PROMOTION OF FLOWERING IN THE PINACEAE[1] BY HORMONES -- A REALITY

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ABSTRACT.--The effectiveness of gibberellins as promotors of flowering in Cupressaceae and Taxodiaceae is historically reviewed, emphasizing that these groups of plants respond to polar gibberellins and produce sexual structures after exogenous application of GA_3. Species in Pinaceae, on the other hand, can be brought into flowering with non-polar gibberellin treatments. Admixtures of gibberellins applied with auxin and stimulatory cultural treatments such as strangulation, nitrate fertilization, root pruning, or drought stress are particularly successful. Each species, age, even each clone and each site may require highly "specific" treatments in forms of dosage level, frequency of application, hormone mixture, and adjunct cultural treatment.

In this paper I will discuss the effects of certain of the gibberellins on flowering of young seedlings, grafted ramets, and older trees in the Pinaceae. I will also give you some history of the work that went into obtaining the results. I do this for two reasons: One is to show you that the induction, or promotion of flowering in Pinaceae is not a simple procedure; gibberellins are not a patent medicine. You can't just walk through a seed orchard and spray the hormones on the trees and stand back and wait for the flowering. It takes a knowledge of the period of "sexual" differentiation, and may require other cultural treatments in addition to the hormones. Results from a given dosage rate, or application

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[1] This paper draws heavily on individual and collaborative work by the following: A. Dunberg, J. Glenn, G. Kiss, C. G. Kuo, W. Morf, J. Owens, R. M. Rauter, G. Sweet, R. Wample, and N. Wheeler.

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STRUCTURES OF GIBBERELLINS

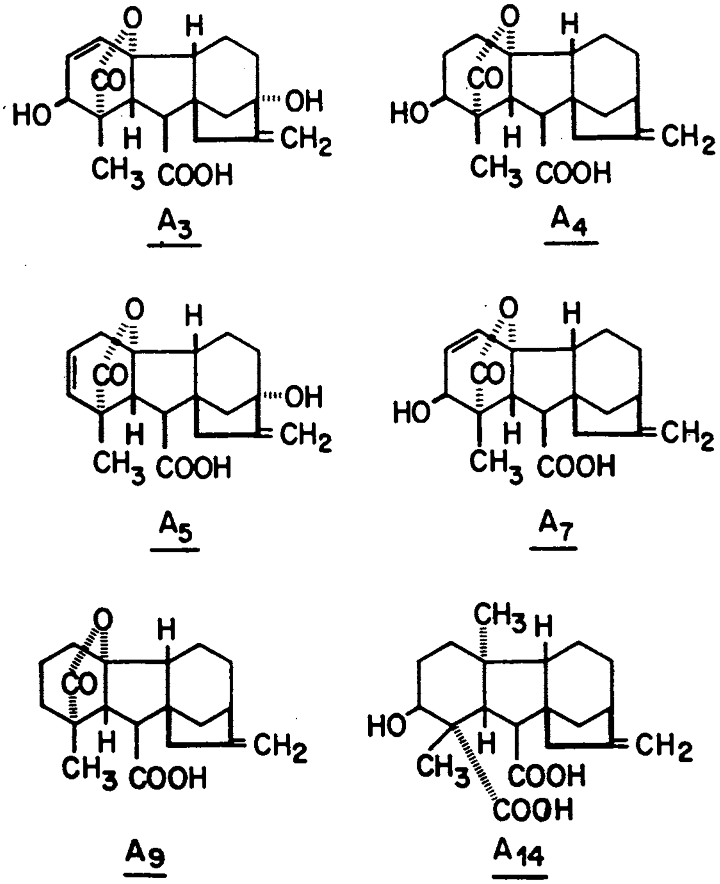

Fig. 1. Structures of 6 gibberellins (GAs) known to promote flowering in conifers. Interconversion occurs between GAs from a less-oxidized state (i.e., GA$_4$, GA$_5$, GA$_7$, GA$_9$, and GA$_{14}$) to a more oxidized state (i.e., GA$_3$) in both the fungus, (*Gibberella fujikuroi* (Saw.) Wollenw. and higher plants (see reviews by West, 1973, and MacMillan, 1974). All except GA$_{14}$ are known to be native to higher plants and GA$_3$, GA$_4$, GA$_7$, GA$_9$, and GA$_{14}$ can be produced in quantity by *G. fujikuroi*. Pathways of interconversion in the fungus are well-known, but are only now being ascertained in higher plants. Figure taken from Pharis *et al.* (1976).

frequency may vary from year to year and from clone to clone. Each species may have specific requirements with regard to type, mixture, and dosage of hormone, timing of treatment, frequency of application, and best type of additional cultural treatment.

A bit of history will also show that the hormones in question are discrete entities, not just a "class" of chemical compounds called gibberellins of which 51 are currently known (for structure, see review by Pharis and Kuo 1977).

Those of you who are not chemists or physiologists probably tend to think of auxins, cytokinins, and gibberellins as classes of hormones, individual compounds within a class having the same effect. Especially with gibberellins, this is not the case. Different gibberellins have different effects, and it is probable that plants regulate growth and differentiation by controlling the level and type of endogenous gibberellin (and other hormones).

The structures of six gibberellins known to promote flowering in conifers are shown in Fig. 1: Notice the presence or absence of the hydroxyl (-OH) group. The gibberellin that you can buy in the horticultural stores to put on your begonias is GA_3; it has two hydroxyl groups on different rings. Another gibberellin, GA_9, has no hydroxyl groups whatsoever. Gibberellins A_4 and A_7 (as a mixture) can be purchased in quantity from Plant Protection (ICI) Ltd., Fernhurt, Haslemere, Surrey, England. Gibberellin A9 is available in small quantities only for research purposes. The other gibberellins shown are not currently available.

Gibberellins are either polar or non-polar. The polar gibberellins are those with two or more hydroxyl groups. The non-polar gibberellins have no (i.e., GA_9) or only one hydroxyl (i.e., GA_4, GA_5, or GA_7) group. Hydroxyl groups are indications of the state of oxidation. Gibberellins, as potent hormones, may be "gotten rid of" by the plant by oxidative metabolism. When you administer a gibberellin to a plant, one of its first reactions is to hydroxylate! The oxidative sequence goes from no or few hydroxyls to many hydroxyls--from a low state of oxidation to a high state of oxidation.

Secondly, the plant may begin to conjugate the gibberellins with glucose and perhaps other sugars (or sulphur, or propyl or peptidyl groups). I think that many of our past studies on gibberellins in conifers have been failures, because conifers turn out to be extremely adept in getting rid of gibberellins (c.f. ^3H-GA_4 metabolism in Douglas-fir; Wample _et al._ 1975).

Tree breeders need "sexy" trees for breeding and seed production--trees with lots of cones and lots of seeds. An example of this is shown for some ramets in a Douglas-fir seed orchard on Vancouver

Island. These grafted ramets flowered profusely; there was only one trouble, they died shortly thereafter from graft incompatibility. This profuse, or early flowering resulting from stress or trauma is a most important reaction exhibited by most woody perennial plants. It (a) gives us a tool by which we may study the physiology and biochemistry of flowering, and (b) offers us an adjunct treatment by which we may "amplify" hormone-induced flowering.

The history of gibberellins in the flowering of conifers began with the Japanese work (Y. Kato and H. Hashizume; see review by Pharis and Kuo 1977) on species of Cupressaceae and Taxodiaceae in 1956 and 1957. As examples of the high efficacy of GA_3 within these families, a seedling of Thuja plicata only 4 cm high can be made to produce staminate strobili; an ovulate strobilus was produced on a 3-month-old Sequoiadendron seedling; and for Cupressus arizonica, a 12-month-old plant sprayed with GA_3 over a period of several months, produced 8,007 strobili out of 8,090 meristems. These conifers, by the way, are nice tools for the physiologist: on just one plant there can be 8,000 to 10,000 meristems, most of which can be transformed quickly from the vegetative to reproductive state.

In addition to being "sexy" hormones, gibberellins in conifers do a lot of other things. As endogenous hormones, they affect stem elongation and diameter growth, and exogenous applications of various gibberellins will increase internode elongation, affect the apical form and apical dominance (enhance or decrease, depending upon the species), and increase diameter growth and shoot dry weight (see review by Pharis (1976a) and Pharis and Kuo (1977).

Thus, we are looking at hormones that are native to conifers, and do a lot of things besides making conifers "sexy". In fact, they appear to do most, if not all, of the vegetative growth "jobs" before doing any "reproductive work." One can perhaps generalize that gibberellins are "sexy hormones" only after they have accomplished certain vegetative growth processes; only after they build up to a certain threshold concentration do they cause sexual differentation. I propose that in the juvenile plant the gibberellins are preferentially used for vegetative purposes, sexual maturity occurring when there are enough gibberellins left over. This will generally occur on the odd meristem here and there, and only in the case of stress will a young conifer flower profusely. In each case--normal maturation, or stress-induced flowering--I propose that the flowering is caused by "left-over" endogenous gibberellins that have not been used or cannot be used for vegetative growth.

We knew from the early work of the Japanese, and from our own work, that we could readily get *Cupressaceae* and *Taxodiaceae* to flower with gibberellin A_3. We also knew that other gibberellins were effective for these two families. But *Pinaceae* species would not flower in response to gibberellin A_3. Furthermore, certain stress, or cultural treatments that are successful on *Taxodiaceae* and *Cupressaceae* work well in *Pinaceae* (see Literature Cited in Pharis (1976)), thus lending credance to a "common cause". Now, what are some of the possible reasons for this conundrum?

First, the growth habit or a meristem production habit in *Cupressaceae* and *Taxodiaceae* species is indeterminant. In other words, about every 15 to 30 days a new meristem is produced. Thus, 10 to 20 times a year each actively growing meristem has a "chance" to initiate a reproductive structure from a previously vegetative meristem (see Owens and Pharis 1967, 1972).

Thus, in the *Cupressaceae* and *Taxodiaceae*, we have access to large numbers of meristems that are continually turning over and making themselves available to possible hormonal effects resulting from exogenous hormone application. In most *Pinaceae* species, on the other hand, meristems can only be induced once a year, and the period of possible induction takes place during a few weeks. During these few weeks, then, its lateral primordia are trying to "decide" whether they will be lateral vegetative, lateral "latent", will abort, or whether they will become reproductive. This, then, is the time one has to hit them with the message, the hormonal signal. Perhaps many of our past experiments with hormone application missed the "differentiative" stage. In essence, an effector treatment that will induce sexual differentiation in the *Pinaceae* is doing so by acting upon pre-existing, undifferentiated primordia (Owens and Molder 1975), unlike *Cupressaceae* and *Taxodiaceae* whose reproductive structures are initiated from previously vegetative meristems.

In addition to the wrong timing in our experiments with *Pinaceae* species, we may have had the wrong gibberellin (i.e., perhaps GA_3 was a "flowering" gibberellin in *Cupressaceae* and *Taxodiaceae*, but not in *Pinaceae*). We therefore went back and looked at situations that would allow us to determine whether we had the right or wrong gibberellins.

We examined cultural treatments, which often (but not always), when used as the sole treatment, cause flowering. There are a number of these: starvation, nitrogen or phosphorus fertilization, girdling or strangulation, root pruning, drought stress, nitrate fertilization (in Douglas-fir, at least) are good examples. In three separate tests we applied certain of these cultural treatments to trees and then determined the endogenous gibberellins of control versus treated trees.

In Cupressus arizonica we repeated (Kuo 1973) the classic work of Lyr and Hoffman (1964), inducing both male and female flowering on Cryptomeria japonica by nitrogen starvation. In Douglas-fir, flowering can be promoted with either drought stress or calcium nitrate fertilization (Ebell 1967, 1972). On a low nitrogen site, Dr. Ebel fertilized a stand of Douglas-fir trees with calcium nitrate. Buds were sampled from treated and untreated trees, freeze-dried, extracted, analyzed for endogenous gibberellins, and the results correlated with induced flowering. Finally, Douglas-fir seedlings were subjected to drought stress.

In each experiment trees given the "flower-inducing" cultural treatment had large amounts of non-polar gibberellins compared with the controls. Conversely, the later fractions, the GA_3-like fractions and other more polar "bioassay active" fractions, were lower in trees that flowered and higher in the control trees that stayed vegetative.

So, three different cultural treatments produced similar results. Now, remember that trees that build up non-polar gibberellins are possibly trees that are not able to metabolize (i.e., hydroxylate) these non-polar gibberellins to more polar gibberellins. Thus, there may be a blockage of oxidative metabolism (or else increased synthesis over and above the ability of the tree to readily interconvert the extra quantities).

With these results in mind, we went into the seed orchards with an increased spectrum of gibberellins. We applied polar GA_3 (which we knew did not work from earlier experiments) as well as a number of the non-polar gibberellins (GA_4, GA_7, GA_9, GA_5) to grafts and seedlings of Douglas-fir and seedlings of lodgepole pine. The results were spectacular, to say the least (see appropriate figures and tables in Ross and Pharis (1976), Pharis et al. (1975), Pharis et al. (1976), Pharis 1975), Pharis and Kuo (1977)). Non-polar gibberellins, especially the $GA_{4/7}$ misture with GA_9, and a low concentration of the auxin, NAA, causes highly significant flowering--GA_3 is essentially ineffective! These earlier results have now been extended to a number of other Pinaceae species (see review by Pharis and Kuo 1977).

The strobili induced by the non-polar gibberellins can be manipulated with regard to sex. In Douglas-fir, high auxin concentrations promote male cone differentiation, low auxin increases females (just the opposite from what one would expect from work with herbaceous plants). We don't yet understand why this is the case, but for Douglas-fir, at least, work by Dr. S. D. Ross of Weyerhaeuser has confirmed this phenomenon in many experiments.

Thus, in the *Pinaceae* we have only a single period, perhaps 6 to 10 weeks, to "tell" previously undetermined buds what they are to become, whether to be male or female, vegetative, latent, or to abort. An effective treatment during this 6- to-10-week period may require that a certain threshold concentration of hormone be maintained during the entire period, and further, that photosynthate and other nutrients be readily available, not only during the 6 to 10 weeks of early differentiation, but subsequently as well. If any of these "promotive factors" is not maintained at an appropriate level, sexual differentiation ceases and abortion, or increased "latency" may occur.

Now, what about juvenility *per se*? Initially, our results were best on saplings or grafted ramets, and nil on young seedlings. Some of Dr. Ross's early work with 4-year-old seedlings showed only a non-significant" tendency" for $GA_{4/7}$ mixture to promote flowering. But, when he used cultural treatments in addition to the $GA_{4/7}$ mixture, flowering was appreciably enhanced on young seedlings.

If one wants to get young seedlings, or recalcitrant grafted clones to flower, I recommend most strongly the use of a $GA_{4/7}$ mixture with an auxin treatment and an additional cultural treatment (such as modest water stress, nitrate fertilization, or non-destructive cambial girdling). I further recommend future location of all seed orchards in warm, sunny locations (especially during early differentiation) where modest water stress in spring can be alleviated by irrigation in summer.

One of our more recent studies into just how a cultural treatment may "assist" the $GA_{4/7}$, or may cause flowering on its own, was done by Dr. A. Dunberg, at the Royal College of Forestry in Umeå, Sweden on *Picea abies*. Grafted ramets of *Picea abies* can often be made to flower by surrounding them with plastic on a light frame. Dr. Dunberg also got flowering with the non-polar gibberellins--the $GA_{4/7}$ and GA_9 treatment being most effective. Thus, as with Douglas-fir, a non-hormonal "flower-inducing" cultural treatment existed for *P. abies*, and *P. abies* also responded to treatment with non-polar gibberellins.

Trees surrounded by plastic were given a radioactive gibberellin (^3H-GA_4) of very high specific activity (i.e., the actual amount of GA_4 was so low as to be metabolized by the tree as if it were a "native" gibberellin). The GA_4 was not enough for the tree to "see" physiologically, but was radioactive enough for us to see in terms of how the tree metabolized it. After 48 to 96 hours of incubation in the field, extracts of control and "plastic-treated" branches were made and analyzed by gas chromatography-radiochromatogram scanning. Under conditions that cause flowering (i.e., plastic on frames), metabolism of ^3H-GA_4 to more polar gibberellins was depressed, relative to metabolism in control branches. Thus, a cultural treatment that causes flowering, results in a build-up of the non-polar GA_4, relative to non-flowering control branches--a situation analogous to the work on endogenous gibberellins of Doulas-fir and *Cupressus arizonica* given flowering promotive cultural treatments!

Some other examples of flowering promotion in *Pinaceae* species are as follows:

Pinus contorta grafted ramets. The $GA_{4/7}$ mixture dripped onto the bud; Prince George, B.C., N. Wheeler, B.C. Forest Service, Research Division. For the treated terminal shoot, frequency of flowering: control-33%; low $GA_{4/7}$--54%; high $GA_{4/7}$--42%. Notice, the supraoptimal effect of $GA_{4/7}$. If we look at the number of females: 0.67--control; 1.79--low $GA_{4/7}$; 1.17--high $GA_{4/7}$. Again, the supraoptimal effect. But, if we look now at females on lateral branches adjacent to the treated shoots: control--17%; low $GA_{4/7}$--21%, high $GA_{4/7}$--25. If we look at number of females on adjacent lateral branches: 0.17--control; 0.71--low $GA_{4/7}$; 0.67--high $GA_{4/7}$. If we look at all other branches on the ramets, flowering frequency is control--25%; low $GA_{4/7}$--29%; high $GA_{4/7}$--38%. Number of flowers for "all other branches" of the ramet: 1.08--control; 1.04--low $GA_{4/7}$; 1.54-$GA_{4/7}$. And, if for this experiment we pull a "sexy" clone out; (SK5) clone comes out: For treated terminals, control--11%, low $GA_{4/7}$--27%, high $GA_{4/7}$--37%; for number of females on the terminals, control--0.18, low $GA_{4/7}$--0.55, high $GA_{4/7}$--0.82.

Picea glauca grafted ramets. Work by G. Kiss, B.C. Forest Service, Research Division, Prince George, B.C. shows a female flowering frequency of: control--0 out of 48; low $GA_{4/7}$--7 out of 48 females; high $GA_{4/7}$--5 out of 48. For number of female strobili (total): control--0.0; low $GA_{4/7}$--15.0; high $GA_{4/7}$--14.0. But, because of the adjunct girdling treatment many branches were lost to snow breakage. For example, control without girdling--0 of 48 branches lost; control with girdling--15 out of 48; low $GA_{4/7}$--15 out of 48; high $GA_{4/7}$--21 out of 48 branches lost. However, although we lost almost half of the branches in the high $GA_{4/7}$ treatment, we still got reasonable flowering (i.e., 14 females).

Similarly good results on *P. glauca* were obtained by R. M. Rauter (using GA_4 on grafted ramets) in both 1976 and 1977, and in an experiment on 55-year-old *P. glauca* trees. $GA_{4/7}$ plus auxin plus girdling proved to be an effective treatment, control flowering being essentially zero.

In summary, flowering promotion in many *Pinaceae* species is now a reality, using certain of the non-polar gibberellins, especially in admixture, and with an auxin and adjunct "cultural"treatments. However, each species, each age, each clone, and each site (i.e., geographic location) may have to have highly "specific" treatments (in terms of dosage level, frequency of application, hormone mixtures, and adjunct cultural treatments) developed to insure success. Results thus far lead me to strongly recommend the "hormonal" approach for the breeder, but it is still too early to make such a recommendation to the seed orchard manager--much additional research work is required in this latter area.

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