SEED MATURATION PRECEDES CONE RIPENING IN NEW ZEALAND PINUS RADIATA

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ABSTRACT

Early collection of **Pinus radiata** D. Don cones may be of considerable benefit for seed production in New Zealand, especially in seed orchards where controlled pollination is used. In this study on the development of seed germinability, cones matured more slowly than the seeds inside them. Thus seeds ripening on the tree were fully germinable and of high vigour by the end of July, much earlier than previously thought, although at this stage they were difficult to extract from the cones without several weeks of air-drying. Cone colour and moisture content were thus ruled out as indices of seed development, but specific gravity may prove a useful guide.

Keywords: seed; cone; maturation index; germination; **Pinus radiata**.

INTRODUCTION

In New Zealand, *Pinus radiata* is the predominant commercial forest species, often performing better than it does in its natural habitat on the Californian seaboard (Bannister 1973). Under North Island conditions there is a 22-month interval between pollination in early Spring (August/September) and attainment of maximum cone size in June 2 years later (Sweet & Bollman 1971). Little attention has been paid to the timing of seed maturation within the ripening cone, picking being carried out under normal practice in open-pollinated seed orchards when the cones have turned brown in November or December. Because of the serotinous nature of *P. radiata* cones there is no real risk of losing germinable seed by cone opening in the North Island, but this can be a major problem in Canterbury, South Island, if cones are collected later than the end of December.

Controlled-pollinated, hedged, clonal, seed orchards of *P. radiata* have been developed in answer to the increasing demand for seed of improved genetic quality. This system

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requires plants to be hedged to a convenient working height of approximately 1.5 m for manual isolation and pollination. In order to ensure new vigorous apical growth, the necessary trimming of 2-year-old cone-bearing shoots must be carried out no later than July/August. Cones are thus collected in an immature state. Fielding (1964) and Wilcox & Firth (1980) reported that seeds extracted from immature green cones harvested at this time and then artificially ripened gave successful plant establishment. However, they did not look at the initial germinability of seeds when the cones were first harvested. Thus it was felt timely to look at the development of seed germinability in relation to cone maturation.

In particular, this paper reports studies on the germinability of freshly extracted seeds in relation to various physical indices of cone maturity which might then be used as a guide to timing early harvests. The value and application of maturation indices was well reviewed by Edwards (1980) who concluded there was great scope for further applied research in this area. Generally, the physical parameters used to provide these indices are cone specific gravity and moisture content (Schubert 1956; Cram & Worden 1957; Ching & Ching 1962; Krugman *et al.* 1974); cone firmness (Crossley 1953), and cone colour (Gordon *et al.* 1970; Wilcox & Firth 1980; Arisman & Powell 1986). It is increasingly clear that there is little scope for generalisation in this area. For instance, the use of cone colour as a maturation index was rejected by Lindquist (1962) and Cram & Lindquist (1979) for *P. sylvestris* L. because cone colour changes varied considerably amongst different trees, and earlier work by Maki (1940) and Fowells (1949) had also rejected such an index for *P. ponderosa* P. et C. Lawson and *P. lambertiana* Douglas (sugar pine).

MATERIALS AND METHODS

Cone Collections

A preliminary comparison between open-pollinated (OP) and controlled-pollinated (CP) second-year cones was carried out on collections made in July 1985 from ramets of the best clones of the Forest Research Institute (FRI) "268" series grown in the open-pollinated seed orchard at Kaingaroa (latitude 38°24'S; longitude 176°34'E; altitude 444 m) located 40 km south-east of Rotorua, and from controlled-pollinated ramets at the Long Mile Archive, FRI (latitude 38°10'S; longitude 176°16'E; altitude 300 m). The number of cones required for the main studies precluded sampling from controlled-pollinated orchards and thus bulked samples of cones were obtained from open-pollinated trees of the FRI "850" series in the Kaingaroa Seed Orchard. Major collections of second-year cones were made at approximately monthly intervals from March to July 1986. On each occasion cones were taken from the same three productive ramets of each of 10 high-performance clones. Additional smaller collections were made from the same series in September 1986, and the previous season's second-year cones were collected on three occasions in the second half of 1985.

Four randomly selected cones were taken from the bulked samples of each collection for use in the studies reported here. Each cone provided a replicate seedlot for the germination studies.

Cone Assessment

Cone features measured included fresh and dry weights, moisture content, cone colour, specific gravity, scale opening, and seed extractability. Moisture contents were determined by the air oven method at 105 °C for 24 h and specific gravity by the water displacement method (Schopmeyer 1974).

A five-stage colour coding scheme was developed to provide a colour-based maturation index using standards from the British Colour Council (B.C.C. 1957) chosen as most closely representing the main colour categories of ripening cones. Early in the year cones are green (Category 1, B.C.C. 174: Moss Green), progressing as they mature to Category 5 (B.C.C. 67, Almond Shell). Intermediate categories were: 2, B.C.C. 233 (Tuscan Yellow); 3, B.C.C. 204 (Cinnamon), and 4, B.C.C. 168 (Nutmeg).

Seed extractability was determined as the number of seeds extracted by the standard kilning method (60°–63 °C for 6 h) as a percentage of the total yield of developed seeds (Bramlett *et al.* 1976).

Seed Extraction

One of the reasons why there is such limited information on seed development in *P. radiata* is that seed is very difficult to extract from immature cones. The cones of this species are serotinous and thus the scales are bonded by resin, requiring exposure to high temperature for seed release. Oven-kilning can be done only when the cones have low moisture content and cannot be used for green cones. Through a series of preliminary tests it was decided that the only practicable way to extract seeds from freshly harvested cones was by dipping the cone in hot (~80 °C) water for up to 1 minute which then made it possible to break open the cones manually and remove the seeds. Extracted seeds were dewinged by hand and then cleaned. Only morphologically sound seeds were used throughout the study. In early collections seeds were screened by a flotation technique to remove empty seeds using the ethanol flotation method of Simak (1973). In the 1986 collections this evaluation was done using low dosage X-ray radiography (20.0 kV at 2.8 mA for 60 seconds, using the Hewlett-Packard Faxitron 43804N system).

Seed Germination and Vigour Testing

Seed germination was tested in accordance with the I.S.T.A. Rules (1985). Four replicates of 25 seeds were dusted with Thiram fungicide and placed in air-tight plastic boxes on germination blotters over cut sheets of paper towelling moistened with 20 ml distilled water. Boxes were kept at a constant 20 °C with continuous low light. Seed dormancy is very common in conifer species and prechilling is often a recommended treatment for *Pinus* (I.S.T.A. 1985). In order to clarify whether prematurely harvested *P. radiata* seeds exhibited a stratification-sensitive dormancy mechanism, two replicated germination tests were carried out for each collection of the 1986 harvested seeds. One test was set up at 20 °C immediately, seeds for the other being kept moist in their germination boxes for 1 week at 5 °C before transferring to 20 °C for the 28-day test.

Numbers of seeds showing normal germination or (in the preliminary comparison) cotyledon emergence were scored every 2 days until trials were terminated 28 days after sowing. A seed was considered to be germinable when the radicle length had reached four times the length of the seed. Median germination or emergence times (T50's) were calculated as described by Coolbear *et al.* (1984). These provide a good indication of seed vigour under unstressed conditions (A.O.S.A. 1983). Percentage data were analysed using the arcsin transformation ($\theta = \arcsin^{-1}\sqrt{\%/100}$).

RESULTS

The results of the comparative measurements made between OP and CP cones of the FRI "268" series harvested in July 1985 are given in Table 1.

TABLE 1—Comparison between open-pollinated (OP) and control-pollinated (CP) cones of the FRI "268" series harvested in July 1985

	OP Cones	CP Cones	(LSD p=0.05)
Cone colour category	1	1	–
Cone specific gravity	1.15	1.05	–
Seed moisture content (%)	22.2	21.8	(1.17)
Seed germinability (%)	96	90	(8.8)
Time to 50% cotyledon emergence (days)	14.6	15.8	(1.20)

Cone Development

Fresh and dry weights of the sampled cones are shown in Fig. 1. Through late April, May, and June there was high sample variation although it appears that maximum cone fresh and dry weights were attained during these months. Similarly, there was high variation in cone volume (Fig. 2), but these data indicate that maximum cone size was attained in June.

Whilst cone moisture content decreased from 50% in March to 37% in September, there was little change noted through May, June, and July (Fig. 3). Specific gravities

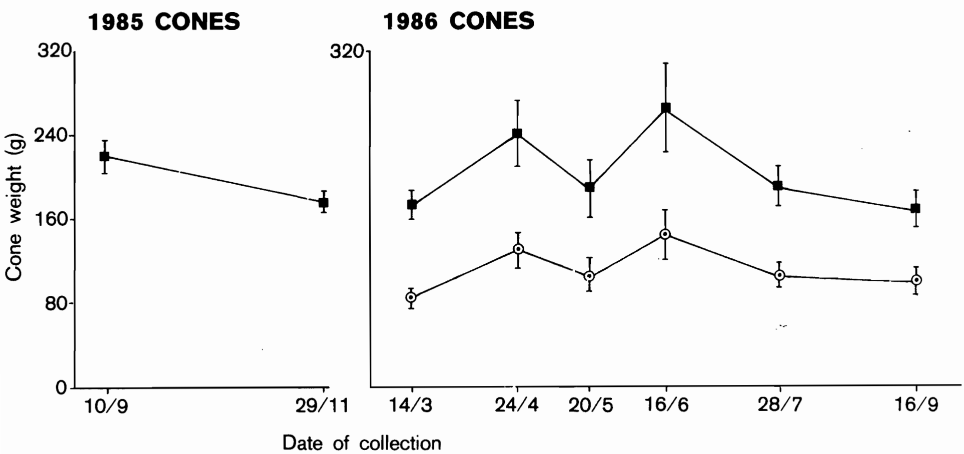

FIG. 1—Changes in fresh (■) and dry (O) weight of cones during the later stages of development. Bars indicate standard errors of individual means.

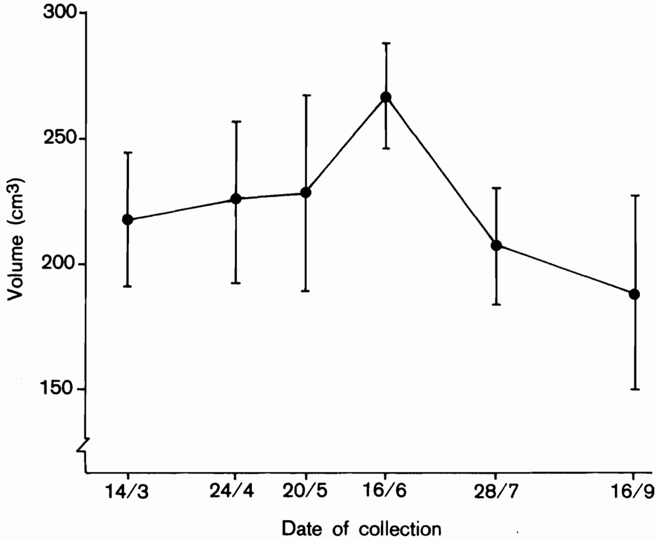

FIG. 2—Volume changes of tree-ripened cones maturing in 1986. Bars indicate standard errors of individual means.

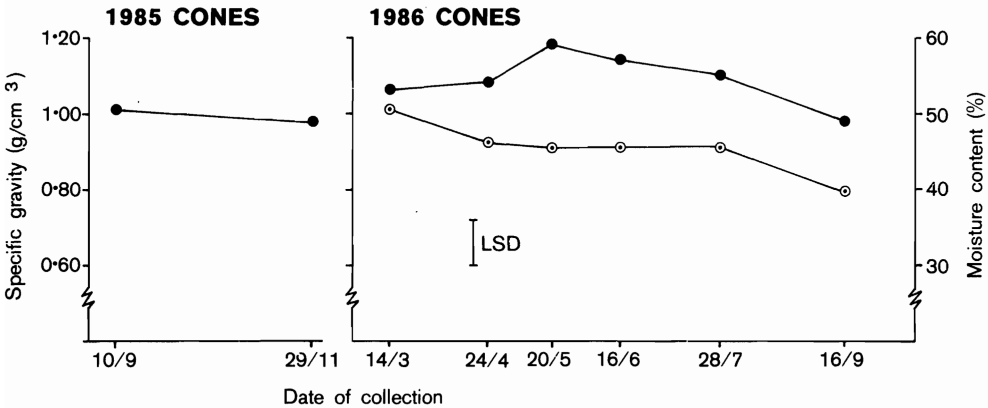

FIG. 3—Chronological changes in the moisture content (O) and specific gravity (●) of cones during the later stages of development. Standard errors of individual means for specific gravity are smaller than the symbols used. LSD for moisture content is calculated at the p = 0.05 level.

of cones collected on the same date showed little variation, this parameter increasing from 1.13 in March 1986 cones to 1.19 in May. Then followed a gradual and relatively steady decline until the specific gravity of September 1986 cones was 0.98. Maturing cones collected in 1985 showed very similar values (1.01 in September, 0.98 in late November).

Through all the collections, March to July 1986, cones remained green in colour. Some colour transition began in September, although most of the cones still remained in colour Category 1. As expected, cones collected in November 1985 were brown in colour (Categories 3 and 4) and ripe enough for immediate extraction of seeds by kilning, although in no cone was scale opening observed before the oven treatment. The following year's cones from the March to September collections remained un-extractable after immediate kilning, requiring at least 6 weeks of dry storage before the seed extractability was 70% or greater.

Seed Germinability and Vigour

The proportion of empty seeds found in cones at each collection is given in Table 2, and the germinability of complete seeds extracted manually after the hot water treatment of developing cones (transformed data) in Fig. 4. In both years maximum seed germinability was attained from July onwards. Seeds extracted from cones collected in mid-March 1986 were not capable of germination and many seeds, from the April collection especially, showed radicle emergence but failed to develop enough to meet the normal germination criteria (Table 2). As can be seen from the graph, the critical period for the

TABLE 2–Percentage of empty seeds and abnormal seedlings (as a percentage of the remainder) from different cone collections in 1986. Values are the means of four replications. Abnormal seedlings showed impaired growth after radicle emergence

Collection date	Empty seeds (%) (±S.E.)	Abnormal seedlings (%) (±S.E.)
24 April	16 (±5.0)	12 (±4.3)
20 May	10 (±3.1)	6 (±4.8)
16 June	12 (±1.6)	4 (±2.0)
28 July	11 (±1.8)	2 (±2.0)
16 September	8 (±1.2)	0

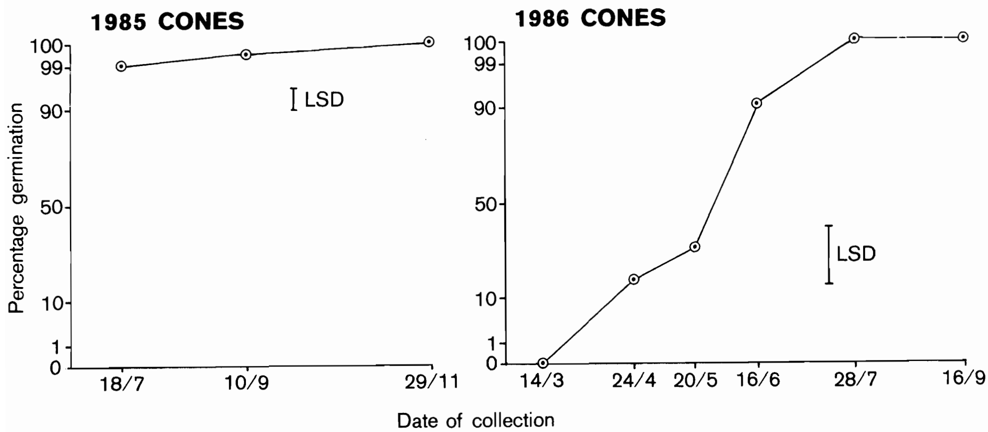

FIG. 4—Chronological changes in the germination capacity (arcsin transformed data) of seeds extracted from cones collected at various stages during the later stages of their development. LSDs are calculated at p = 0.05 level.

development of germinability is between late May and mid-June when the proportion of normal germinants increased from 28% to 90%. By the end of July germination of sound seeds was effectively 100%.

Comparison of data for stratified and non-stratified seeds showed no significant effects of chilling on freshly harvested material; thus, the median germination time (T50) data for the 1986 collections is presented as the combined means for both treatments (Fig. 5). No stratification treatments were carried out on the seeds of the 1985 collection. Clearly seed vigour improves as cone development continues through from May until September. Mean T50 for seeds from the September 1986 collection was approximately 11 days, a very similar value to that of the September 1985 collection. Seeds of the late November collection in 1985 showed a slight, but not significant increase in median germination time. Because data are calculated on numbers of seeds germinated, the T50 for the April collection may be artificially low as the very few germinants probably represent one extreme end of the vigour range of the total population of seeds at an early stage of maturation.

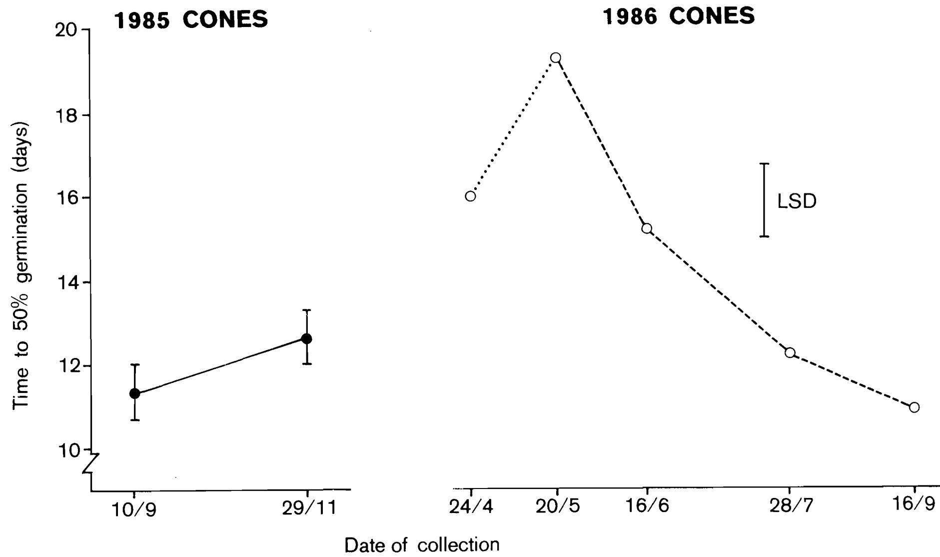

FIG. 5—Median germination times (T50) of seeds extracted from cones collected at various stages during the later stages of their development. Bars on data for 1985 cones are standard errors of individual means. LSD for 1986 cones is calculated at the p = 0.05 level.

DISCUSSION

In *P. radiata,* cone development takes around 28 months from female strobili initiation until maturation. Broadly, development may be divided into two principle phases – firstly, initiation until just before fertilisation and, secondly, from fertilisation to maturation (Bramlett *et al.* 1976). This present study investigated the later stages of the second phase.

Apart from the differences in specific gravity, the preliminary comparison of OP and CP "268" series cones harvested in July 1985 indicated that the two sets of cones were at comparable stages of development. The assumption is then made that results from the following year's detailed study on OP cones can be extrapolated to CP cones. What was surprising from the preliminary study was that immature cones collected in July already contained highly germinable seed.

Allowing for high sample variation, cone dry weight and size can be considered to reach a maximum in June (Fig. 1 and 2). These results are consistent with the earlier study on the development of *P. radiata* in New Zealand by Sweet & Bollmann (1971). Cone moisture contents decrease from mid-March onwards (Fig. 3), before maximum cone size is attained, earlier than might have been expected from comparison with other pine species such as *P. resinosa* Aiton (Dickmann & Koslowski 1969).

Edwards (1980) suggested that for a number of pine species maturity is attained when cone specific gravity falls below 0.9 and the moisture content is below 50%. Clearly both this suggested moisture content and specific gravity are too high for *P. radiata,* and major developmental changes occur in the seeds as the cone moisture content falls from 50% to 40% between March and September (Fig. 3). In practice, too, the specific gravity of mature cones varies with species and probably environment. A specific gravity of 0.89 was suggested by Wakeley (1954) for southern pines, and Maki (1940) and Fowells (1949) reported 0.84 and 0.80 as appropriate specific gravities for *P. ponderosa* and *P. lambertiana,* respectively. For *P. radiata,* mature brown cones have a specific gravity close to 1.0. This parameter was the least variable of those measured in this study, a maximum of nearly 1.2 being reached in May, suggesting dry matter was accumulating more rapidly than increases in volume at this stage. The decline in specific gravity from mid-June onwards roughly parallels losses in cone moisture content and is close to 1.0 in mid-September. This loss of water is also responsible for the decrease in cone volume (cf. Harlow *et al.* 1964).

Pinus radiata seeds show 90% germinability in mid-June (Fig. 4) and are approaching maximum vigour (in terms of rate of germination) by the end of July (Fig. 5), during which time the cones are still green. Therefore it is clear that seed maturation within the cones is well ahead of the cone itself and cone colour cannot be used as an index of seed development. Nor would cone moisture content provide a reliable index because of relatively high variation during a period of relatively low moisture loss (Fig. 3). On the other hand, there may be potential for using cone specific gravity as an index of seed development.

Seeds from green cones cannot, of course, be extracted by conventional kilning methods, but immature *Pinus* cones can be successfully prepared for kilning by air-drying (Wilcox & Firth 1980; Arisman & Powell 1986). Taking this into consideration with our findings on the earliness of seed development in *P. radiata.* there could well be much scope for increased flexibility in planning and operation of commercial cone collection for both CP and OP plantations. Furthermore, if artificial ripening techniques can be applied when the seeds themselves are still at an early stage of maturity, cone collection may be successfully brought forward to even earlier dates. This possibility will be investigated in our subsequent studies.

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