A PHYSIOLOGICAL STUDY OF SEED CONE PRODUCTION IN *PINUS RADIATA*

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ABSTRACT

Individual grafts in two **Pinus radiata** seed orchards were categorised (a) as being "good flowerers", or (b) as flowering poorly, **either** because (i) many of their long shoots failed to develop ("non-developers") **or** because (ii) most of their long shoots differentiated into branches ("mainly branch"). At one site buds in trees of the 3 categories were treated with a mixture of gibberellin A4/7 with naphthaleneacetic acid (GA/NAA) at the stage of long shoot differentiation. At the second site buds were harvested at that stage and extracted to assay for endogenous gibberellins and cytokinins, carbohydrates, nitrogen and amino acids.

In all categories of tree an increased number of seed cones were differentiated following GA/NAA application. This resulted in part from an increase in total long shoot numbers, and in part through a developmental "switch" from branches to seed cones.

Buds of good flowering trees had high levels of soluble carbohydrates and amino acids, but low levels of total and non-polar gibberellins, and cytokinins, relative to trees whose long shoots mostly developed into branches.

The hypothesis is proposed that undifferentiated long shoot primordia tend to differentiate as seed cones rather than as branches when their rate of growth is high. Treatments which increase seed cone differentiation may involve a temporary diversion of metabolites from the apical meristem of a bud to its developing long shoots at the time when differentiation is occurring.

INTRODUCTION

General

Most tree breeding programmes utilise clonal seed orchards to provide genetically improved material. Much of the physiological work carried out into the reproductive processes of forest trees has as its direct or indirect aim, the increased production of seed from orchards. This study is no exception.

Seed cone production in the Pinaceae has been increased experimentally in a number of ways, although the physiology is not always well understood. Site has a profound effect, with the indication being that favourable sites have warm temperatures and are dry and sunny at the time of seed cone initiation and/or differentiation (Sweet, 1975). Artificially-applied drought stress has also promoted seed cone production (Ebell, 1967) and there is a belief that reproductive differentiation may be associated with a short-term

check to some aspect of vegetative growth. Greenwood (1978) sees it as a check to bud activity.

High carbohydrate levels also are believed to promote reproductive differentiation (Ebell, 1971), and while most researchers also accept a role for nitrogen (N), it is poorly understood (*see* e.g., Sweet and Hong, 1978). While there is some evidence that a ratio of high carbohydrate to low nitrogen favours seed cone production, a number of researchers have been successful in increasing reproductive activity by applying N to seed orchards. The forms of N and the times of application have been said to be critical (Ebell and McMullan, 1970; Ebell, 1972). Finally, the application of appropriate gibberellins (GAs) has increased seed cone production in a number of members of the Pinaceae (Pharis and Kuo, 1977). In that family the appropriate GAs are non-polar ones, with GA4/7 mixtures being almost universally used. Naphthalene-acetic acid is frequently combined with gibberellins to enhance their effect (Ross, 1976). The suggestion has been made (Pharis and Kuo, ibid.) that a high ratio of non-polar to polar GAs may favour reproductive development, with the reverse ratio favouring vegetative growth.

In all reports of increased seed cone production with treatment, there is evidence of high clonal variability. In general the clones which already have high reproductive capability respond best (author, unpub.; S. D. Ross and R. F. Piesch, in prep.) — and there is a major requirement to develop treatments which will increase seed cone production in the reproductively less successful clones.

Reproductive Development in Radiata Pine

The process has been reported in detail by Bollmann and Sweet (1976). The apical meristem of a leading shoot or other vigorous branch initiates bud scales (cataphylls) in the axils of which primordia are initiated. The majority of these primordia develop into needle fascicles. After a number of needle fascicles has been differentiated, the pattern of development changes, and a series of some 12 or so axillary primordia develop into long shoots. The subsequent differentiation of long shoots may be into branches or seed cones. Alternatively, long shoots may abort or cease development at a number of different stages. The sequence of needles plus long shoots is designated a cycle and several cycles may be formed by one branch on an annual shoot. The final cycle does not contain seed cones.

There is considerable variability in seed cone production in a radiata pine orchard and, by looking at the previous season's growth and reproduction, trees representing 3 broad categories were recognised for this study:

(i) the "good flowerer" — in which a high proportion of the total long shoots terminating the first cycle of the annual shoot developed as seed cones.

(ii) the "non-developer" — in which a high proportion of the long shoots terminating the first cycle failed to develop into either seed cones or branches by the spring following initiation, but remained as small buds.

(iii) the "mainly branch" category — in which the majority of the long shoots terminating the first cycle of the annual shoot developed as branches.

Both categories (ii) and (iii) were classed as poor flowerers relative to category (i).

The intent of the study reported here was (a) to establish whether the reproductive behaviour of trees fitting one of these 3 categories would be repeated the following

season; (b) to investigate the extent to which the categories differed in their response to GA/NAA applications aimed at increasing seed cone production; and (c) to compare the categories with respect to their endogenous levels of gibberellins, carbohydrates, total N and specific N compounds. In the event, endogenous cytokinin levels were also examined, although they have not been implicated seriously in the literature on conifer seed cone production.

MATERIALS

The only trees suitable for use in the study were in seed orchards; and production requirements necessitated carrying out separate aspects of the study in different seed orchards.

1. *GA Application*

This part of the experiment was set up in a 6-year-old block of the Kaingaroa grafted seed orchard (Sweet and Thulin, 1969). In the main part of the experiment, 30 grafts whose previous years' seed cone production fitted *each* of the 3 categories defined above were marked. The 90 grafts represented 12, 14 and 16 clones respectively in categories (i), (ii) and (iii). On each ramet three uniform first-order branches were selected which were sufficiently vigorous for seed cone differentiation to occur. In a subsidiary trial, branches were selected on 13 grafts of a single clone known to display marked periodicity of seed cone production — an unusual trait in *P. radiata.* The clone had flowered heavily the year prior to selection.

2. *Trees for Extraction*

The grafts from which buds were to be collected were located at Waimihia seed orchard, 50 km to the south of the Kaingaroa orchard (Bollmann and Sweet, 1976). They were 9 years old. Apical buds were collected from first order branches with sufficient vigour for seed cone differentiation. They were from trees whose previous year's behaviour fitted one of the 3 categories. Seventy buds were collected from each category as follows:

Category	No. clones involved	Total no. ramets involved	Total no. buds collected
"Good flowerer"	13	19	70
"Non-developer"	11	18	70
"Mainly branch"	15	19	70

METHODS

1. *Timing*

Treatment application at Kaingaroa and bud harvest at Waimihia were carried out at a time when bud development was at the same morphological stage — one where long shoots were recognisable but had not differentiated. This stage was believed, from preliminary experiments (author, unpub.) to be one at which gibberellin application should increase seed cone production. At Kaingaroa, treatment was on 7 February, repeated on 14 February. At Waimihia, bud collection was on 16 February.

The period between long shoot initiation and the first recognisable signs of differentiation is some 6 weeks, varying from tree to tree and branch to branch (Bollmann

and Sweet, 1976; in prep.). Buds dissected and examined at the 2 sites at the time of treatment or harvest all had long shoots initiated. None however had recognisable differentiation of these.

2. *GA Application and Assessment*

In each of the Kaingaroa trees one of the matched branch buds was treated at its base with 400 μg of GA 4/7 mixture (46% GA4 : 54% GA7) and 50 μg naphthaleneacetic acid (NAA) in 100 μl of 70% ethanol (Ross, 1976). Treatment used a micropipette to flood approximately 1 cm of stem tissue with the solution. A second branch acted as a control (receiving 100 μl of 70% ethanol), while the third received 400 μg GA4/7, 50 μg NAA, 200 μg benzyladenine (BA) in 100 μl of 70% ethanol. All treatments were applied on 2 dates (see Timing).

The next spring (at anthesis) the following counts were made: (i) number of branches, (ii) number of seed cones and (iii) number of undeveloped long shoots in the long shoot cluster terminating the first cycle (—that which had been initiated but not differentiated at the time of treatment). Together these gave a value for total long shoots. In addition the length of the main shoot axis distal to the long shoots assessed was measured. Finally, seed cone counts were repeated 3 months later to provide information on seed cone loss (Sweet and Thulin, 1969). The statistical significance of all information was examined by t-test. Six of the 90 treated trees could not be assessed because one of the branches had been broken or damaged.

3. *Harvesting, Extraction and Bioassay*

The buds at Waimihia were collected on 16 February and harvested into liquid nitrogen. On return to the laboratory they were freeze-dried and stored at —20°C.

(a) *Plant growth substance extraction and analysis.* A random 50-bud sample was taken and bulked from each of the 3 groups (freeze-dried weights: good flowerer 28.8 g; non-developer 27.3 g; mainly branch 25.0 g).

The freeze-dried buds were extracted with 80% methanol and then, as an aqueous fraction at pH 9.0, partitioned with diethyl ether (discarded), and at pH 3.0 with ethyl acetate. The ethyl acetate fraction was purified successively with PVP, charcoal : celite and silicic acid (Reid *et al.,* 1974), before estimating gibberellins with the lettuce hypocotyl bioassay (Crozier *et al.,* 1970). Statistical results are derived from analysis of variance of 3 bioassays each utilising a different amount of extract.

The fraction left after partitioning the pH 3.0 aqueous phase with ethyl acetate was brought to pH 7.0 and partitioned with n-butanol. The butanol fraction was purified by PVP, Dowex 50W-X4 and paper chromatography (Hewett and Wareing, 1973) before being bioassayed by the soybean callus method. The bioassay was at one concentration only and statistical analysis was not possible.

(b) *Carbohydrate extraction and analysis.* The 20 harvested buds per category remaining after plant growth substance extraction were bulked and then ground and used for carbohydrate and N determinations. All procedures for carbohydrate extraction and assay were as reported by Cranswick and Zabkiewicz (in press). Quadruplicate 100 mg samples of the freeze-dried tissue were extracted with 60% ethanol at 60°C for 8 hours. This was followed by TMS derivatisation and gas chromatographic analysis of the soluble carbohydrates. Two aliquots of each extracted sample were examined by gas chromatography. Starch was enzymatically hydrolysed to glucose and quantified

colorimetrically after oxidation by glucose-oxidase. Statistical examination was by analysis of variance.

(c) *Amino acids and total N.* The techniques used have been described by Sweet and Hong (1978). Duplicate 1 g samples of bud tissue were homogenised with 80% ethanol, and loaded onto a Dowex 50W-X8 column. After an ethanol wash (discarded) the amino acids were eluted with ammonia, chromatographed in quadruplicate on thin layer plates with butanol : acetic acid : water (4 : 1 : 1), developed with ninhydrin and their optical density at 510 nm recorded. Significance levels were determined by analysis of variance. Total N determinations were made on duplicate samples of bud tissue by Kjeldahl digestion followed by NH_4^+ estimation by an automated colorimetric procedure.

RESULTS

1. *Efficacy of the Categorisation of Trees*

A comparison of seed cone numbers on the *control* branches of grafts of the 3 categories (Table 1) indicates that the grafts chosen (on the basis of their previous year's flowering history) as good flowerers did have more seed cones at the time of anthesis than the other two categories (chosen as being poorer seed cone producers). The difference between the good flowerer and the mainly branch categories was clear cut and highly significant statistically, in terms of both seed cone number and ratio of branches to seed cones (1.13 for good flowerers and 3.18 for mainly branch).

The identification of non-developers, however, was less effective — in particular there was no evidence that the number of undeveloped buds was higher in this category than the other categories.

2. *The Effect of Exogenous GA Application*

The results of assessment in the spring following treatment are presented in Tables 1 and 2.

Table 1 indicates that application of GA/NAA significantly increased seed cone production in all 3 categories of graft, the average increase being 60%. The GA/NAA-caused increase in seed cone production relates in part to an increase in the total number of long shoots present, but apparently also represents a conversion of some potential branches to seed cones. The relative importance of these two effects varied: good flowering grafts increased total long shoot number greatly in response to treatment, while in the mainly-branch grafts GA/NAA apparently converted potential branches to seed cones.

The greatest increase in seed cone production in Table 1A occurred in the mainly-branch trees. Table 1B, however, shows (from the subsidiary experiment) that GA/NAA was very effective in increasing seed cone production in a periodic flowering clone in an off-flowering year. The effect again was attributable to an increase in total long shoot number, and a "conversion" of potential branches to seed cones.

Both Tables 1A and 1B indicate an increase in shoot length distal to the seed cones, following GA/NAA treatment.

Table 2A indicates that the increased seed cone production resulting from GA/NAA application was followed, in the two poor-flowering categories, by an increase in the percentage of cones lost during the 3 months following anthesis.

Table 2B shows that a GA/NAA/BA application influenced neither seed cone production nor subsequent loss of cones significantly, compared with GA/NAA alone.

TABLE 1—A. The effect of GA/NAA on long shoot development on branches of three different seed cone production categories (values are means for the first cycle of the annual shoot — see footnote for symbols)

n	Seed cone production category	Seed cone No.		Branch No.		No. of undev. buds		Total long shoot No.		Shoot length (cm)	
		GA/NAA	Control	GA/NAA	Control	GA/NAA	Control	GA/NAA	Control	GA/NAA	Control
29	Good flowerer	3.17**	2.31 a	2.83	2.62 ab	2.38**	1.62 a	8.38***	6.55 a	35.9**	29.0 a
25	Non developer	3.20**	2.00 ab	1.54	2.35* b	1.69	1.73 a	6.43	6.08 a	25.5***	22.1 b
30	Mainly branch	2.13***	1.03 c	2.17	3.27**a	2.07	1.70 a	6.37	6.00 a	26.3	25.7 ab
84	Weighted mean	2.81***	1.76	2.21	2.77**	2.06	1.68	7.08**	6.21	28.1**	24.8

B. The effect of GA/NAA on long shoot development in a single clone with a periodic flowering habit (the clone was treated in off-flowering year — values are means for the first cycle of the annual shoot. n = 13)

Seed cone No.		Branch No.		No. of undev. buds		Total long shoot No.		Shoot length (cm)	
GA/NAA	Control	GA/NAA	Control	GA/NAA	Control	GA/NAA	Control	GA/NAA	Control
2.62***	0.38 (3.57)†	2.00	2.92	2.07	1.38	6.69**	4.61	26.5	22.9

The * indicates the statistical significance of differences between treated and control branches within a seed cone production category (5%*, 1%** and 0.1%***).

Letters a, b, c indicate the statistical significance of differences between the control treatments of each of the 3 categories. Significance is at the 2% level or better.

† This value is the mean number of seed cones on the same branches the previous year.

TABLE 2—A. Percent loss of seed cones during the 3 months following anthesis†[1,2]

Category	n	% seed cone loss	
		GA/NAA	Control
Good flowerer	22	36.2	38.2 a
Non developer	21	69.7*	47.0 a
Mainly branch	15	62.5*	39.5 a
Weighted mean (n = 58)		55.1*	41.7

† [1] The difference in n values between this table and Table 1a reflects the fact that branches with zero flowering were excluded from % seed cone loss calculations.

[2] For an interpretation of the statistical significance see Footnote to Table 1.

B. An examination of the effect of adding benzyl adenine (BA) to GA/NAA applications
The values are means for the first cycle of the annual shoot — no differences are statistically significant

n	Category	No. seed cones differentiated		% seed cone loss[0]	
		GA/NAA/BA	GA/NAA	GA/NAA/BA	GA/NAA
31	Good flowerer	3.39	3.39	33.7	34
30	Non developer	1.83	1.53	58.2	56.5
17	Mainly branch	2.18	2.29	59.0	72.5
78	Weighted mean	2.53	2.44	48.6	51.0

[0] Loss was assessed 3 months after anthesis.

3. *Endogenous Gibberellin Levels*

These are presented in Fig. 1. There were no interactions with amount of extract bioassayed; the data presented are means of 3 bioassays using respectively 3/10, 1/10 and 1/100 of the 50 bud extract from each category. The 25 fractions are numbered in the order they were collected from the silicic acid column, and the elution points from the column of synthetic GA9, GA4/7 and GA3 are shown.

Buds from all 3 categories of tree contained both non-polar and polar gibberellins. Summing for each category all peaks in Fig. 1, the ratio of GA's in good flowerer : non developer : mainly branch was 81 : 92 : 100. The largest amounts in each fraction were non-polar gibberellins; the mainly branch category had significanly (2% level) more gibberellins in fractions 2,3,4 and 5 than did good flowerers and the non developers. The non-polar gibberellins in fractions 2,3,4 and 5 made up 50% of all gibberellins in the buds of good flowering trees, and 38% and 58% respectively of those in the non-developer and mainly branch categories.

4. *Endogenous Cytokinin Levels*

High cytokinin levels were present in extracts from all three of the flowering categories (Fig. 2), the ratio of good flowerer : non-developer : mainly branch being 64 : 57 : 100.

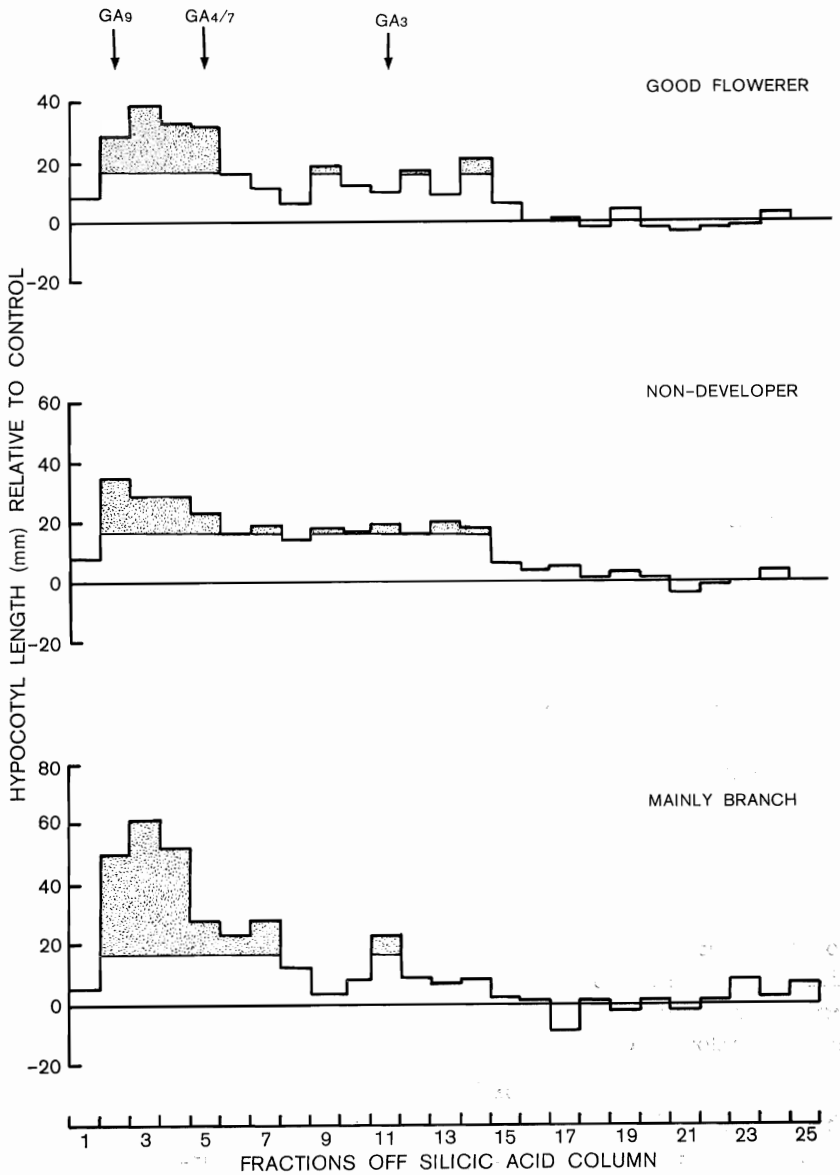

FIG. 1—Results of Gibberellin bioassays (lettuce hypocotyl) of silicic acid fractions of the 3 seed cone production categories. The values are means of 3 bioassays at different concentrations. The shaded area represents GA level greater than 10 ng GA_3 equivalent. The fractions in which 3 synthetic GAs are eluted are indicated.

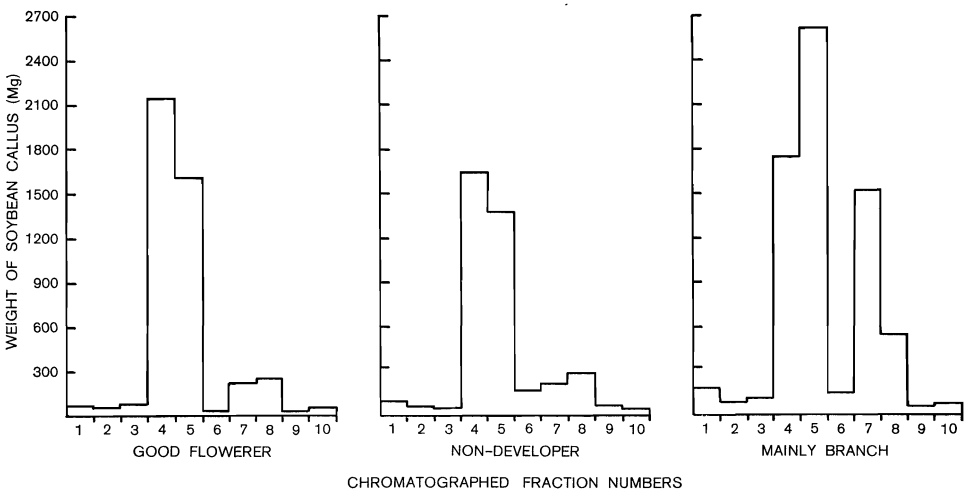

FIG. 2—Results of cytokinin bioassays (soybean callus) of paper chromatogram fractions of the 3 seed-cone production categories. The data are unreplicated. Zeatin and zeatin riboside have Rf values of 0.7 to 0.8 in this solvent system.

5. *Carbohydrate Levels*

The buds from good flowering trees contained higher levels of the soluble carbohydrates examined than did those from non-developers and the mainly branch trees (Table 3).

The differences were statistically significant for glucose, fructose, and total monosaccharides plus cyclitols. There were no apparent differences between the 3 categories of trees in either sucrose or starch levels.

6. *Total N and Amino Acid Levels*

Buds of good flowering trees contained significantly higher arginine and total free amino acid levels than those from mainly branch trees (Table 4). The ratio of free arginine to total free amino acids, however, did not differ between the 2 categories. The highest arginine level, and the highest proportion of arginine to total amino acids was in the non-developer buds.

7. *Carbon/nitrogen (C/N) Ratio*

The ratios of total monosaccharides to total free amino acids (a form of C/N ratio expressing available nutrients) were good flowerer 50.1, non-developer 38.3, mainly branch 49.0.

DISCUSSION

It is pertinent to reiterate that the trees selected as good flowerers did in fact, on their untreated branches, produce more seed cones than those selected as non-developers; and more than twice as many as those selected as having mainly branch buds. The non-developers did not, however, in the year of the experiment, have significantly more *undeveloped buds* on their control branches than the other 2 categories. Thus their choice as a category was not completely successful.

TABLE 3—Carbohydrate levels in buds (mg/g bud tissue)

	Glucose	Fructose	Cyclitols	Total monosaccharides + cyclitols	Total soluble acids	Sucrose	Starch	% seed cone increase in response to GA/NAA†
Good flowerer	36.56	8.55	51.84	96.95	62.2	16.7	17.2	37%
Non developer	20.08	5.92	48.62	74.62	57.6	16.2	15.9	60%
Mainly branch	19.87	6.15	44.38	70.4	58.2	16.9	17.2	107%
Statistical sig. (see Table 1 for interpretation)	***	*	NS	***	NS	NS	NS	

† from Table 1.

TABLE 4—Nitrogen levels in buds

	Free Arginine (μg/5 mg dry wt)	Total free amino acid (μg/5 mg dry wt)	Arginine as % total amino acid	Total N (%)
Good flowerer	3.60 b	9.68 a	37.2 b	1.359
Non developer	4.92 a	9.74 a	50.5 a	NA
Mainly branch	2.60 c	7.18 b	36.2 b	1.338
Statistical sig.	***	***	***	†

*** = significant at 0.1%
\+ = insufficient sample for statistical analysis — mean of 2 replicates only

In part the Discussion will relate the levels of endogenous hormones and nutrients of the three categories of tree to their seed cone production behaviour. It thus needs reiterating that while the buds extracted were collected from Waimihia, it has only been demonstrated for Kaingaroa that the division into the three flowering categories was successful. While there is every reason to believe that it was just as effective at Waimihia, this supposition is unproven.

The Use of GA to Increase Seed Cone Production in P. radiata

The results indicate clearly that the GA/NAA applications which have been shown to increase seed cone production in other conifers (Pharis and Kuo, 1977) can do this also in *P. radiata.* Further, the treatments were successful on a range of genotypes with considerable variation in ability to produce seed cones. Contrary to some previous experience the poor-flowering clones in this experiment responded more to treatment than the good flowerers.

While the percent seed cone loss was generally higher in GA-treated buds than in controls, this in no way negated the effect of the GA. Three months after anthesis (when considerable cone loss had occurred on both treated and control trees) the number of remaining seed cones on GA-treated branches was slightly more than twice that on their matching controls.

GA/NAA applications clearly have potential to increase seed cone production in *P. radiata* both in breeding orchards and in intensively managed seed orchards (*see* Sweet and Krugman, 1977).

The Mechanism of Response to Exogenous GA

The increase in seed cone numbers in this experiment, was associated with an increase in total long shoot numbers and a decrease in branch numbers. At the time of GA/NAA application, all long shoots in the cycle had been initiated (*see* Methods). One must thus accept that GA/NAA operated solely on the differentiation phase. Some long shoots which would otherwise have developed as branches must have been converted to seed cones, and other long shoots which would have either aborted or reverted to short shoots (Bollmann and Sweet, in prep.) must have continued development as long shoots. This broad mechanism is confirmed by 2 previous years' application of GA at the time of long shoot *initiation* which were largely unsuccessful in increasing seed cone production (author, unpub. data).

The success of GA/NAA in increasing seed cone production seems to be at variance

with the endogenous GA levels recorded. Those levels would appear to indicate that higher quantities of non-polar GAs are associated with long shoot development as *branches.* (That information is supported by an unpublished study carried out some years previously by the author and R. P. Pharis which also showed considerably higher GAs both polar and non-polar, in buds of poor flowering as compared with good flowering *P. radiata*).

It is clear that the amounts of GA added to increase seed cone production were considerably above physiological levels (e.g., a total of 400 μg GA4/7 was applied to each bud at each of 2 dates in these experiments, compared with maximum endogenous levels recorded of some 50 ng GA_3 equivalent per bud). That is, the application rate exceeded the natural level by some 8000 times. Thus, assuming reasonable entry of exogenous GA into the buds, the prospect must be considered that exogenously-applied GA is operating in some way distinct from that of endogenous GAs.

In a recent (unpublished and as yet unconfirmed) experiment with *P. radiata,* S. D. Ross, R. P. Pharis, M. P. Bollmann, and the author have indications that GA 4/7/NAA applied exogenously as in this experiment may be acting in the short term to alter the distribution of carbohydrates between the apical meristem of a bud and the developing long shoots within it. The result is a considerable increase in the nutrient allocation to, and relative growth rate of, the latter relative to the former. Such a finding fits well with the implication from this paper that GA/NAA may restrict the abortion or reversion of developing long shoots — a restriction likely to be associated with adequate nutrient levels. And if one considered evidence of Greenwood (in press) to suggest that the differentiation of long shoots into seed cones may require a faster *rate* of growth than differentiation into branches, exogenous GA could be expected to increase seed cone development at the expense of branch development — a factor for which there was also evidence in the experiments.

The Role of Carbohydrates, Nitrogen and Arginine

Sweet and Hong (1978) in a series of experiments have failed to directly promote seed cone production in *P. radiata* grafts and rooted cuttings by application of a number of forms of N, although such application successfully increased N, arginine and total free amino acid levels several-fold in buds at the times of long shoot initiation and differentiation.

And indeed an (unpub.) adjunct to this study, where NH_4NO_3 was applied to good flowering, non-developer and mainly branch trees *again* did not increase seed cone production. Thus the fact that in this study buds from good flowering trees did have higher N, amino acids, and arginine levels than the mainly branch buds would, in the light of the other studies, have to be regarded as *other than* a cause and effect relationship. Particularly as the highest levels were found in the non-developers. The fact that no differences in the ratio of free arginine : total free amino acids were found between the good flowering and the mainly branch buds, and the fact that the highest ratio was in the non-developers, again is in accord with Sweet and Hong's findings that arginine has no special role in seed cone production in *P. radiata* (compare Ebell and McMullan, 1970 for Douglas fir).

Monosaccharide levels, like N levels, were significantly higher in the good flowering than in the mainly branch buds. However, in light of the fact that the flowering response

to exogenous GA application in the 3 groups was negatively correlated with their soluble carbohydrate levels (see Table 3) it would seem that high soluble carbohydrate levels are *not* a prerequisite for GAs to be effective.

CONCLUSIONS

Suggestions have been made in the biological literature that the early rate of growth of a structure may effect the subsequent *direction* of its differentiation (*see* e.g., Mittwoch, 1970). Thus following GA application, if it is possible to regard the temporarily increased supply of metabolites to long shoots (shown by Ross *et al.)* to be a critical factor influencing their differentiation toward seed cones rather than branches. If so it would also seem possible that the temporary check to apical meristematic growth given for example by drought stress, could perform the same role — to divert in the short term, metabolites to differentiating long shoots.

In *P. radiata,* those buds whose long shoots largely differentiate into branches (and thus which at that time perhaps have high apical meristematic activity) have high endogenous gibberellin and cytokinin levels, low levels of soluble carbohydrates and low levels of N and amino acids — compared with buds, in which a higher proportion of long shoots become seed cones. It may thus be possible to regard these as attributes of a largely vegetative bud.

Reproductive buds in contrast are those in which at the time of long shoot differentiation, apical meristematic activity is not favoured. Such buds in this case had lower hormone levels, and higher soluble carbohydrate and amino acid levels. The higher carbohydrate and amino acid levels in the bud could well *result* from a situation which restricted the activity of its apical meristem in initiating new primordia.

To summarise, indications are that to increase seed cone production in *P. radiata* requires an environmental situation, or chemical treatment which will temporarily divert metabolites from the apical meristem of the bud to the developing long shoots within it. Geographic locations with high solar insolation and probably a degree of water stress constitute an appropriate environmental situation; GA4/7 is apparently an appropriate chemical.

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