

POLLINATION IN *PINUS RADIATA*

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ABSTRACT

Strobili of *Pinus radiata* D. Don were receptive over a total period of 5 weeks, but within that time there was variation between clones and between successive cycles of a shoot. Individual cones remained receptive for 2 to 13 days; cone closure occurred after rain. Pollination drops were observed in humid conditions only. Pollen movement in an artificial drop was by flotation but, *in vivo*, non-floating pollen was also transferred to the micropyles.

Pollen can move into the micropyles before the cone scale complexes have visibly separated, but most is progressively transferred from the micropylar arms during a period lasting 12 days from the onset of visible cone receptivity. Individual ovules can put out repeated pollination drops.

The capacity of the micropyle limits the amount of pollen which can reach the nucellus and germinate. Within a cone, micropyle size varies according to the position of the ovule; ovules further from the apex of the cone tend to have smaller micropyles. Mean micropylar capacities also vary between and within clones. When the total pollen caught in a cone averaged some seven pollen grains per ovule, most micropyles were completely filled.

INTRODUCTION

Since the end of last century the morphology and development of *Pinus* have been described in a number of publications, but little of the literature has related specifically to *Pinus radiata*. Because of this, and the need to produce large amounts of genetically improved seed of this species in New Zealand, detailed knowledge of its reproductive development is required. A study of shoot development with some emphasis on cone* initiation has been presented (Bollmann and Sweet, 1976), as have a description of the seasonal growth of the seed cone (Sweet and Bollmann, 1971) and a report of ovule and seed development (Lill, 1976). This paper deals with pollination.

Pollination may be defined as the process by which the male gametophytes of a species are transferred to the proximity of its female gametophytes. In *Pinus* it involves pollen-shed and transport of pollen by air currents, the catching of air-borne pollen by the female cone, and the transfer of pollen in the cone to the micropyles of the ovules.

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* The word cone is used here to mean the ovule- and seed-bearing structures of pines, regardless of the stage of development.

As Sarvas (1962) pointed out, the receptive pine cone is an excellent device for catching pollen. It consists of an axis bearing spirally-arranged scale complexes with channels between them (Fig. 1a and 1b). A scale complex consists of an upper ovuliferous scale and a lower, initially more delicate, bract scale (Fig. 1c).

Immediately prior to and during the pollination period cones emerge from the bud scales and their axes elongate so that the cone scale complexes are pushed apart. At the same time the ovuliferous scales swell and become darker red. Cones are regarded as fully receptive when their scale complexes are horizontal and the channels between them are wide. The ovuliferous scales in the upper part of the cone bear two ovules at their bases and the ovules project fine micropylar arms into the channels between the scales (Fig. 1c and 1d). The ovuliferous scales of the lower part of the cone bear rudimentary ovules or none at all. Many of the pollen grains carried into the channels are intercepted by the micropylar arms of the ovule and pollen remains attached to them, either because they are sticky (Doyle and O'Leary, 1935) or through a bioelectric effect (McWilliam, 1959). In order to germinate, pollen must be transferred to the micropyle, a tube leading from between the micropylar arms to the cavity in the nucellus cap of the ovule (Fig. 1d). The means of transference is assumed to be the pollination drop (Doyle and O'Leary, 1935; McWilliam, 1958; Sarvas, 1962), to which no alternative function has been ascribed. Pollen grains in the micropyle are shown in Fig. 1e.

Cone closure after receptivity is due to the continued swelling of the ovuliferous scales which eventually meet and seal the cone. Stages in the process from cone emergence to closure can be expressed in a descriptively-based classification similar to those used by Cumming and Righter (1948) and Pattinson, Burley and Geary (1969). The subjective classification (in Figs. 3 and 9) used by Lill (1974) allows fine distinctions to be made, based on daily observations of cone development.

OBSERVATIONS AND RESULTS

Timing and Duration of Cone Receptivity

At the Forest Research Institute clonal archive in Rotorua eleven trees were climbed daily during August and September 1972, and the degree of receptivity was recorded for marked cones. The trees chosen were grafted ramets of clones 19, 55, 89, 274, and 372, planted in 1968. *Pinus radiata* is a polycyclic species with female cones often arising in more than one position on the annual shoot. Forty female cones terminating the first cycle of the current season's shoot growth were observed from each clone and 20 cones terminating the second cycle were observed from clones 55, 89, and 274 only. An estimate of pollen-shed was made by recording the number of clones, from a total of 44, which were shedding pollen at a given time. Pollen-shed and female cone receptivity occurred over 5 weeks (Fig. 2), but within this period peak pollen-shed in each clone was at different times, as was peak cone-receptivity (Fig. 3). Observation in 1973 showed that the date of the pollination period varied between years (Fig. 2) as did clonal receptivity within the period. Most of the cones in a second cone-bearing cycle became receptive when those of the first cycle were closing, but some variation occurred within cycles (Fig. 3).

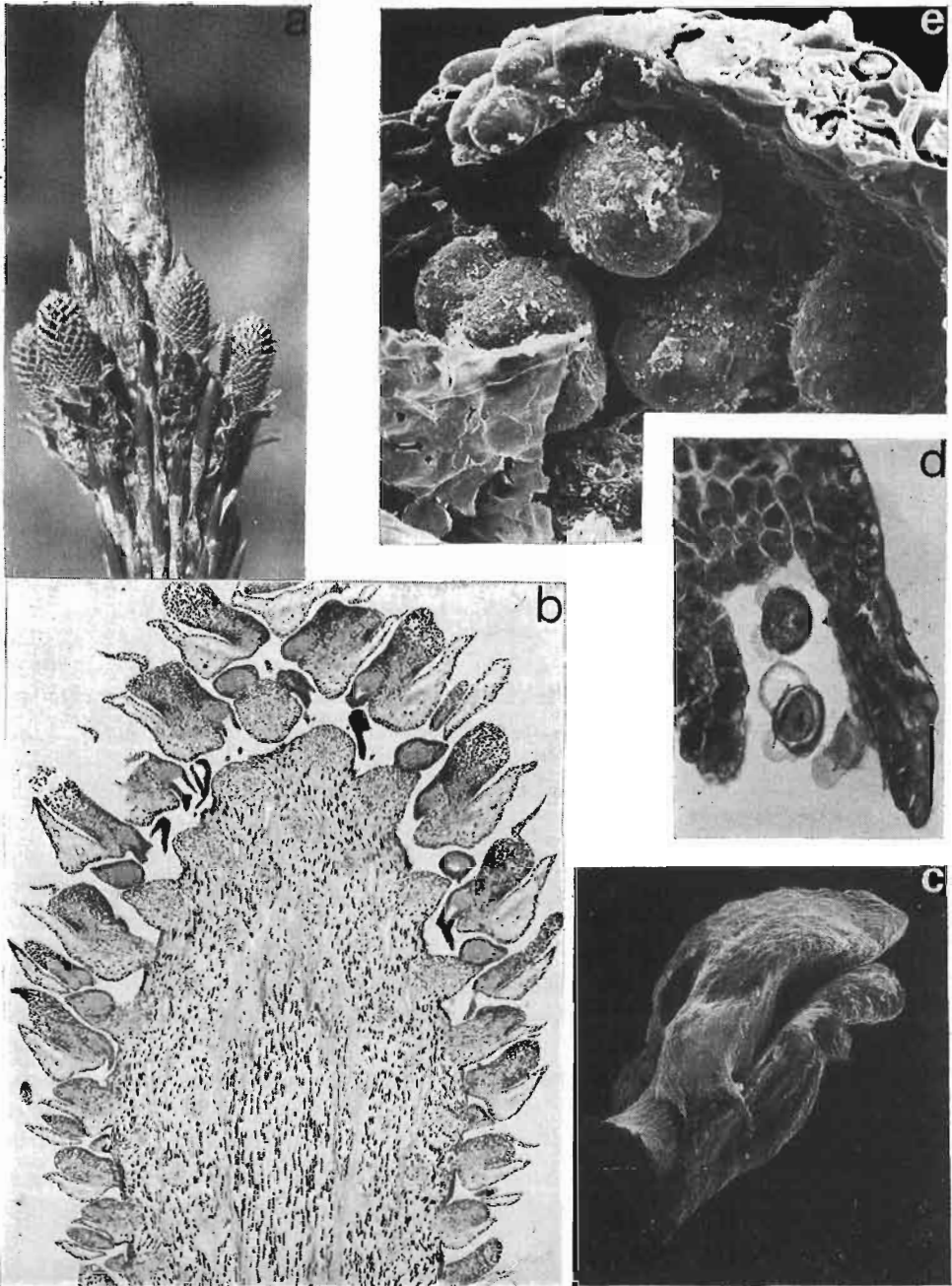


FIG. 1 (a)—Receptive cones near the shoot tip. August. $\times 1$.
 (b)—Section of receptive cone showing scale complexes. August. $\times 12$.
 (c)—Ovuliferous scale complex from cone at late-receptivity showing bract scale, and ovuliferous scale with ovule, micropyle, and micropylar arms. August. $\times 16$. The tissues, viewed with a scanning electron microscope after coating with carbon and gold palladium, show a small amount of collapse.
 (d)—Section of micropyle showing pollen grains between the micropylar arms. August. $\times 146$.
 (e)—Pollen grains in the micropyle viewed with a scanning electron microscope after coating with carbon and gold palladium. August. $\times 424$.

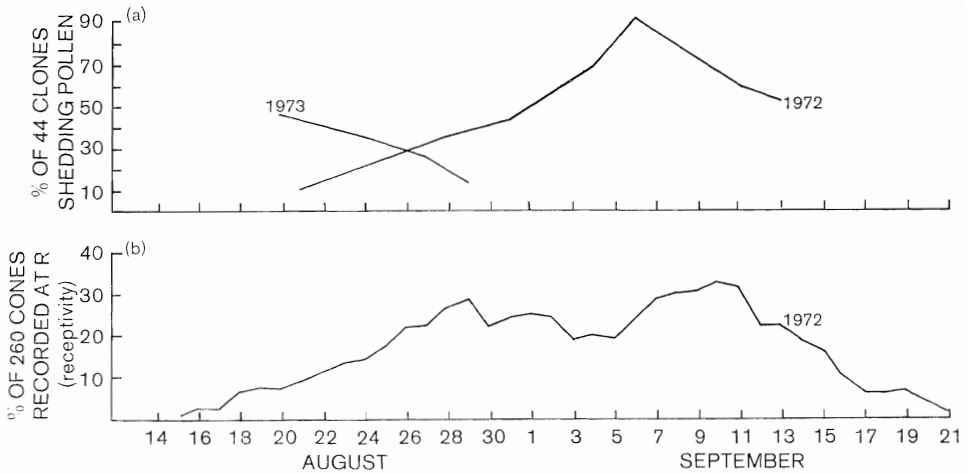


FIG. 2 (a)—The pattern of pollen-shed in 1972, with some data plotted from 1973. (b)—The pattern of cone receptivity in five clones in 1972. All data from Rotorua.

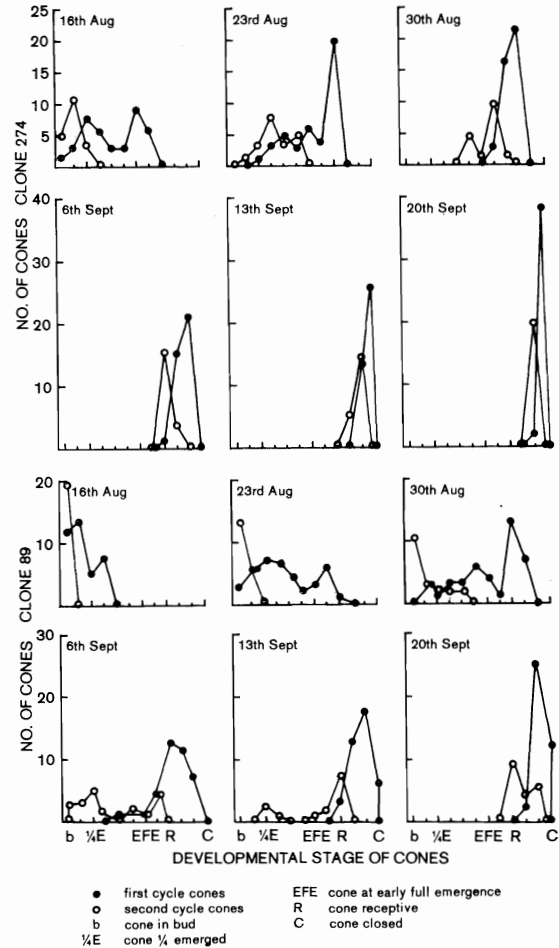
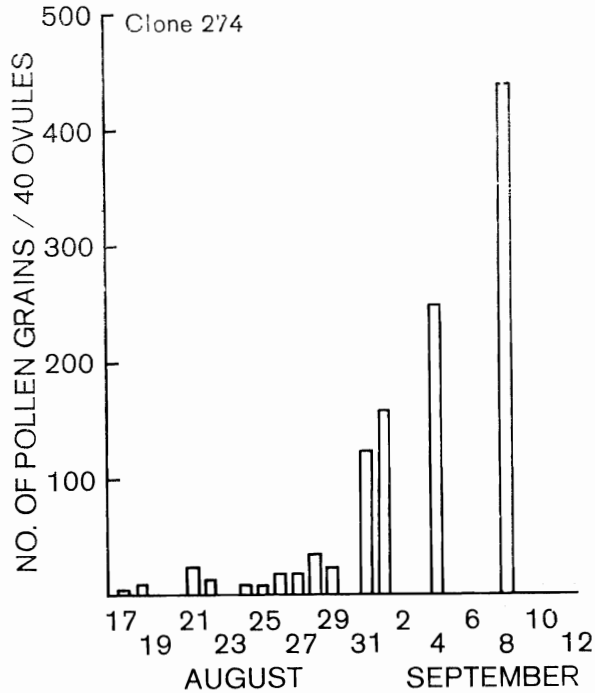
Trapping of Pollen

The amount of pollen arriving in individual cones depends on the time that the cone becomes receptive relative to the peak period of pollen-shed, and on the *duration* of individual cone receptivity. The quantity of pollen caught was determined from fresh dissections of 40 ovules from each cone harvested: for each ovule the number of pollen grains inside the micropyle, on the micropylar rim and arms, and on the ovule surface was recorded. Fig. 4 shows that receptive cones harvested at the beginning of pollen-shed (cf. Fig. 2 for timing) caught small amounts of pollen relative to the amounts caught by a receptive cone harvested near the peak of pollen-shed. But first cycle cones which became receptive at the beginning of the pollination period (Fig. 5) remained receptive for longer than those which became receptive later. The variation in the amount of pollen found in closed cones made it apparent that the receptive duration was not dependent on the amount of pollen caught. Nor did the decreased receptive duration of cones as the pollination period progressed appear to depend on rising seasonal temperatures. Regression analyses (Fig. 6) showed relationship between the duration of receptivity in a cone and the number of days from the time it became receptive until the first period of rain (defined for this regression as being more than 2 mm in 24 hours). The relationship was significant at the 0.1% level for first cycle cones of both clone 274 ($R^2 = 0.83$) and clone 55 ($R^2 = 0.76$). These were both clones with cones which became receptive over a period of several weeks, enabling the relationship to be easily examined.

In prolonged wet weather the tissues of ovuliferous scales became more than usually turgid, and this may allow sufficient swelling to cause cone closure. Wet weather could affect pollination adversely in any year if it occurred early in the pollination period and stimulated cone closure before much pollen had been trapped; this is a factor distinct from the influence of wet weather on the process of pollen-shed which has been well documented (e.g., Sarvas, 1962; Ebell and Schmidt, 1964).

FIG. 3 (right)—Progressive cone development for two clones over the receptive period at Rotorua in 1972. The numbers of cones at different developmental stages are shown for different dates. Counts were of 40 first-cycle and 20 second-cycle cones for each clone.

FIG. 4 (below)—Pollen counts in individual receptive cones harvested on a number of dates at Rotorua in 1972.



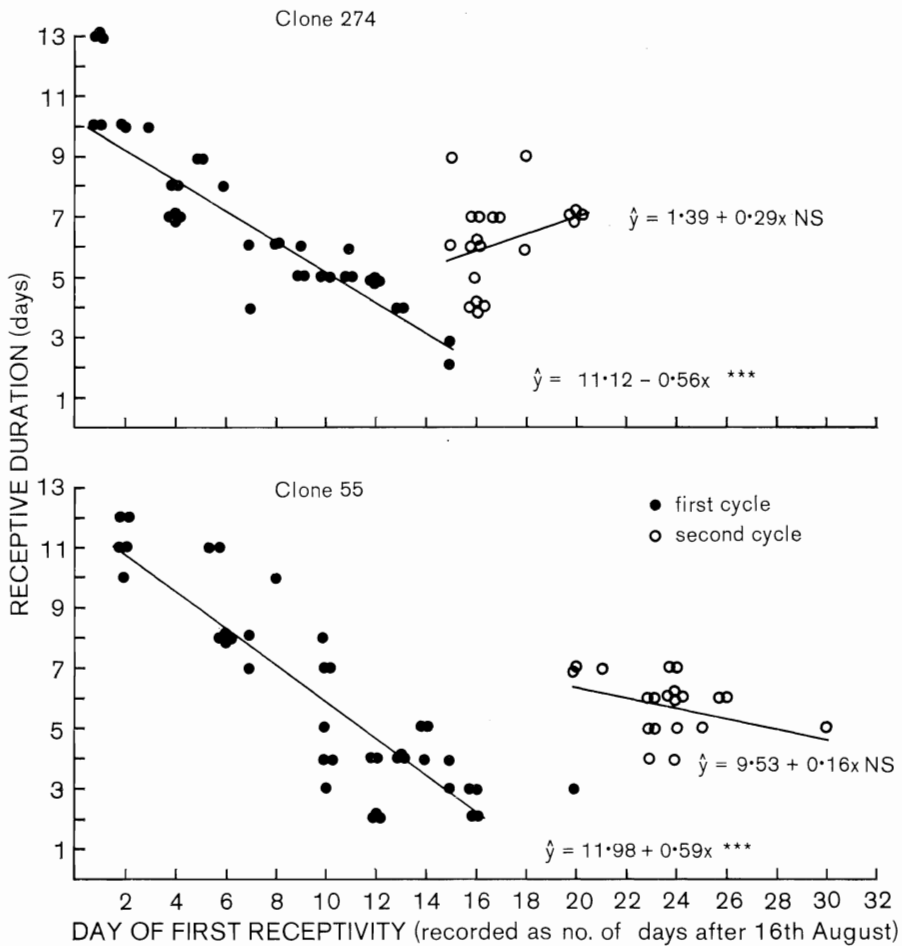


FIG. 5—Receptive duration of individual first- and second-cycle cones plotted against the date they became receptive. The longer period of receptivity shown here by early second-cycle cones than by late first-cycle cones is not representative of all clones examined.

The immediate success of pollination of a cone is measured by the number of pollen grains in the micropyles and this, in turn, is determined both by the amount of pollen caught by the cone, and by the micropylar capacity. Figure 7 shows that as the total amount of pollen caught by the cone increases to an average level of seven pollen grains per ovule, so the number of grains reaching the micropyles increases. As long as pollen can reach the cone in amounts in excess of this figure, pollination success is probable.

Pollen Transfer

Pollination drops were observed between the micropylar arms of ovules in cones on potted grafts kept in a glasshouse at high humidity. For observation the grafts were laid on their sides so the cones could be viewed with a stereoscopic microscope. Drops

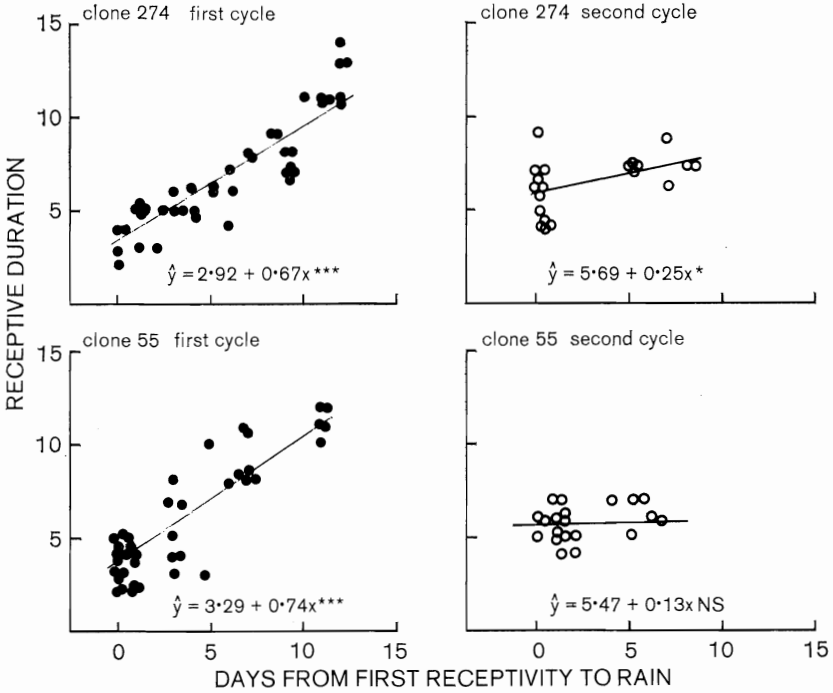


FIG. 6—Receptive duration in first- and second-cycle cones plotted against the time interval between first recorded receptivity and the first subsequent day of rain.

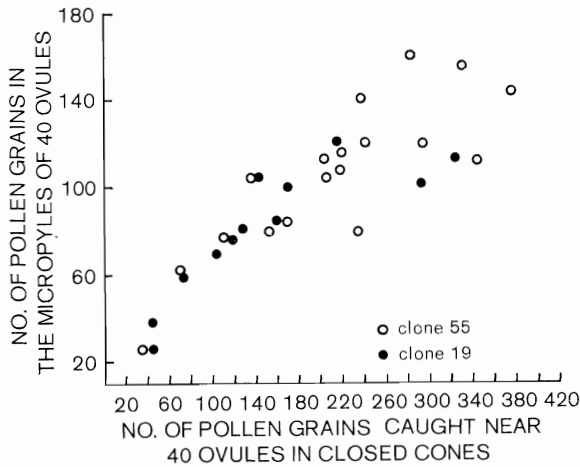


FIG. 7—Pollen grains dissected from the micropyles plotted against the total trapped in the cones for 2 clones at Rotorua in 1973.

were also seen in receptive cones harvested from the field after several days' rain, but on other occasions they were not seen during the day. Drops were seen on a few ovules from three cones at different stages of emergence and receptivity, harvested from the field at 4 a.m., but earlier (at 10 p.m.) and later in the morning drops were not found.

When pollen was allowed to fall onto pollination drops in cones on the potted grafts it floated on the surface of the drop and remained there. An hour later, when observation was discontinued, the drops had not disappeared. This was possibly because of the high humidity in the glasshouse chamber.

A simulated micropyle made from a $1\ \mu\text{l}$ capillary tube with two "arms" (hairs, attached to the lower rim with melted wax) was set up. The tube contained a solution of glucose (33 mM), fructose (40 mM) and sucrose (2.5 mM) (McWilliam, 1958). The arrangement was clamped vertically and viewed through a stereoscopic microscope. When pollen was puffed onto the "arms" and a drop was squeezed from the capillary tube, the pollen immediately moved into the drop and floated upward, either just inside the drop's perimeter or directly through its centre. Many grains continued to float up through the capillary tube, and all moved with their wings uppermost. The movement of pollen to the capillary mouth (the equivalent of the micropylar rim) was rapid, so for successful pollination *in vivo* the drop probably need be present between the micropylar arms for only a short time. If the drop dries quickly or is actively resorbed by the ovule tissues the rarity of drops seen in the field would be explained.

Pollen was shaken in chloroform for 2 hours. After this treatment it did not float in water, and when it was dried and applied to the artificial micropyle it sank to the bottom of the drop and remained there. Such non-floating pollen was applied to bagged cones, which were left on the tree until they had closed. The cones were then harvested, fixed in FAA (formo-aceto-alcohol), embedded in wax, sectioned at $10\ \mu$, and stained with Safranin and Fast Green. Examination of the micropyles showed that non-floating pollen had been transferred into them. Figure 8 shows that the relationship between the amount of pollen in the micropyle and the amount caught by the ovules was similar for non-floating and "normal" pollen.

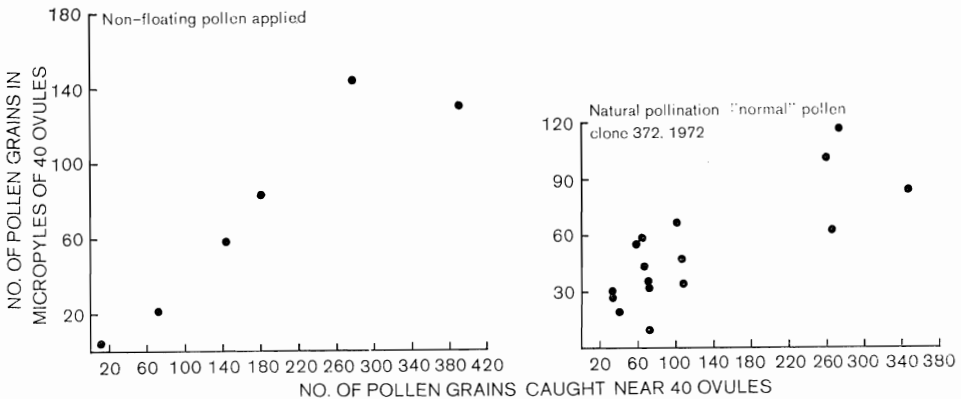


FIG. 8—Pollen grains in the micropyles in relation to the total number caught by the ovules. Left: non-floating pollen applied artificially. Right: natural pollination by "normal" pollen.

Pollen grains from *Pseudotsuga menziesii* are wingless, do not float and have volume four to five times greater than those of *P. radiata*. This pollen was applied to bagged cones of *P. radiata* and a small number of ovules were later found with it in their micropyles, confirming that non-floating material of a considerable size can reach the nucellus.

Although observations showed that pollen floated upward in a pollination drop and in a capillary tube, the ability of non-floating pollen to reach the micropyles suggests that uptake must also be possible by resorption of fluid by the ovule or by evaporation of the drop. When we allowed these processes to occur in our experimental system, surface tension held the pollen onto the receding drop but no firm evidence was obtained to suggest which process might occur naturally. Nor was the ovule tissue which secretes the pollination drop identified, although the changes in the nucellus cap cells lining the micropyle indicate that they are particularly active while the cone is receptive.

Timing of Pollen Transfer

The amount of pollen caught by cones which are just beginning to emerge is limited, mainly by the short time which pollen has had to accumulate in the cone. Hence in emerging cones, whether naturally or artificially pollinated, usually only one or two micropyles contain pollen.

To determine the timing of pollen transfer to the micropyles, large quantities of marked pollen (blackened by staining with hair dye) were applied to unbagged cones of one clone at a number of recorded stages of cone development. Cones were harvested either 2 or 6 days after the application of blackened pollen, or after cone closure. In each case the amounts of natural and blackened pollen in the micropyles of 30 ovules were examined (Fig. 9). Because the blackened pollen was applied in excess, it is

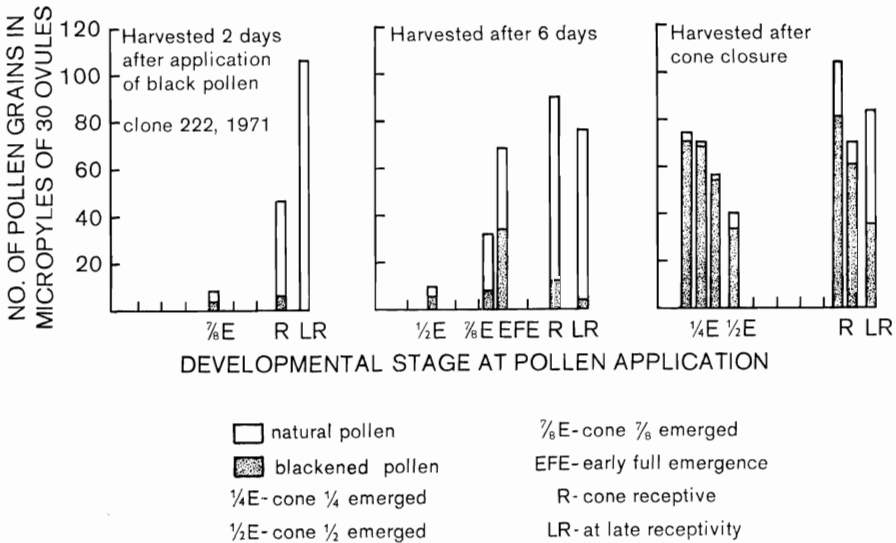


FIG. 9—Number of naturally caught and artificially applied pollen grains in the micropyles of 30 ovules. Artificial pollination was at different stages of cone development and harvesting was at 3 different dates after pollination.

expected that most of the yellow pollen in the micropyle was taken up before the blackened pollen was applied. Figure 9 indicates that pollen can move into the micropyle before cones are visibly receptive. Nonetheless most of the pollen is transferred to the micropyle while the cones are visibly receptive and even while they are closing. Complete transfer of pollen to the micropyles did not occur during the first 6 days after application, but at some time subsequent to this.

Bagged cones, pollinated with large amounts of pollen on their first recorded day of receptivity, were harvested at daily intervals. Dissection showed that overall the amount of pollen transported into the micropyles increased during at least 12 days (Fig. 10a). In the first few days the majority of ovules remained unpollinated (Fig. 10b) but after a week most of the ovules contained pollen. The increase in total pollen in the micropyles shown in Fig. 10 after all ovules were pollinated illustrates that pollen continues to be transported into micropyles which already contain pollen grains.

Accepting that the pollination drop is the medium of transport, the pattern of pollen uptake can be interpreted to indicate that in any one cone a few ovules initially secrete a drop and gradually more start secretion. Over the period of drop production individual ovules must produce a drop on more than one occasion.

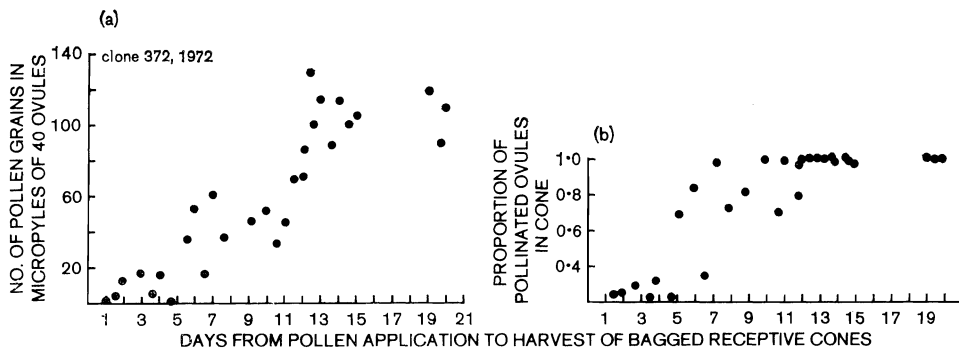


FIG. 10—Data from bagged cones artificially pollinated on the day of first-recorded receptivity. Pollinated ovules were those containing at least one pollen grain.

Micropylar Capacity

While tree breeders wish to understand the mechanism and timing of pollination, they also need to know how many pollen grains normally reach the micropyles. This is because multiple pollination offers the opportunity for fertilisation of more than one archegonium, a factor which is important in maintaining heterozygosity (Sarvas, 1962).

Variation in the quantity of pollen in micropyles exists within cones, between cones, and between clones. In the presence of ample pollen this is attributable to variation in the micropylar capacities.

When ovules were dissected from apical, middle, and basal regions of the ovule-bearing zone, most of those in the basal zone were found to contain 0, 1, or 2 grains, whereas those from the other zones often accommodated more than two grains (Fig. 11). Functional ovules were smaller in the basal than in the apical region (Lill, 1974).

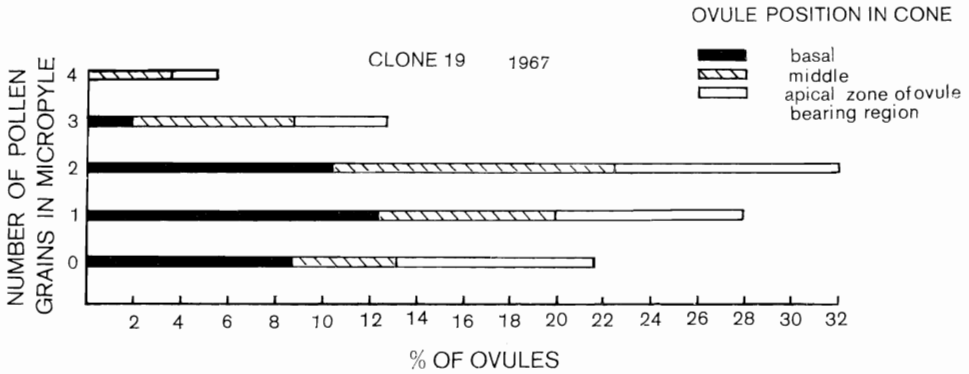


FIG. 11—The percentage of ovules which have 0, 1, - - - 4 pollen grains in the micropyles. For each pollen count the proportion of ovules from different parts of the cone is shown. The sample is based on an examination of all the ovules in five cones of one clone.

Cones harvested from grafts of two clones planted in 1957 and 1958 at Kaingaroa, were used to examine clonal and year-to-year differences in the amount of pollen found in the micropyle (Table 1). The data showed a significant clonal difference (0.1% level) in the quantity of pollen within the micropyles. Significant between-year differences (1% level) and a clone × year interaction (0.1% level) were also shown. Clone 55 micropyles occasionally contained six, and often five, pollen grains. In clone 19 they rarely had this number.

TABLE 1—Mean number of pollen grains trapped in the micropyles of 30 ovules from the ovule-bearing zone. Sampling was based on five cones for each of two clones in 5 separate years

Clone	Year					Mean
	1967	1968	1969	1970	1971	
55	99	101	108	92	68	94
19	48	56	62	51	64	56
Mean	74	78	85	71	66	

Success of Natural Pollination

Although the micropylar capacity may limit the number of pollen grains accommodated, this happens only when there is a plentiful supply of pollen trapped in the cone (an average of more than seven grains per ovule). Table 2 shows the mean number of pollen grains caught per ovule for cones at two sites over several years. At the seed orchard site at Kaingaroa, 30 of the 34 cones examined averaged more than five pollen grains per ovule, whereas at the Rotorua clonal archive (with few trees to provide pollen) only 20 of the 43 clones averaged more than five pollen grains per ovule. It is

clear from Figs. 7 and 10 that to obtain one pollen grain in the micropyle of each ovule (the minimum necessary for further development — Sarvas, 1962), requires an average of about two grains per ovule in the cone as a whole. Table 2 shows that averages of two or less occurred infrequently in our studies, indicating that lack of pollen is not often limiting to subsequent cone development in *P. radiata*.

TABLE 2—Variation in the mean number of pollen grains per ovule caught by naturally-pollinated cones at two sites. (The mean value for each cone was based on a sample of 40 ovules taken from the ovule-bearing zone)

Site	Year	Clone	No. cones examined	Pollen grains caught		
				0-1	2-5	above 5
Kaingaroa (seed orchard)	1968	19	6	0	1	5
	1968	55	6	0	0	6
	1970	19	6	1	0	5
	1970	55	6	0	0	6
	1971	19	5	5	0	5
	1971	55	5	5	0	2
Rotorua (clonal archive)	1971	19	5	2	2	1
	1971	55	5	0	5	0
	1971	274	3	0	3	0
	1971	372	5	0	3	2
	1972	19	6	0	0	6
	1972	274	8	1	1	6
	1973	55	6	0	1	5
	1973	19	5	5	2	3
Total			77	6	21	50

DISCUSSION

The study reported here was undertaken to provide information necessary for seed orchard management. *Pinus* displays polyzygotic polyembryony, i.e., an ovule can be pollinated by several pollen grains each of which can fertilise an archegonium. In *P. radiata* the number of pollen grains held in the micropyle may range from none to six, and the number of archegonia ranges from one to four (Lill, 1974). After fertilisation of these archegonia there is generally considerable pro-embryo or embryo abortion and normally only one embryo develops to maturity in each ovule. This is believed to be important in maintaining heterozygosity by discriminating against related matings (e.g. Sarvas, 1962). If the level of selfing is to be minimised, it is thus important that a seed orchard produces large amounts of pollen. In most currently managed seed orchards it is also desirable that a wide range of pollen parents have the opportunity to pollinate any given female parent.

These considerations raise questions as to the effectiveness of the earliest- and latest-flowering clones in the orchard. They also lead to queries about (i) how much pollen is required to fill the micropyles; (ii) whether ovules are pollinated only by the

first pollen which arrives on the micropylar arms; (iii) whether all the ovules of a cone are pollinated at the same time; and (iv) what factors influence the duration of receptivity.

The results from the study answer these questions. It is clear from examination of Figs. 2 and 4 that clones which have cones receptive when maximum quantities of pollen are available are more heavily pollinated than those with cones which become receptive prior to that time. To ensure that all ovules have sufficient pollen available to completely fill their micropyles it is necessary, at least for some clones, that an average of some seven grains be caught in the vicinity of each ovule (cf. clones 19 and 55 — Fig. 7). For the clone illustrated in Fig. 4 this point was not reached until a time well into the pollination period when some 70% of the clones on the site were shedding pollen (Fig. 2).

Pollen applied to cones which are only partially emerged can ultimately reach the micropyle in normal quantities, as can pollen applied at late receptivity (Fig. 9). Because the movement of pollen from the micropylar arms to the nucellus occurs over a period of time (Fig. 10), late-arriving pollen, provided it lands on the micropylar arms, may have as good an opportunity to reach the micropyle as early-arriving pollen. This could be important for those early-flowering clones which remain receptive for an extended period of time.

The duration of receptivity was shown to be dependent on the rainfall pattern (Fig. 6). This finding has important implications for the siting of seed orchards: provided that cones can remain receptive for long enough, it does not matter if they become receptive early in the season. But rapid closing after receptivity is restrictive, in terms of both the total quantity and the genetic variability of the pollen they obtain.

Assuming that the pollination drop is the method of transfer of the pollen grains from the micropylar arms to the micropylar chamber (an assumption which, while unproven, is generally accepted and appears sound from the experiments reported here) then the results (Fig. 10) suggest that (a) drops may be produced at different times by different ovules in a cone, and (b) they may be produced repeatedly by the same ovule. The earliest drop must be produced before the cone scales are fully open, and some must still be produced at late receptivity (Fig. 9). Clearly if there is appreciable pollen on the micropylar arms, then production of a large drop should enable the micropyle to be filled immediately with pollen. The fact that this did not happen in our studies (Fig. 10) implies that only small drops were being produced — so small that only one or two pollen grains at a time were reached by the drop and moved into the micropyle. If the pollination drop is dependent for its existence on a low level of water stress in the tree then small drops might be expected in a season in which there were a number of consecutive days without rain (Fig. 6). Under such circumstances the pollen grains reaching the micropyle would be those nearest. One would expect that the distribution of pollen on the arms should be random and not determined by the time the pollen was shed: thus late-arriving grains would have the same opportunity to land there as early ones. Consequently the chance of a grain from a given clone reaching the micropyle should be in direct proportion to the concentration of grains present in the atmosphere from that clone.

It may be that under wet conditions, when the cones are receptive for a shorter time, pollination drops would be larger than under dry conditions. If so, the micropyles could still be well filled with pollen provided that adequate quantities of pollen were present on the arms when the drop emerged. Thus, wet weather in the middle of the pollination period should be less harmful than at the beginning.

In 1972, pollen-shed occurred over a 40-day period, and the maximum time for which any one cone remained open and receptive was 13 days. At one stage 90% of the clones in the orchard were shedding pollen at the same time, but clearly the percentage contribution from each individual clone varied continuously over the whole period. Clones with more than one cycle of female cones on the annual shoot must have been pollinated by a wider range of clones than those with a single cycle, but within a cycle there was also a substantial variation between cones in the timing of receptivity (Fig. 3).

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