, their very vigorous growth appears to predispose them to a reduced ability to differentiate female strobili. However, vigorous vegetative growth is not totally antithetical to flowering, since the GA<sub>4/7</sub> promotion of female flowering frequently is accompanied by enhanced vegetative bud and/or shoot elongation (46,60,108,142).

On the other hand, a severe top pruning which removes the upper third to half of live crown may actually increase the production of female strobili for the tree as a whole (71,83,111). In this instance the residual crown is comprised of many intermediate-vigor shoots in what was previously the female-male transition and male zones. Such shoots will respond to top pruning with only a moderate increase in vegetative vigor, and this apparently is sufficient to predispose them to differentiate female strobili.

Pollen production, in turn, may be depressed by this severe top pruning treatment, as the weaker shoots on which male strobili normally are borne are elevated to a vigor class where they remain vegetative or begin producing female strobili. But there exist numerous other shoots in the lowermost crown that are normally too suppressed to produce even male strobili. Top pruning can affect a portion of these, but an even more effective treatment appears to be a terminal pruning or disbudding of the lower branches themselves (48,73). A combination of annual top and side pruning has proved highly effective in increasing both female and male flowering potential in potted and field-grown Tsuga heterophylla orchard trees (111).

Despite its attractiveness in explaining the effects of various treatments on sex expression in conifers, there is little direct evidence for the bud vigor hypothesis. We (97,99,115,119) review elsewhere the evidence that GAs of a less polar nature play a direct morphogenic role in flowering of conifers, a role that is independent of any effect on bud vigor or nutritional status. Furthermore, it now appears that the flowering response to many cultural treatments may be, at least in part, hormonally mediated. Promotive drought, rootpruning, girdling, heat and



nitrate fertilization treatments have all been shown to result in elevated endogenous levels of less polar GAs in shoots at time of strobilus differentiation (18,20,96,97,141).

#### 4. ROLE OF PLANT GROWTH REGULATORS

##### 4.1. Lessons from angiosperms

Sex expression in many angiosperms appears to be under strong control, and to involve the interactions between various classes of PGRs, including GAs, auxin, cytokinins, ethylene and abscissic acid (ABA) [see reviews by Vince-Prue (139), Pharis and King (92) and Sladky (130)]. While the analogy between angiosperms and gymnosperms with regard to sex expression is no doubt fraught with exceptions, there are many similarities and some valuable lessons for manipulating the sex of conifers to be learned from our experience with angiosperms. For example:

- (i) A PGR may function either as an apparent male or female "sex hormone" in different species or even cultivars of the same species. GAs are quite variable in this regard (92,139), but differential responses have also been reported for cytokinins and ABA (27,28). High auxin and ethylene levels generally are associated with femaleness, although again there are exceptions (92,130).
- (ii) The effect of a PGR on sex expression may vary with the plant's developmental stage (78). This is most common with monoecious species which initiate male and female flowers at different stages of ontogeny (92).
- (iii) Timing of PGR application in relation to stage of floral development is important. For example, in corn the tassel may become pistillate, male sterile or remain male fertile as  $GA_3$  treatment is applied progressively closer to the onset of microspore meiosis (45).
- (iv) The female or male role of a PGR may be concentration dependent and also dependent on the presence of other applied PGRs and/or environmental factors, such as photoperiod, which may influence endogenous hormone levels (27,28,92,139).
- (v) PGRs may influence the sex ratio directly through the sex of flowers initiated or indirectly, either through differential abortion of stamens or pistils, or by altering the growth and branching pattern in such a way as to favor the production of flowers of one sex over another (92).

These lessons learned from angiosperms no doubt apply to the study of PGRs in relation to sex expression in conifers. When applying PGRs or sampling tissues for endogenous contents the temporal and spatial patterns of sex expression for the species in question must be taken into account. Whereas usually the PGR is applied at only one concentration, a graded series of concentrations will probably be necessary to establish its effect on sex expression. It is furthermore necessary to examine the interactions between different PGRs (GAs and auxin especially), as sex expression may be controlled more by their balance than the absolute level of any one PGR. Photoperiod and temperature can also influence sex expression (discussed later), and should therefore be taken into account in designing treatments.

When assessing treatment responses it is just not sufficient to count the female and male strobili initiated. These should be closely examined for differential abortion (and the possible timing of its occurrence); for changes in their normal distribution along the shoot and within the crown; and for the occurrence, type and location of any sex transformations. Finally, close attention should be given to changes in growth rate and branching patterns that may indirectly influence the sex ratio.

Unfortunately, the above criteria are seldom met, and consequently the existing literature provides a very confused understanding of the potential role and practical uses of PGRs to control sex expression.

#### 4.2 Gibberellins

As already noted, there is compelling evidence that GAs, especially those of a less oxidized or polar nature (e.g. GA<sub>4</sub>, GA<sub>5</sub>, GA<sub>7</sub>, GA<sub>9</sub>), play a direct morphogenic role in floral initiation/differentiation in conifers (17,20,92,93,96,97,99,115,119,141). A role for GAs in sex expression is also implied. In the Cupressaceae and Taxodiaceae, the threshold concentration of GA<sub>3</sub> required to initiate male strobili is lower than that for female strobili, whose absolute number and proportion, however, increases with increasing concentration (47,62,63,81,82,93,94). In *Thuja plicata*, it appears as well that the minimum duration of GA<sub>3</sub> treatment required to induce male flowering is shorter than that for inducing females (S.D. Ross, unpubl.). Treatment with GA<sub>3</sub> was reported by Hashizume (48) to cause masculinization of female strobili in *Cryptomeria japonica* seedlings. However, timing of the GA<sub>3</sub> treatment can also determine whether male (June-Aug. application) or female (Aug.-Sept. application) flowering is primarily promoted in this species (47,82,122).

The role of GAs in sex expression of Pinaceae species is less clear. Bioassays of extracts from male versus female clones of *Picea abies* (53) and *Pinus sylvestris* (141), crown zones in *P. sylvestris* (56), and shoot regions in *Abies sibirica* (77) indicate that in these species, as in Cupressaceae and Taxodiaceae, male flowering is associated with higher levels of endogenous GAs as opposed to female flowering. However, the responses to exogenously applied GA<sub>4/7</sub> do not necessarily support this conclusion.

In several studies with *Pseudotsuga menziesii*, treatment with GA<sub>4/7</sub> alone has preferentially promoted female flowering (8,98,107,108,110), although there are exceptions (72,105). Timing of treatment has not affected the sex ratio in this species, although increasing the dosage of GA<sub>4/7</sub> can increase the proportion of male strobili initiated (108).

Treatment with GA<sub>4/7</sub> alone also has primarily promoted female flowering in *Tsuga heterophylla* (117,118). However, by using adjunct cultural treatments (e.g. drought, rootpruning or heat) plus GA<sub>4/7</sub> it is possible to obtain adequate male flowering (10,103,117,118). Male strobili differentiate 2-3 weeks before female strobili in this species (Fig. 1), and it is possible that a very early GA<sub>4/7</sub> treatment (as vegetative buds are beginning to swell) will preferentially promote male flowering (A. Colangeli, per. comm.).

The situation with regard to GA<sub>4/7</sub> effects on sex expression in *Pinus* is somewhat confused. In *P. sylvestris*, GA<sub>4/7</sub> treatment timed to the normal periods of male (early) and female strobilus differentiation (late) has primarily promoted that sex (16; O. Luukkanen and R. Hagqvist, per. comm.). The optimal GA<sub>4/7</sub> application times also differed for promotion of male and female flowering in accelerated-growth *P. banksiana* seedlings, although not in a manner that seemed to relate to the known timing of strobilus

differentiation in this species (13). On the other hand, treatment with GA<sub>4/7</sub> alone has only promoted female flowering in P. taeda, regardless of age<sup>4/7</sup> of trees, cultural conditions or timing and application dosage (42-44,112).

In Picea, exogenously applied GA<sub>4/7</sub> has tended to preferentially promote female flowering, both in seedlings and<sup>4/7</sup> mature trees (19,64,68,70,100,135). However, it is possible that in many of the studies the GA<sub>4/7</sub> was applied past the optimal time for influencing male flowering (15,109,136), or was applied to that portion of the crown with an inherently high potential for producing female strobili. Using mature field-grown P. glauca trees, Marquard and Hanover (1984a), found that GA<sub>4/7</sub> promoted primarily female flowering when applied to the female-male transition<sup>4/7</sup> zone but male flowering when applied to the lower, strictly male zone.

Bonnet-Masimbert (6) reported a similar differential response by upper- and lower-crown branches of Larix leptolepis and L. decidua to applied GA<sub>4/7</sub>. However, both in his study and Katsuta's (55) on L. leptolepis, the GA<sub>4/7</sub> elicited a relatively greater female response for the tree as a whole, even though the treatment apparently was more optimally timed to influence male strobilus differentiation.

Given the experience with angiosperms (see above), it is not surprising that exogenously applied GAs can affect the sex ratio differently in different conifer species and even in different studies on the same species. Although it appears that GAs may preferentially promote male flowering in some conifers (e.g. Cupressaceae and Taxodiaceae) and female flowering in others (e.g. Pseudotsuga and Tsuga), there are yet others (e.g. Pinus, Picea and Larix) for which this PGR does not seem to specifically influence sex expression. Thus, it may be as Chalupka (14) suggests, that GAs function as a triggering agent to induce initial changes in the apical meristem which lead to reproductive as opposed to vegetative development. Whether the GA-promoted meristem differentiation occurs in a female or male direction is then a function of other factors, such as crown position, timing, photoperiod, and/or interaction of the GA with other PGRs.

#### 4.3. Auxin and ethylene

Heslop-Harrison (51) notes that sex expression in many plants may be controlled by auxin gradients originating in the stem and branch apex during the course of normal differentiation. Ethylene, well as auxin, seem to have a relatively consistent role in sex expression in herbaceous and woody angiosperms (130,139)-- high contents of each being associated with femaleness. It is known that exogenously applied indoleacetic acid (IAA) can increase ethylene production in vegetative tissues of several higher plants, and the degree of stimulation increases with increasing auxin concentrations (52). This effect of exogenous auxin is apparently brought about by increasing the activity of ACC-synthase (ACC= 1-aminocyclopropane-1-carboxylic acid), an immediate biosynthetic precursor of ethylene. The auxin- increased ACC synthase activity can be synergized by a cytokinin (BA) and reduced by ABA (52). Hence, even the relatively clear-cut effect shown by auxin and ethylene may involve other PGRs.

Some work with Pinus species also has noted a high endogenous auxin content of shoots from the crown region that is normally female, relative to the region that is normally male (35,48), and similarly clones that tend to produce mainly female relative to male strobili (56,58,141). In Abies sibirica, Minina and Belyaev (76) found that the upper, female side of the diageotropic shoot had a higher endogenous auxin content than the lower, male side.

In the Cupressaceae and Taxodiaceae, exogenously applied auxins have generally but not always promoted femaleness (48,61,63). Ethrel, an ethylene-generating compound, also has had variable effects on the sex ratio when given with GA<sub>3</sub> (4,49). Bonnet-Masimbert (4) found with Cupressus arizonica that Ethrel<sup>0</sup> stimulated female flowering under photoperiods (short day) favoring femaleness but males under long days favorable to male flowering. Nagao et al. (81) reported that Ethrel enhanced both the female and male response to GA<sub>3</sub> in Chamaecyparis obtusa.

Some early reports indicated that exogenously applied auxins will increase the proportion of female strobili produced in Pinus (48,93) and Larix (48). However, in subsequent studies on Pinaceae species, auxins alone generally have had little promotive effect on flowering (15,75,10,105).

On the other hand, auxins can modify the flowering response by some Pinaceae species to applied GAs. Contrary to the situation in Cupressaceae, and also the evidence for endogenous shoot contents (see above), there is some evidence that auxins [naphthaleneacetic acid (NAA) being more effective than IAA] can preferentially enhance the male response to GA<sub>4/7</sub> in Pseudotsuga menziesii (96,98,107) and Picea sitchensis (133). Treatment with NAA alone also has caused the reversion of female to male strobili in L. leptolepis and Pinus densiflora (48), and inhibited female flowering in P. banksiana (13). And, in P. taeda, branch applications of triiodobenzoic acid (TIBA), an inhibitor of polar auxin transport, slightly increased the production of female strobili (112).

These male-promotive results for auxin have not, however, been noted for Tsuga heterophylla (S.D. Ross and R.F. Piesch, unpubl.), or in all studies on Pseudotsuga (105), Pinus (13,65) or Picea (70,100). Frequently the auxin treatment resulted in depressed female and/or male flowering, which may be a phytotoxic response. Furthermore, in all these studies the auxin was tested at only one, or at most two concentrations, either or both of which may have been outside an optimal range for affecting sex expression.

#### 4.4. Cytokinins

It has been noted that many of the treatments which promote flowering (e.g. rootpruning, drought and flooding of roots, high temperatures and excesses or deficiencies of N) could retard root growth. This has led to the suggestion (8,101) that actively growing roots export chemical substances inhibitory to flowering. Zaerr and Bonnet-Masimbert (146) present results for potted Pseudotsuga menziesii grafts which suggest that this inhibitory substance(s) may be a cytokinin. The promotion of flowering by GA<sub>4/7</sub> alone, and especially with flooding of roots, caused a marked decrease in endogenous (immunoassay active) cytokinin-like substances in shoots harvested during the period of strobilus differentiation. From a comparison of distal and proximal shoot segments, it also appeared that the threshold concentration of cytokinin-like substance that was presumably inhibitory to male flowering was lower than that required to inhibit female flowering.

However, Bonnet-Masimbert and Zaerr in Chapter 2 present additional results where the relationship between endogenous cytokinin-like substances and flowering is less clear. Furthermore, in contrast to their trends for endogenous cytokinin-like substances, the synthetic cytokinin, benzyladenine (BA), when coadministered with GAs, has promoted female flowering in P. menziesii (113) but male flowering in Picea sitchensis (133). Thus, the picture for cytokinins and sex expression in conifers is by no means clear. The situation is not unlike that in some herbaceous angiosperms, where

cytokinins and GAs both may be involved in sex expression (92,139).

##### 5. ROLE OF PHOTOPERIOD AND TEMPERATURE

That photoperiod can influence sex expression in some Cupressaceae and Taxodiaceae conifers is well established (4,63,79-82,87,88). Long days (LD) generally maximize the absolute production of strobili of both sexes. But, whereas male flowering has a quantitative requirement for LD, shortening the day length usually increases the proportion of female strobili initiated. Thus, the control of sex expression by photoperiod generally is not absolute, although in one study with Cryptomeria japonica (79), treatment with  $GA_3$  induced only female strobili under 8-h days and only male strobili under 24-h days.

In nature male and female strobili of Cryptomeria differentiate under LD and SD, respectively, whereas this apparently is not the case for all members of the Cupressaceae (87). In Chamaecyparis, Cupressus and Thuja, males are initiated under LD and increasing day length and female strobili also under LD, but when day length is decreasing. Thus, for these species sex expression may depend on the direction of change in day length and not the actual photoperiod (87). However, the importance of photoperiod (or its direction) in influencing sex expression in nature is still not clear. In field and greenhouse studies conducted under natural photoperiods on Thuja plicata (S.D. Ross unpubl.) and Chamaecyparis nootkatensis (R.C. Bower and S.D. Ross, unpubl.),  $GA_3$  treatment became less effective in promoting both female and male flowering the progressively later the  $GA_3$  was applied following flushing in spring. This is consistent with the observation (81) that LD maximize the production of strobili of both sexes in C. obtusa.

Giertych (31) has equated male strobilus initiation in Pinus with LD flowering behavior, and female flowering with SD flowering behavior. Evidence for this based on the southward and northward movement of provenances and clones, in which trees experience shorter and longer day lengths, respectively, in summer than in their natural range is inconclusive, however. In most such trials with Pinus species (29,124,126,128,144), the southward movement of provenances and clones has significantly increased not only female but also male flowering. This suggests a response to warmer, more favorable temperatures, rather than to changing photoperiod per se (34,128). In one comparison of 50 Pinus ponderosa var. scopulorum provenances from latitudes 32.3°N to 47.6°N (106), the northern provenances as a group produced more female strobili when planted in the middle of the range (lat. 41.0°N), whereas the southern provenances in the same plantation produced more male strobili, thus implying a photoperiod causality.

Kosinski and Giertych (57) found with Pinus sylvestris and Picea abies that increasing natural (white) light in the vicinity of the terminal bud apical dome using an optical fiber inserted through the bud scales increased the probability of that bud differentiating female strobili. The authors suggest that since bud scales absorb more red (R) than far-red (FR) (104), complete decay of the Prf to Pf form of phytochrome might not occur under the white light treatment rich in R, thereby signaling a long

night (SD) situation. However, there may be an even more direct effect. FR enrichment of normal photosynthetically active radiation can stimulate GA metabolism (2,84), so that R (e.g. white light) enrichment may result in decreased GA hydroxylation. This could lead to the build up of less polar GAs, the precursors of polar GAs upon whose adequate availability flowering appears to depend (97,99).

Durzan et al. (1979) reported the inhibition of female flowering in field-grown Picea glauca trees using night-interrupted exposures to R via a laser during the period of strobilus differentiation. However, the fact that there was a year lag between treatment application and its effect on strobilus initiation raises questions about its photoperiodic basis. Furthermore, there was no mention of male flowering being affected by the LD treatment.

The issue is further complicated by the finding (37,91) that night interruptions of FR were more effective than R in mimicking the LD-enhancement of male strobili in GA<sub>3</sub>-treated Cupressus arizonica seedlings. However, there is good precedent for FR being promotive of flowering in LD plants (26), and if FR were increasing overall GA content and metabolism within the plant (2,84), then it might be expected to interact with exogenous GA<sub>3</sub> application in an additive or synergistic way to increase flowering (males in this study on Cupressus). It may be noted, however, that in Chamaecyparis obtusa night breaks with R were more effective than with FR in promoting both female and male flowering (81), whereas in Cryptomeria japonica R increased only females and FR increased only males (82). For both species, blue-filtered light promoted only male flowering.

There is evidence from recent growth chamber experiments that photoperiod can influence sex expression in at least two Pinaceae conifers, Pinus contorta (60) and Tsuga heterophylla (103). In conjunction with GA<sub>4/7</sub> treatment, SD favored female flowering and LD male flowering in both species (photoperiods of 10 & 19.6 h for Pinus, 13 & 18 h for Tsuga). Each study also compared a warm and cool temperature regime (22/15°C & 15/8°C for Pinus, 30/30°C & 20/20°C for Tsuga). With Tsuga, female and male flowering were each significantly better (both photoperiods) under the warm regime. With Pinus, however, female flowering also was slightly better under the warm regime, whereas male flowering was significantly better in the cool regime. This contrasts with the situation in Cryptomeria, and to a lesser extent in Chamaecyparis, where the optimal temperature was higher for induction of male than female strobili (81,82).

Greenwood (40,42) has shown that when potted grafts of Pinus taeda which have overwintered in a greenhouse under continuous light are then exposed to naturally SD in spring, female flowering is dramatically enhanced. He attributes this not to a direct photoperiod effect on flowering, but rather to the premature setting of a terminal resting bud which occurs under the shortened photoperiod. Female strobilus differentiation in this species coincides with the end of shoot elongation, and the suggested effect of 'out-of-phase dormancy' is to enable sexual differentiation to occur before the onset of normal winter dormancy. However, the treatment enhances male flowering to an even greater extent, even though male strobilus differentiation normally occurs much earlier and, based on the results noted above for P. contorta (60), might be expected to be inhibited under the shortened photoperiod. Ross and Pharis (115) suggest that the effectiveness of out-of-phase dormancy may have a basis similar to drought, rootpruning and other stress treatments, which is to arrest vegetative growth and thereby

allow the build up within the shoot of less polar GAs (possibly also nutrients) essential to flowering.

In Pseudotsuga menziesii, both female and male strobili are normally differentiated under a relative short but lengthening photoperiod in early-mid spring (Fig. 1). However, both sexes can also differentiate under relatively long but shortening photoperiods on lammas shoots which may form following a mid-summer drought (5). Bonnet-Masimbert (5) therefore concluded that flowering in this species depends on internal inductive conditions occurring at a specific stage of lateral primordium development, rather than at a precise time of the year.

Yokoyama and Asakawa (145) classed Larix leptolepis as day-intermediate on the basis that female strobili were produced under photoperiods of 12-16 h but not under shorter or longer photoperiods. Unfortunately, the authors used only a single clone which did not produce any males, and so their classification may be premature.

In the Cupressaceae, both the duration of the day period and intensity of light are important in a quantitative sense for male flowering especially (37,80,91,94). Thus, extending the photoperiod by low intensity supplemental light or by night breaks is not nearly as effective in promoting male flowering as is a longer exposure to high light. The implication is that a higher level of photosynthate (or GA) production favors quantitative increases in male over female flowering for Cupressaceae species. However, this is in contrast with the finding for Cryptomeria japonica (79) that initiation of female strobili requires a higher light intensity than initiation of male strobili.

Studies on Cupressus arizonica (37,91) also show that the endogenous GA content of shoots increases with day length, reaching maximum levels under continuous light of high intensity. Reconciliation of these promotive effects of LD/high endogenous GA on male flowering with the fact that increasing the concentration of exogenous GA<sub>3</sub> increases the female:male ratio (47,61,82,94) can only be accomplished if one involves a high dosage threshold of GA for female flowering, or if one brings in other interacting factors such as auxin (e.g. high GA increases auxin production, and increased auxin:GA yields increased femaleness).

One further effect of photoperiod on sex expression bears mentioning. Following differentiation, there is generally the requirement for SD followed by LD for continued strobilus development through to anthesis (4,37,38,88,91,95,103). In the Cupressaceae and Taxodiaceae, it appears that neither female nor male strobili will develop without a SD treatment, which is most effective if administered at about 5°C (4,94). However, the male and female strobili have differential requirements for photoperiod and/or cold, this requirement being satisfied sooner for males than for females (37,91,95). Also, in Pinus taeda, male strobili which are in a state of arrested development will resume growth under imposed LD, whereas female strobili require SD, unless GA<sub>3</sub> also is given under the LD (39).

## 6. CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

The way by which sex expression in conifers is regulated is poorly understood. This lack of understanding is as much a consequence of lack of critical study as a reflection of the complexity of the process. We suspect that, as is probably the case with with angiosperms, the process is under strong hormonal control, where balances between GAs, auxin, cytokinins and possibly inhibitors such as ABA are responsible for the observed gradients of sex expression along the stem, branch and shoot. However, the nature of this control and the possible involvement of non-hormonal aspects of shoot

vegetative vigor and nutritional status remain obscure. For many conifers strobilus expression of sex depends on timing of the inductive stimulus, apparently in relation to stage of bud development. Nevertheless, there is evidence that for some species female and male flowering do have different photoperiod requirements.

Prospects for the operational control of sex expression in seed orchards to facilitate pollen and crop management also remain to be established. Yet, there are several potentially promising approaches, especially for use in indoor, containerized orchards where environmental and treatment conditions can be controlled. These include: (1) crown pruning to favor the production of specific types of shoots which have a high potential for differentiating either female or male strobili; (2) timing of treatments to coincide with the critical developmental stages for effecting female or male strobilus differentiation; (3) control of photoperiod for those species so responsive; and (4) manipulation of endogenous hormone balances through exogenous PGR applications under appropriate conditions noted in (1-3) above.

However, the successful development of these and other possible approaches for operational use requires that we have a better basic understanding of the natural control of sex expression. For example, it is still not clear when, during differentiation/early development of a strobilus, its sex is determined. There is some evidence from studies on Pseudotsuga menziesii (S.D. Ross, unpubl.) and Tsuga heterophylla (103) that sex expression can be influenced well after reproductive differentiation in lateral primordia is presumed to occur. Nor do we know if a particular effective treatment is influencing the sex ratio through a direct effect on floral initiation, through the subsequent differential development/abortion of previously initiated female and male strobili, or by altering branching and growth habits.

Answers to all of these questions will require the development of better techniques (anatomical, histochemical, and perhaps immunoassay) for detection of the very earliest stages of differentiation for female and male strobili. Finally, future research also should seek to characterize and quantify those positional, morphological and physiological attributes of shoots and buds which are constantly associated with sex expression. Such a characterization will aid, for example, in the practical development of sex-specific pruning regimes. It will also provide needed insight into designing sampling regimes for analysing PGRs and the carbohydrate and nitrogen status of shoots in relation to sex expression, and assessing flowering responses to different PGR and cultural treatments.



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