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Controlled Pollination without Isolation – a New Approach to the Management of Radiata Pine Seed Orchards

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Abstract

This paper records a series of experiments, in hedged and meadow seed orchards, aimed at evaluating and improving the techniques of controlled pollination without prior isolation of strobili.

The results indicate that in a New Zealand hedged *Pinus radiata* seed orchard, multiple pollination of strobili with pollen suspended in water is effective at excluding unwanted pollens from entering the ovules. The effect is further improved by the prior use of compressed air to "blow off" any unwanted, naturally dispersed pollen grains from the surface of the strobili. With these methods it has proved possible, without isolating strobili, to ensure that only pollen applied by the orchard manager enters the ovules.

Key words: Seed orchard, meadow orchard, controlled pollination.

Introduction

For many years now there have been concerns expressed about the reduced levels of genetic gains from radiata pine orchard seed in New Zealand, relative to those expected on the basis of progeny trials. The deficiencies of conventional seed orchards in this respect have been reviewed by Sweet and Krugman (1977). They indicated a major problem arising from the lack of control over pollination. Radiata pine seed orchard technology in New Zealand has been progressively modified since that time to improve genetic gains (see e. g. SHELBOURNE et. al., 1989). Thus current practice by the major seed orchard company in New Zealand is to produce a high proportion of its orchard seed by controlled pollination on hedged rows of grafted clones.

Since 1989, new seed orchard plantings by that company (Proseed New Zealand) have been with meadow orchards (SHELBOURNE et al, 1989; SWEET et. al., 1990). These consist of high density (5000 sph) clonal blocks of grafts, which will bear strobili on their leading shoots one or two years after planting, and will produce high yields of seed per hectare at a young age. Their attraction, vis a vis the hedged orchards which they will replace, is economic. In financial terms, the seed they produce is much less costly; and in terms of genetic improvement, they allow improved genetic material to come into production much faster (see SWEET et. al., 1990; ARNOLD, 1990).

Controlling pollination is seen as the key to increased genetic gain from seed orchards but, if done with isolation of strobili, there is a significant cost component to it (see ARNOLD, 1990). The intent of the research reported in this paper was to explore the genetic effectiveness of controlled pollination carried out without isolation. Should sound technique permit this without significant reduction of genetic gain, then seed orchard management would be both cheapened and simplified logistically.

There has been considerable research reported on the process of pollination in *Pinus*. The classical work by SARVAS (1962) with *Pinus sylvestris* has been important in the development of controlled pollination technology. In particular, from the point of view of the questions asked in this paper, it drew attention to the finite capacity of the micropyle to contain pollen, and to the method by which pollen was moved (against the force of gravity) into the micropyle. The pollination droplet (first reportet by DOYLE and O'LEARY, 1935) was seen as the key to pollination. Work by LILL and SWEET (1977) explored for *Pinus radiata* some of the issues explored by SARVAS for *Pinus sylvestris*. A synthesis of the significant parts of the above research, for the problem reported in this paper, would indicate that:

1. The micropyle of *Pinus radiata* has a maximum capacity of 7 or 8 pollen grains, and on average holds fewer than 5 grains.
2. Everything else being equal, the pollen grains in the micropyle reflect in number and constitution the mix found on the micropylar arms; which in turn reflect the mix in the air, over time.
3. Once the micropyle is full of pollen, any subsequent pollen events in the orchard are of no relevance to the manager.

Thus the pollination droplet has for long been seen as critical to controlled pollination technology. If the

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controlled pollen could be applied early, in large quantities relative to any background pollen, and if it could be taken up immediately to fill the micropyle, then it should be possible to dispense with isolation. The limiting factor, however, was expected to be the activity of the pollination droplet, which LILL and SWEET (1977) had found to be very infrequent in operation, particularly on the low rainfall sites where seed orchard location is favoured. There was some promise offered, however, by a paper by M. S. GREENWOOD (1986), in which he illustrated that in *Pinus taeda*, pollen grains could be transferred from the micropylar arms to the micropyle by raindrops. Clearly, if that could happen in *Pinus radiata*, the prospects for a more rapid uptake of pollen into the micropyle would be greatly improved.

The experiments reported here used the information in the literature to explore techniques to optimise the effectiveness of controlled pollinations, without isolation, under quantifiable conditions.

Materials and Methods

With one exception (in Experiment 3), all experiments were carried out at the Amberley seed orchard, in Canterbury in the South Island of New Zealand. The 72 ha site, in a 650 mm rainfall area, was converted from a conventional seed orchard to a hedged one in 1985, and is currently managed for the production of controlled pollinated seeds, with isolation and hedging. Since 1989, 7.5 ha of meadow orchard have been established per year, and it is that orchard from which the company seeks to produce controlled pollinated seed without isolation. The ramets used in the experiments were located in both the hedged and meadow sections of the orchard.

The major technique employed involved the use of dyed pollens. A number of dyes were used (see SOMERVILLE and SWEET, 1978; and GREENWOOD, 1986 for details). Dyed pollen grains, while inviable, are transported in air and in liquids in the same way as normal pollen. Because their air sacs are unaffected by the dyeing process, they float up in the pollination droplet into the micropyle. When ovules are dissected under a binocular microscope, the colour of individual pollen grains is easily assessable.

The controlled application of pollens to strobili was done (a) with normal dry pollen; and (b) with pollen suspended in water. The dry application was the standard one of blowing a mix of pollen and air through a hypodermic needle onto the strobili. In the "wet" application, compressed air was again used to force a suspension of pollen in water onto the strobili.

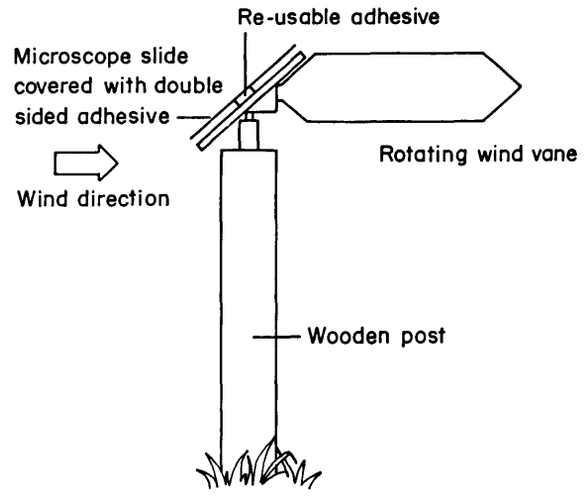

Figure 1. — **Trap designed to collect pollen moving in the prevailing wind.**

The Experiments: Design and Results

Experiment 1. A definition of background pollen levels in the Amberley orchard

1. Design

Simple pollen traps (see *Fig. 1*) were constructed and installed at strobilus height at three locations within the orchard. They were maintained and their slides collected daily (other than for weekends) from 2 August to 25 September, 1989, the major part of the seasonal period of pollen shed. Counts were made of the number of pollen grains on an area of 2 cm^2 of each slide, approximating to the surface area of a receptive strobilus.

It was possible by relating the location of each trap to the wind direction each day, to obtain an approximation of the amount of pollen trapped from inside the orchard, relative to that from outside.

2. Results

Figure 2 illustrates the total number of pollen grains trapped per day by the three traps. The mean number of grains per 2 sq. cm. of trap averaged 90 per day over the season, with a maximum of 550 on the day of 7, September 1989. A strobilus receptive for (say) 10 days is thus likely to receive several hundred pollen grains, at least, during that time. Just under half (47%) of all pollen trapped was categorised as having definitively come from outside the orchard, but the real figure is likely to be higher as it was not possible to differentiate between external and internal

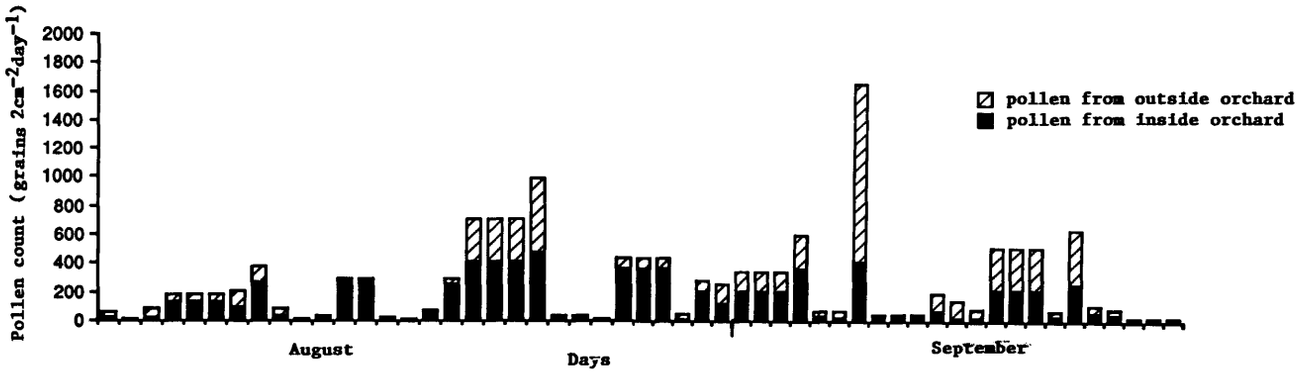

Figure 2. — **Daily pollen counts, Amberley seed orchard. Data plotted are sums from 3 traps. (Slides were not collected on weekends, and Monday pollen counts were divided by 3 to adjust for this. 3 days data were lost during the period).**

pollen on traps under the wind conditions when they collected a mix. Clearly if controlled pollination in Amberley seed orchard is to be managed without isolation, the techniques will need to be effective.

Experiment 2. The effectiveness of "shelter" as a pollen filter

1. Rationale and Design

The pollen trapping reported in Experiment 1, as well as that reported by KEMPE (1989), gave strong indications that the ramets in the seed orchard themselves were effective filters of outside pollen. Frequently, pollen trapped inside the orchard was substantially less than that trapped on the edge, when a pollen laden wind was blowing from outside. This experiment thus set out to explore the effectiveness of "shelter", by comparing the pollen trapped down-wind from a release point in a seed orchard and an open pasture. On 3 occasions, at times outside the period of natural pollen shed, and with wind velocities between 3 m/sec. and 3.5 m/sec, pollen was released (a) within the hedged part of the seed orchard, and (b) on bare ground adjacent to the orchard. Pollen traps in a line at 10 m spacing, to a maximum of 80 m distance downwind from the release point, were collected and counted after each release. The pollen counts were expressed as percentages of the pollen caught on the first (10 m) trap, to cope with the differences between replicates in wind velocity. Mean values are presented in *table 1*. It is clear from the significant differences in pollen trapped inside and outside the orchard, that the trees in a seed orchard are effective at filtering out pollen from the air, relative to the situation in open ground. The effectiveness of trees as filters of pollen provides an incentive to establish shelter around and within a controlled pollination seed orchard, to reduce natural pollen levels.

Experiment 3. The impact of timing on the effectiveness of controlled pollination without isolation

1. Rationale and Design

The intent of this preliminary experiment (which was carried out at 3 different locations in New Zealand, differing in the amount of background pollen), was to determine whether controlled pollination of strobili in the absence of isolation could be effective enough to have any practical value. Dyed pollen was applied in excess to more than 100 marked clusters of strobili at different stages of receptivity. Some 6 to 8 weeks later, when the scales were closed, the strobili were collected and a number of ovules at random were dissected out. The micropyles were opened and the pollen grains inside counted and categorised as dyed or natural.

Table 1. — Trapping following pollen release inside and adjacent to Amberley seed orchard. Data are numbers of pollen grains trapped, expressed as % grains caught by a trap 10 m downwind from the release point. Values are means of 3 replicates.

Distance (m) Downwind from Release Point	Inside Seed Orchard*	On Bare Ground Adjacent to Seed Orchard*
20	22.3	34.5
30	10.8	25.5
40	2.6	12.9
50	1.0	15.8
60	1.0	11.3
70	1.1	8.3
80	0.6	4.5

*) Differences between sites are significant at 0.001% by DUNCAN's (1955) test.

Table 2. — Pollination with dyed pollens in the absence of isolation. Values are counts of dyed pollen as percent total pollen grains in micropyles.

Trial Site	Background Pollen Levels	Bud stage* at which Pollen Applied 1	2	3	4	5	Total Number of Ovules Counted
A. Rotorua (Central North Island)	Median	91	85	83	85	23	155
B. Kaingaroa (Central North Island)	Highest	43	80	83	73	26	290
C. Amberley (South Island)	Lowest	77	67	85	78	87	50

*) 1 = just emerged from scales
2 = one half to three quarters emerged from scales
3 = approaching full emergence from scales
4 = early receptivity
5 = late receptivity

At trial sites A and B (SOMERVILLE and SWEET, 1978, unpub.), pollination was repeated over three successive days. At site C (KEMPE, 1989, unpub.), pollination occurred only once.

2. Results

These are presented in *table 2*. They indicate that, even in areas of substantial levels of background pollen (the central North Island contains New Zealand's major areas of plantation forest), controlled pollination without isolation can be moderately effective. An inference from the effectiveness of pollen application over a range of bud stages must be that the pollination droplet did not emerge very frequently. Equally, if raindrops can be effective in transferring pollen grains into the micropyle, there was little evidence of rain during the period of the studies. (Unfortunately, no records were kept of this).

Apparently, with quite a wide latitude in timing, controlled pollen, if applied in large amounts, could be very competitive with natural pollen. The implications with regard to pollination droplet behaviour, however, suggested that the effectiveness of pollen application might be improved.

Experiment 4. Substitution for the pollination droplet

1. Rationale and Design

The production of a pollination droplet appears to be sporadic (Experiment 3). Successful, controlled pollination without isolation requires applied pollen to be taken up to fill the micropyle as soon as possible. It is therefore appropriate to consider whether a substitute for the droplets can be developed, and to that end this experiment explored the application of water to strobili at pollination time.

Four treatments were tested, each on different whorls of strobili approaching receptivity. The strobili had been isolated prior to pollination. The first pollination in each treatment was as follows:

1. Standard pollination using dry, dyed pollen.
2. As for 1, but pollination was followed by a fine spray of water.
3. As for 1, but pollination was preceded by a fine spray of water.
4. Dyed pollen was sprayed on as a suspension in water at a concentration of 3% (w/v).

Approximately 1 hour after pollination, and again 24 hours after that, strobili in all treatments were re-pollinated using dry pollen. Each of the three pollinations used pollen which was dyed with a different colour.

Approximately 2 weeks after the strobilus scales had closed, strobili were harvested. The micropyles of 25 ovules

Table 3. — Mean number of coloured pollen grains in the micropyles of 25 ovules: data from Experiment 4.

Application Method for First Pollination	Mean number of pollen grains from: 1st Pollination*	2nd (dry) Pollination	3rd (dry) Pollination	Total†
Standard (dry) pollination	2.27 a	1.52	0	3.79
Standard pollination followed by water spray	3.68 ab	0.18	0	3.86
Water spray followed by standard pollination	3.72 ab	0.20	0	3.92
Pollen suspended in water	4.42 b	0	0	4.42

*) Values without a common letter differ significantly at 1% by DUNCAN's (1955) test.
†) Values do not differ significantly.

per treatment, selected at random, were dissected and the pollen grains recorded by colour.

2. Results

These are presented in *table 3*. The use of water, either as a spray, or as a medium in which to suspend pollen, improved pollen uptake. While all application methods involving water were effective, the suspension of pollen in water proved most effective. The number of pollen grains in the micropyles resulting from the first pollination, relative to the subsequent ones, indicates that water droplets can substitute for the pollination droplet in *Pinus radiata*. The dissections indicated that within 1 hour of application with a pollen suspension in water, the micropyles must have been filled with pollen grains. The total number of pollen grains in ovules pollinated with a water suspension were significantly higher (1% level) than those pollinated with dry pollen.

Experiment 5. Definition of an appropriate concentration at which to apply pollen suspended in water

1. Rationale and Design

While Experiment 4 had indicated the effectivess of a pollen suspension in water of 3% w/v, it was possible that much lower concentrations of pollen could be equally effective. This experiment set out to test that proposition.

Three concentrations of pollen suspension were used: 0.03%, 0.3% and 3.0% w/v. Application of dyed pollens was made with a 1.5 litre horticultural sprayer that allowed application to be made as a fine mist. All strobili had been isolated to ensure that there was no pollen on the micropylar arms prior to pollination. Treatment consisted of the removal of isolation bags and pollination with the appropriate pollen suspension. Approximately 1 hour later, all strobili were pollinated again, this time with quantities of normal dry pollen of a different colour. Some 2 weeks later when the strobili were no longer receptive, they were harvested. Randomly selected ovules were dissected and the pollen grains in their micropyles counted.

2. Results

These are presented in *table 4*. The results from the 3% pollen concentration support those from Experiment 4 in indicating that the droplets of water applied with the pollen must act as pollination droplets. At that concentration, not a single grain of the dry pollen applied one hour later was able to reach the micropyle. This suggests the probability that uptake into the micropyle occurs immediately following pollination. The results do indicate, however, that to ensure the micropyle is filled, a concentration of (probably) greater than 1% pollen is necessary. (Subsequent research by the authors, not reported here, suggests that a concentration of 2.5% to 3.0% is in fact optimal for *Pinus radiata*).

Table 4. — *Mean* number of coloured pollen grains in the micropyles of 25 ovules: data from Experiment 5.

Pollen concentration in suspension	Mean number of pollen grains from 1st Application (in water)*	2nd Application (dry)	Total†
0.03%	0.34 a	3.28	3.62 d
0.3%	2.92 b	1.23	4.15 de
3.0%	4.46 c	0	4.46 e

*) Values without a common letter differ significantly at 0.01%.
†) Values without a common letter differ significantly at 5%.

Experiment 6: The development of an operational method for controlled pollination without isolation

1. Rationale and Design

It is anticipated that, in practice, as strobili emerge from their scales they will be pollinated with pollen suspended in water. This may occur three times, at perhaps two-day intervals. With seasonally early clones there may be little external pollen in the air, but by the time most strobili start to emerge, external pollen levels will be high.

This experiment thus set out to explore the necessity for:

(i) removal of pre-existing pollen
(ii) pollination at the earliest possible time
(iii) repeated pollinations.

The experiment, which utilised a single clone, was based on a split-split-plot design with two replicates. The main plot incorporated prior pollen removal from the strobili. the sub-plot incorporated the timing of pollen application in relation to strobilus development, and the sub-sub-plot

Table 5. — Mean number of applied pollen grains per micropyle, as affected (a) by amount of strobilus development at the time of first pollen application (¼ to¾ emerged from bud scales); and (b) by the presence or absence of a compressed air treatment to remove existing pollens prior to pollination.

Comp. Air Treatment	Degree of Strobilus Development ¼E	½E	¾E	Mean†
Treated	4.33	3.69	4.28	4.10 a
Not treated	2.78	3.42	3.90	3.33 b
Mean	3.56*	3.56*	4.10*	

*) These values do not differ significantly.
†) Values without a common letter differ significantly at 1%.

Table 6. — Mean number of applied pollen grains per micropyle, as affected (a) by the number of pollen applications made; and (b) by the presence or absence of a compressed air treatment to remove existing pollens, prior to pollination.

Comp. Air Treatment	No. Pollen Applications 1	2	3	Mean†
Treated	2.53	4.80	4.97	4.10 a
Not treated	1.50	3.58	4.61	3.33 b
Mean*	2.06 d	4.19 e	4.79 f	

*) Values without a common letter differ significantly at 5%
†) Values without a common letter differ significantly at 1%

explored the number of pollen applications. The pollen applied had previously been stained black. Four weeks after pollination, the strobili were harvested, and the number of black pollen grains was counted in the micropyles of two ovules, from each of the strobili in each treatment.

2. Results

These are presented in *tables 5* and *6*. They indicate the following:
(i) the timing of pollen application relative to the stage of strobilus development was not of significance (*Table 5*);
(ii) the use of compressed air to remove existing pollen from strobili was highly significant (*Tables 5* and *6*);
(iii) the number of pollen applications was significant, especially in the absence of a compressed air treatment (*Table 6*).

Discussion and Conclusions

The aim of the six experiments was to explore the problem of controlled pollination without isolation and develop techniques which would improve its effectiveness.

Experiments 1 and 2 respectively quantified the amounts of pollen in Amberley seed orchard, and indicated the potential of shelter for reducing these levels. Experiment 3 indicated that, even in areas with high background pollen levels, the prospect of controlled pollination without isolation had sufficient potential to be worth pursuing. Experiments 4, 5 and 6 sequentially developed a technique, based on the use of pollen suspended in water, which effectively excluded all unwanted pollens.

As indicated by the experiments, the keys to successful controlled pollination without isolation are to:

1. reduce background pollen levels as much as possible, both within the orchard and on the strobili to be pollinated;
2. make controlled pollinations *before* any background pollen has been taken into the micropyle;
3. make pollinations in such a way, and at such a time, that the applied pollen is taken up immediately to fill the micropyle.

With respect to Point 1, the experiments indicate the likely effectiveness of shelter around the orchard in reducing the levels of pollen from outside. At Amberley seed orchard, however, around half of the pollen which was trapped came from inside the orchard. While it proved possible to remove at least some pollen from strobili prior to controlled pollination, internal shelter may also be advantageous in restricting pollen movement. There may also be scope for improvement to the simple compressed air method used to remove background pollen from strobili in these experiments.

With respect to point 2. it is probably fortunate that most seed orchards today are located on low rainfall, low humidity sites. This is certainly true for Amberley, and on that site it is unlikely that the humidity regularly reaches a level appropriate for pollination droplet formation. It is suspected that raindrops may constitute the main natural method of transfer of pollen to the micropyle at Amberley, but even rain is relatively infrequent during the pollination season. The experiments indicate that sequential pollinations are effective in placing applied pollens in micropyles, and it is not necessary to time these to catch the earliest stages of strobilus emergence.

With respect to point 3, the experiments indicate that in Amberley orchard, the application of optimal pollination treatments can fill the micropyles to a level such that there is no physical space for subsequent pollen to arrive. The mean value of 4.97 applied pollen grains per micropyle achieved by the best current technique (*Table 6*) is higher than the figure of 4.46, in *table 4*. But even in the experiment reported in *table 4*, no further pollen (from subsequent dry pollinations) was able to get into any of 25 micropyles which had an average of 4.46 pollen grains in them.

On the basis of the experiments reported above, the best current technique would be as follows:

1. Monitor clones as they approach anthesis.
2. As individual clusters of strobili reach the stage of 1/4 emergence from their bud scales, pollinate them, and repeat this on two subsequent occasions at 2 to 3-daily intervals. Prior to each pollination, use compressed air to remove any existing pollen from the scales. Pollinate with a 3% (w/v) suspension of pollen in distilled water.

An important factor which does need discussion at this point is that, to date, no seed produced from pollen suspended in water has been matured, collected and germinated. Because pollination is suspected to occur naturally by rain splash, there is no reason to believe that the application of pollen in water will have any negative implications for pollen germination or seed set. Nonetheless, it will be prudent to await collection of the first such seed, in mid-1992, before utilising the procedure on a large scale.

A further issue for a commercial seed company is whether its clients will be prepared to purchase controlled pollinated seed which has been produced without isolation. It is our expectation that it may be necessary, for commercial reasons, to have the genetic quality of such seed determined, and certified by an independent organisation. A number of methods exist by which this could be done, and they are currently being evaluated.

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