ARTIFICIAL RIPENING OF PREMATURELY HARVESTED CONES OF NEW ZEALAND *PINUS RADIATA* AND ITS EFFECT ON SEED QUALITY

A. RIMBAWANTO*, P. COOLBEAR†
Seed Technology Centre, Massey University,
Private Bag, Palmerston North, New Zealand

and A. FIRTH
Ministry of Forestry, Forest Research Institute,
Private Bag, Rotorua, New Zealand

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ABSTRACT

Second-year **Pinus radiata** D. Don cones harvested as early as April ripened successfully in dry storage and produced seeds of high germinability and vigour. This is 3 months earlier than previously recorded. The limiting factor was not the development of the seeds themselves, but the point at which cones became amenable to efficient extraction by conventional kilning techniques. In most trials this took between 6 and 9 weeks. Prolonged artificial ripening beyond 12 weeks caused some loss of seed vigour, but no index of cone maturation during storage formed a sound basis for assessment of viability and/or extractability of the seeds.

Artificial ripening triggered a change from developmental to germinative mode and there was a concurrent loss of seed moisture but this was not the only change involved in this process. Seeds from artificially ripened cones showed a limited response to stratification treatment, suggesting a small degree of residual dormancy.

Keywords: seed; cone; germination; ripening; stratification; seed vigour; **Pinus radiata**.

INTRODUCTION

Premature harvesting of cones and then allowing them to ripen off the tree, or "artificial ripening", has been used in several conifer species as a means of bringing forward or increasing the flexibility of cone-collecting operations. The success of artificial ripening depends on both the stage of seed development when the cones are collected (Fowells 1949; Bevege 1965; Krugman 1966; Barnett & McLemore 1970) and the conditions under which the cones are stored. Optimum artificial ripening

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* Current address: Directorate of Reforestation, Ministry of Forestry, Forestry Centre Building, Jalan Gatot Subroto, Senayan, Jakarta Pusat, Indonesia.
† To whom all correspondence should be addressed.

conditions have been reported to vary considerably from species to species. Silen (1958), for example, found that storage of cones in damp peat moss was necessary for the ripening of *Pseudotsuga menziesii* (Mirb.) Franco seeds, as did Matyas (1973) for *Pinus sylvestris* L. These results indicated that moisture is required to promote metabolic changes necessary for maturation of seed in these species. On the other hand, Franklin (1965) and Pfister (1966) found that this kind of storage was harmful to *Abies* species, damp anaerobic storage conditions promoting fungal infection. Dry storage has been found effective for many *Pinus* species, including *P. elliotti* Engelm. (Bevege 1965), *P. taeda* L. (Cobb *et al.* 1984), and *P. merkusii* Jungh. et de Vriese (Arisman & Powell 1986).

Fielding (1964) and Wilcox & Firth (1980) reported success in dry artificial ripening of green cones of *P. radiata.* The latter workers found that cones collected in July and artificially ripened for 10 weeks were ready for seed extraction and produced good quality seed in time for normal sowing in October. Thus the usual 1-year delay in establishing progeny tests for naturally ripened seed (which are not available until November or December) could be avoided. Both these studies were, however, incomplete in that neither identified the initial germinability of seeds from prematurely collected cones, nor characterised optimal artificial ripening conditions. Matyas (1973) and Barnett (1976), working with *P. sylvestris* and *P. palustris* Miller, respectively, have both shown the dangers of prolonged after-ripening, causing losses in seed germinability and quality.

The use of controlled-pollinated, hedged, clonal seed orchards for *P. radiata* in New Zealand is increasing. This practice demands earlier cone collection so that the necessary pruning can be carried out in the winter months. Our recent studies have shown that seed maturation in *P. radiata* is well ahead of cone ripening on the tree (Rimbawanto, Coolbear, Dourado & Firth 1988). Thus this present study was undertaken to extend previous work and investigate procedures for artificial ripening, especially with respect to the timing of cone collections and the optimal duration of the artificial ripening period.

MATERIALS AND METHODS

Cone Collections

Major collections of second-year cones from open-pollinated trees of the FRI "850" series in the Kaingaroa Seed Orchard were made at approximately monthly intervals from March to July 1986. At each collection time, four cones were taken from each of three randomly selected ramets from 10 of the potentially best clones chosen on the basis of FRI progeny tests. Ramets were all grafts checked for good health at the commencement of the trial. Cones from one ramet of each of the 10 clones were then bulked together for each storage study.

Artificial Ripening Conditions

Cones of two sets of ramets from each collection were stored for up to 21 weeks at a constant temperature of 20°C, 70–80% RH. One set was extracted using the hot water method (described below), four cones being randomly selected at each sampling time. Each cone was treated as an individual replicate providing a separate sample of seeds for the quality assessments reported in this paper. The second set of cones stored under these conditions were extracted by kilning to provide the extractability data. The final set of cones bulked after each collection were used for a comparative storage study under ambient conditions (temperatures ranging from 12° to 23°C) and extracted by kilning.

Cone Assessment and Seed Extractability

Cone specific gravity was determined by the water displacement method (Schopmeyer 1974). Cone colour was assessed on the basis of a colour coding scheme chosen from colour standards provided by the British Colour Council (B.C.C. 1957). 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 Category 2, B.C.C. 233 (Tuscan Yellow); 3, B.C.C. 204 (Cinnamon), and 4, B.C.C. 168 (Nutmeg).

For the major study, seeds were extracted by dipping the cones in hot water ($\cong$ 80°C) for up to 1 minute, then breaking them and extracting manually. Only morphologically complete seeds (as determined by X-radiography) were used in the germination studies. Seed extractability from cones 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 Assessment

Moisture contents and dry weights of seeds were determined by the air oven method with samples held at 103°C for 17 h as recommended by ISTA Rules (I.S.T.A. 1985). Moisture contents were expressed as percentages of fresh weight. Germination tests were conducted at a constant 20°C for 28 days in continuous low light. Each replicate consisted of 25 seeds which were dusted with Thiram fungicide and placed in airtight plastic boxes containing germination blotters over cut sheets of paper towelling moistened with distilled water. The response of seeds to stratification was tested by holding a duplicate set of germination boxes at 5°C for 1 week before transferring to 20°C. Numbers of seeds showing radicle emergence, normal germination (defined as when radicle length has reached four times the length of the seed), and cotyledon emergence were scored every 2 days. The median time for each event (T50) was calculated by linear interpolation as described by Coolbear *et al.* (1984). These indices provide a good assessment of vigour under unstressed conditions (A.O.S.A. 1983).

RESULTS

Physical Changes in Cones During Artificial Ripening

As cones are held in dry storage their specific gravity falls (Fig. 1) reflecting losses of moisture. A highly significant reduction in specific gravity occurred during the first 3 weeks of storage, thereafter there were only slight decreases to a range between 0.95 and 0.99 g/cm^3, comparable with the value of fully mature cones (Rimbawanto, Coolbear, Dourado & Firth 1988).

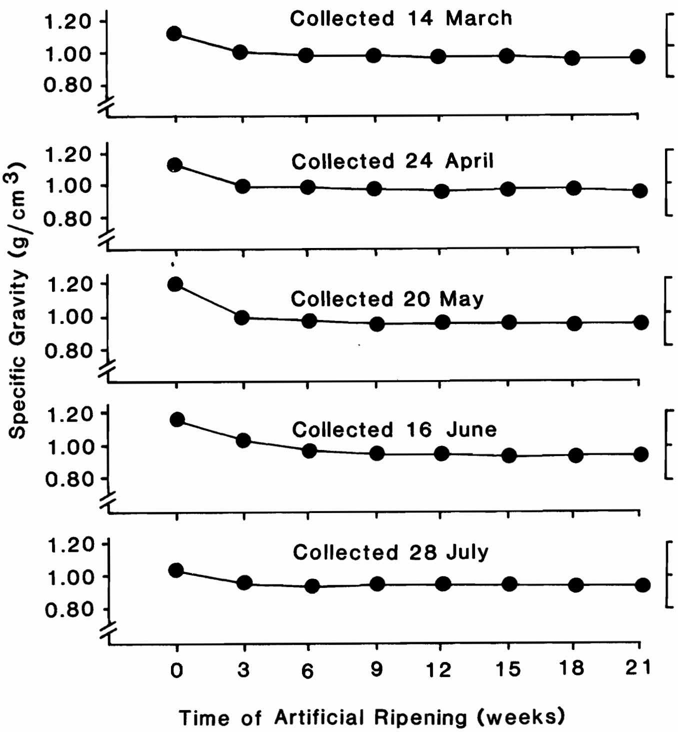

FIG. 1—Changes in the specific gravity of cones collected on different dates during artificial ripening at 20°C constant. Each value is a mean of four replications; SEs are smaller than the symbols used.

Definite cone colour changes occurred during the artificial ripening period (Fig. 2), browning of the cones being most rapid in the June–July collections. Ripening cones began to respond to kilning after 6 weeks' storage, when the scales of all tested samples opened completely. Extractability of seeds did not, however, correlate well with the degree of scale opening after kilning. Cones ripened for 9 weeks usually gave better extraction efficiencies than those kilned 3 weeks earlier (Fig. 3). In general, extractability increased in a similar pattern to development of cone colour, but there was often very high sample variation for samples ripened for 6 or 9 weeks, and the extractability of seeds from cones collected in June developed much later than the other collections.

Physical Changes in the Seeds During Artificial Ripening

There was no clear evidence for any progressive changes in seed dry weight during artificial ripening of the cones, but small changes may have been masked by variation between clones and individual cones. Seed moisture content declined with artificial ripening, especially over the first 9–12 weeks (Fig. 4) although it is evident that in the May, June, and July harvests there was little moisture loss over the first 3 weeks

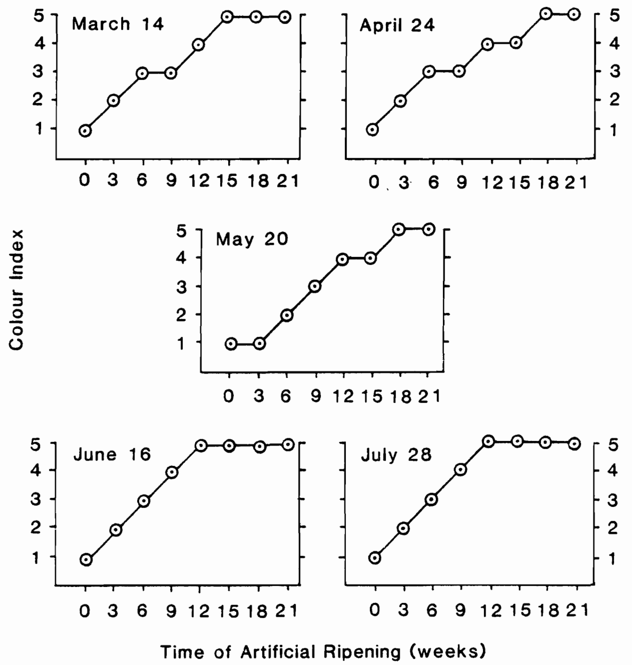

FIG. 2—Cone colour changes during artificial ripening at 20°C for different 1986 collections as indicated. The colour index is based on British Colour Council colours.

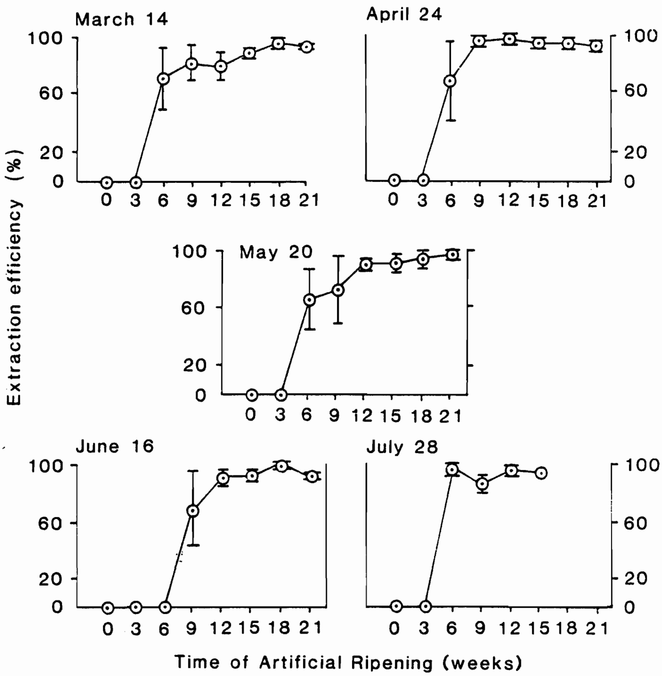

FIG. 3—The extraction efficiency (%) of cones from different 1986 collections after artificial ripening at 20°C. Each value is the mean of four replicates; individual SEs are shown.

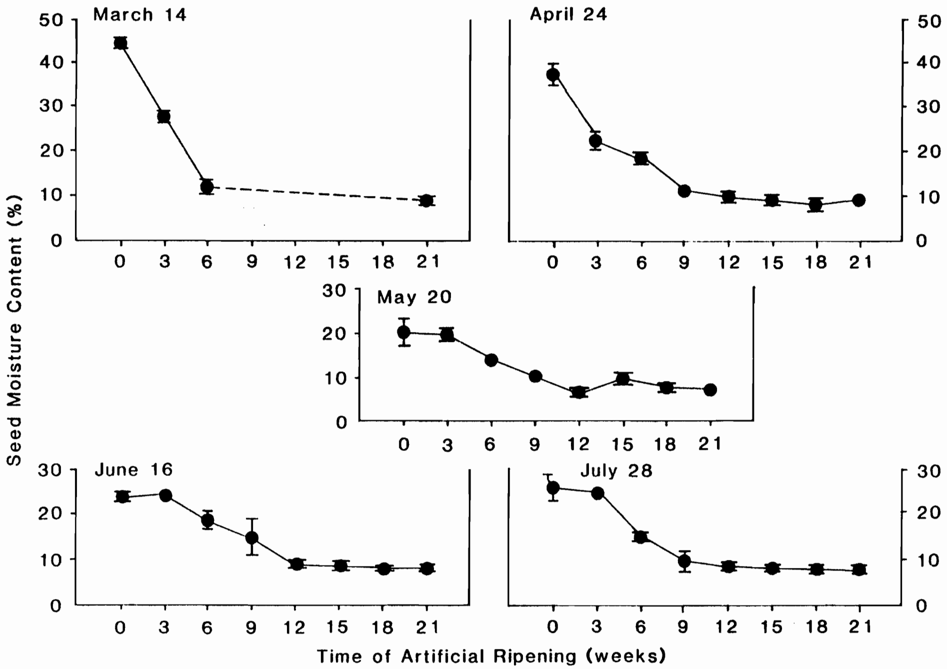

FIG. 4—Changes in seed moisture content during artificial ripening at 20°C, for different 1986 collections as indicated. Each value is the mean of three or four replications; individual SEs are shown where larger than the symbols used.

of storage. Mean moisture contents after 12 weeks or more were 10% or less, much lower than that of mature tree-ripened seed collected in September (21.5%) (Rimbawanto, Coolbear & Firth 1989).

During artificial ripening, seed coat colour changes occurred in material from all collections, following a similar pattern to that found in tree-ripening seeds (Rimbawanto, Coolbear & Firth 1989). The rates of darkening, however, depended on the degree of maturation of the seeds at cone collection: seeds from cones harvested in March, April, or May, and then ripened for 21 weeks, did not match the seed colour from cones of June and July samples stored for 12 weeks. Despite these changes in seed coat colour during ripening, the seed coat dry weight remained a constant proportion of seed dry weight irrespective of collection date or storage time (data not shown). Similarly, X-ray examination of prematurely harvested seed showed no obvious anatomical changes taking place during artificial ripening.

Germinability and Vigour of Seeds

Data on the germinability of seeds from cones collected in March, April, and May, and then artificially ripened, are shown in Fig. 5. None of the fresh seeds collected in March germinated, but 3 weeks' storage increased the germination to 36%. Further

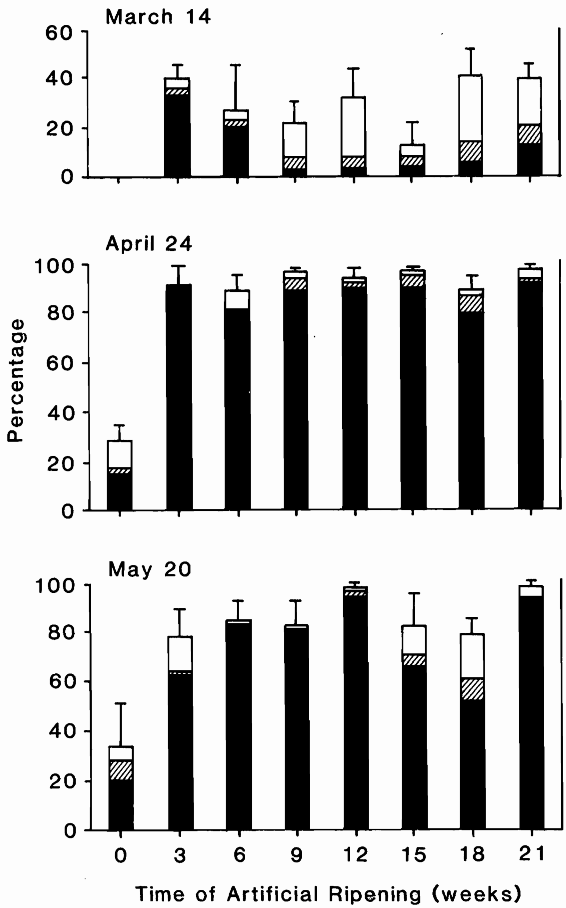

FIG. 5—Final percentage radicle emergence (□), germination (▨), and cotyledon emergence (■) of seeds extracted from cones collected on the dates shown and artificially ripened for varying times at 20°C. Each value is the mean of four replications of non-stratified seeds, individual SEs are shown for radicle emergence data.

storage had an adverse effect on germinability, a large proportion of seedlings failing to achieve normal germination because of retarded radicle development. Variation between replicates was very high.

Seeds from cones of the April collection showed 16% germination before artificial ripening, but this value rose to 90% after 3 weeks' storage and remained high as storage was prolonged further. A very low proportion of abnormal seedlings was observed. The increase in germinability of this collection coincided with a very large decrease in moisture content of the seeds during artificial ripening, from 37.6% to 22.5% (Fig. 4). A similar pattern of improved germination performance after a short period of artificial ripening was observed in the May collection, but effects were less pronounced and the variation and proportion of abnormals were greater than in seeds from cones harvested in April. Note that in May the moisture content of freshly harvested seeds was already low (20.5%).

The initial germinability of seeds collected in June and July was already 89% and 97% respectively. Storage of cones increased germination only slightly and no effects of prolonged artificial ripening on germinability were noted (data not shown).

Although we found no evidence for stratification treatment affecting the final germination capacity of seeds from artificially ripened cones, there were significant effects on seed vigour assessed on the basis of time to reach 50% (T50) radicle emergence, normal germination, or cotyledon emergence. There were, however, no significant interactions between stratification and time of artificial ripening; thus, data on vigour changes with time of cone collection and storage have been pooled over stratified and non-stratified seeds (Fig. 6). Prolonged artificial ripening periods clearly impaired the vigour of developing seeds, except in those of the July collection which were already of very high vigour when freshly harvested and appeared resistant to deterioration during storage in the cones for at least up to 9 weeks.

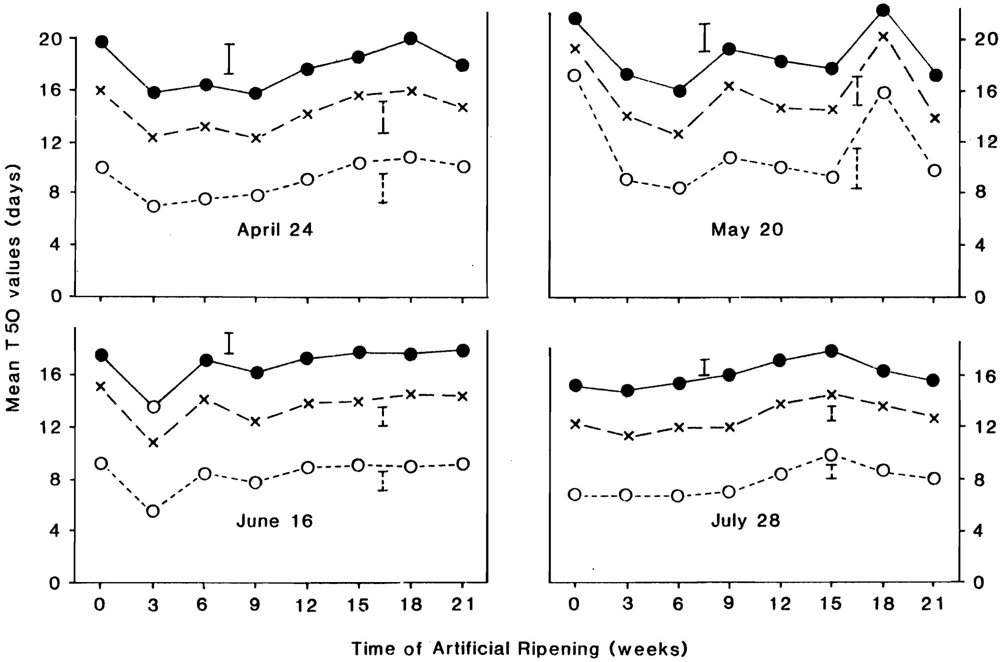

FIG. 6—Mean times to reach 50% (T50) radicle emergence (●——●), germination (× - - - - ×), or cotyledon emergence (O - - - - - O) of seeds from cones of different collections artificially ripened for varying times at 20°C. Data are pooled over stratification treatments and thus each value is the mean of eight determinations. Bars are least significant differences calculated at the p = 0.05 level.

The overall effects of stratification treatment on seeds from different cone collections pooled over artificial ripening period are given in Table 1. Except for seeds from the July cone collection, stratification advanced each event of seedling development. The magnitude of response appears greater for the less developed seeds, while in the July collection the stratification treatment significantly impaired the rate of radicle growth.

The comparison of final germinability of kiln-extracted seeds from cones collected in March, April, May, and June, and stored for between 6 and 21 weeks, showed that storage at ambient temperatures (between 12° and 23°C) was as effective as constant temperature 20°C storage (data not shown).

TABLE 1–Effects of stratification on seed vigour of artificially ripened seeds collected at different times. Data pooled over storage times

	Date of cone collection			
	April 24	May 20	June 16	July 28
*Time to 50% radicle emergence** (days)				
– Stratification	10.4b	12.4b	9.2b	8.4b
+ Stratification	7.8a	10.3a	7.6a	7.1a
Time to 50% normal germination (days)				
– Stratification	15.9b	16.6b	14.5b	13.7a
+ Stratification	12.8a	15.0a	13.0a	15.4b
Time to 50% cotyledons (days)				
– Stratification	19.4b	19.4b	17.6b	16.8b
+ Stratification	16.2a	18.1a	16.3a	15.4a

* Pairs of means followed by different letters are significantly different at $p<0.05$

DISCUSSION

We have already shown that seed development is well ahead of cone development in New Zealand *P. radiata.* Seeds from green cones collected in July were already of high germinability and vigour (Rimbawanto, Coolbear, Dourado & Firth 1988). From the present study it is clear that cones collected as early as the second half of April contain seeds which can be induced to germinate well by artificial cone ripening in dry storage. This is 3 months earlier than had previously been recognised. The limiting factor is the ripening of the cones themselves rather than that of the seeds within them. Prematurely harvested cones generally need 9 weeks of dry storage before seeds can be efficiently extracted by normal kilning techniques (Fig. 3).

After this period of storage, seeds from all collection dates in this study showed improved vigour, except for the July sample, where no improvement was observed over freshly extracted seeds which were already of high vigour. There was, in fact, no significant difference between the germination rates of seeds from cones of the April collection and those from cones collected in July, when both had been artificially ripened for 9 weeks (Fig. 6). Prolonged artificial ripening of cones, especially beyond 12 weeks, did, however, lead to losses of vigour, even though moisture contents of the seeds were around 10%. Unfortunately, neither cone colour nor specific gravity changes provided an ideal maturity index for cones during artificial ripening (compare

Fig. 1 and 2 with 3, 5, and 6). Assessment of extraction efficiency at intervals after 6 weeks' storage is probably the most practical way to decide the best time to terminate artificial ripening.

Physiological Changes During Artificial Ripening

Edwards (1980) pointed out that, although artificial ripening procedures have been applied over the past two decades to more and more seeds, there is very limited information on the processes involved. Data obtained in this study show that, for earlier cone collections, artificial ripening for seeds of *P. radiata* is effectively an imposed maturation process, rather than one promoting further development. There were no significant increases in seed dry weight, suggesting that reserves did not move from cone to seed during the storage period. Indeed, the moisture level of seeds within the stored cones (Fig. 4) rapidly became too low for such transport to be likely.

Our studies on development in tree-ripening *P. radiata* seeds (Rimbawanto, Coolbear & Firth 1989) clearly show that, in the March of the second year after pollination, the majority of seeds have yet to develop sufficiently to be germinable and thus artificial ripening is only marginally successful at this stage (Fig. 5). In contrast, by late April, seeds are much more advanced developmentally and the artificial ripening conditions are now an effective trigger for the change from developmental to germinative mode (cf. Kermode *et al.* 1985). Loss of seed moisture content is an important component of this process (comparing moisture content and germination of seeds from the April collection, Fig. 4 and 5), but it is not the only factor involved: seeds from cones of the May collection were already at just above 20% moisture content when harvested, but germinated very poorly without artificial ripening. It is not clear why seeds from cones collected in May showed greater variation and a poorer response to artificial ripening than those from the April collection. This could reflect sampling error, but may be due to the stage of seed development. By the second half of May, seeds are at a stage when the progression between developmental and germinative mode is occurring naturally on the tree. Because of this they may possibly have been at a period of maximum susceptibility to the environmental stresses imposed by harvesting and subsequent handling.

In our previous work (Rimbawanto, Coolbear, Dourado & Firth 1988) we found no significant effect of stratification on freshly harvested seeds. This was not true of the artificially ripened seeds in this study, where a stratification treatment improved rate of germination (Table 1) although not over-all germinability. Possibly the more abrupt artificial ripening treatment did not completely overcome all the dormancy mechanisms present in the developing seed which would be naturally alleviated during the course of tree ripening. It is unlikely that it would be economically worthwhile to stratify artificially ripened seeds in commercial practice, however. The germination delay incurred by not carrying out this treatment is at the most 3 days, and decreases rapidly for later cone collections.

It should, perhaps, be emphasised that all the germination and vigour testing carried out in this study was done under optimal conditions. We have little information on the ability of early collected, artificially ripened seeds to germinate under stress, or on variation in response from year to year. However, on the basis of these results, successful plantings of artificially ripened seeds from early collected cones have been made in the nursery this year (1988).

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