Increase of Seed Yield and Physical Quality in a *Pinus radiata* Control-Pollinated Meadow Seed Orchard

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Abstract

Experiments were initiated to increase seed yield and physical quality in a New Zealand *Pinus radiata* controlled pollinated (CP) seed orchard. To do this an irrigation treatment; and other treatments such as girdling, foliar fertiliser and hormones, were applied during the period of rapid cone growth commencing some 14 months after pollination.

Irrigation significantly increased cone growth and seed yield; giving e.g. an increase of 53% in cone fresh weight, 39% in seed weight, 31% in the number of filled seeds per cone and 53% in seed germinative energy. Girdling and foliar fertiliser application also increased cone and seed parameters to comparable levels, but only in the absence of irrigation. Hormone application was ineffective unless combined with girdling or foliar fertiliser treatments.

While the results were based on only 2 clones, the magnitude of the increase in seed yield would indicate a potential for significant returns from commercial application of these treatments.

Key words: seed, seed orchard, control-pollinated, germination, irrigation, nutrition, *Pinus.*

FDC: 232.311, 174.7 *Pinus radiata;* (931).

Introduction

The concept of seed orchards was initially proposed by LARSEN (1956), and since that time they have been the subject of considerable research and development. But this research has been almost entirely aimed at developing technologies which maximised the genetic quality of seed. There has been little research aimed at improving the *physical* quality of the seed coming out of orchards. Yet the relationship between seed size, and germination rate and seedling quality has been well established for many years (e.g. WILCOX, 1983).

The experiments reported here were aimed at improving the yield, size and germination ability of seed from cones in a *Pinus radiata* seed orchard. In New Zealand, cones of *Pinus radiata* show an increase in dry weight of some 250% during their second year of seasonal growth, between mid-October and mid-December (SWEET and BOLLMANN, 1970); and fertilisation of the ovule occurs early in that period (LILL, 1975). A high proportion of seed failure is also known to occur at the time of fertilisation and during embryo development (SWEET, 1973; SETIAWATI *et al.,* 1993). High growth rates in developing cones obviously create high demands for nutrients, both mineral and carbohydrate (see e.g. DICKMANN and KOZLOWSKI, 1968). The rationale for the experiment reported here was thus to increase the availability of water, carbohydrates and mineral nutrients to developing cones during their rapid growth phase; and examine the consequent impacts on cone size, seed size and seed yields.

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Materials and Methods

Experimental site

The experimental area was located in a control-pollinated meadow seed orchard of *Pinus radiata* at Amberley, in the South Island of New Zealand. The concept and developmental history of meadow orchards in New Zealand has previously been reported by CARSON *et al.* (1992). Essentially, grafted ramets are planted at high stocking on sites which favour initiation of strobili close to the ground. The grafts are pruned to optimise production of strobili at heights of less than 2 m. Control-pollination is practiced and the orchard is managed on a rotation of less than 10 years. The Amberley orchard contains some 20 clones. Amberley is at a latitude of 43°10'S. It has a 650 mm rainfall and the predominant soil type is silt loam lying over a clay loam. The soil is imperfectly drained.

Ramets

Three-year old ramets (planted in 1990) of 3 clones, spaced at 1 m x 2 m were utilised in this experiment. The selection criteria for the clones was that they contained sufficient ramets with developing cones entering their second year of growth, after pollination in August 1991.

Treatments

Irrigation, foliar fertiliser, hormone and girdling treatments were applied during the period of rapid cone enlargement, i.e. between mid-October 1992 and the end of February 1993. The timing of treatment in relation to the sequence of cone development is shown in *figure 1*.

Irrigation

A drip irrigation system was supplied to each treated ramet. Irrigation commenced when the soil moisture content dropped to 75% of field capacity and applied water at 10 l/ramet/day to reach and maintain field capacity. The irrigation was scheduled during the night, to reduce evaporation. Soil moisture content was measured, largely on a weekly basis, using time domain reflectometry (TDR) at 20 cm depth in the soil.

Foliar fertiliser

A major rationale for applying fertiliser to the foliage (as distinct from the soil) was to minimise increases in vegetative growth of the ramets. A complete liquid foliar fertiliser with N:P:K:Ca composition 10:10:7:0.5; and micronutrients Mg, Fe, Cu, Mn, Zn, B and Mo was utilised. Each treated ramet received 500 ml of foliar fertiliser, at each application, containing 1 g each of N and P, 0.7 g K and 0.05 g Ca. The foliar fertiliser was applied weekly from mid-October 1992 until mid-December 1992 (8 applications in total). A week after the last fertiliser application, foliage samples from fertilised and unfertilised treatments of the three clones were collected and ground, and their nutrient contents analysed.

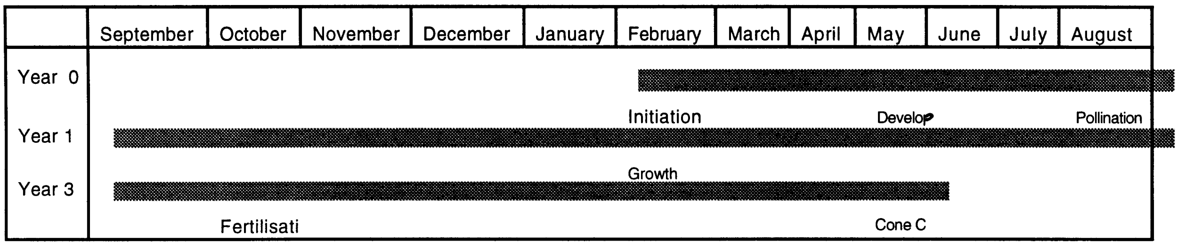

Fig. 1. – Diagram of time sequence from female initiation of *P. radiata* to cone collection.

Girdling

Girdling has been shown to increase carbohydrate levels above the girdle in shoots of *P. radiata* (CAMERON, 1970). This is normally associated also with the accumulation of plant growth substances (KRAMER and KOZLOWSKI, 1979). Locking nylon cable ties 368 mm x 7.6 mm in dimension were used for girdling the treated ramets. Girdling was carried out by fastening the cable ties tightly around the stem, 20 cm below the developing cones (*Fig. 2*). The girdles were applied in mid-October and removed 16 weeks later.

Fig. 2. – A locking nylon band was placed 20 cm below developing cones to restrict phloem movement.

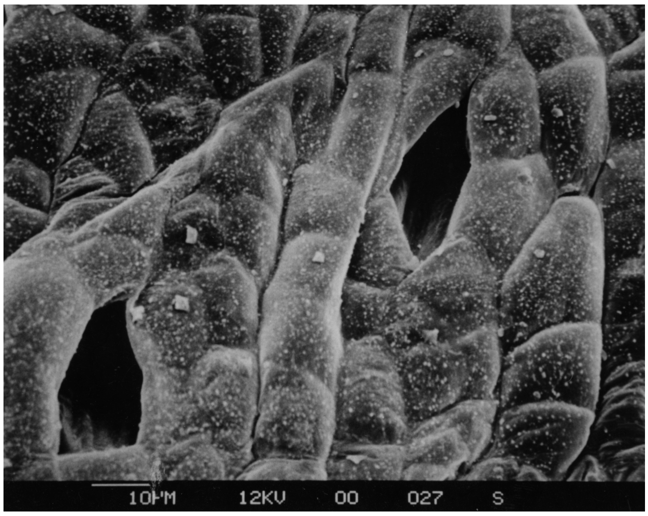

Fig. 3. – Scanning electron micrograph of cone surface showing "stomata-like" apertures.

Hormone application

Two hormones, benzylamino-purine (BAP) and gibberellin A4/7 were combined in an aqueous solution at a concentration of 100 ppm for each hormone. The commercial surfactant Pulse was added to the hormone solution at a concentration of 1 ml/10 l. The hormone solution was sprayed weekly on to the developing cones using a hand sprayer, from mid-October 1992 until mid-December 1992 (8 applications). Previously, cone samples of each clone were taken for scanning electron microscopy (SEM). The SEM results showed "stomata-like" apertures on the surface of the developing cones (*Fig. 3*), offering a possible entry route for externally applied substances.

Experimental design

The treatments were applied in factorial combination as a split-plot design. Irrigated and non-irrigated treatments were randomly allocated within each of three clonal blocks. Each of these main plot treatments was then split into 8 subplots with cultural treatments, i.e.: (1) control; (2) fertilised (F); (3) girdled (G); (4) hormone (H); (5) fertilised + girdled (FG); (6) fertilised + hormone (FH); (7) girdled + hormone (GH); (8) fertilised + girdled + hormone (FGH). The total combination was 3 clones x 2 irrigation treatments x 8 cultural sub-treatments making 48 experimental units. Each experimental unit was represented by 3 ramets, making a total of 144 ramets. Most ramets carried at least 3 developing cones.

Cone and seed analysis

Cones were collected at the end of May 1993 and all of the physical cone characteristics (fresh weight, volume, length and diameter) were measured. The cones were then cured for eight weeks at 20 °C and 70% RH (RIMBAWANTO et al., 1988). The seeds were extracted by placing the cured cones in an oven at 60 °C to 63 °C for 6 hours. De-winging was done manually and empty seeds were separated with an agricultural seed cleaner. The total number of developed seed and filled seed was counted, and the weight of 100 seeds obtained. The seeds were stratified by soaking in distilled water for 24 hours, and germinated on moist blotting paper in a petri dish. The germination conditions were a temperature of 20 °C and an RH of 85% for 28 days (ISTA, 1985). Germinated seeds were observed every 2 days. Germinative energy and germination capacity were calculated (WILLAN, 1985).

Data analysis

Statistical analysis was done using Proc ANOVA, t-tests and DUNCAN's Multiple Range Test (SAS Institute, 1987).

Results

Soil moisture measurements and foliage analyses are presented in Appendices 1 and 2 respectively.

Cone and seed results are presented for only 2 clones out of the 3 clones treated. That is because the seeds of the third clone, irrespective of treatment, were so severely infected by

Table 1. – Analysis of variance for cone parameters.

Source	Pr > F			
	Fresh Weight	Volume	Diameter	Length
Main-Plot				
Clone (Cl)	0.0049	0.0024	0.0399	0.0009
Irrigation (I)	0.0110	0.0041	0.0767	0.0044
Sub-Plot				
Cultural Treatment (CT)	0.0001	0.0001	0.0001	0.0001
CT x Cl	0.0001	0.0001	0.0001	0.0007
CT x I	0.0001	0.0001	0.0001	0.0088
CT x Cl x I	0.0019	0.0044	0.0031	0.3715

Table 2. – Analysis of variance for seed yield parameters.

Source	Pr > F			
	Filled Seed/Cone	Empty Seed/Cone	Total Seed/Cone	% Filled Seed/Cone
Main-Plot				
Clone (Cl)	0.5953	0.0229	0.2822	0.2058
Irrigation (I)	0.8033	0.0880	0.5991	0.6240
Sub-Plot				
Cultural Treatment (CT)	0.0039	0.0001	0.0794	0.0001
CT x Cl	0.0281	0.1302	0.0026	0.3191
CT x I	0.0027	0.0172	0.1486	0.0019
CT x Cl x I	0.4705	0.2373	0.6566	0.2823

Table 3. – Analysis of variance for seed physical quality parameters.

Source	Pr > F		
	Seed Weight	Germ Energy	Germ Capacity
Main-Plot			
Clone (Cl)	0.0302	0.0966	0.1928
Irrigation (I)	0.0459	0.0332	0.2233
Sub-Plot			
Cultural Treatment (CT)	0.0017	0.0001	0.0001
CT x Cl	0.0455	0.0001	0.3731
CT x I	0.0001	0.0001	0.0001
CT x Cl x I	0.1900	0.0001	0.4580

fungi that seed yield and physical quality parameters could not be assessed.

The analyses of variance on cone and seed parameters are presented in *tables 1, 2* and *3*. Those tables indicate generally high levels of significance for clones, irrigation and cultural treatments; along with quite substantial interactions between them. When the data were examined critically, the significance of irrigation as a Main Plot treatment was seen to be very heavily confounded by its interaction with the cultural treatments. An illustration of that is given in *table 4* which looks at the effect of treatments on filled seeds per cone. The number of filled seeds in the control, non-irrigated treatment is increased 31% by irrigation, 34% by fertiliser application and 25% by girdling. But the number of filled seeds in the control irrigated treatment was increased very little by fertiliser (1%) and girdling (7%). And the *mean* irrigated treatment did not differ from the mean non-irrigated treatment.

Because of the strong interaction between irrigation and the cultural treatments, it is necessary to examine them independently, as is done in *tables 5* and *6*. The values given in these tables are means of values for the cones collected from 6 ramets (2 clones x 3 ramets per clone).

The effect of irrigation

A comparison of irrigated and non-irrigated control treatments shows that irrigation had a significant effect on almost all of the cone and seed parameters recorded (*Table 5*). Irrigation not only increased cone size but also the yield and the physical quality of the seeds. Irrigation increased cone weight by 53%, cone volume by 57%, the number of filled seeds per cone by 31%, total seeds per cone by 20%, seed weight by 39% and seed germinative energy by 53%. These differences were statistically significant at the 5% level by t-test.

The effect of cultural treatment

In the absence of irrigation; girdling and foliar fertiliser treatments significantly affected all of the cone and most of the seed parameters assessed (*Table 6*).

Girdling increased cone fresh weight by 49%, cone volume by 50%, the number of filled seeds per cone by 25%, seed germinative energy by 74%, and it reduced the number of empty seeds per cone by 39%. The application of foliar fertiliser increased cone fresh weight by 35%, the number of filled seeds per cone by 34%, and reduced the number of empty seeds per cone by 23% (*Table 6*). The data for hormone application are not presented: hormone application on its own did not significantly change the value of any cone or seed parameter assessed.

The relative effectiveness of irrigation and cultural treatment

A comparison of cone and seed parameters between control irrigated values and the highest non-irrigated values (*Table 7*) indicated that the "best" non-irrigated treatment was more effective than irrigation in terms of increasing seed yields per cone, and seed germination ability; but not in other respects. It also showed that the highest non-irrigated values always involved a girdling treatment. In no comparison was fertiliser more effective than girdling.

Table 4. – Numbers of filled seeds per cone – the interaction of irrigation and cultural treatment.

Treatment	Number of filled seeds per cone	
	Non-Irrigated	Irrigated
Control	64 b	84 a
Fertilised ($)	86 a	85 a
Girdled (G)	80 b	91 a
Hormone (H)	76 a	76 a
F G	87 a	87 a
F H	70 a	74 a
G H	95 a	73 b
F G H	80 a	84 a
Mean	80 a	82 a

The statistical significance indicated is at the 5% level by t-test

Table 5. – The effect of irrigation on cone and seed parameters.

Parameters	Control Irrigated	Control Non-irrigated	% Difference
Cone Fresh Weight (g)	185 a	121 b	53
Cone Volume (cc)	166 a	106 b	57
Cone Diameter (mm)	59 a	51 b	16
Cone Length (mm)	102 a	89 b	15
Filled seed/cone	84 a	64 b	31
Empty seed/cone	29 a	31 a	-6
Total seed/cone	113 a	94 b	20
% filled seed/cone	75 a	66 b	14
100 Seed Weight (g)	4.37 a	3.15 b	39
Germinate Energy (%)	52 a	34 b	53
Germinative Capacity (%)	82 a	73 a	12

The statistical significance indicated is at the 5% level by t-test

A further review of the interaction between irrigation and cultural treatment

Table 8 indicates how little gain is achieved by girdling or fertilising in the presence of irrigation. None of the differences in *table 8* are statistically significant.

Discussion

While all of the treatments reported above have been frequently applied in seed orchards, they have traditionally been used to increase the *initiation* of strobili. The unique aspect of this experiment is that the efforts to enhance seed yield and quality were focused on the time of rapid cone enlargement, immediately following the process of fertilisation (SWEET and BOLLMANN, 1970). The magnitude of the response to treatment indicates clearly the high level of demand for nutrients at that time.

Although this study set out to include 3 clones in each treatment, the high levels of fungal infection in one clone precluded its use. Infection of *Pinus radiata* cones by pathogenic fungi is not uncommon, with *Sphaeropsis sapinea* having been confirmed as one primary pathogen (GRBAVAC and DARLING, 1993). Clonal variability in sensitivity to pathogens is well documented (e.g., BRAMLETT, 1974) and unfortunately one clone in this experiment was particularly sensitive.

While the results apply specifically to only 2 clones, the data indicated that in those clones substantial gains could be obtained in seed yields; and seed size and germinability. These were obtainable *either* through increased water availability, *or* through treatments aimed at increasing the availability of carbohydrates or mineral nutrients. The fact that treatment responses were not additive indicates limits to gains in these parameters.

While plant water stress measurements were not made, it can reasonably be presumed that the maintenance of soil at field capacity (an average of 17% above non-irrigated soil – see *Appendix 1*) reduced plant water stress, and increased photosynthesis and consequent carbohydrate availability. *Appendix 2* shows that it also increased foliar levels of all mineral elements analysed, other than magnesium. It is clear from *Appendix 2* that, in terms of foliage mineral nutrient levels, irrigation equated to the application of foliar fertiliser to non-irrigated ramets. It is unfortunate that carbohydrate levels were not measured, but it is a reasonable supposition that irrigation would also have increased these through increased photosynthesis. Thus, in terms of their effects, irrigation on

Table 6. – The effect of girdling and fertilizer treatments on cone and seed parameters in the absence of irrigation.

Parameters	Control	Girdled	% Difference	Fertilizer	% Difference
Cone Fresh Weight (g)	121 b	180 a	49	163 a	35
Cone volume (cc)	106 b	159 a	50	144 a	36
Cone Diameter (mm)	51 b	60 a	18	58 a	14
Cone Length (mm)	89 a	100 a	12	95 a	7
Filled seed/cone	64 b	80 a	25	86 a	34
Empty seed/cone	31 a	19 b	-39	24 b	-23
Total seed/cone	94 b	99 ab	5	110 a	17
% filled seed/cone	66 b	82 a	24	78 a	18
100 Seed Weight (g)	3.15 b	4.04 a	28	3.65 ab	16
Germinative Energy (%)	34 b	59 a	74	36 b	6
Germinative Capacity (%)	73 a	82 a	12	80 a	10

The statistical significance indicated is at the 5% level by t-test

Table 7. – The comparison of control irrigated and highest non-irrigated values of cone and seed parameters. The statistical significance indicated is at the 5% level by t-test.

Parameters	Control Irrigated	Highest Non-irrigated *	% Difference
Cone Fresh Weight (g)	185 a	181 GH a	-2
Cone Volume (cc)	166 a	159 G a	-4
Cone Diameter (mm)	59 a	60 G a	2
Cone Length (mm)	102 a	101 GH a	-1
Filled seed/cone	84 b	95 GH a	13
Empty seed/cone	29 a	19 G b	-34
Total seed/cone	113 a	120 GH a	6
% filled seed/cone	75 b	82 G a	9
100 Seed Weight (g)	4.37 a	4.04 G b	-8
Germinative Energy (%)	52 b	59 G a	14
Germinative Capacity (%)	82 a	87 FG a	6

*) G=girdled; GH=girdled with hormone treatment; FG=girdled with fertilizer application

Table 8. – The comparison of control irrigated with 'best' irrigated values containing girdling (G) or fertilizer (F) of cone and seed parameters.

Parameters	Control Irrigated	'Best' Irrigated + G	% Increase	'Best Irrigated + F	% Increase
Cone Fresh Weight (g)	185	189	2	188	2
Cone Volume (cc)	166	167	1	165	-1
Cone Diameter (mm)	59	60	2	59	0
Cone Length (mm)	102	105	3	103	1
Filled seed/cone	84	91	8	85	1
Empty seed/cone	29	21	-28	21	-28
Total seed/cone	113	114	1	106	-6
% filled seed/cone	75	81	8	80	7
100 Seed Weight (g)	4.37	4.26	-3	4.33	-1
Germinative Energy (%)	52	51	2	56	8
Germinative Capacity (%)	82	85	4	90	10

this site may well have equated to the effects of girdling and mineral nutrient application on non-irrigated ramets.

The responses to increased nutrient availability included cone size, the number of both total and filled seeds per cone, and germinative energy of the seeds. There is abundant literature evidence for a number of crops (e.g. WARDLAW, 1968; SOMMER and SCHULTE, 1985) that developing fruits and seeds are particularly strong nutrient sinks; and that increasing the availability of nutrients in plants, also increases their supply to developing fruits and seeds. There is also evidence that seeds which have developed in the absence of nutrient stress have increased germinative energy. WEST and LOTT (1993) e.g., showed in 11 species of pines that there was a strong correlation between seed weight and embryo weight and that large seeds had substantially larger mineral nutrient reserves than smaller seeds.

The magnitude of the gains achieved in this experiment is such that seed orchard managers can be expected to seek to capture them in practice. The management of CP meadow orchards for a species such as *P. radiata* is both intensive and complex, with much of this complexity being driven by the multi-year development sequence of cones (see *Figure 1*). If the ramets are to be used for the collection of successive cone crops in successive years, then the prospect exists that treatments which are promotive for one age-class of developing cones may be negative in their impact for another age class on the ramet. The period of irrigation as carried out in this experiment overlapped in time with initiation of a new flower crop (*Figure 1*); and there is considerable literature evidence (e.g. OWENS *et. al.*, 1992) that water stress promotes strobilus initiation. Thus irrigation, if it is to be the preferred method for improving the physical quality of seed may need to be terminated earlier than in this experiment.

Equally, there are strong economic and logistical reasons to aim to minimise the vegetative growth of ramets in a CP meadow orchard. The impact on vegetative growth of both irritation and mineral nutrient sprays needs to be evaluated.

While girdling may appear from the above analysis to be the most appropriate tool to use, it again has the potential to impact on ramet health in the longer term. In that context, however, the locking nylon cable ties which were used in this experiment to restrict the movement of solutes in the phloem appeared very effective; and they can be removed simply without (it is believed) any permanent damage to the ramets.

While further research will be needed to broaden these narrow clonally-based experimental techniques and develop them into a routine management tool, the gains from doing so appear to be at such a level that seed orchard managers will wish to capture them. There is some irony in the recognition that the current move to locate seed orchards in areas of soil moisture stress may be having a negative impact on the physical quality of their seeds.

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Appendix

Appendix 1. – Soil moisture measurements at 20 cm soil depth during the study. The statistical significance indicated is at the 5% level by t-test.

	Soil Moisture Content (%)	
Date	Irrigated	Non-irrigated
5 Nov 92	37 a	24 b
12 Nov 92	35 a	24 b
19 Nov 92	35 a	23 b
26 Nov 92	38 a	18 b
10 Dec 92	35 a	25 b
16 Dec 92	32 a	15 b
4 Jan 93	34 a	17 b
5 Feb 93	34 a	17 b
12 Feb 93	33 a	16 b
20 Feb 93	35 a	17 b
27 Feb 93	34 a	17 b

Appendix 2. – Result of foliage analysis of irrigated and non-irrigated trees. The statistical significance indicated is at the 5% by t-test.

	Foliar Nutrient Levels			
	Irrigated		Non-irrigated	
Elements	+ F*	- F*	+ F*	- F*
N (%)	2.05 a	2.05 a	2.06 a	1.98 b
P (%)	0.17 a	0.17 a	0.17 a	0.14 b
K (%)	1.01 a	1.01 a	1.01 a	0.80 b
Mg (%)	0.08 a	0.08 a	0.08 a	0.07 a
Ca (%)	0.36 a	0.36 a	0.36 a	0.31 b
B (ppm)	28.8 a	28.6 a	29.9 a	22.0 b

*) + F = treatment with foliar fertiliser application
– F = treatment without foliar fertiliser applied