

RAMET SIZE AFFECTS THE TIMING OF FEMALE STROBILUS FORMATION IN A *PINUS RADIATA* SEED ORCHARD CLONE

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ABSTRACT

There was substantial variation in the timing of lateral long-shoot initiation in a *Pinus radiata* D. Don clone, associated with ramet size. Grafted meadow orchard ramets aged 2, 3, and 4 years from planting (with respective heights approximately 1, 2, and 4 m) were sampled on 19 February and 31 March. On 19 February, microscopic examination revealed that vegetative and female strobili primordia were differentiating on 4-year-old ramets, but 2-year-old ramets had not initiated any lateral long-shoot primordia. The 3-year-old ramets had vegetative and undifferentiated long-shoot primordia but no female strobili. On 31 March, buds on the 2-year-old ramets contained lateral long-shoot primordia in early stages of differentiation. At this time strobili primordia of the 2-year-old ramets were similar in size and state of bract formation to those of the 4-year-old ramets 6 weeks earlier. Application of GA_{4/7} on 19 February stimulated only the 2-year-old ramets to form female strobili.

Keywords: bud development; cone formation; gibberellin (GA_{4/7}); histology; meadow orchard; megasporangiate strobilus; seed orchard; *Pinus radiata*.

INTRODUCTION

In *Pinus radiata*, shoot formation at the apical meristem of polycyclic branches proceeds through the production of bud scales, cataphylls, and then cataphylls with axillary short

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shoots or needle fascicles. After several needle fascicles have differentiated, a series of lateral long shoots is initiated; the cycle is then repeated. These long shoots may develop into either branches or female strobili; alternatively, development may be arrested, resulting in lateral latent buds (Bollmann & Sweet 1976). The time interval between the initiation of a lateral long-shoot primordium and its recognisable differentiation into a branch or female strobilus is approximately 6 weeks. The process of “decision making” during that 6 weeks has been called determination (Romberger 1963). There is evidence from *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) that a changing environment, even quite late during that period, may affect the outcome of determination (Silen 1973).

Studies of the timing of lateral long-shoot initiation have been restricted to a few sites only, in a few years (Bollmann & Sweet 1976). It appears that in New Zealand much of the 6-week determination period for strobili in the bud's first cycle of growth occurs during the month of February. Thus, hormonal treatment with gibberellin ($GA_{4/7}$) to stimulate female strobilus development is carried out in clonal seed orchards between mid-February and mid-March to coincide with this period of determination. The effectiveness of treatment varies with year and clone (Sweet 1995). There is also evidence that the effectiveness of $GA_{4/7}$ stimulation of female strobilus development declines with age of the ramets (Siregar 1994; Siregar & Sweet 1996).

This study was carried out on a single clone of *P. radiata* in a commercial seed orchard. We investigated the influence of ramet size on the timing of lateral long-shoot initiation and determination, and response to commercial application of $GA_{4/7}$.

MATERIALS AND METHODS

On 19 February and 31 March 1993, terminal buds were collected from three separate blocks of seed orchard plantings made respectively in 1989, 1990, and 1991. The scions used to establish these ramets were collected from other seed orchard ramets between 10 and 15 years of age. They were grafted and grown in the graft nursery for 1 year before being planted in the seed orchard. All plantings were at Amberley in the South Island of New Zealand, and all material collected was from a single clone which was considered a good cone producer. Approximate heights of the ramets in the three age-classes were 4, 2, and 1 m respectively. At each collection date, buds were taken at random from five control ramets of each age-class. Material from one typical ramet per age-class was photographed. On 19 February only, gibberellin $A_{4/7}$ was applied to ramets of each age-class; controls were not treated. Application to the 1989 and 1990 ramets was by stem injection at the rate of 2.5 mg GA in 0.5 ml 95% ethanol. The 1991 ramets (which were regarded as too small for stem injection) were treated by application to the buds at the rate of 0.5 mg GA in 0.5 ml ethanol.

After each collection, the buds were sliced longitudinally and fixed in FAA (40% formalin/acetic acid/95% ethanol/water, 1/1/14/1 by vol.) under vacuum for 24 h. Where lateral long-shoot buds were present, they were dissected out and processed separately. The buds were dehydrated in a tertiary-butyl alcohol series and embedded in paramat (BDH Chemicals). Sections were made with a rotary microtome at 10–12 μ m and stained in safranin-fast green (Johansen 1940) or toluidine blue (Berlyn & Miksche 1976). Sections were examined under a compound microscope and primordia were classified on the basis of shape and the presence of lateral primordia as reproductive, vegetative, or undifferentiated

(Bollmann 1983). In the spring following collection, the mean date of anthesis (defined as the date when the most female strobili of the age-class were receptive for pollination) was recorded for each age class of ramet, and counts of female strobili, vegetative long shoots, and latent buds were made on 20 ramets of each treatment and age-class.

Data were analysed by analysis of variance and, where appropriate, subjected to the Duncan's multiple range test. Because of the unequal numbers of observations of long-shoot primordia in the different classes, the PROC GLM procedures were used (SAS Institute 1987).

RESULTS AND OBSERVATIONS

Status of the Shoot at Harvest

A typical bud cluster from one ramet from each of the 1989, 1990, and 1991 planting dates is illustrated in Fig. 1A and 1B. Clearly, seasonal extension growth at both 19 February and 31 March was greater in the older larger ramets. While the 1989-planted ramet had extended 14 cm above its basal branch cluster on 19 February, the 1991-planted ramet only reached that length on 31 March.

Apical Development

Bollmann (1983) found that, compared with vegetative buds, seed cone (strobilus) buds developed more slowly, but they had an apical dome which was larger, both in height and diameter. These differences in bud size and apical dome were also evident in the material we studied (Table 1; Fig. 2 and 3).

There were significant differences in developmental stage between buds of different-aged ramets (Table 1; Fig. 2A–F). On 19 February differentiating strobili (height(h) = $588 \pm 47 \mu\text{m}$; diameter(d) = $730 \pm 59 \mu\text{m}$) were present in buds of the 1989-planted ramets (Fig. 2C); vegetative buds were present on 1989- and 1990-planted ramets (Fig. 2B and 2E); and the 1991-planted ramets had no lateral long-shoots (Fig. 2F).

Primordia continued to develop on ramets of all ages between the two sample dates. By 31 March, buds from all planting ages had differentiating strobili, but there were considerable differences in their size and stage of development (Fig. 3C, 3D, and 3E). Strobili primordia on 1989 ramets ($h = 1645 \pm 62 \mu\text{m}$; $d = 1294 \pm 69 \mu\text{m}$) had increased significantly in size from 19 February (Table 1) and bracts were evident and expanding along the flanks of the primordia (Fig. 3E). Strobili primordia on the 1990 ramets ($h = 558 \pm 36 \mu\text{m}$; $d = 753 \pm 38 \mu\text{m}$) were similar to those of the 1989 ramets on 19 February with little evidence of bract initiation (compare Fig. 2C and 3C). Strobili primordia on the 1991 ramets ($h = 776 \pm 69 \mu\text{m}$; $d = 894 \pm 135 \mu\text{m}$) were intermediate in size and bract initiation (Fig. 3D). In addition, in the 1991 ramets development of vegetative buds had not proceeded as rapidly as development of reproductive buds. Although the reproductive buds were similar to those on the 1989 ramets on 19 February, the vegetative buds showed no evidence of axillary meristems (compare Fig. 2B and 3B) and there were many undifferentiated primordia (Table 1; Fig. 2A and 3A).

The pattern of bud formation was the same for all three age-classes; the first-initiated lateral long-shoots were usually reproductive, with the later-initiated ones being vegetative.

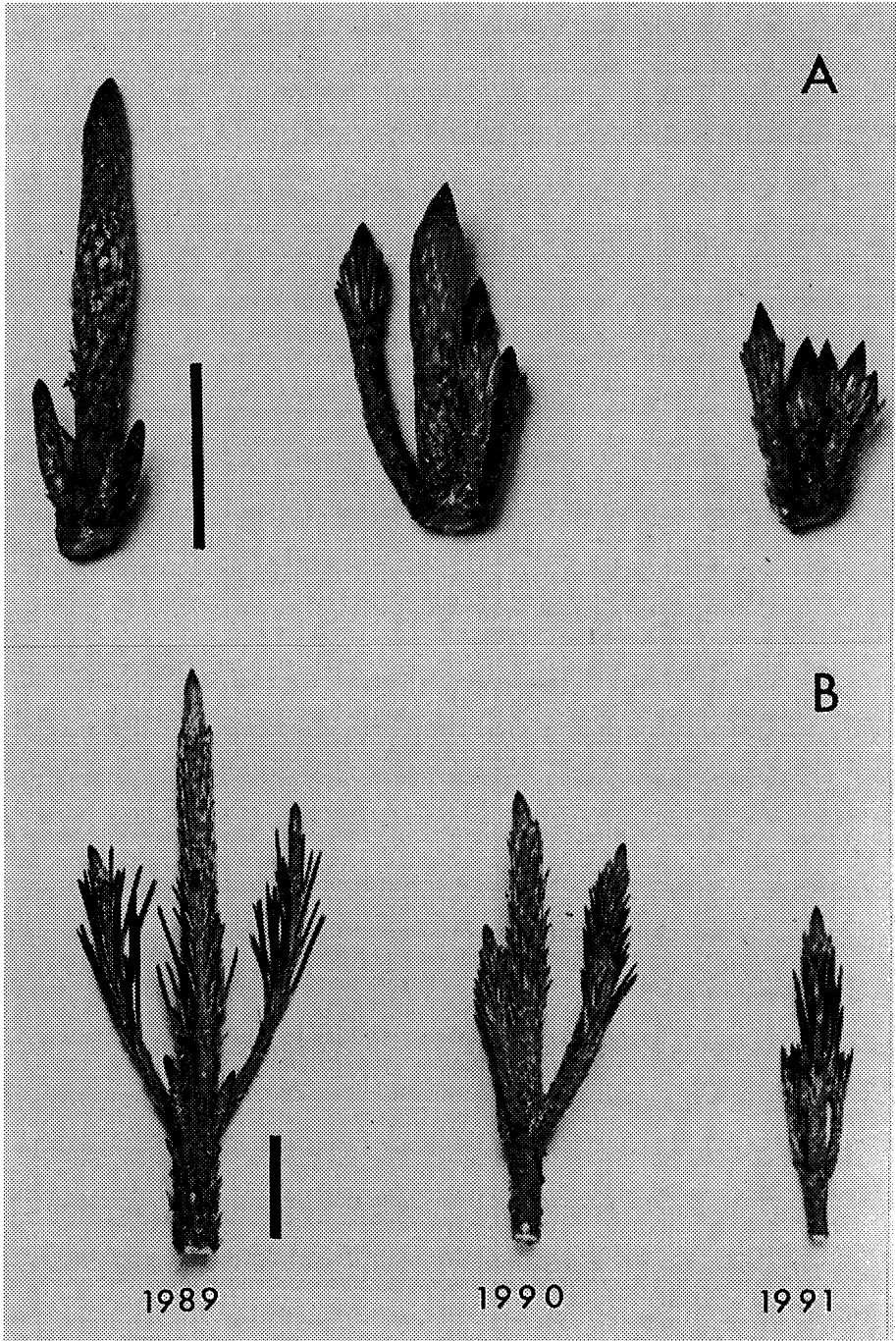


FIG. 1—Photographs of long-shoot terminal bud clusters of representative ramets from each age-class, A 19 February and B 31 March 1993. Bar = 5 cm.

TABLE 1—Size (μm) of long-shoot primordia above the last-formed cataphylls*

Year of planting	Sample date	Strobilus		Vegetative		Undifferentiated	
		Height	Diameter	Height	Diameter	Height	Diameter
1989	19 February	588 ^b	730 ^b (8)†	212	400(17)	282	565(2)
	31 March	1645 ^a	1294 ^a (6)	212	518(7)	—‡	—
1990	19 February	—	—	188	447(6)	235	400(4)
	31 March	776 ^b	894 ^b (5)	141	376(4)	165	306(1)
1991	19 February	—	—	—	—	—	—
	31 March	558 ^b	753 ^b (5)	165	329(7)	305	447(8)

^{ab} Within columns, means followed by different letters are significantly different ($p = 0.01$, Duncan's multiple range test)

* These measurements would be the same as the apical dome of Bollmann & Sweet (1976) except for strobili once they started forming ovuliferous scales and then the whole strobilus was measured

† Number of primordia measured given in parentheses

‡ No primordia in classification



FIG. 2—Buds from ramets collected on 19 February and stained with safranin-fast green (except as noted). A–E are lateral buds from terminal buds.

A–C = primordia from 1989 ramet ($\times 55$): A undifferentiated, B vegetative (stained in toluidine blue), C reproductive.

D and E = primordia from 1990 ramet ($\times 55$): D undifferentiated, E vegetative.

F = bud from 1991 ramet ($\times 6$). a = Lateral bud (lower left side in B and E).



FIG. 3—Lateral buds from terminal buds of ramets collected on 31 March and stained with safranin-fast green (except as noted).

A–C = primordia from 1991 ramet ($\times 55$): A undifferentiated (stained in toluidine blue), B vegetative, C reproductive.

D = reproductive primordia from 1990 ramet ($\times 55$).

E = reproductive primordia from 1989 ramet ($\times 31$).

Status After Shoot Expansion

The dates of anthesis and the nature of the first cycle lateral long-shoots for the 1989, 1990, and 1991 ramets in the spring of 1993 are summarised in Table 2. Anthesis for the 1991 ramets occurred 4 weeks after the 1989 ramets. The 1991 ramets also had more latent buds than the older ramets ($p < 0.001$). The application of GA_{4/7} enhanced strobilus production only in the 1991 ramets. This appeared to occur through the formation of more lateral long-shoots as there was no change in the number of branches or latent buds.

TABLE 2—Date of anthesis and effect of ramet age and gibberellin treatment on the number of vegetative branches, latent buds, and strobili borne laterally in the first potential cone-bearing cycle of 1993.

Year of planting	Date of anthesis	Bud type	−GA _{4/7}	+GA _{4/7}	SEM*
1989	22 July	Branch	1.5	1.9	0.56
		Latent	0.25	0.08	0.18
		Strobilus	5.0	4.1	0.41
		Total	6.7	6.1	
1990	27 July	Branch	1.1	1.0	0.46
		Latent	0.41	0.58	0.17
		Strobilus	5.7	5.8	0.35
		Total	7.2	7.4	
1991	15 August	Branch	1.5	1.5	0.46
		Latent	1.4	1.6	0.35
		Strobilus	2.6	4.2†	0.49
		Total	5.5	7.3	

* Standard error of the mean.

† For the 1991 plantings the number of strobili produced by ramets treated with GA is significantly greater than the controls ($p < 0.001$).

DISCUSSION

Ramet size and age influenced the timing of initiation and differentiation of primordia. Larger ramets displayed earlier initiation and differentiation of lateral long-shoots. Thus, by 19 February lateral long-shoot primordia were already present and differentiating on the 1989 ramets. The long-shoot primordia were at a stage similar to those reported for older conventional seed orchard trees on 27 February 1979 (Bollmann 1983). On the 1990 ramets initiation had taken place and differentiation of vegetative buds was evident (Fig. 2E) while a number of the first-initiated lateral long-shoots were undifferentiated (Fig. 2D). These primordia were similar in appearance to those reported by Bollmann (1983) for older trees on 24 January. This would indicate that developmentally the 1990 ramets were approximately 4 weeks behind the 1989 ramets on 19 February. Long-shoot primordia were not evident on the 1991 ramets (Fig. 2F). Ramet size did not alter the pattern of differentiation of long shoot primordia which was the same as that reported for *P. radiata* by previous workers (Bollmann & Sweet 1976; Bollmann 1983; Ross *et al.* 1984). The delay in initiation and differentiation of strobili primordia was carried over to the timing of anthesis. Thus, differentiation of strobili primordia in the 1991 ramets was 6 weeks behind the 1989 ramets and anthesis was delayed about 4 weeks.

The rate of differentiation of vegetative shoots on the 1991 ramets was slower than for the 1989 ramets (compare Fig. 2B and 2D with Fig. 3B). Delayed development of vegetative buds has been reported for cultural treatments which stimulate cone production (Ross *et al.* 1984; Owens *et al.* 1992). This might indicate that the younger smaller ramets would be more susceptible to the enhancing effects of GA_{4/7} than older larger ramets. Alternatively, the difference between the 1989 and 1991 ramets in rates of primordium development might reflect environmental conditions at the time of initiation, with earlier initiation occurring when conditions favoured vegetative growth and later initiation coinciding with conditions which would favour reproductive development. In other pines development of reproductive primordia continues later in the season than vegetative bud development (Greenwood 1981).

The effectiveness of GA application in stimulating cone production is confounded by differing methods of application, ramet size and age, and developmental state. However, Siregar & Sweet (1996) found that, for stimulation of cone formation by GA_{4/7}, stem injection was more effective than spraying; and so our results on stimulation by GA_{4/7} should be conservative. On the basis of this study, it would appear that GA application should be made close to the time of initiation of primordia for the greatest stimulation of cone production. The increase in cone production was accompanied by an increase in the number of long-shoot primordia—not the conversion of vegetative primordia to reproductive primordia. Similar results have been noted for *P. radiata* in the past (Sweet 1979).

This study was limited to one clone which was representative of a “good” cone producer. The results indicate that it may be good to expand observations to other clones. The effectiveness of GA in enhancing cone production may be increased by earlier application on larger ramets as they appear to initiate cones earlier than smaller ramets. However, future studies on timing of GA application should include a measure of bud morphology and the stage of differentiation of lateral long-shoot primordia.

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