# OPTIMAL TIMING OF GIBBERELLIN $A_{4/7}$ APPLICATION TO INCREASE FEMALE STROBILUS NUMBERS IN A *PINUS RADIATA* SEED ORCHARD

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## ABSTRACT

Timing of $GA_{4/7}$ application was investigated for the 1993 year at the Amberley and Waikuku seed orchards in Canterbury. February was confirmed as being a more favourable month for application than March. The response "window" was considerably wider when $GA_{4/7}$ was applied by stem injection than when it was applied externally to buds. At the dosages used, stem injection was also confirmed as being a much more effective way to increase female strobilus yield than bud application. The optimal application time for different clones varied little: in the three clones studied the mean range was only 7 days. Also, response to the timing of application did not differ appreciably between ramets of different size and age.

**Keywords**: gibberellin; strobilus production; seed orchard; *Pinus radiata*.

## INTRODUCTION

The capacity of a mixture of gibberellin $A_4$ and $A_7$ ($GA_{4/7}$) to increase strobilus initiation in members of the family Pinaceae is very well established (e.g., Pharis *et al.* 1987; Pharis & Ross 1986). In New Zealand, Sweet (1979) demonstrated that female strobilus numbers in open-pollinated orchards of *Pinus radiata* D.Don could be increased by the application of $GA_{4/7}$. This resulted in part from an increase in the numbers of total long shoots initiated, and in part from a developmental "switch" from branches to seed cones. The level of increase was moderate only, however, averaging some 60%. Ross *et al.* (1984) showed that, relative to controls, topical $GA_{4/7}$ application significantly enhanced the movement of $^{14}C$ and subsequent dry matter diversion to potential female cones.

When control-pollinated meadow orchards were established, Mathers (1989) transferred the technology to the much smaller grafts, with considerably improved results. While substantial clonal variability existed, increases in strobilus number of between 100% and 200% were commonplace in some clones. Following Mathers' work, the commercial application of $GA_{4/7}$ in meadow orchards in New Zealand has become relatively routine (Carson *et al.* 1992).

The chemical nature and dosage of the gibberellin $A_{4/7}$ mixtures tested by Sweet (1979) and Mathers (1989) was based on considerable prior research by a number of workers, spearheaded by R.P. Pharis and S.D. Ross. Much of this work has been summarised by Pharis & Ross (1986) and Pharis *et al.* (1987). Mathers' studies confirmed that $GA_{4/7}$ applied to buds with a potential reproductive capacity at a dose of 400 ug in 95% ethanol, or injected in 95% ethanol into the base of a tree at a dose of 15 mg/tree, constituted near-optimal treatments.

Commercially in New Zealand, $GA_{4/7}$ is now routinely applied in mid-February. The original rationale for this timing was that in the central North Island first-cycle long shoots are actively differentiating into branches or female strobili at that time (e.g., Bollmann & Sweet 1976). That seemed a logical time to apply the $GA_{4/7}$ and, because this has subsequently proved effective on a wide range of sites in New Zealand, timing of application has subsequently received little further investigation.

What is unclear, however, is whether mid-February is in fact the optimal time at any given site. Also unclear is the extent to which the optimal timing varies between sites, between clones, and with different application methods. The impact of ramet size on optimal timing of application is also unknown. For logistical reasons, commercial applications tend to be carried out over a period of at least 3 weeks in large orchards. Thus the size of the "window of effectiveness" of $GA_{4/7}$ is also important.

The study reported here was designed to explore these issues as far as possible. For logistical reasons, however, it was only possible to work at two sites, which are 15 km apart. Thus, effectively the issue of site has not been explored in the experiments reported here.

## MATERIALS AND METHODS

### Gibberellins

In both experiments a mixture of 46% $GA_4$, 54% $GA_7$, produced by Abbott Laboratories, North Chicago, Illinois, USA, was used. During application the ethanolic solutions were kept in a cool, insulated container at all times.

Two methods of application were used in the experiments—bud application and stem injection. In the former, a dose of 400 μg $GA_{4/7}$ in 200 μl of 95% ethanol was applied to individual buds. It was micropipetted on to the proximal part of every bud on a ramet which, based on its size and position, was judged to be potentially capable of bearing strobili. In the latter, 15 mg $GA_{4/7}$ in 1 ml of 95% ethanol was injected into a hole drilled into the main stem, just below the lowest branch whorl. The hole was drilled on a downward slope to half the stem diameter, using a 3-mm bit for 1-year-old ramets, and a 4.7-mm bit for ramets aged 2 years and older.

### Selection of Clones and Ramets

All ramets used in the two experiments were growing in Proseed's Waikuku and Amberley seed orchards, located in Canterbury some 25 and 40 km respectively north of Christchurch.

*Experiment 1: To determine the most effective application time for $GA_{4/7}$, using different clones and application methods.*

Ramets of four clones, in their second growing season after planting, were selected (on the basis of prior knowledge) to cover a range of strobilus initiation capacity, from heavy to light (*see* Table 1). Of the two poor-flowering clones, the ramets of one had been pruned after their first growing season to stimulate the development of potentially strobilus-bearing branches. Ramets of the three other clones were unpruned (*see* Table 1).

Gibberellin $A_{4/7}$ was applied to 10 ramets of each clone on each of the following dates in 1993: 1, 5, 9, 13, 17, 21, 25 February, and 1, 5, 9, 13, 17, 21, 26, 30 March. The 10 ramets were divided into two sub-treatments of five ramets each. In sub-treatment 1, bud application was used and in sub-treatment 2 stem injection.

Ten additional ramets in each clone were selected as non-treated controls, to provide a baseline from which to measure treatment effects. In five of these, 95% ethanol was applied to buds on 21 February, and in the other five it was injected into the stem on the same date.

TABLE 1–Clones used in Experiment 1.

| Clone No. | Planting year (& age) | Form | Previous strobilus prodn. | No.control ramets | No.treated ramets | Location |
|---|---|---|---|---|---|---|
| 1 | 1991 (2) | Unpruned | Good | 10 | 150 | Amberley |
| 2 | 1991 (2) | Unpruned | Intermediate | 10 | 150 | Amberley |
| 3 | 1991 (2) | Unpruned | Poor | 10 | 150 | Amberley |
| 4 | 1991 (2) | Pruned | Poor | 10 | 150 | Amberley |

*Experiment 2: Determination of the impact of ramet age on optimal GA application time.*

A single clone only was used, Number 1 from Experiment 1. Information on the ramets used is presented in Table 2. The planting years for the ramets used were 1990 (age 3), 1991 (age 2), and 1992 (age 1). It is evident from Table 2 that ramet age was confounded with pruning, and with seed orchard location. While unfortunate, this situation was unavoidable; the trial was established in managed seed orchards, and fully-matching ramets were not available.

Gibberellin $A_{4/7}$ was applied to the three age-classes of material on 1, 9, 21 February and 1 and 9 March 1993. On each date the $GA_{4/7}$ was applied to 10 ramets of each class. There were two sub-treatments: on five of the ramets buds were treated, and on the other five stem injections were utilised. Dosages were as for Experiment 1. Again, as for Experiment 1, there were 10 untreated control ramets to provide a baseline for each treatment. On half of these, buds were treated with 95% ethanol on 21 February, and on the other half 95% ethanol was injected into the stem on that date.

TABLE 2–Ramets of Clone 1 used in Experiment 2.

| Planting year (& age) | Form | Approx. height (m) | Previous strobilus prodn. | No.control ramets | No.treated ramets | Location |
|---|---|---|---|---|---|---|
| 1992 (1) | Unpruned | 0.5 | Good | 10 | 50 | Waikuku |
| 1991 (2) | Unpruned | 1.0 | Good | 10 | 50 | Amberley |
| 1990 (3) | Pruned | 2.5 | Good | 10 | 50 | Amberley |

## Experimental Design

Experiment 1: The design was a split-plot, with clones and dates of application as the main plot factors, and mode of application as the sub-plot factor.

Experiment 2: The design was a split plot, analysing ramet age (size) and date of application as the main plot factors, and method of application as the sub-plot factor.

Counts of female strobili were made at anthesis after treatment, and these data were statistically analysed using PROC ANOVA and Duncan's multiple range test (SAS Institute 1987).

# RESULTS

## Experiment 1

Clone 4 was excluded from the analysis and is not presented in the Tables, because none of the 160 ramets treated with $GA_{4/7}$ or ethanol alone produced strobili.

The ANOVA is presented in Table 3. Most of the treatment effects and their interactions were statistically significant at greater than the 1% level.

Female strobilus counts meaned across clones are presented in Table 4, and expressed in Fig. 1 as a percentage increase over the untreated controls. It is clear from both data sets that for these 2-year-old ramets, stem injection was a much more effective means of treatment than bud application. Not only did it give considerably higher yields of female strobili, but the period during which it was effective was considerably longer. With bud application, the "window" of significant increase in strobilus production ran from 17 February to 5 March. With stem applications, in contrast, it ran from 1 February to 9 March. The major increase in strobilus yield (from stem application on 21 February) was 217%. Application after 9 March was ineffective.

Clonal variation in percentage increase of female strobili is illustrated in Fig. 2, meaned across application method. The data indicate considerable clonal variability, with peak increases in strobilus production ranging from 44% (Clone 3) to 179% (Clone 1): the higher the intrinsic strobilus production ability of a clone, the more it responded to $GA_{4/7}$. The pattern of response to timing of application was broadly similar between clones.

TABLE 3–ANOVA of strobilus number, Experiment 1.

| Source | df | Pr>F | Significance |
|---|---|---|---|
| Main-plot (A) | | | |
| Clone (C) | 2 | 0.0001 | ** |
| Date (D) | 14 | 0.0002 | ** |
| Error A | 28 | | |
| Sub-Plot (B) | | | |
| Appl. method (M) | 1 | 0.0001 | ** |
| M*D | 14 | 0.0002 | ** |
| M*C | 2 | 0.0007 | ** |
| M*D*C | 28 | 0.1199 | ns |
| Error B | 360 | | |
| Total | 449 | | |

TABLE 4–Total female strobilus numbers per ramet in Experiment 1 by date and method of application. Data are meaned across clones

| Date | Bud application | Stem application |
|---|---|---|
| 1 February | 5.8 | 10.9 |
| 5 February | 5.7 | 11.9 |
| 9 February | 6.7 | 14.8 |
| 13 February | 5.1 | 13.5 |
| 17 February | 11.7 | 11.6 |
| 21 February | 9.4 | 17.1 |
| 25 February | 9.8 | 14.4 |
| 1 March | 7.5 | 14.2 |
| 5 March | 8.7 | 10.4 |
| 9 March | 5.5 | 8.3 |
| 13 March | 4.0 | 4.3 |
| 17 March | 5.4 | 5.9 |
| 21 March | 5.7 | 7.1 |
| 26 March | 3.7 | 5.7 |
| 30 March | 4.7 | 5.0 |
| Mean | 6.6 b | 10.3 a |

Untreated control ramets (n=30) had a mean strobilus number of 5.4. Values enclosed by boxes differ significantly from controls at 5% level.

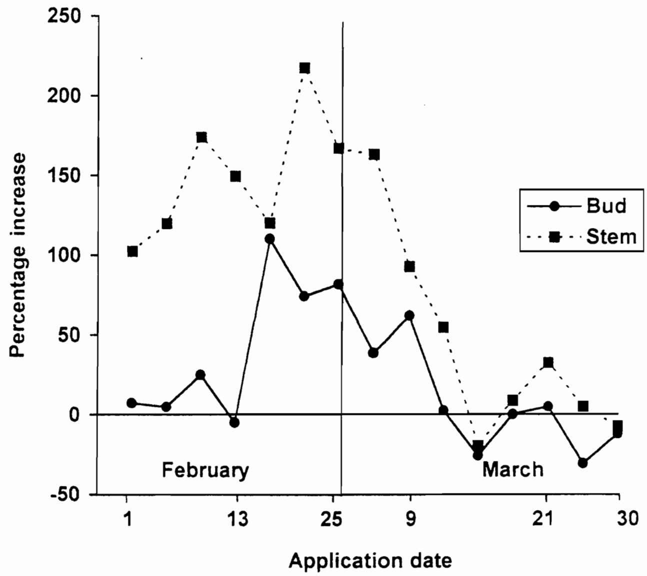


FIG. 1–Experiment 1: percentage increase in female strobili over untreated controls, by application date and method of application.

## Experiment 2

The ANOVA is presented in Table 5. The treatment effects and most of their interactions were significant at greater than 1%.

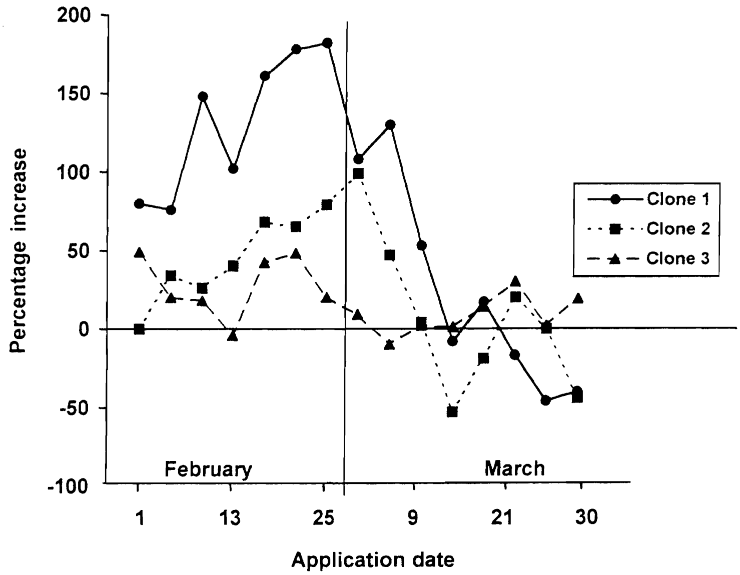


FIG. 2–Experiment 1: percentage increase in female strobili over untreated controls, by clone and application date.

TABLE 5–ANOVA of strobilus number, Experiment 2.

| Source | df | Pr>F | Significance |
|---|---|---|---|
| Main-plot (A) | | | |
| Size (S) | 2 | 0.0001 | ** |
| Date (D) | 4 | 0.0064 | ** |
| Error A | 8 | | |
| Sub-Plot (B) | | | |
| Appl. Method (M) | 1 | 0.0001 | ** |
| M*S | 2 | 0.0003 | ** |
| M*D | 4 | 0.0102 | * |
| M*S*D | 8 | 0.2023 | ns |
| Error B | 120 | | |
| Total | 149 | | |

Female strobilus counts, by age class and application method, are presented in Table 6. It is clear that, irrespective of application method, the 1-year-old ramets had a greater percentage increase of strobili (175%) in response to $GA_{4/7}$ than did the 2-year-old (115%), which in turn were higher than the 3-year-old ramets (11%).

In terms of total numbers of strobili induced by $GA_{4/7}$, however, the picture is different. The 1-year-old grafts "added" an extra 1.4 strobili per ramet, the 2-year-old ramets an extra 9.2, and the 3-year-old ramets an extra 2.6 strobili per ramet over the untreated controls.

As in Experiment 1, stem injection was in general a much more effective application method than bud application. This generality, however, did not apply to the 3-year-old ramets where stem injections did not differ significantly from bud application.

TABLE 6–Female strobilus numbers per ramet in Experiment 2, and percentage increase over the controls. Data are means across application dates

| Planting year (& age) | Bud application | | Stem injection | | Mean of two methods | |
|---|---|---|---|---|---|---|
| | Number | Incr. (%) | Number | Incr. (%) | Number | Incr. (%) |
| 1992 (1) | 1.7 | 112.5 | 2.6 | 225.0 | 2.2 c | 175.0 |
| 1991 (2) | 11.8 | 47.5 | 22.6 | 182.5 | 17.2 b | 115.0 |
| 1990 (3) | 21.9 | 2.8 | 25.9 | 21.6 | 23.9 a | 10.9 |
| Means | 11.8 b | 54.7 | 17.0 a | 143.9 | 14.4 | |

Untreated control ramets had a mean strobilus number of 0.8 (1 year old), 8.0 (2 years old), and 21.3 (3 years old).

The number of branches which are potentially capable, on size grounds, of bearing strobili is of course greater on larger ramets. Using experience from previous research regarding branches which are capable of becoming reproductive, mean values for these were assessed for each ramet age as follows: 3-year-old (pruned) 4.9; 2-year-old (unpruned) 4.1; 1-year-old (unpruned) 1.0. The effect of the $GA_{4/7}$ was both to increase the number of potential strobilus-bearing shoots which actually bore strobili, and also to increase the number of strobili per cluster.

The relationship between ramet age and application date, in terms of percentage response of strobilus number to GA application, is shown in Fig. 3. During the first 3 weeks of February there was little interaction between ramet age class and application date; with the 1-, 2-, and 3-year-old ramets having optimum application dates of 21, 21, and 9 February, respectively. After 21 February, however, the response pattern of the 1-year-old ramets changed somewhat, relative to the other two age-classes.

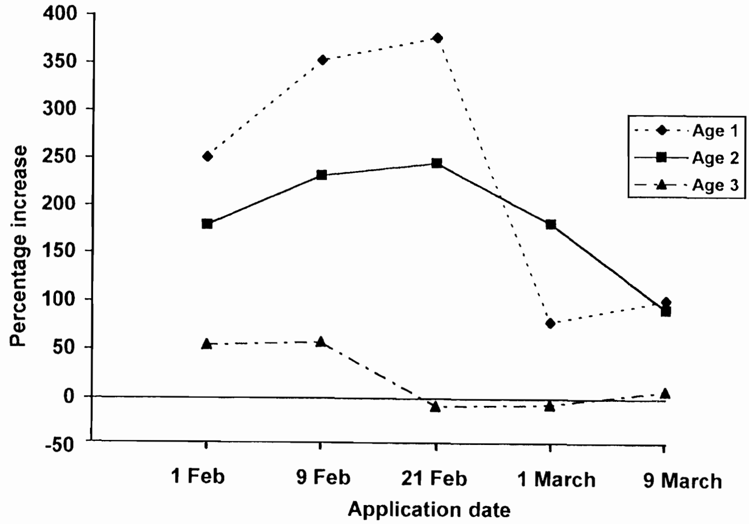


FIG. 3–Experiment 2: percentage increase in female strobili, by ramet age and application date.

## DISCUSSION

On the results of the experiments, February was a more effective month than March for the application of $GA_{4/7}$ at the Amberley and Waikuku seed orchards in 1993. While the pattern of $GA_{4/7}$ response did vary clonally, the peak response date varied by only 8 days over the three clones studied. The optimal timing was also affected by ramet size (in the one clone studied), but differed by only 12 days between the different-aged ramets.

It has been widely been believed that a key to understanding the role of $GA_{4/7}$ in increasing the numbers of female strobili is a knowledge of the morphological state of the buds at the time of optimum response to the $GA_{4/7}$ mixture.

In that context the results of a bud morphological study by Dickson *et al.* (1993) carried out at Amberley at the same time as Experiment 2 are of interest. Using Clone No. 1 ( the same clone used in Experiment 2 in this paper) it provided information on the timing of differentiation of long shoots on ramets of that clone aged respectively 2, 3, and 4 years from planting. Sampling on 19 February 1993 showed that buds on 4-year-old ramets contained differentiating female strobili. At that date the 2-year-old ramets had not even initiated any long shoots which could subsequently differentiate. It was 6 weeks later (31 March) before those buds contained differentiating female strobili. The 3-year-old ramets were intermediate in their timing between the 2- and the 4-year-old ramets.

It is thus clear that the maximum response to $GA_{4/7}$ application in the 2-year-old ramets used in Experiment 2 occurred some weeks before long shoots were differentiating into strobili; and in fact before any long shoot initiation had occurred at all. The general concept that $GA_{4/7}$ should be applied at the time of long shoot differentiation was thus not supported by the study of Dickson *et al.* (1993). At present there is no clear indication as to why February is an optimal month for application of $GA_{4/7}$ across material at very different stages of bud development, but the topic offers an opportunity for further research.

On a percentage basis, increased strobilus production due to $GA_{4/7}$ application was most effective on the smaller ramets, i.e., 1- and 2-year-old ramets. In terms of number of female strobili per ramet "added" by the $GA_{4/7}$ treatment, the 2-year-old ramets clearly gave the greatest response. Despite possible confounding due to location and pruning (*see* Table 2), this is believed to be a real effect.

The difference between 1- and 2-year-old ramets in the number of strobili "added" is interpreted as a difference in the number of buds per ramet which, on size grounds, are potentially capable of becoming reproductive. Such an explanation, however, does not explain why the 3-year-old ramets "added" fewer strobili than the 2-year-old ones. Taking the percentage response data into account, and comparing the responses with those achieved in the 6-year-old ramets studied by Sweet (1979), it seems that there may be an overall decrease in GA response with increasing ramet size. Because GA dosage remained constant with increasing ramet size, dose-response studies will be needed to determine whether this is a response to the (presumed) reduced amounts of GA reaching the buds in larger ramets, or whether its basis is more complex.

In terms of method of application, the "timing window" was considerably wider when $GA_{4/7}$ was applied by stem injection than when it was applied to the buds. Stem injection was also confirmed as a much more effective way to increase strobilus numbers than bud application. In the light of this strong finding of the operational superiority of stem injections of $GA_{4/7}$, it is necessary to comment that no research has been done since 1989 on the relative dose rates of the two application techniques. It is possible that, with modified dose rates for the bud application technique, its performance could be improved. However, an important factor in determining dose rates is to minimise the development of gibberellin-induced toxicity in the ramets, and the trend today is to try to reduce rather than increase dose rates.

Based on the behaviour of three clones in a single year, it seems to be desirable in operational $GA_{4/7}$ application at the Amberley seed orchard to concentrate the time of application in the middle and end of February. If that is not logistically possible, it may be preferable to move earlier into February than into March. Logically, the timing should be most optimal for those ramets which can be expected to give the greatest returns in terms of actual response to treatment. The results of Experiment 2 indicate that ramets that were 2 years old from planting gave the greatest response in terms of increased numbers of female strobili. One-year-old and 3-year-old ramets responded at a comparable, but much lower absolute level. Thus it would be sensible to treat 3-year-old ramets first in the season (on the basis of the evidence in Fig. 3), to optimise the timing of 2-year-old ramets, and then finally to treat 1-year-old ramets.

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