MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN DEVELOPING *PINUS RADIATA* D. DON SEED AND THE EFFECTS OF EARLY CONE COLLECTION AND POST-HARVEST TREATMENT ON SEED QUALITY

A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF AGRICULTURAL SCIENCE IN SEED TECHNOLOGY
AT MASSEY UNIVERSITY, PALMERSTON NORTH
NEW ZEALAND

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1987

To Mum and Dad who have always inspired
me to pursue knowledge

ABSTRACT

Morphological and physiological changes in developing **Pinus radiata** D. Don seed and the effects of early cone collection and post-harvest treatment on seed quality

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This study aimed to characterise the sequence of cone and seed development, investigate morphological and physiological changes occurring during artificial ripening and assess the potential of artificial ripening in commercial practice.

In general, the results show that cone and seed development of Pinus radiata exhibit a pattern similar to other coniferous species, but seed development and the acquisition of germinability proceed at a rate much faster than the maturation of the cone itself. Seed germinability is attained in June when cone dry weight and size are at maximum; cone colour and specific gravity changes occur much later, thus lessening the effectiveness of these two parameters as indices of maturity. Therefore, a cone with a green colour and high specific gravity does not necessarily contain poorly germinable seeds.

The aquisition of germination capacity is closely associated with the level of moisture within the seed. The seed requires a low level of moisture to switch on the germination programme. A moisture level of between 20 - 25% is suggested as the 'required' level. The seed needs to remain at this critical moisture level for a period of time to allow the developing seed to complete the process of switching. The more developed the embryo and megagametophyte, the better the germination performance. Immature seeds collected in March are not capable of germinating despite desiccation during artificial ripening.

Artificial ripening of P. radiata seed for three weeks substantially improves the germinability of early collected seeds (April and May). For the late collected seeds (June onwards) artificial ripening has little scope to improve it since initial germination was high. Although further storage has little effect on the final germination, it reduces the speed of germination indicating a process of deterioration. During artificial ripening, no further development of embryo and megagametophyte of the early collected seed is observed nor are there any increases in dry weight. Moreover, the main protein complement of the seeds remains proportionally the same irrespective of time of collections and artificial ripening. These suggest that artificial ripening of P. radiata seed is a maturation process rather than a developmental one.

The practical implications of these findings are potentially good. Brown cone colour is no longer a pre-requisite indicator to commence cone harvesting. Infact cone collection as early as autumn/winter is justifiable provided that the cones are allowed to dehydrate at a temperature not exceeding 20°C for at least six weeks, or until the specific gravity drops below 1.00 because at this point seed extraction can be successfully done by the kilning method. Cone storage for more than nine weeks would not be advisable since the seed will begin to deteriorate owing to unfavourable storage conditions. At this stage seed should be extracted from the cones and stored separately at 5°C.

ACKNOWLEDGEMENT

I would like to extend my sincere appreciation and thanks to many people and organization who have supported me during the period of my study. I am particularly grateful to the New Zealand Government for financial support and to the Department of Forestry of the Government of the Republic of Indonesia, the Seed Technology Centre, Massey University and the Forest Research Institute of the New Zealand Forest Service who made it a reality.

I am deeply indebted to Dr P. Coolbear of the Seed Technology Centre for two years of interaction, guidance, enthusiasm, fertile discussion and most of all encouragement which have culminated in the production of this thesis. I am also deeply grateful to Dr M.J. Hill, the Director of the Seed Technology Centre for his tremendous support, advice and concern in ensuring that my study proceeded well. I also thank Dr A.M. Dourado for her involvement in the early preparation of this study.

My deep appreciation also goes to Mr T. Firth of the New Zealand Forest Research Institute, Rotorua for his most helpful assistance in the cone collection and constructive criticism during the course of the experiment. I also appreciate the valuable discussion and suggestions made by Dr C.J.A. Shelbourne of the New Zealand Forest Research Institute, Rotorua. Thanks also due to Dr R. Burdon of the New Zealand Forest Research Institute, Rotorua for reading the manuscript.

As a foreigner, I have appreciated the opportunity to study at the Seed Technology Centre, Massey University and I am particularly happy with the friendly and helpful reception by the staff which have made my staying a pleasant time. I particularly thank Mrs A.M. Davies for typing this report.

Last but not least, tremendous moral support, love and encouragement from home has been a great benefit for me.

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1. INTRODUCTION

In modern history the economic importance of conifer wood is very significant. Millions of hectares of conifer forest have already been planted in many areas of the world to replace the large scale utilisation of indigenous forest and meet the increasing demand for wood. New Zealand is no exception, with a current established exotic conifer plantation of well over one million hectares. Of the species used, Pinus radiata is predominant, making it by far the most important commercial species and contributing substantially to the nation's economy. Radiata pine frequently performs better in New Zealand than in its native habitat despite the fact that many plantings experience more severe environmental conditions (Bannister, 1973).

Seed production plays an essential role in this success owing to the fact that seedling propagation remains the most efficient and economical method of plant production. The current national requirement of P. radiata is around 3500 kg per annum, all of which is supplied by state and privately owned open pollinated first generation clonal seed orchards. The demand will continue to grow as more over-logged and unproductive areas need to be replanted. There is also an increasing demand for seed of greater improved genetic quality which has stimulated the development and improvement of seed orchard management systems. A major recent advance has been the establishment of controlled pollination hedged clonal seed orchards to ensure maximum genetic gain in seed produced through complete control of both male and female parents. This system requires plants to be trimmed (hedged) to a convenient working height of approximately 1.5 m. Following isolation and pollination in spring the vigorous female flower bearing shoots are permitted to grow above this height. Cone bearing shoots are cut back to 1.5 m in May/June some twenty three months later and the immature cones collected. This cutting back operation and its timing are essential to enable recovery of the lower plant and stimulate production of vigorous new shoots for pollination a year later. The prematurely harvested cones then have to be artificially ripened to facilitate seed extraction and obtain good quality seeds.

In addition to the great importance of artificial ripening to controlled pollination seed orchards, its potential for open pollinated seed orchards is equally high. Generally, in commercially important gymnosperms, cone collection commences when the cones are fully mature. A

number of parameters such as cone colour, specific gravity and moisture content have been found a very useful basis for judging seed maturity. However, in many species the period between cone ripeness and seed dispersal is often only a few weeks duration, requiring maximum effort to obtain maximum use of the available cone crop. Silen (1958) proposed that a viable alternative approach could be to harvest cones prematurely. Such cones, if suitably handled, could be artificially ripened to produce high quality seeds. The major benefit will be in the flexibility in planning and organizing cone collection since the period of collection can be extended. Above all, by harvesting earlier and artificially ripening the seeds for sowing in October, the cycle from seed production to seedling production of P. radiata is reduced by one year.

In recent years a number of studies have been carried out on some aspects of artificial ripening. Despite this, however, little is known about the physiological processes underlying the success of artificial ripening. It appears that the optimum conditions for artificial ripening differ among species. Some species, such as Douglas-fir (Silen, 1958), respond well to wet storage conditions, while others, such as slash pine (Bevege, 1965), prefer dry storage. The artificial ripening period for some species appears crucial owing to deterioration following prolonged storage of cones. Nevertheless, one thing seems to be certain, in that the key factor for successful artificial ripening relies on determining the stage of maturity at which point collection can safely be commenced. Unfortunately, little work has been done on accurately characterising the developmental process in Pinus spp. seed. In P. radiata for instance the time when the seed reaches maturity within the cones is unknown, with a resulting gap in information on how early cones can in fact be collected.

This study was intended to characterise the sequence of cone and seed maturation of P. radiata during the second season of development and to utilize this information in determining the optimum conditions for artificial ripening. For this purpose the study was designed to:

a) Study the course of seed development in conjunction with the acquisition of germination capacity.

b) Examine various conditions favourable for artificial ripening, notably combinations of storage temperatures and duration of storage to obtain high quality seed and facilitate seed extraction.

c) Determine parameters of maturity applicable to successful extraction and high germination.

d) Study some of the physiological aspects of development and maturation in tree-ripened and artificially ripened seed.

The overall study was carried out over two years, commencing in July, 1985, at the Seed Technology Centre in collaboration with the Genetics and Tree Improvement Group of the Forest Research Institute, Rotorua. The study was divided into two phases: a preliminary study, and a more detailed study covering artificial ripening and seed development. The object of the first part was to study the response of seeds produced by two seed orchard management systems, i.e. conventional open pollinated (OP) and hedged controlled pollinated orchards (CP), to different artificial ripening conditions and extraction methods. The second part of the study concentrates on the second phase of seed development of OP orchard seed and the effects of artificial ripening on cones harvested at intervals from March to July when the seeds are ripe within the cones.

2. LITERATURE REVIEW OF CONE AND SEED DEVELOPMENT, PARAMETERS OF MATURITY AND ARTIFICIAL RIPENING

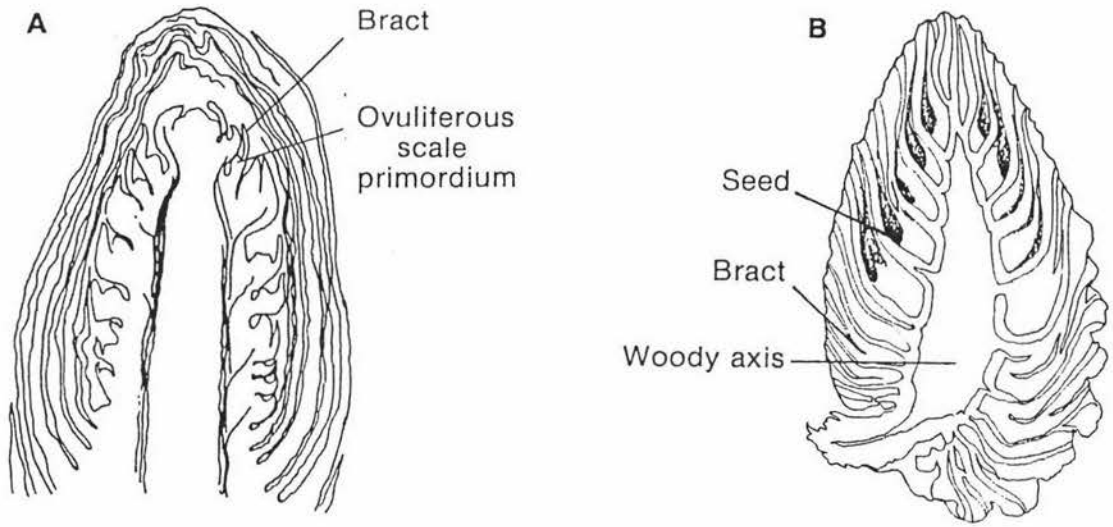
2.1 Cone Development

The study of cone development, especially the interpretation of the structure and evolution of the cone, represents, perhaps, one of the most difficult and controversial problems in plant morphology (Foster and Gifford, 1959). It is concerned with the various processes and stages from the stage of cone initiation, until when it is fully mature. The word cone is used here to mean the ovule- and seed-bearing structures of pine, regardless of the stage of development (Lill and Sweet, 1977). The literature published to date is far from clear in distinguishing maturity and ripeness. Maturity is often associated with a stage where accumulation of reserves is completed (i.e. maximum dry weight is achieved) as in physiological maturity. However, in some species many of the embryos may be immature when physiological maturity, as defined by Harrington, has been achieved and may continue to develop after removal from the parent plant. Furthermore, other physiological changes still occur afterwards, such as moisture reduction and increase tissue sensitivity to hormones. In some conifer species the situation may be even more complex because cone and seed development proceed at a different rate. Seeds may be ripe before cone maturity is reached. To avoid confusion, therefore, the terms maturity and ripeness are used interchangeably.

2.1.1

Morphologically, Foster and Gifford (1959) described the cone as having a woody axis bearing a spirally arranged series of bracts (Fig. 2.1). In the axil of each bract is a thick, woody scale upon which two ovules are borne, though not all scales produce functional ovules. Only those in the central part of the cone have the potential to produce ovules and eventually seeds (Bramlett *et al.*, 1976). The general pattern of cone development in temperate *Pinus* is well-documented (Stanley, 1958; Foster and Gifford, 1959; Bramlett *et al.*, 1976). Generally, the primordia of

Fig. 2.1 Morphology of ovuliferous structures of young megasporangiate strobilus (A) and morphology of fully developed cone (B) of **Pinus** spp.



the cones are initiated in the summer, and emerge in the following spring. Pollination takes place in the spring and fertilization will not occur until the subsequent spring. Cone maturity occurs in the autumn, twenty eight months after cone initiation. In some other pine species, the maturity period is much shorter; for example, the period from pollination until maturity in P. merkusii is twelve and a half months (Kingmuangkow, 1974).

2.1.2 Chronologically, Ching and Ching (1962) identified five distinct developmental stages in Pseudotsuga menziesii cones, i.e. 1) receptive; 2) enlarging; 3) fertilization and seed development; 4) maturation; 5) senescence. With P. radiata cones, the stage of development from strobilus emergence to cone maturity can be divided into three stages (Sweet and Bollmann, 1971). The first stage involves pollination, then followed by fertilization, and finally a period of cone maturation. A prominent feature of the reproductive cycle of Pinus is the relatively long interval between pollination and fertilization. In P. radiata this interval is almost fourteen months (Sweet and Bollmann, 1971), thirteen months in P. resinosa (Ferguson, 1904).

However, the time elapsed during the pollination, fertilization and ripening stages of cones can be readily modified by climate. Mirov (1962) emphasized that as the equator was approached, the reproductive development of pine was modified. In the mountains of Java, P. merkusii pollen was produced and shed intermittenly throughout the year. Ovulate strobili and mature cones were recorded throughout the year. In Nicaragua, ovulate strobili of tropical pines continued to develop during the winter and matured in a little over a year. In Southern Finland fertilization in P. sylvestris usually takes place in July, some thirteen months after pollination. However, when early summer temperatures are high fertilization occurs in June. At northern limits of the range, pollen tube development is so slow that fertilization may not take place before winter (Sarvas, 1962).

In those conifers in which cones and/or seeds mature over a two year period, the main increase in size and dry weight of cones occurs during the second year of their development, i.e. prior to and after fertilization. In a few species, however, such as western juniper (Juniperus occidentalis) and Alaskan cedar (Chamaecyparis nootkatensis) the cone attains practically full size during the first growing season, even though its seed ripen a season or more later (Schopmeyer, 1974). Sweet and Bollmann (1971) reported that P. radiata cones grew from about 1.5 cm to 4.0 cm during the one year period following pollination. Subsequently, tremendous increases in size and weight were found during the fertilization period. The cone reached full size and weight in June, 22 months after pollination, but ripening did not take place until another 5-6 months later. In P. ponderosa, maximum growth in length occurs during mid June of the second year (Roeser, 1941), whereas in P. resinosa, growth of cones is essentially complete by the end of July when cones reach a final length of 40-50 mm (Lyons, 1956); this maximum size is attained a month before dry weight accretion is completed (Dickmann and Kozlowski, 1969a).

Cone development exhibits many of the same features as fruit development - substantial increase in size; increase in moisture content and daily size fluctuation due to hydration and dehydration; high respiratory rates; accumulation of carbohydrates, minerals and other chemical constituents; and steady increase in dry weight (Katsuta and Satoo, 1964; Kozlowski, 1971). In the later stages of cone development, however, many of these processes reverse; water content decrease markedly, carbohydrates and minerals move from the cone to the seed, and respiration declines (Kozlowski, 1971).

2.1.3 The visible growth of the cone reflects morphological and physiological events taking place within the developing ovule. Thus, Dickmann and Kozlowski (1969b) found that seasonal changes in size, fresh weight and dry weight of first-year P. resinosa strobili were slow and gradual as pollen tube growth and megagametophyte development proceeded at a low pace. Size and dry

weight increased sharply just before and after fertilization signalling a rapid development and enlargement of the seed. At the same time, there was a sharp increase in the weight per cone of extractable reserves which include mostly carbohydrates with small amounts of fats, fatty acids and amino acids, lignin, hemicellulose, and cellulose fractions.

In discussions of seed development, the moisture content is generally recorded as being high just before or after pollination. Following fertilization which involves cytological and morphological development, the size of the seed increases but its moisture content remains constant and high. During later stages of seed development, there is an actual loss of water not accompanied by accumulation of food reserves (Pollock and Ross, 1972).

In cone development, however, the pattern of moisture content does not follow the same trend. Percentage moisture content of cones increases after their emergence and then gradually decreases. In the second year, moisture content again increases until late June (summer), after which time a sharp decrease occurs through maturation (Dickmann and Kozlowski, 1969a). However, the moisture attainment and moisture loss of the scale and the seed proceed at different rates. The moisture content of the seed is reduced at a faster rate than that of the cone scale (Ching and Fang, 1963). This is because nutrients which are required for the growth of the seed are temporarily stored in the cone scale, and mobilization of the nutrient will require high ambient moisture.

When changes in the water balance of tissue are observed, Kozlowski (1964, 1968) emphasized that these should be evaluated not only in terms of moisture percentages but also relative changes in water weight and dry weight. Thus, Clausen and Kozlowski (1965) found that immediately after pollination the percentage of moisture of Picea and Larix cones increased, this was followed by a significant decrease although the weight of water per cone remained the same or increased because there was a greater proportional increase in dry weight.

2.1.4 Apart from substantial increases in length, width, volume, moisture content and dry-weight, cone development also exhibits physiological and biochemical changes. Linder and Troeing (1981) found that in central Sweden the respiration rate of one-year old P. sylvestris cones reaches maximum at the time of fertilization (early July). Thenceforth it gradually decreases indicating the increasing amount of structural tissue. In Pseudotsuga menziesii it is found that the respiratory activity is high following pollination, providing energy for anabolic activities of tissue within the cone, and gradually decreases with maturity (Ching and Ching, 1962; Ching and Fang, 1963). Similarly, Kozlowski and Keller (1966) emphasized that seasonal respiration of fruits of woody plants is generally highest at the time of fruit setting and decreases on a weight basis during the growing season. In many cases, the metabolic activity of the cone scale and the seed are not the same. This becomes obvious when respiratory activity is measured separately. The respiratory rate per unit dry weight of the seed is higher than that of the scale irrespective of stage of development, but when respiration is measured on the basis of total nitrogen content the reverse relation is observed (Ching and Fang, 1963). The lower respiratory rate per unit dry weight of scale was attributed partially to the lower rate of synthesis for cellular material and partially to its photosynthetic activity, while the reverse relation may be due to the differential accumulation of nitrogenous compounds in the two kinds of tissues.

There have been few reports concerning the biochemical changes which take place during cone development. Dickmann and Kozlowski (1969b) in their study on changes of reserve components in P. resinosa cones, reported that, during the first year and the early part of the second year, reserves increased at a rate approximately parallel to the overall weight increment of young cones. In the latter part of the second season of development, however, a marked shift was observed to the conversion of carbohydrate reserves to structural components, especially cellulose.

As the cone reaches maturity, it is reasonable to assume that reserve materials of cones will decrease as a result of mobilization of these materials by maturing seeds. Apparently, starch grains in the scales of Pseudotsuga menziesii cones become concentrated close to the seeds in each scale. Gradually levels of starch are reduced in the scales but begin to increase in the embryo indicating a movement of carbohydrate into developing seeds (Ching and Ching, 1962).

2.1.5 It is clear that cone development features a pattern of growth involving various changes which proceed at different rates than the changes within the developing seed. However, there is no doubt that cone maturity ultimately provides some guide to seed maturity. Consequently, the recognition of ripening characteristics of cones is an essential part of seed production.

2.2 Seed Development

Seed development in gymnosperms differs from that in angiosperms in several aspects. One key difference is the absence of true endosperm in gymnosperm seeds. The tissue in which the embryo is embedded, and which functions as endosperm, is the female gametophyte or embryo sac. In angiosperms, the endosperm is triploid because it is formed by the fusion of two nuclei in the embryo sac plus a sperm nuclei.

From the time of strobilus initiation until seed maturation, seeds undergo various changes. The following review attempts to describe seed development in three major areas, i.e. cytological, physiological and biochemical development.

2.2.1 Cytological development

Singh and Johri (1972) described a coniferous seed as originating from an ovule which comprises a nucellus and an integument surrounding the nucellus. The integument is extended to form a micropylar canal through which pollen grains are transported

into the micropyle during pollination. It is suggested that pollen floats through the liquid pollination drop (Lill, 1974). When pollen grains reach the nucellus tip the micropylar arms elongate across their axis and swollen tissues close the micropylar canal.

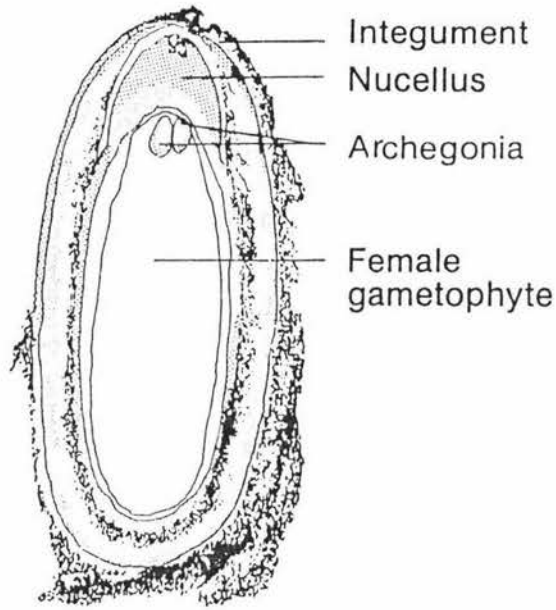
At the time of pollination the male gametophyte has not completed its development, and megasporogenesis has just begun in the ovules (Foster and Gifford, 1959). Following pollination the male gametophyte develops slowly during which time the pollen tube emerges from the spore and the generative cell divides to form a stalk and a body cell. Subsequently, the single functional megaspore within the nucellus of the ovule enlarges rapidly, becoming vacuolate, and the cytoplasm and nucleus move to the periphery of the cell. A series of free nuclear divisions also takes place prior to winter dormancy. In P. radiata, the growth of the functional megaspore occurs between six and ten weeks after pollination (Lill, 1976). Foster and Gifford (1959), noted that at the end of the first period of development (during autumn and winter) the megaspore of Pinus contains up to 32 free nuclei in pariental cytoplasm; 256 nuclei was observed in the megaspore of P. radiata (Lill, 1976). This structure can now be described as the megagametophyte.

In the following spring there is a very rapid formation of free nuclei within the megagametophyte, and up to 2000 free nuclei may be produced depending on the size and shape of the megaspore (Ferguson, 1904; Foster and Gifford, 1959; Lill, 1976). In the latter part of the spring, cell wall formation begins and a massive central body develops in the eroded nucellus. Lill (1976) pointed out that this formation occurs at different times in different ovules of one cone or even of one ovuliverous scale.

Archegonia may develop before the female gametophyte is completely cellular (Ferguson, 1904; Thomas, 1951), although early formation of archegonia is not seen in P. radiata (Lill, 1976). The initial which is a surface cell differentiated at the micropylar end of the gametophyte divides into a small outer primary neck cell and a larger inner central cell. The primary

neck cell forms four neck cells by means of two successive anticlinal divisions. Apparently, the number of neck cells varies among species and even in the same species. The central cell enlarges with the nucellus remaining close to the neck of the archegonium. Ultimately, this divides to form a small ventral canal cell which eventually degenerates and the nucleus of the egg cell enlarges and descends to a central position in the egg. When mature, the egg of the archegonium is jacketed by a distinct layer of gametophyte cells (Foster and Gifford, 1959). The number of archegonia in pines ranges from one to seven, but in P. radiata the range is from one to four with clonal differences in the frequency of ovules with more than two archegonia (Lill, 1976). A median longisection of an ovule is shown in Figure 2.2.

Fig. 2 .2 Median longisection of ovule showing female gametophyte with two archegonia; **Pinus** spp. (from Foster and Gifford, 1959)



As the female gametophyte resumes its development, the stalk and spermatogenous cell of the male gametophyte move down toward the lower end of the pollen tube. Two sperm nuclei (male gametes) are produced by the final nuclei divisions about a week before fertilization. One of these nuclei is larger than the other and will become the effective sperm nuclei. The pollen tubes of several pollen grains grow actively through the nucellus, but only two to three will reach the female gametophyte (Ferguson, 1904; - Foster and Gifford, 1959). The mechanism of fertilization is marked by one pollen tube discharging the two sperm nuclei, the stalk nucleus and the tube nucleus into the egg cytoplasm. The larger of the nuclei fuses with the egg nucleus; other nuclei eventually degenerate (Foster and Gifford, 1959).

In the post-fertilization phase, the fertilized egg cell undergoes several changes (Fig. 2.3). Following fertilization the nucleus of the fertilized egg cell yields two nuclei, each of which promptly divides forming a total of four free nuclei located near the middle of the cytoplasm of the egg. The four nuclei move to the lower end of the archegonium and the third nuclear division occurs which is accompanied by wall formation. In the early stages of embryo development, the embryo consists of a lower tier of four cells, completely bounded by walls and an upper tier without walls adjacent to cytoplasm of the egg. The next division usually occurs in the cells of the upper tier and is followed by a similar transverse division in the lower tier. The proembryo, now consists of sixteen cells arranged in four superposed tiers. In the later development, some of these cells disintegrate and abort; the others which are apical tiers, suspensor tiers and rosette tiers develop producing polyembryonic cells (Foster and Gifford, 1959).

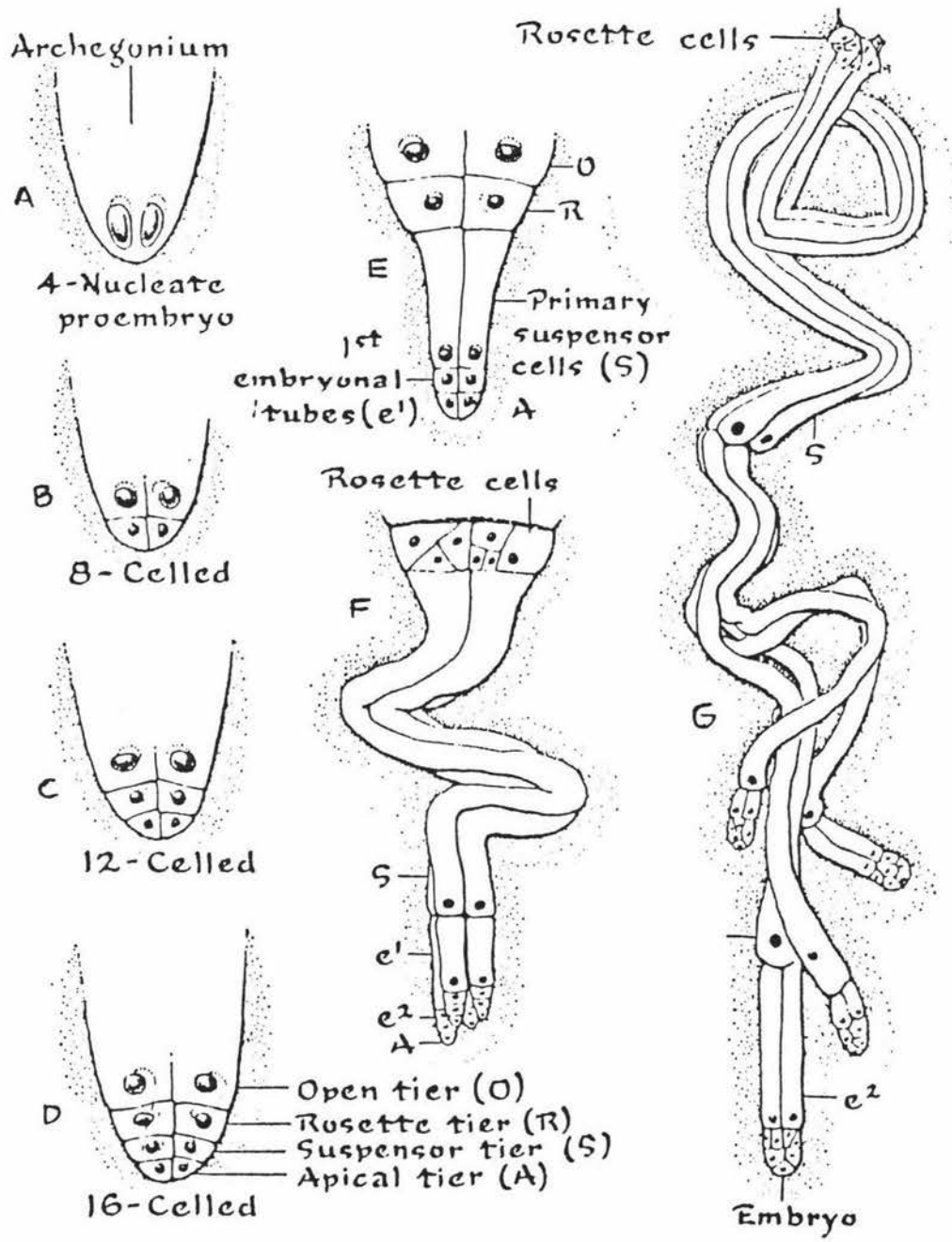


Fig. 2.3 Early embryogeny in Pinus. A, free nuclear phase in embryogeny with four nuclei; B - D, development of the four superposed cell tiers of the proembryo; E, elongation of primary suspensor cells and formation of first embryonal tubes (e^1); F, the ultimate separation of the lower cell tiers into four vertical series of cells; G, series of four competing embryos formed by cleavage. (from Foster and Gifford, 1959).

One of the important aspects of polyembryony in Pinus, as described by Foster and Gifford (1959), is the result of the ultimate separation of the lower cell tiers (suspensor and apical tiers) of the proembryo into four fillamentous embryos, each will give rise to a separate embryo. This process is termed as cleavage embryony. Another type of polyembryony is rosette polyembryony where embryos arise from the rosette cells behind the primary suspensor cells; this also produces four embryos. Thus each proembryo is theoretically capable of forming eight embryos. These embryo systems grow in competition for variable periods of time, but only a single embryo among them normally reaches a fully developed condition in the ripe seed. The surviving embryo which is normally formed from cleavage polyembryony continues to develop and becomes the differentiated embryo of the pine seed. When the primary proembryo is developing, the middle integument cells at the micropylar end of the seed develop thick walls.

The last stage in seed development is the growth and the differentiation of the successful embryo. The fully developed embryo of Pinus consists of a whorl of cotyledons (the number of which in some species is proportional to the size or the bulk of the embryo apex), a short hypocotyl and a primary root. The embryo is embedded in the massive female gametophyte (Foster and Gifford, 1959). As the seed develops toward maturity, the embryo and the female gametophyte (the endosperm) grow to occupy the entire cavity. This implies that the cavity develops for the embryo and the gametophyte to fill, rather than being formed by embryo and gametophyte growth (Rediske, 1961; Edwards, 1980). These organs are protected by a thin papery inner membrane and a hard outer seed coat.

2.2.2 Physiological development

Physiological changes are, of course, concomitant processes of the anatomical and morphological changes taking place during cone and seed development. They normally depend on and are regulated by growth substances produced by the developing ovules (Krugman et

al., 1974). Processes include variations in seed moisture content, increase in seed dry weight, colour changes in the seed coat, changes in respiratory activity, and the onset of and increase in seed germination capacity.

Water plays a dynamic role in seed development. Developing ovules undergo an initial increase in size shortly after pollination, followed by a sharp increase after fertilization and the initiation of the embryo during which time cell division, enlargement, and differentiation proceed to form the visible structure of the seed. Pollock and Roos (1972) indicated that during this period the size of the seed increases but its moisture content remains constant and high. Deposition of reserve materials continues after morphological development so that dry weight increases more rapidly than fresh weight; thus the percentage of water in the seed declines. During later stages of seed development there is a rapid moisture loss. The pattern of moisture reduction, however, is not merely determined by atmospheric conditions. A number of studies emphasized that the loss of water during seed maturation is more an inherent phase of seed development than implied by the passive concept of seed drying (McIlrath et al., 1963; Dodds and Pelton, 1967; Pollock and Ross, 1972). For most seeds, desiccation or maturation drying is the terminal phase of seed development. It has been suggested that this loss of water from seed tissues plays some role in the transition from a developmental programme to a programme oriented toward germination and growth (Dasgupta and Bewley, 1982; Kermode et al., 1985a). For example, Kermode and Bewley (1985b) suggested that drying in Ricinus communis L. seeds appeared to be a pre-requisite for the induction of hydrolytic enzymes essential to the post-germinative (growth) phase of seedling development.

A relationship between moisture loss and germinability in Pinus seeds is not known. Nevertheless, the pattern of reduction of seed moisture and the importance of maturation drying are similar to most other seeds (F.T. Bonner, 1986 - pers. comm.). Krugman et al., (1974) showed that shortly after fertilization the

female gametophyte and embryo of P. lambertiana contained as much as 700 percent moisture based on oven-dry weight. At the time of natural dispersal the moisture content was 30 and 60 percent respectively. The level of moisture content at maturity varies depending upon species. Generally, Pinus seeds can tolerate extreme desiccation without losing their viability.

A major feature of seed development is accumulation of nutrient reserves which is expressed in the steady increase of seed dry weight. This process is particularly evident in the female gametophyte, which serves the dual function of bearing gametes and nourishing the embryo. Seed dry weight reaches its maximum when nutrient reserves cease flowing into the seed, at which time the seed is regarded as physiologically mature (Shawn and Loomis, 1950; Pollock and Roos, 1972). Little is known of the dry weight status during development of conifer seeds, and information on seed dry weight has so far proved to be of little practical value in cone harvesting. However, data are available on the chronological changes in cone dry weight of Pseudotsuga menziesii (Ching and Ching, 1962). Dry weight of the cones increased steadily and slowly, and the maximum was not reached until six weeks before natural seed dispersal. In P. radiata cone dry weight progressively increases from the beginning of pollination and reaches a maximum six months before natural ripening (Sweet and Bollmann, 1971). It is believed that the trend in seed dry weight is similar, although the time to reach the peak may be different.

Another important indicator of metabolic activity during seed development is the rate of respiration. A need for respiration is obvious in tissues which are performing net metabolic work (Opik, 1980). Seeds of woody plants show wide variations in respiration during their development. Young developing seeds have very high rates of respiration similar to the cone scales, but as the seeds mature the rate decreases to a minimum in fully mature seeds. Little is known of the respiratory rate of Pinus seeds. However, Ching and Fang (1963) reported that the O_2 uptake of Pseudotsuga

menziesii seeds at 75 days after pollination was 2.67 μl O_2 mg dry weight $^{-1}$ hour $^{-1}$. This O_2 uptake steadily decreased and at 105 days after pollination it reached 0.76 μl O_2 mg dry weight $^{-1}$ hour $^{-1}$. According to Opik (1980) the high respiratory rate in developing seeds is associated with an increase in protoplasmic mass (cell number and size increasing), including increasing amounts of respiratory enzymes and organelles.

Much has been written about respiratory processes in plants where the seed is an important item for food. Duffus and Rosie (1977) found that steep increases in the activities of some glycolytic and Pentrose Phosphate Pathway enzymes in the endosperm of ripening barley (Hordeum vulgare) coincide with maximum respiration rate. Opik (1980) also reported that in pea cotyledons, peaks in the activities of some mitochondrial enzymes coincide with the high O_2 uptake. Based on the need for respiration for metabolic work in developing seeds, it seems reasonable to assume that similar events could be observed in Pinus seeds, although it should be noted that the interval between pollination and fertilization is relatively long, and rapid growth takes place just before and after fertilization.

2.2.3 Metabolic and hormonal development

Developing seeds are a rich source of natural growth-regulating substances which are essential for the development of the seed and the growth of surrounding tissue (Kramer and Kozlowski, 1979). Hormones which have been most often isolated and identified in tree seeds are various auxins, gibberellins, and cytokinins (Nitsch, 1965). The hormone levels fluctuate as the seed develops. Krugman (1965) found that in pine the principal hormone in the ovule prior to fertilization is auxin. Following fertilization auxin initially increased in concentration and then decreased to a low level in the mature seed. In another study, Krugman (1967) found that a gibberellin-like substance first

appeared after fertilization and increased in concentration only to decrease at seed maturity. Increase in gibberellin concentration is associated with active embryo development in P. lambertiana Dougl. (Krugman, 1967) and with the endosperm development in Ginkgo biloba L. (Banerjee, 1968).

Concurrent with anatomical development of the embryo, numerous food-storage activities are taking place. Soluble organic compounds such as simple sugars, fatty acids, and amino acids are gradually converted into more complex carbohydrates, proteins, oils and fats (Konar, 1958a,b). In an oily seed such as noble-fir (Abies procera Rehd.) a reduction in reducing sugars, starch, and soluble nitrogen and an increase in crude-fat occurs during maturation (Krugman et al., 1974). In pines, lipid reserves increase rapidly in the developing seed, first appearing in the gametophyte, then in the embryo (Konar, 1958a,b). Nitrogenous reserve compounds also increase rapidly. Katsuta (1961) found that in Japanese black pine (P. thunbergiana Parl.), an initial accumulation of glutelin is followed by an accumulation of albumin. In another study, Katsuta (1959) found that albumin is the major protein of the fully mature embryo, and glutelin is the major protein in the endosperm in Japanese red pine (P. densiflora) and black pine (P. thunbergii).

2.3 Parameters of Maturity

2.3.1 Introduction

The decision when to harvest seeds or cones is usually a compromise between several factors such as the state of maturity, past and expected weather, and the availability of man-power and machinery. The best time for cone harvesting is when most of the cones are fully mature. In some species this may be too late because the final stage of seed ripeness is very short and ripening is then followed by seed dispersal (non-serotinous cone). However, when cones are collected before the seeds are fully mature, it will result in uncertain seed quality. Because of their importance in

successful cone and seed collection, maturity indices have been studied intensively. Maturity indices can be classified into three main categories, physical parameters, physiological and morphological parameters, and biochemical parameters.

For some species criteria established for cone maturity are not applicable as indicators of seed maturity. This is partly because the morphological and physiological maturation of cones and seeds are completed at different times. Rediske and Nicholson (1965) observed that in noble-fir seed even in late collections, when seeds had reached maximum dry weight and the embryo had fully elongated, the seeds had low germination. They suggested that an after-ripening period was required; in this case the seeds should be left in cones for some time before being extracted. Consequently, it is important to examine closely the relationship between maturity of cones and the ripeness of seed.

2.3.2 Physical indices of maturity

In general, the physical parameters of maturity for cones and (or) seeds which are commonly used are the specific gravity and moisture content of cones (Schubert, 1956; Cram and Warden, 1957; Ching and Ching, 1962; Krugman et al., 1974), cone firmness, colour and brittleness of seeds (Crosley, 1953), and cone colour (Gordon et al., 1970; Wilcox and Firth, 1980; Arisman, 1984).

While specific gravity and moisture content are two interrelated physical parameters which can be objectively measured, cone colour is not quite so definable a variable. Nevertheless, cone colour has been employed as a useful indicator for several species, both in temperate and tropical regions. The fact that it is easy to assess and that it gives generally satisfactory results has probably led to its being widely used. The general pattern of colour change in many pine species is the change from green to various shades of yellow and brown as maturity advances. Arisman

(1984) recommended that for P. merkusii only brown and green/brown cones be collected, as these cones are superior in many ways compared to green cones. The use of cone colour to judge maturation has also been reported by Fenton and Sucoff (1965) for P. virginiana. For this species the best time of cone collection is when the cone colour has changed from green to purple.

Despite the fact of its applicability for some species, colour change has not always been a satisfactory index. In P. sylvestris, cone colour varies considerably among individual trees and it is not a reliable measure of maturity (Lindquist, 1962; Cram and Lindquist, 1979). Maki (1940) and Fowells (1949) also discounted cone colour as a useful index for P. lambertiana and P. ponderosa respectively. Apart from cone colour variation among individual trees, it is also possible that cone colour can be distorted by external factors unrelated to maturity such as insect damage (Edwards, 1980).

Specific gravity is a more objective index of seed maturity and is based on the water loss during maturation. Once the relationship between seed maturity and specific gravity of freshly harvested cones has been established, then the easiest way to determine whether the cones have reached a standard specific gravity range is to carry a container of a suitable liquids of known specific gravity to the collection site. Edwards (1980) suggested that for a number of pine species maturity is reached when cone specific gravity falls below 0.9 and moisture content is below 50%. In practice, the standard cone specific gravity varies depending on species. A specific gravity of 0.89 was suggested by Wakeley (1954) for southern pines (P. echinata, P. elliottii, P. palustris and P. taeda). For P. ponderosa and P. lambertiana Maki (1940) and Fowells (1949) suggested a specific gravity of 0.84 and 0.80 respectively.

Although the physical and physiological condition of the cone proves to be a reliable basis for judging seed maturity, a necessary precaution needs to be taken when using ripeness criteria. Rietveld (1978) pointed out that the specific gravity -

cone maturity relationship holds true for cones in a population of trees, not for the population of cones in a single tree. Because cones on different trees may resist moisture loss, or dry prematurely due to differences in thickness of cuticle, exposure, or surface area, the relationship in individual trees may not follow exactly the same pattern as the mean of the population. Thus early collections of cones from 'mature' individual trees which pass the test will yield lower proportion of mature seeds than indicated by their cone specific gravity.

2.3.3 Physiological and morphological indices of maturity

Physiological and morphological indices of maturity are more elaborate and difficult to obtain, and the analysis requires skills and equipment which may not be readily available. They also have little value in practice as compared to the physical indices. Nevertheless, these parameters have a high practical value for angiosperm seeds, and some studies have shown that these indices prove reliable for gymnosperms.

Morphologically, a mature seed has an embryo which fully occupies the cavity, a firm megagametophyte with no separation from the testa, and a fully developed seed coat (Edwards, 1980). In some species, embryo development has been employed as a maturity index for the initiation of cone collection. Finnis (1950) concluded that collection of Pseudotsuga menziesii cones could be commenced when the ratio embryo length of the seeds to the embryo cavity had reached 70%. Later, Ching and Ching (1962) recommended delaying the collections until the embryo had reached a relative length of 90% or more, since seedling vigour was then at a maximum.

In Japan, Katsuta (1975) claimed that when the embryos of P. thunbergii and P. densiflora reached a relative length of 60%, they appeared to possess a latent capacity for germination that was convertable into actual germination by stratification. Edwards (1980) suggested that a relative embryo length of 75% is widely accepted in British Columbia as the point at which to begin cone

collections in most species. Although it may sound relatively impracticable, measurement of embryo development can be readily accomplished by field personnel equipped with a 10 x magnifying glass, a sharp knife and minimum training (Dobbs et al., 1976). If facilities are available it can also be determined on X-ray film.

In contrast, Bevege (1965) found that even when the embryo of P. elliottii seed has fully filled the cavity (morphologically mature) the seed germinated unsatisfactorily. Thus, although the embryo is morphologically mature it may not have reached physiological maturity, that is, high germinability.

There is a general consensus that physiological maturity does not necessarily refer to germinability. Some researchers have described a state of physiological maturity that is complete before embryo development is completed. In this case, physiological maturity has been described as the completion of accumulation of storage reserves within the seed as marked by achievement of maximum dry weight (Shawn and Loomis, 1950; Harrington, 1972). For a similar process, Anderson (1955) used a term morphological maturity. In some crop species such as carrot, many embryos are immature at physiological maturity and continue to develop after the seed is removed from the plant (Harrington, 1972). In maturation studies of bromegrass, Grabe (1956) showed that maximum germination took place when dry weight was at maximum.

There is very little literature on the relationship between the points of maximum dry weight and maximum germinability in pine seeds. Cram and Worden (1957) noted that maximum seed weight was reached concurrently with maximum germination and seed yield per cone, just before natural seed fall, in Picea glauca. However, in many species the relationship does not hold true because of dormancy problems, or after-ripening requirements.

2.3.4 Biochemical indices of maturity

The assessment of maturity judged by biochemical changes is more elaborate and requires special equipment and well-trained

personnel. It is more complicated and time-consuming to perform, and therefore, it is less practicable. The principle is based on the fact that as the seed matures a number of measurable chemical changes take place. By correlating the relative amounts of selected biochemical constituents with seed germination, a biochemical index of ripeness can be developed.

Rediske (1961) stressed that the most important biochemical process is the change from mobile to storage forms of food within the embryo and megagametophyte. He conducted an analysis on seven constituents of Pseudotsuga menziesii seeds, i.e. crude fat, iodine number, reducing sugars, non-reducing sugars, starch, soluble nitrogen and protein nitrogen. A high correlation was found between reducing sugar content and maturity as determined by germination. Reducing sugar content decreased significantly as the seed matured, while the other constituents changed more haphazardly, and therefore, are not suitable as indices of maturity. It was then concluded that seeds with a reducing sugar level of 22 mg/g seed were immature, and maturity was achieved when the level had fallen to 13 mg/g seed.

The high correlation between the level of reducing sugars and seed maturity has also been reported by Barnett (1976) for Southern pines, namely P. taeda, P. palustris, and P. elliottii. The levels decreased to about 0.20% at maturity, when the specific gravity dropped to 0.88, the normal index of maturity. The other chemical constituents, i.e. lipids, insoluble nitrogen and total sugars showed no significant pattern which could be used as maturity indices. The levels of lipids and total sugars were relatively constant for all three species as maturation progressed. The levels of insoluble nitrogen remained stable in P. taeda and P. palustris, but increased in P. elliottii. Rediske and Nicholson (1965) examined similar chemical constituents of Abies procera seeds as in Rediske's earlier study. Crude fat was found to be the main storage form and this constituent increased rapidly and

sufficiently uniformly to be used as a maturity indicator. It was concluded that at a crude fat level of 22 mg/g in the seeds, A. procera cones were mature enough to collect, even though artificial ripening was necessary to achieve maximum seed quality.

Other biochemical changes that have been used as indices of maturity are the changes of component levels in the cone scales. Dickmann and Kozlowski (1969b) examined the changes in reserve materials, lignin, hemicellulose, and cellulose of first year conelets and second year cones of P. resinosa. During the first eleven months after cone emergence, reserve materials (primarily carbohydrates) constituted approximately half of the cone dry weight. Rapid cone growth occurred from mid-June to early August and was accompanied by transformation of reserve materials into cellulose and, to a lesser extent, into hemicellulose and lignin. In the first year, the concentration of extractable reserves remained fairly constant, fluctuating between 44% and 57%. In the second year, however, the concentration rose rapidly until mid-June, and then decreased rapidly until late September, and was accompanied by a corresponding rise in cellulose levels.

2.3.5 Many possibilities for obtaining maximum use of the available cone crop thus depend upon a knowledge of the ripening process so that the level of seed maturity can be determined. The various maturity indices that have been discussed offer good prospects for the considerable improvement of seed production and seed quality. As Rediske (1961) pointed out, knowledge of when seed is mature would make possible the maximum extension of the season for cone collection, the processing of only mature seed, and the storage of seed under optimum conditions when mature.

2.4 Artificial Ripening of Conifer Seeds

Basically, artificial ripening is a way to improve germinability of prematurely harvested seeds by keeping the cones under appropriate conditions for a period of time before extraction. This idea was particularly triggered by the need to

employ a practical means of extending the period of seed collection in order to maximize the use of the existing cones. Silen (1958) suggested three benefits of adopting artificial ripening in seed-production schemes, viz. 1) more flexibility in cone-collecting operations, 2) the collection period can be extended and 3) allowing salvage of immature cones from logging operations. Sometimes, it is considered to have practical and economical value for seedling production. Wilcox and Firth (1980) reported that artificial ripening of P. radiata seeds could avoid the usual one-year delay in establishing a progeny test from naturally ripened seed which are usually ripe for harvesting in November or December. Artificially ripened seeds which were harvested in July were ready to be extracted in time for normal sowing in October.

Artificial ripening of seeds has been proved to be feasible in most of the conifers in which it has been investigated. Silen (1958) recommended that storage in damp peat moss was the best means of storage for Pseudotsuga menziesii cones collected up to five weeks prior to natural seed dispersal. A similar recommendation was made for P. sylvestris in Hungary (Matyas, 1976). Barnett (1976) had found that Southern pines, namely P. taeda, P. palustris and P. elliottii can be artificially ripened. Traditionally, Southern pine cone collections begin within the two - three week period after specific gravity drops below 0.9, the normal index of maturity (Wakeley, 1954). Because of the lack of man power for collecting cones, this period became too short to allow collection of enough cones to provide an adequate seed supply. By extending the collection period by two to three weeks, the number of seeds collected might easily be doubled.

In Southern Queensland, the study of artificial ripening for P. elliottii var. elliottii Engl. was undertaken by Bevege (1965) in an attempt to avoid cone loss due to strong cyclonic winds which occur during seed maturation period (January to March). Good germinable seeds were obtained from collections made in late January after storing the cones for a month. Fielding (1964) and Wilcox and Firth (1980) also found that artificially ripened seeds

of P. radiata produced satisfactory germination as compared with naturally ripened seeds, though these studies were incomplete in that they did not ascertain the germinability of seeds from prematurely collected cones before artificial ripening.

From the information gathered to date, the storage conditions for successful artificial ripening are still ill-defined. There are variations among species and even within species. This is due, at least in part, to a lack of understanding of the maturation process in conifer seeds. Silen (1958) had found that dry storage did not permit any ripening of Pseudotsuga menziesii seeds, indicating that moisture is required to promote requisite metabolic changes. In contrast Franklin (1965) and Pfister (1966) found that storage in peat moss was harmful to noble fir (Abies procera) and grand fir (Abies grandis) cones respectively. This was attributed to anaerobic conditions caused by excessive moisture and development of injurious fungi. However, Pfister (1966) observed that seed dry weight increased suggesting that maturation probably progressed satisfactorily during the early stage of storage.

In other species, dry storage conditions seem to be suitable. Wilcox and Firth (1980) stored green cones of P. radiata in paper bags and placed them in an unheated room with temperatures fluctuating between 20-24°C. Working on the same species, Fielding (1964) stored the cones at a temperature of approximately 10°C for a period of time before transferred to a room with temperatures of 13-27°C. However, no indications were given concerning the interaction between low and high temperatures. Nevertheless, both studies produced similar results. Cone storage on a shaded rack at temperatures between 66°F and 83°F has been reported as the most effective condition for seed maturation of P. elliottii var. elliottii Engl. (Bevege, 1965), while Krugman (1966) found that storage in sealed plastic bags at 10°C is suitable for P. lambertiana seeds. Cobb et al. (1984) reported that P. taeda cones artificially ripened in muslin sacks and air-drying in the shade of orchard trees produced high quality seeds.

Although there seem to be inconsistencies in results determining the most effective conditions for artificial ripening, the studies cited suggest that temperature, relative humidity and time are important to allow physiological processes to take place within the seeds for further maturation. Matyas (1976) emphasized that prematurely collected P. sylvestris cones should not be stored for more than one month as the germination will increase only slightly, and dropped significantly after three months storage. A similar result has been reported for P. palustris whereby storage for longer than eight weeks reduced germinability (Barnett, 1976). Wilcox and Firth (1980) obtained high germination radiata pine seeds after ten weeks storage, but, it is difficult to confirm whether ten weeks is the optimum time because there was no variation in storage time and no information on initial germinability was available. McLemore (1975) demonstrated that both seed yields and germinability increased for P. elliottii and P. taeda by storing cones for up to five weeks.

Equally important is the stage of seed development when the cones are harvested. Ideally, the cone should be detached from the tree when the seed is no longer dependent on the mother plant for further maturation. This is probably the point where physiological maturity as defined by Harrington (1972) takes place. This stage of development may vary depending on environmental factors, clone and species. For instance, Krugman (1966) observed that when cones of P. lambertiana had reached a specific gravity between 0.85 and 0.87, they contained seeds advanced enough in their maturation to be artificially ripened in the cone. In another study, Fowells (1949) also recommended that P. lambertiana cones having a specific gravity of 0.80 or less be collected to obtain mature seeds. Cones of P. elliottii, P. echinata, P. palustris and P. taeda can be artificially ripened only when the cone specific gravity had fallen below 1.0 (Barnett and McLemore, 1970).

In other work on conifer species, the stage of maturity has been evaluated in terms of calendar date. In Australia, Bevege (1965) found that if cones of P. elliottii were collected in late

January, storing the cones in shade for a month will result in high germination. For a mid-February collection, two weeks of similar storage should be sufficient. Zasada (1973) successfully ripened Picea glauca seeds from cones collected in Alaska four weeks ahead of natural seed fall. McLemore (1975) demonstrated that for P. elliottii and P. taeda grown in Louisiana, cones collected two to three weeks before they ripened on the tree yielded fairly good seeds after the cones had been stored.

The optimum time for cone collection of P. radiata is not yet known. Sweet and Bollmann (1971) found that cone maximum dry weight was reached in June, some five to six months before natural ripening, but this does not necessarily imply that cone and seed reach physiological maturity at the same time.

3. MATERIALS AND METHODS

3.1 Introduction

Studies on the artificial ripening and seed development of P. radiata were carried out at the Seed Technology Centre, commencing in July 1985 till September 1986. During these studies various aspects of seed production, seed development and seed quality testing were investigated. Outlines of the experiments are presented in the form of flow-charts (Figs 3.1 and 3.2) and details described in the following sections:

3.2 Cone Procurement

3.2.1 Collections in 1985 (Preliminary study)

The P. radiata cones for the preliminary study were collected from two sites: Long Mile Archive plantations in the vicinity of the Forest Research Institute (lat. 38°07'S, long. 176°19'E alt. 300 m) where controlled pollination (CP) had been carried out, and Kaingaroa Seed Orchard, a commercial scale open-pollinated (OP) seed orchard, 40 km south-east of Rotorua (lat. 38°24'S, long. 176°34'E alt. 444 m). Premature harvesting was done in July.

Twenty ramets were randomly selected at each site from ten top-ranked clones of the '268' series to provide sufficient cones. From each clone, five cones, evenly distributed over the crown, were taken from each ramet, packed separately in plastic bags and transported to Palmerston North.

One of the major constraints in designing this experiment was the limited availability of CP cones which restricted the intention to conduct a large-scale study investigating clonal variation. Accordingly, the experiment was designed to accommodate this constraint without sacrificing the validity of the design. This was achieved partly by taking ten top-ranked clones to represent

Figure 3.1: Flow chart of the experimental procedures of the preliminary study

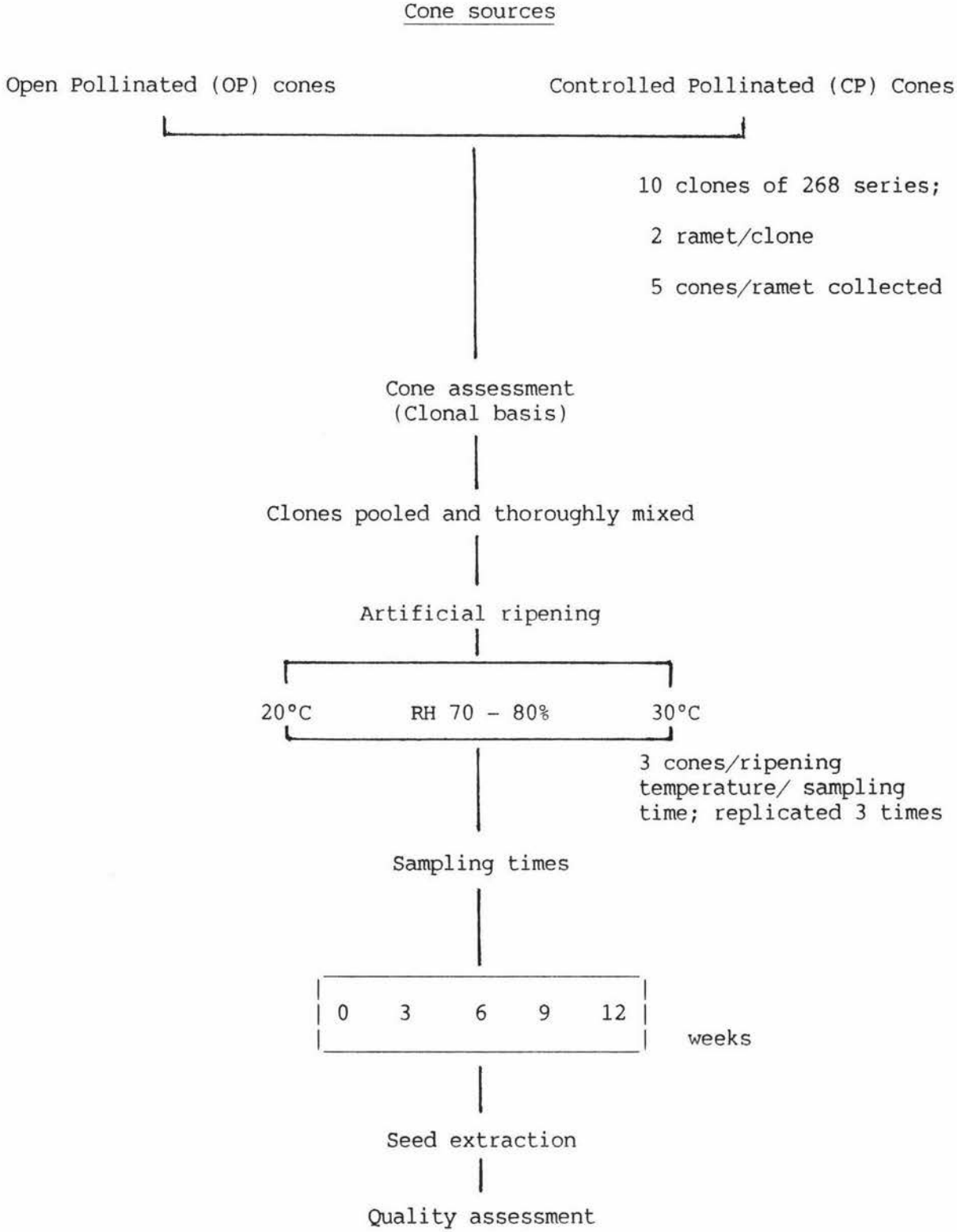
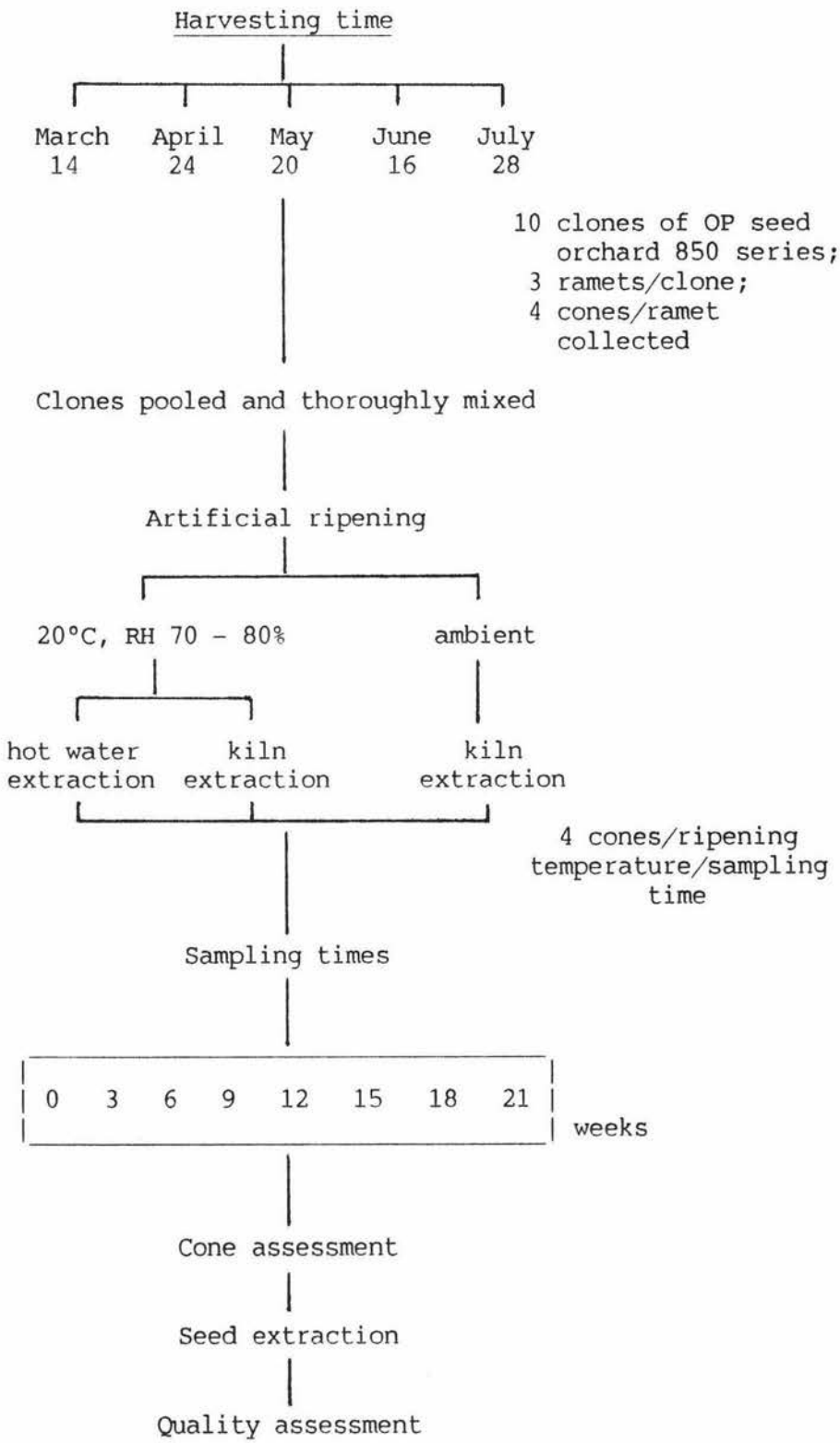


Figure 3.2: Flow chart of the experimental procedures of the detailed study



the population. Based on the Forest Research Institute's experience, ten clones were considered to be the minimum number of clones if a representative sample of the clonal population is to be achieved. Seed quality assessments were done on a bulked sample containing three-four cones for each treatment replicated three times. On the other hand, physical characteristics of the cones, such as size, specific gravity and yield, were done on a clonal basis.

3.2.2 Collections in 1986 (Detailed study)

In this study, P. radiata cones were collected from Kaingaroa Seed Orchard. To provide a range of seed maturity, collections were made at approximately monthly intervals, commencing in March and finishing in July, 1986. An additional collection was made in September 1986. Prior to these collections, in July, September and November 1985 cone collections were carried out; thus providing a full-one year crop cycle.

Ten of the potentially best clones were chosen from the '850' series clonal population planted in the orchard. The clones were selected on the basis of their performance in the progeny test conducted by the Forest Research Institute (A. Firth, 1985- pers. comm.). From each clone, three ramets were randomly selected. Although the ramets used in this study were selected randomly, this initial selection was modified as necessary to assure that the ramets had enough cones to be used throughout the study. Four cones, evenly distributed over the crown, were hand-picked from each ramet. Care was taken, as far as possible, to ensure that the collected cones were from the same pollination year. The selected ramets were marked to prevent cones being taken from these ramets during commercial operations. In total, 120 cones were collected each time, packed separately according to their ramets and clones, transported to Palmerston North and stored at 5°C for up to three days before sorting.

Because of the limited supply of cones, inevitably the sample had to be mixed; therefore ignoring clonal variations. Thus the cones were split into three groups, one group for each storage treatment. One ramet, from each of the ten clones was included in each group. For each storage time four cones were then randomly selected from each of the three bulked samples using a random number table. Each cone was treated as individual replicate; therefore whenever materials were available every quality test was done on four replicates.

3.3 Experimental Strategy

The starting point of this study was the brief report by Wilcox and Firth (1980) that prematurely collected seeds will undergo further maturation in the cone if the cone is stored under conditions suitable for the seeds to continue to ripen. Their storage conditions, broadly described as warm, humid and with good ventilation were adapted for this study.

3.3.1 The preliminary study

Wilcox and Firth (1980) compared the performance of seeds from July harvested controlled pollinated cones which had been artificially ripened for ten weeks at 20-24°C to those collected the January before from the previous year's mature crop. The present study was designed to investigate whether or not these ripening conditions were optimal for a July harvest and also to see if the technique could be applied equally well to an OP cone.

Cones from both OP and CP samples of the July 1985 harvest were artificially ripened at two constant storage temperatures, 20°C and 30°C, for 0, 3, 6, 9 and 12 weeks. For both storage temperatures the relative humidity was 70-80%. For each ripening treatment, three replicates of three to four cones were used. To

allow free air circulation to occur the cones were placed in paper bags with the top of the bag remaining unsecured. Because the conditions were suitable for fungal growth, the cones stored at 30°C were dusted with Captan at a rate of 0.25 g per cone.

3.3.2 Detailed study of artificial ripening on cones at different stages of maturity

One of the key factors in successful artificial ripening is the stage of cone/seed maturity at the time when the cone was detached from the parent plant (Krugman, 1966; Edwards, 1980). It has been suggested that in the development of cones and seeds there seemed to be a point prior to which the seeds cease development if the cones are detached from the parent plant, regardless of the post-harvest treatment to the seed. Despite the importance of this question, very little work has been done to study the developmental stages of conifer seeds. The present study was designed to investigate the response of seed at different stages of maturity to artificial ripening and to provide information on the level of seed maturity according to calendar date and cone maturity. Furthermore, the study also attempted to investigate some aspects of the physiological development of the seed both during natural as well as artificial ripening.

Based on the outcome of the preliminary study, an environment with a constant air temperature of 20°C and 70-80% relative humidity was used as the standard storage regime and storage at ambient temperature was used as comparison (temperatures ranged between 12°C and 23°C). The cones from each harvesting time were artificially ripened at the above storage conditions for 0, 3, 6, 9, 12, 15, 18 and 21 weeks. Further, the cones stored at 20°C were subdivided into two lots to study the response of cones to different extraction techniques.

The physiological studies of seed development were carried out using the existing samples. This included the measurement of

respiratory rate and electrophoresis of protein extracted from seeds of different stages of maturity.

3.4 Cone Assessment

In the detailed study, various cone characteristics were assessed at the end of each storage period. These included cone specific gravity which was tested by the water displacement method (Schopmeyer, 1974), cone colour, cone scale opening (Arisman, 1984) and seed extractability after kilning (Bramlett, et al., 1976).

Each cone was assigned to one of five colour indices, classified by the British Colour Council (1957): 1. B.C.C. 174 (Moss green); 2. B.C.C. 233 (Tuscan Yellow); 3. B.C.C. 204 (Cinnamon); 4. B.C.C. 168 (Nutmeg); 5. B.C.C. 67 (Almond shell). The maturity index progresses from one to five. Cone scale opening was scored using a three-point system: score 1 - completely closed, score 2 - partially open, score 3 - completely open. Seed extractability was determined as the 'extraction efficiency' which is the percentage of extracted developed seeds in relation to the total yield of developed seeds (suggested procedures by Bramlett et al., 1976).

3.5 Seed Extraction

One of the major obstacles in this study was the difficulties in seed extraction. P. radiata is known to have a serotinous cone habit in which the outer edges of the cone scale are bonded together by resin and require exposure to high temperature for seed release (Edwards, 1981). Provided that the cone has relatively low moisture content, seed extraction can easily be done by oven-kilning. However, since many of the cones were harvested when they were still green and with a high moisture content, kiln extraction could not be successfully applied.

Through a series of preliminary tests, it was then decided that hot-water extraction was the only practicable way to extract the seed. This was achieved by dipping the cone in near-boiling water (temperature 80-82°C) for ten to sixty seconds, depending upon the dryness of the cone. Following this treatment, the scales could now be separated easily, and the seeds were dissected carefully by means of secateurs and scalpels.

The other extraction technique used was the standard oven-kilning method in which cones were placed in an air-forced oven at temperature 60-63°C for six hours.

In the preliminary study, a comparative test on the effect of those two extraction methods on seed quality was conducted. A sample of CP cones of '268' clonal series harvested in July 1985 and artificially ripened until November 1985 were used. For each extraction method, ten cones, one from each of the ten clones, were exposed to the treatment. The seeds extracted from the ten cones were then pooled and four replicates of fifty seeds were taken from the sample for germination testing. The results are presented on the following table (Table 3.1).

Table 3.1: ANOVA of germination percentage as regards to extraction methods.

Source of Variation	df	SS	MS	F
Treatments	1	278.5	278.5	16.9 **
Error	6	103.0	17.2	
Total	7	381.5		

	Treatments	
	Hot water	kilning
Means @	83 [a]	95 [b]

@ means followed by the same letter are not significantly different

** Significant at 1% level.

It is apparent that the hot water extraction method has somehow weakened seed germinability.

The extracted seeds were dewinged by hand, cleaned, counted and kept in labelled paper packets before being used for quality testing. To avoid bias due to abortion and other damage, only morphologically sound seeds were used throughout the study.

3.6 Seed Quality Assessment

3.6.1 Morphological assessment

In the seeds of many conifer species, the levels of embryo and megagametophyte development have been advocated as a ripeness index and they appear to be particularly useful in assessing the time at which cones may be collected for artificial ripening (Edwards, 1980). For such a morphological assessment, X-ray radiography has been a very useful tool for it is quick, non-destructive and relatively simple. Another simple, but less accurate, method is the flotation technique in very low density liquid which separates sound seeds from empty seeds.

In the preliminary study, seed separation based on morphological development, was achieved by a flotation technique in 95% ethyl alcohol, density 0.81 (Simak, 1973). Sinking seeds, which are sound, were removed immediately and dried. In the detailed study, X-ray radiography was used. The seeds were exposed to a very low dosage of X-ray (20.0 KVP at 2.8 mA for 60 seconds) using the Faxitron X-ray machine. This dosage was adopted after a series of preliminary tests to determine the safe dosage relative to a good quality image. The seeds were placed on a Polaroid film (Polaroid Type 55 pos/neg) and were arranged such that when removed from the film they remained on the same position. Aborted seeds, seeds with disintegrated megagametophytes and seeds with deformed embryos were discarded after being counted.

With the same dosage and film type, 25-50 seeds were exposed to X-ray, and the condition of embryos and megagametophytes of the seeds were examined.

3.6.2 Moisture content and dry weight

Determination of these parameters were carried out using the air-oven method with samples being held at 103°C for 17 hours as advocated by the ISTA Rules (1985). Moisture content of seeds was expressed as a percentage of fresh weight. Seed dry weight was calculated from the same sample.

It was observed that the seed coat of early-collected seeds (March, April and May) was physically immature. It was still yellowish brown and relatively soft. In order to know whether or not the seed coat was physiologically mature, dry weight of embryo and megagametophyte was determined using the same sample. The percentage of embryo and megagametophyte dry weight over seed dry weight was then calculated.

3.6.3 Seed germination and vigour

The standard germination test is perhaps the most familiar method for determining seed quality. It has been considered the standard by which most other seed assessment methods are evaluated because it is an actual measure of growth and, with minor modifications, the results can usually be related to the performance of seeds in the field (Leadem, 1981).

In this study, seed germinability was assessed in accordance with the ISTA Rules (1985). Three replicates of 50 seeds (in preliminary study) and four replicates of 25 seeds (in detailed study), were germinated after being dusted with the fungicide, Thiram at a rate of 0.5 mg per 25 seeds. The seeds were placed in

Tupperware plastic boxes on germination blotters over cut sheets of paper towelling moistened with 20 ml of distilled water. The boxes were kept in a controlled room at a constant 20°C with continuous low light. A seed is considered as a normal germinant when the radicle length has reached four times the length of the seed. The test was concluded after 28 days.

Seed dormancy is very common in conifer species. It is normally the result of the interaction of the hereditary properties of the plant, the environmental conditions prevailing during seed maturation and/or from conditions occuring during harvesting, extraction and storage (McLemore and Barnett, 1966; Krugman, 1966). In order to clarify whether prematurely harvested P. radiata seeds exhibit dormancy mechanism, a stratification treatment was applied. For each sample category, the seeds, prepared in the same manner as the normal test, were kept at 5°C for one week, and then transferred to 20°C for the 28 day germination test.

Germination (or assessment of normal seedlings) as defined in seed testing practice is considered inadequate as the measure of the potential physiological status of a seed because a seed is simply classified as either germinative or not. The nature of this study, however, required more sensitive criteria so that the effects of the treatments can be examined more thoroughly.

In the preliminary study, a germination test on an Inventum table was conducted in addition to the germination boxes. It appeared that the Inventum table created water stress to the developing seedlings. Accordingly, data of this test were used as a measure of seed vigour under stress conditions.

In addition to standard germination criteria, a modified vigour criterion was incorporated into the germination testing. Initial radicle emergence and cotyledon emergence (determined when the cotyledons became visible) were also counted at regular

intervals (every two days). From this data rates of germination could also be calculated as median germination times according to the formula;

$$T_{50} = t_i + \left[\frac{\frac{N+1}{2} - n_i}{(n_j - n_i)} \cdot (t_j - t_i) \right]$$

Where N is total number of germinants; n_i, n_j adjacent cumulative germination counts at times, t_i, t_j; t_i; and t_j are counts where $n_i < \frac{N+1}{2} < n_j$ (Coolbear, et al., 1980).

3.7 Physiological Studies on Seed Development

This study attempted to investigate the course of seed development from a physiological point of view. Such information can be very helpful to assist artificial ripening practice, and also to understand the process of seed maturation.

Two aspects of physiological development of seeds were investigated, viz: rate of respiration and changes in protein content. This study was conducted using the existing samples. Details of the procedures are described below:

3.7.1 Respiratory test

Energy is an essential prerequisite for growth and metabolic energy is derived from respiration. Thus, when overall metabolic activity is related to growth, a correlation between respiration and growth is to be expected. Many observations have found that differences in the respiration of germinating seeds represent the comparative levels of seed vigour (Woodstock, 1968, 1973; Woodstock and Grabe, 1967). Because the degree of seed maturity determines seed vigour it is pertinent to observe whether respiratory activity of developing P. radiata seed can be related to maturity as determined by germination.

Respiratory activity was measured for six replicates of ten seeds. At first, the seeds were disinfected with 1% NaOCl for fifteen minutes, washed with water and transferred into a solution of 0.01N HCl for ten minutes. Then the seeds were rinsed eight times with distilled water. The HCL solution was used to remove residual chlorine since this may affect subsequent metabolism (Abdul-Baki, 1974). The disinfected seeds were then imbibed on wetted Whatman No. 1 filter papers for a period of time (between 24 and 192 hours) before transferring to 15-ml reaction flasks. Oxygen uptake was measured by placing the seeds in 0.5 ml distilled water with the centre well of the flask filled with 0.2 ml 20% KOH and a filter paper wick (Umbreit et al., 1972). In half of the flasks KOH was omitted so that oxygen and carbon dioxide could be measured. The respiratory activity was measured in Gilson differential respirometer at a constant temperature of 20°C. After at least 30 minutes equilibration, the reading was taken and volume changes were recorded. The flasks were shaken at 130 oscillations per minute. It is important to note that respiratory measurements over a period of up to 192 hours could not be done on the same seed sample due to exhaustion of KOH in the respirometer-flasks. To avoid this problem the seeds were imbibed outside the flasks and measurements were taken at each designated time from different samples. Respiratory quotient (RQ) was calculated from the volume ratio of CO_2 evolved to O_2 consumed.

3.7.2 Electrophoresis

The principle behind this experiment is that growth of seed involves a variety of biochemical changes. They include changes in the proportion and composition of storage proteins. Katsuta (1961) observed in Japanese black pine that an initial accumulation of glutelin is followed by an accumulation of albumin. Protein identification by means of electrophoresis is capable of showing the changes.

In brief, the basic concept of electrophoresis can be described as follows. By definition, electrophoresis is used to describe the migration of a charged particle under the influence of an electric field, and is particularly effective for separating and examining the properties of molecules of high molecular weight such as proteins (Andrews, 1981). The migration of proteins is influenced by their molecular weight and the charge density.

The procedure used to extract the seed proteins and for running the electrophoresis was as follows. Five samples of seeds from cones collected and extracted in April, May, June, July and September and one sample of seeds from cones collected in April which had been artificially ripened for twenty one weeks were used in this study. Protein extraction was done using the Urea/SDS method (Quero, 1980). Five decoated seeds from each sample were ground in a pestle and mortar, and placed in a test tube before adding 10 ml of the extracting solution. After shaking with the vortex mixer for 30 seconds the solution was left overnight. When extraction was completed, it was centrifuged at 3000 g for ten minutes, and 5 ml of the supernatant was taken for precipitation with an equal volume of TCA.

Because protein identification was carried out by comparing band intensity, it is important that the amount of protein loaded onto the gel is constant. Consequently, the samples were assayed and total protein concentration determined so an appropriate volume of sample could be loaded. The assay was performed by the Bio-Rad Protein Assay in the following manner (Bio-Rad, 1982). 0.1 ml of each standard and samples was placed in a clean, dry test tube, 5.0 ml of the diluted Bio-Rad reagent was added and mixed using the vortex mixer. After waiting at least 5 minutes, the optical density was measured at 595 nm. From this measurement, a standard curve was plotted and the total concentration of protein (μg) in each sample could then be determined.

The protein extract along with the standards (composition of this standard mixture is given in Appendix 1) was run on polyacrylamide gel (10% PAGE) using the slab gel attached to the Studier-type electrophoresis apparatus (Hames, 1981). The electrophoresis was carried out at constant current of 25 mA for about 2.5 hours. After electrophoresis the gel was removed and stained overnight in an acetic-acid/coomassie blue solution. Destaining was carried out using destaining solution which is a mixture of ethanol, glacial acetic acid and H_2O, and identification of the protein bands could then proceed. It is important to note that SDS-extracted proteins are denatured and all have equal charge densities. The rate of migration of the protein along the gel therefore is solely dependent on molecular weight. Distance migrated is proportional to log. molecular weight and thus the weights of proteins in the samples can be determined by reference to the migration of standard proteins. The amount of each protein in the samples could be estimated from the gel scans measured on an LKB Ultroscan Densitometer, but these assessments are only semiquantitative because the intensity of the coomassie blue staining depends on the nature of the protein.

4. RESULTS OF PRELIMINARY STUDY

4.1 The Effect of Artificial Ripening on OP and CP Seeds

Data on seed quality prior to any artificial ripening of cones is central to the present study since this will be a reference for the effects of subsequent storage treatments. Data gathered included such variables as cone specific gravity, seed moisture content, seed germination and vigour.

At the time of collection, July 18, cones of OP and CP ramets were still green and had an average specific gravity of 1.15 and 1.05 respectively. This appreciable difference was due to the fact that CP cones were collected one week earlier than OP cones. Cones lost weight during storage and thus the specific gravity dropped to a range between 0.90 to 0.95 after three weeks storage.

The difference in the initial moisture content between OP and CP seeds was not statistically significant, being 21.8 and 22.2% respectively (Fig. 4.1). High storage temperature accelerates the loss of seed moisture within both OP and CP cones. Thus seeds from cones stored at 30°C had more abrupt reduction than those at 20°C (Fig. 4.1). The minimum point (around 8%) was reached after only three weeks storage at 30°C when no further changes took place. On the other hand, the moisture of seeds ripened at 20°C progressively declined, reaching below 10% at six weeks storage (Fig. 4.1). In no cases did statistical analysis show significant differences between OP and CP seeds, at either storage temperature or after any period of artificial ripening.

Despite what appeared to be immature cones, the germinability of the freshly extracted seeds for both OP and CP was surprisingly high, i.e. 90 and 96% respectively (not significantly different, Fig. 4.2). Artificial ripening caused significant deterioration (Ps0.05 level) when the seeds were ripened for twelve weeks at 30°C.

Fig. 4.1 Changes in seed moisture content during artificial ripening at 30° C (A) and 20° C (B). Values are means of 3 replicates. LSD is calculated at a probability level 0.05 are shown.

(⊙ = OP seeds; • = CP seeds).

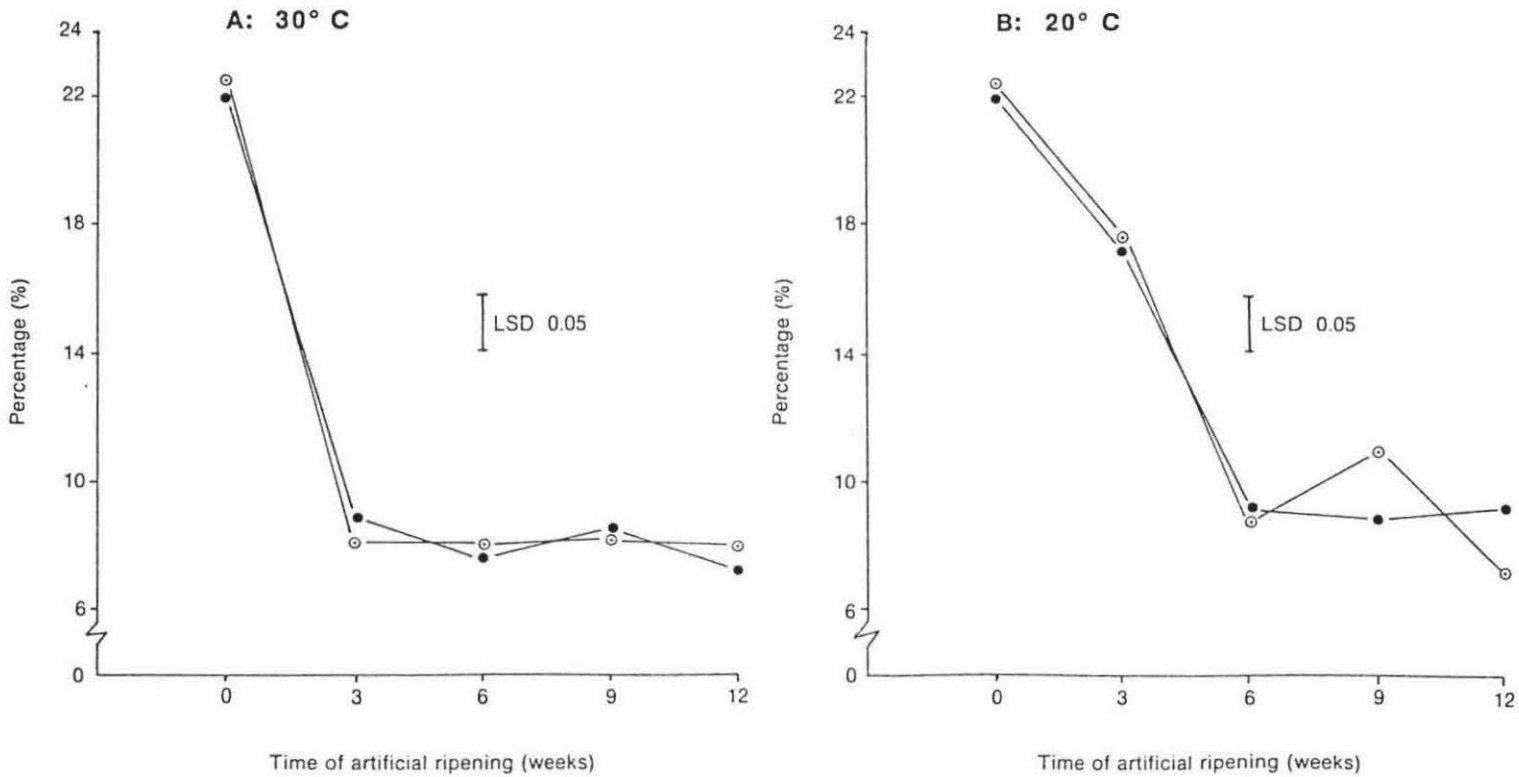
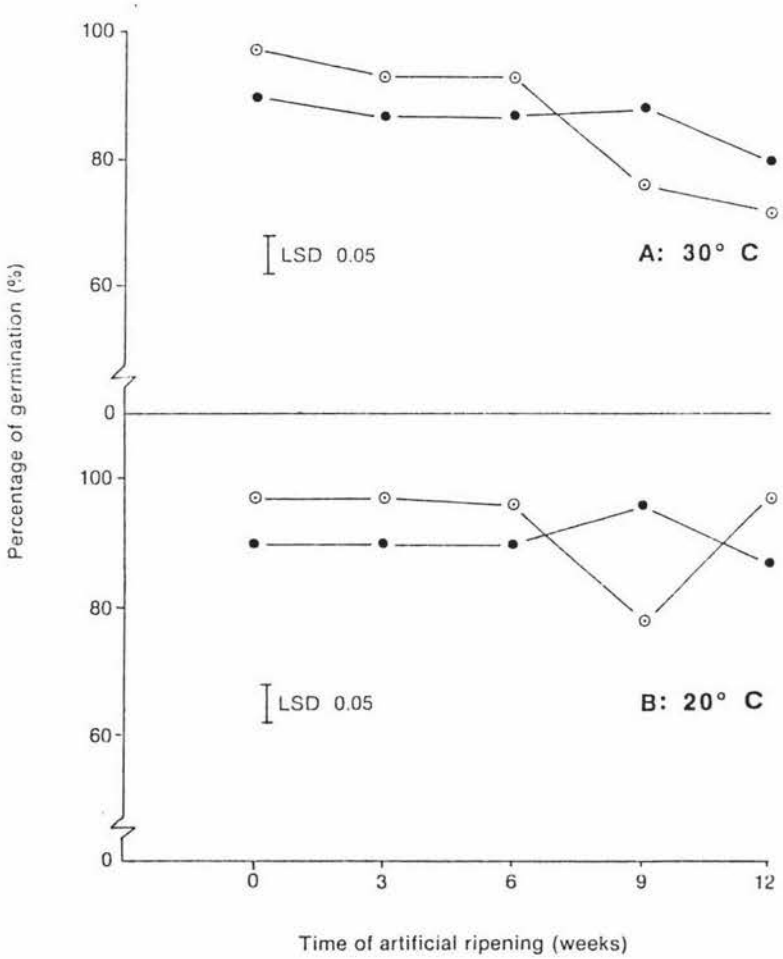


Fig. 4.2 **Changes in the final germination of OP and CP seeds during artificial ripening at 30° C (A) and 20° C (B).** The test was carried out in germination boxes at 20° C and data were obtained from 6 replicates. LSD is calculated at a probability level 0.05 are shown.

(⊙= OP seeds; • = CP seeds).



4.2 Seed Vigour

It was mentioned earlier (3.6.3) that the germination test in the Inventum table was considered as a modified vigour test. The results as presented in Figure 4.3. indicate a rapid deterioration from the first three weeks of storage onwards. Again, there is evidence that the high temperature storage (30°C) significantly accelerated the deterioration compared to cone storage at 20°C. The analyses indicate no significant interactions between pollination techniques, storage time and storage temperatures. However, interactions between pollination techniques and storage temperatures was significant. The germination percentage of OP and CP seeds (data pooled over storage times) stored at 20°C was significantly different, being 87.2% and 73.9% respectively. At 30°C the differences are not significant, being 71.2% of OP seeds and 71.3% of CP seeds.

The other type of vigour test investigated the time to 50% cotyledon emergence. This test was conducted in conjunction with the standard germination test. Once again the results show a pattern of deterioration during artificial ripening as the values of T50 increased (Fig. 4.4). OP seeds artificially ripened at 30°C showed significant deterioration after three weeks artificial ripening whereas CP seeds treated under the same condition significantly deteriorated after nine weeks artificial ripening. On the other hand both OP and CP seeds artificially ripened at 20°C showed significant reduction in vigour after six weeks artificial ripening. The analysis also show that both OP and CP seeds had a similar response to the treatment, and no significant differences were observed between OP and CP or between ripening temperature.

Fig. 4.3 **Final germination percentage under water stress after artificial ripening at 30° C (A) and 20° C (B).** Values are means of three replicates. LSD is calculated at a probability level 0.05 are shown.

(⊙= OP seeds; • = CP seeds).

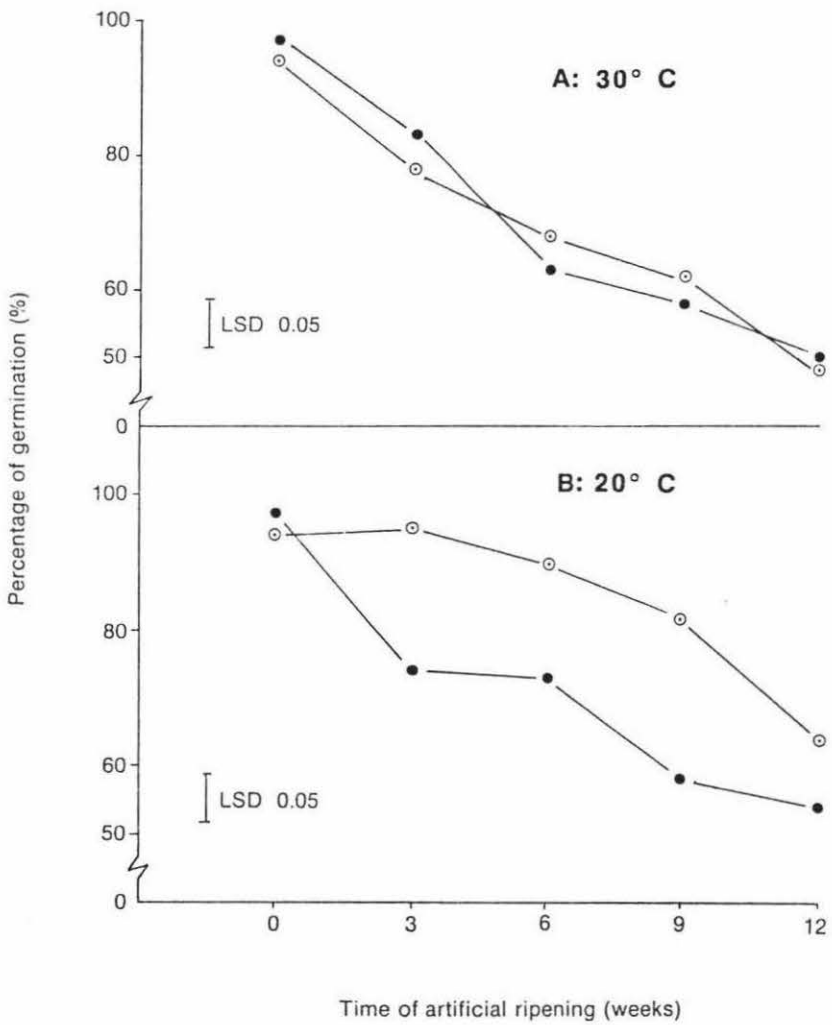
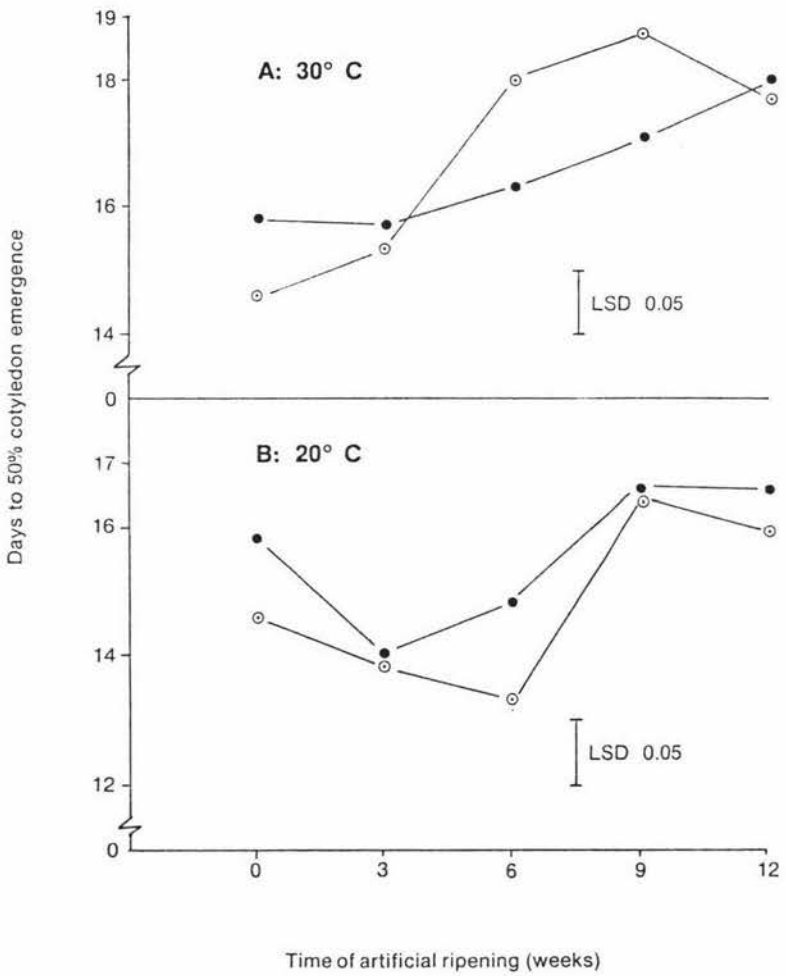


Fig. 4.4 Changes in vigour calculated as times to 50% cotyledon emergence following artificial ripening at 30° C (A) and 20° C (B). Values are means of 6 replicates. LSD is calculated at a probability level 0.05 are shown.

(⊙ = OP seeds; • = CP seeds).



5. RESULTS OF DETAILED STUDY

5.1 Cone Development on The Tree and in Artificial Ripening

The physiological development of cones was observed by measuring variables such as fresh weight, dry weight, moisture content and specific gravity. The fresh and dry weight of cones showed high variation among samples, but there seemed to be a trend of increasing weights from March through June (Fig. 5.1). The cone moisture content, however, showed a slow decreasing pattern as the season progressed (Fig. 5.2). Cones collected in March contained as much as 50% moisture compared to 37% in September. Statistical analysis clearly showed significant differences in cone moisture content between March and September.

Contrary to the trend of moisture content, the specific gravity of freshly collected cones increased from 1.13 in March to 1.19 in May, but then declined to reach 0.98 in September (Fig. 5.2). Mature cones from 1985 collections showed similar specific gravities (1.01-0.98) to cones collected in September 1986. During storage, changes in cone specific gravity reflected drop in moisture content (Fig. 5.3). In all collections, a highly significant reduction took place during the first three weeks of storage. From this point, the specific gravity decreased only slightly to a range between 0.95 to 0.99, comparable with the value for September and November 1985 as well as September 1986 cones.

The use of cone colour was also studied as an indicator of cone development. Photographic appearance and cone colour indexation were noted at different sampling times. Plate 5.1 illustrates cone colour changes as the season advances. The colour of cones ripening on the tree appears to be unchanged throughout, the observation remaining at index 1, although transitions began in September. As storage progresses, significant colour changes occurred accordingly (Fig. 5.4). The trend appeared similar in all collections, but the sequence of changes took place rather more rapidly after collection in cones collected in June and July.

Fig. 5.1 Changes in fresh and dry weight of cones during the second season of development. Bars indicate the standard errors of individual means.

(⊡ = fresh weight; ⊙ = dry weight)

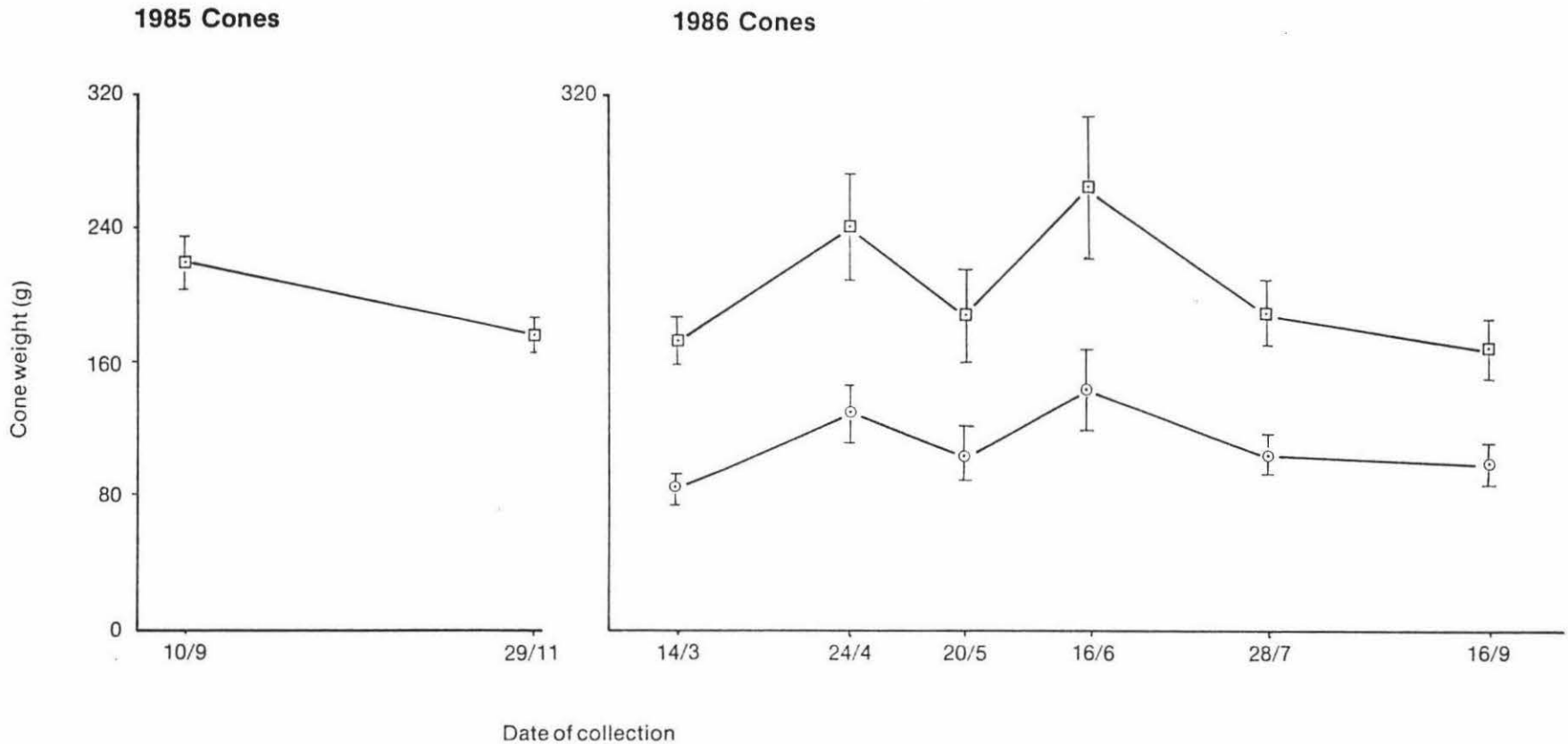


Fig. 5.2 Chronological changes in the moisture content and specific gravity of cones during the second season of development. Standard errors of individual means for specific gravity are much smaller than the symbol used. LSD for moisture content is calculated at a probability level 0.05

(⊙ = moisture content; • = specific gravity)

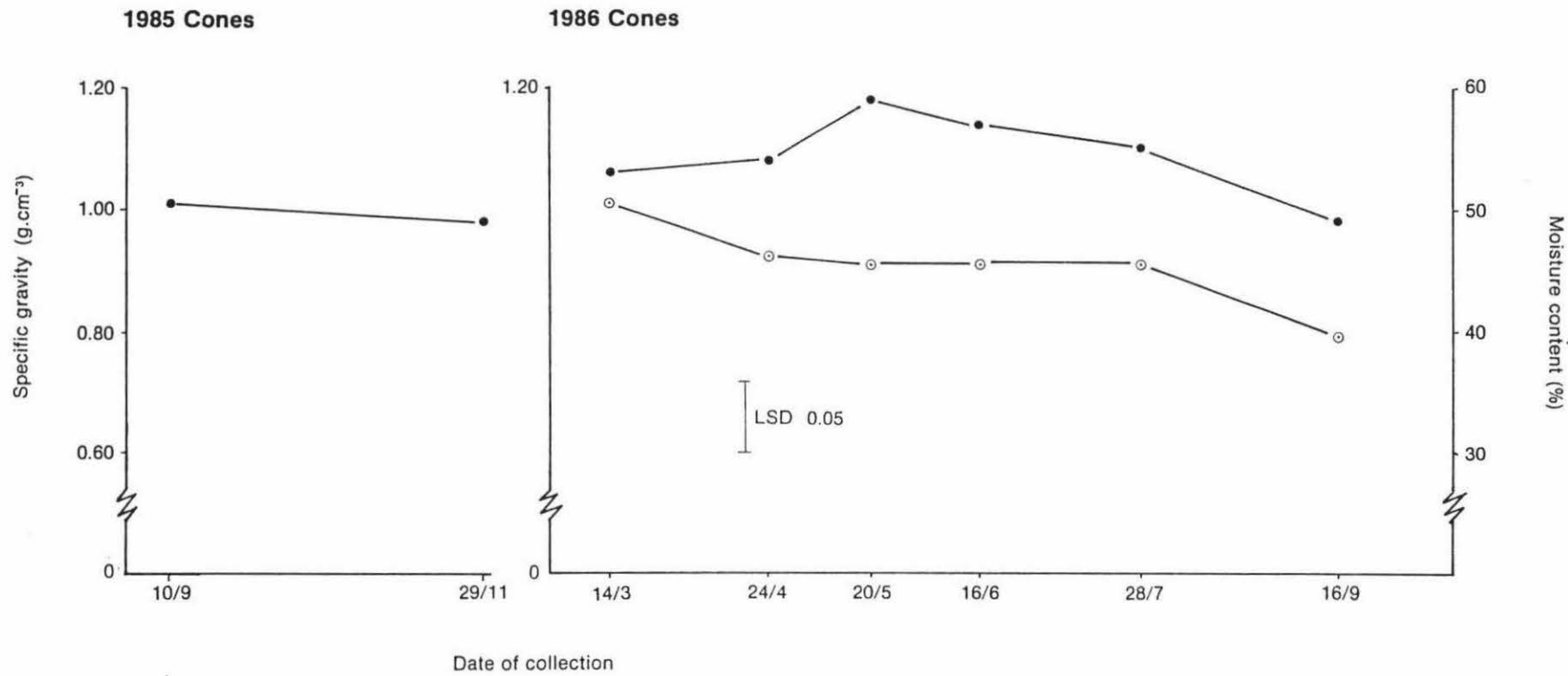


Fig. 5.3 Specific gravity changes during storage of cones from different collections. The standard errors of each mean is much smaller than the symbol used.

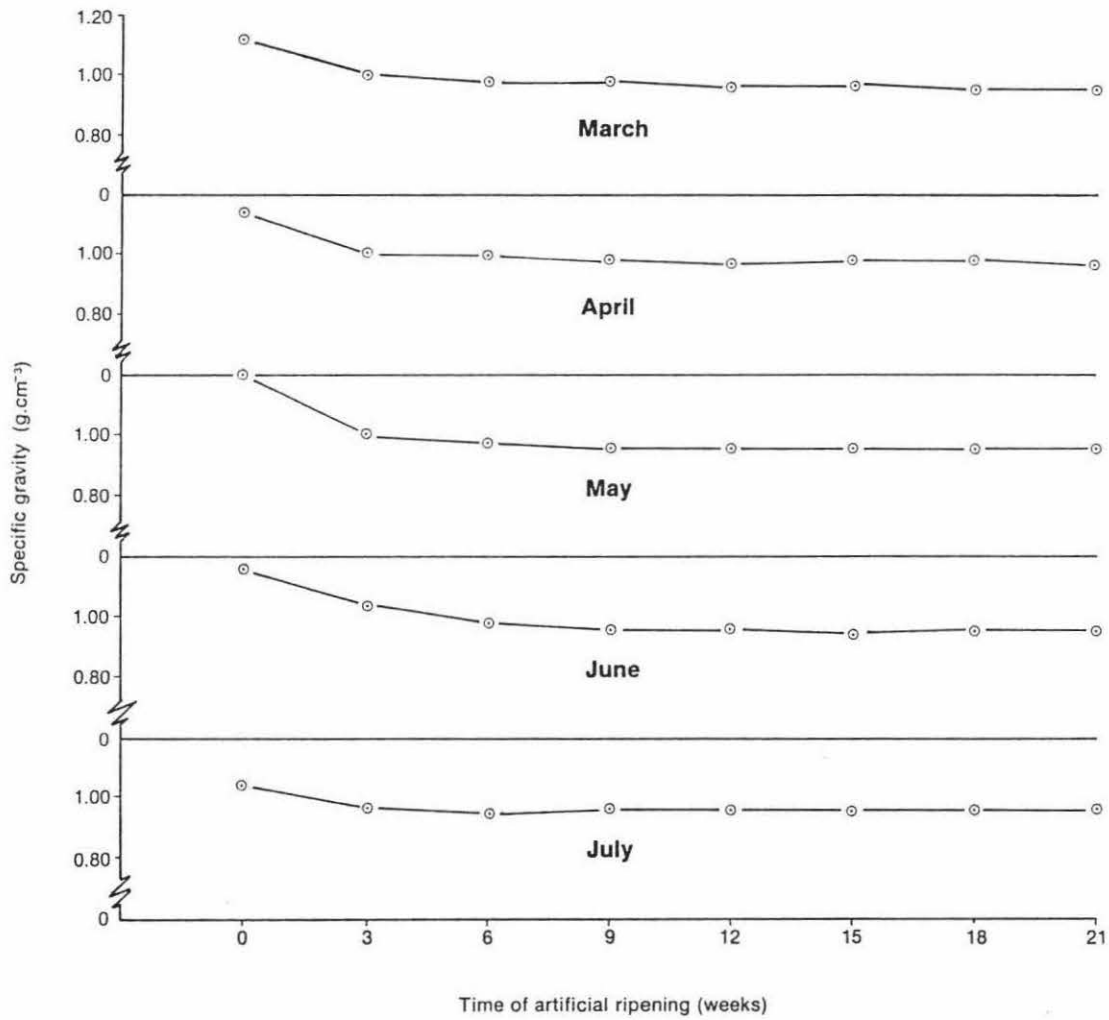


Plate 5.1 Cone colour changes during the second season of development. A - March; B - April; C - May; D - June; E - July and F - September.

A

B

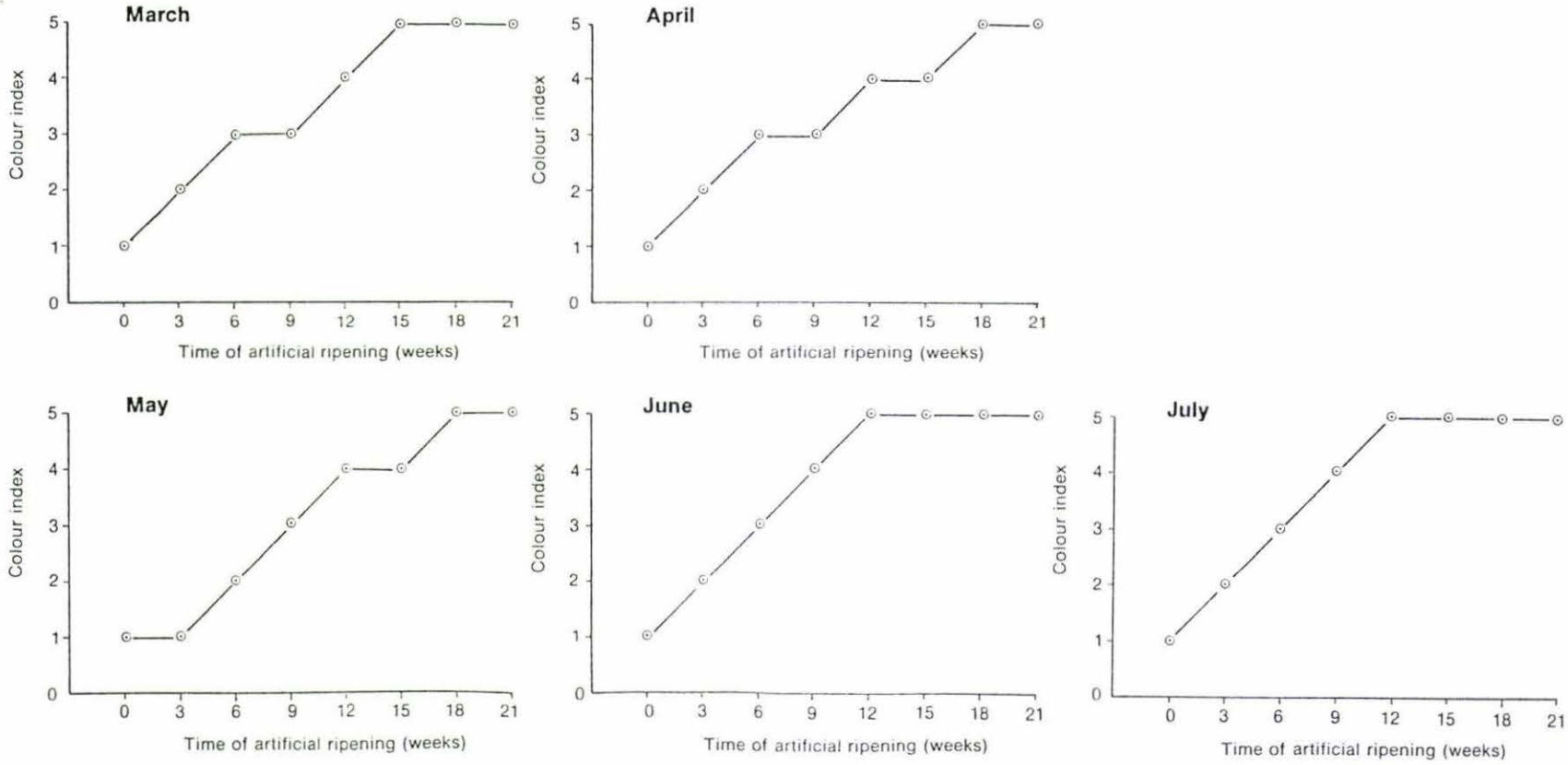
C


D


E
1 2 3


F
1 2 3

Fig. 5.4 **Cone colour changes during storage for different '86 collections as indicated by a colour index based on the British Colour Standards.**



The time of harvesting did not affect the extractability of freshly collected cones. Cones collected from March till September remained unextractable after being exposed to kilning (in the preliminary study it was found that cones collected in November 1985 responded very well to the extraction method). In contrast, cone storage increased the degree of cone scale opening. The cone began to respond to kilning after six weeks storage when all tested cones completely opened their scales. However, the degree of scale opening did not seem to affect the extraction efficiency. Although cone scale opening of all six weeks storage samples rated three (completely open) the extraction efficiency was generally much less than for cones stored for nine weeks or longer. There was high cone/clonal variation in extractability after six/nine weeks storage as well as variation in collections (Fig. 5.5). In general, similar maturation patterns were found us for cone specific gravity and cone colour, although extractability from June cones was only appreciable after nine weeks storage, later than might have been predicted.

5.2 Seed Quality on The Tree and in Artificial Ripening

5.2.1 Fresh weight, dry weight and moisture content

Chronological changes in fresh and dry weight, and moisture content of seed as the seed develops on the tree are presented in Figure 5.6. It is clear that the seed lost moisture during the second season of development. The moisture dropped very dramatically from 44.7% in March to 20.3% in May. Fluctuations in June and July were not significant and moisture content in September was 21.5%. Although moisture loss occurred, seed fresh weight was relatively constant throughout. The loss in moisture was compensated by the increase in seed dry weight, there being a significant increase from March 14 to September 16.

Fig. 5.5 The extraction efficiency (%) of cones from different '86 collections following storage at 20° C. The bars represent standard errors of individual means.

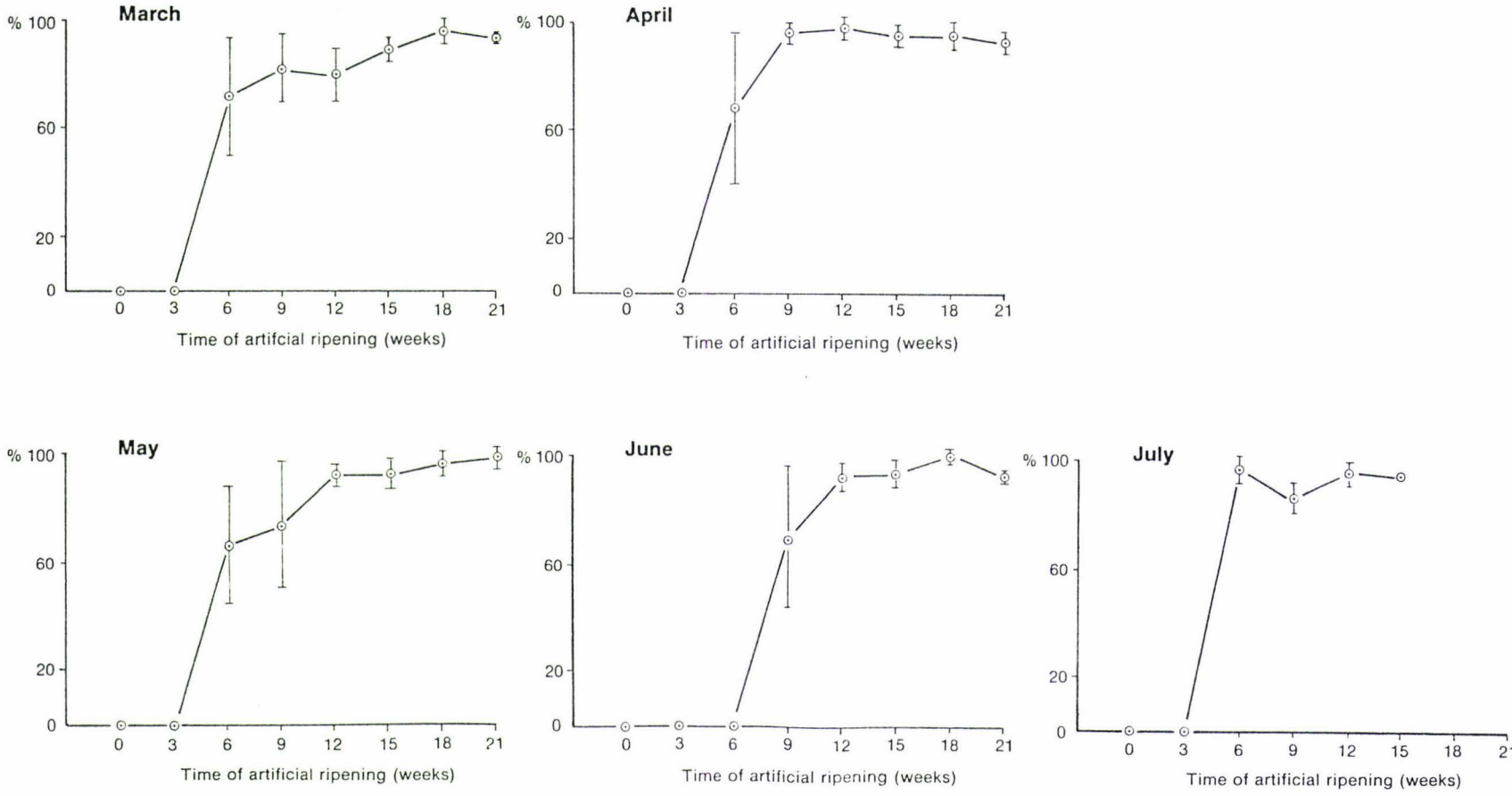
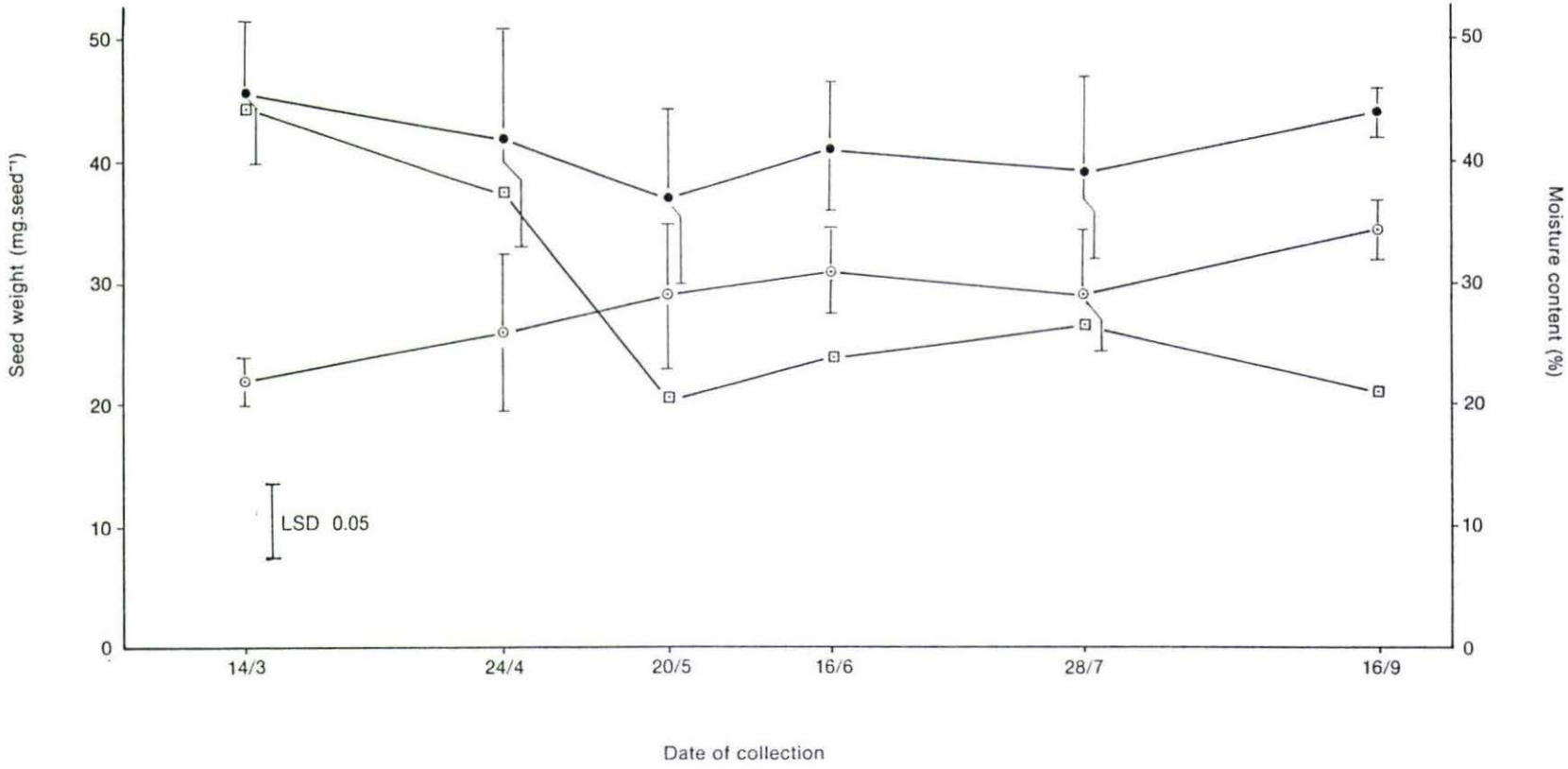


Fig. 5.6 Chronological changes in fresh and dry weight, and moisture content of seeds during the second season of development ('86 collections). The bars indicate standard errors of individual means. LSD for moisture content is calculated at a probability level 0.05

(• = fresh weight; ⊙ = dry weight; ⊡ = moisture content).



Mean values of dry weight and moisture content of seeds following artificial ripening are shown graphically in Figure 5.7 a, b, c, d and e. There was no clear evidence for any progressive change in dry weight during storage. The fluctuation in values clearly reflecting variation between clones and individual cones. Despite these variations, seed moisture content showed a decline with storage. Reduction was rapid during the first nine - twelve weeks storage, but moisture content then remained relatively constant. This trend applies to all collection categories. Moreover, the minimum value of moisture content appeared to be in the same range, viz. between 8-9%. This happened despite the fact that seed extraction was done by the hot-water method. Accordingly, this is good evidence supporting the suggestion that the extraction technique has no pronounced effect on seed moisture content. It is important to note that these low moisture contents are much less those that of tree-ripened seeds harvested in September.

5.2.2 Seed coat colour changes

The physical appearance of the seed coat from freshly collected seeds in March, April, May, June, July and September clearly showed the stage of seed coat development (Plate 5.2). The seed coat of March and April samples was yellowish-brown, soft and the seed was rather firmly stuck to the bract scale. In May and June, it had brownish in colour with black spots and hard. By July and September, the seed coat was predominantly black in colour.

During storage, seed colour changes took place in all samples. However, the rate of colour changes differed depending upon the degree of maturation. Thus, it was observed that even after twenty one weeks storage, the seed colour of March, April and May collected samples did not match the seed colour of June and July samples stored for twelve weeks. However, there were no clear indications that these changes were accompanied by changes in seed coat dry weight as indicated by the percentages of embryo and megagametophyte over seed dry weight which were relatively constant during storage (Table 5.1).

Fig. 5.7 a **Changes in the dry weight and moisture content of seeds during artificial ripening.** The cones were collected in MARCH '86. Bars indicate standard errors of individual means. Data are the means of three or four replications.

(⊙ = dry weight; • = moisture content)

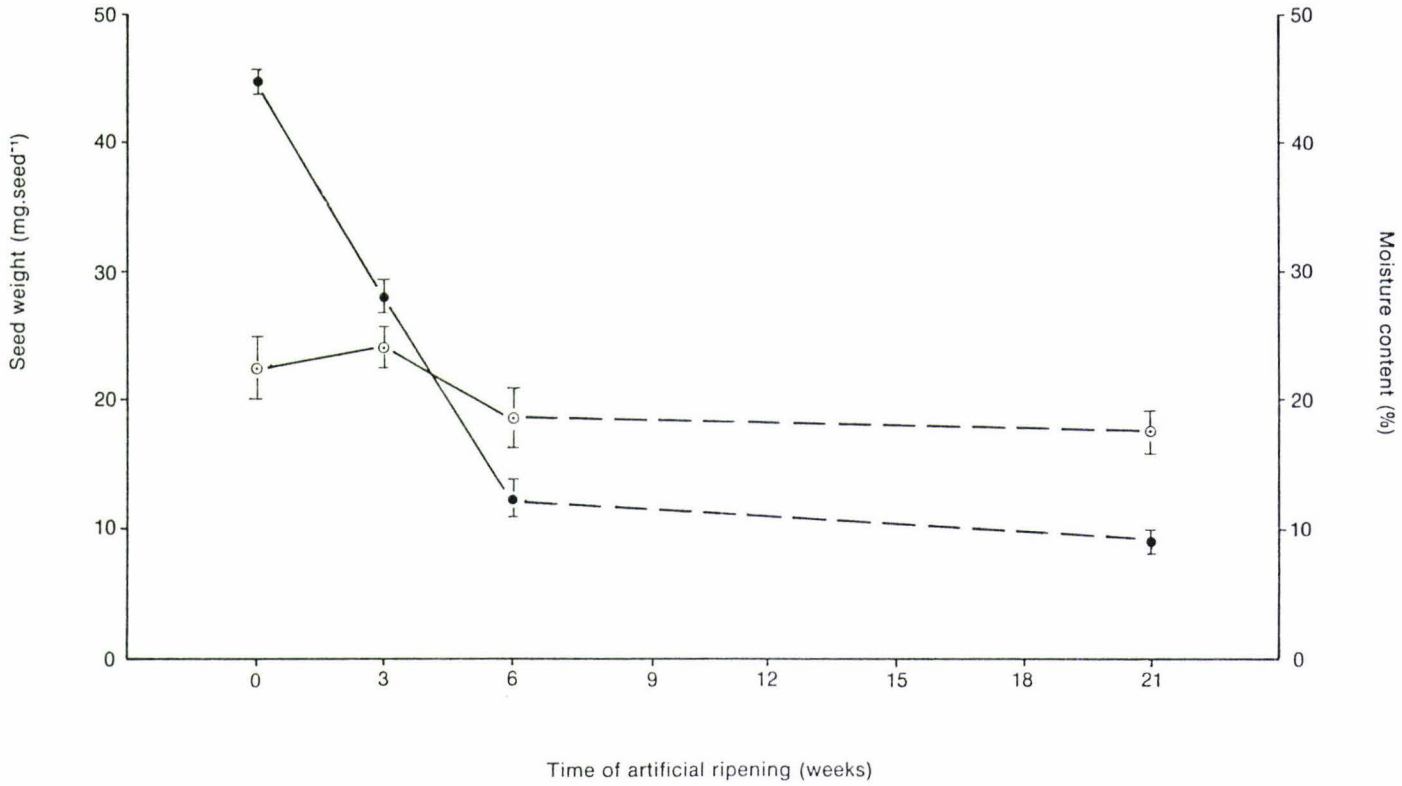


Fig.5.7 b **Changes in the dry weight and moisture content of seeds during artificial ripening.** Cones were collected in APRIL '86. Bars indicate standard errors of individual means where larger than the symbols used.

(⊙= dry weight; • = moisture content)

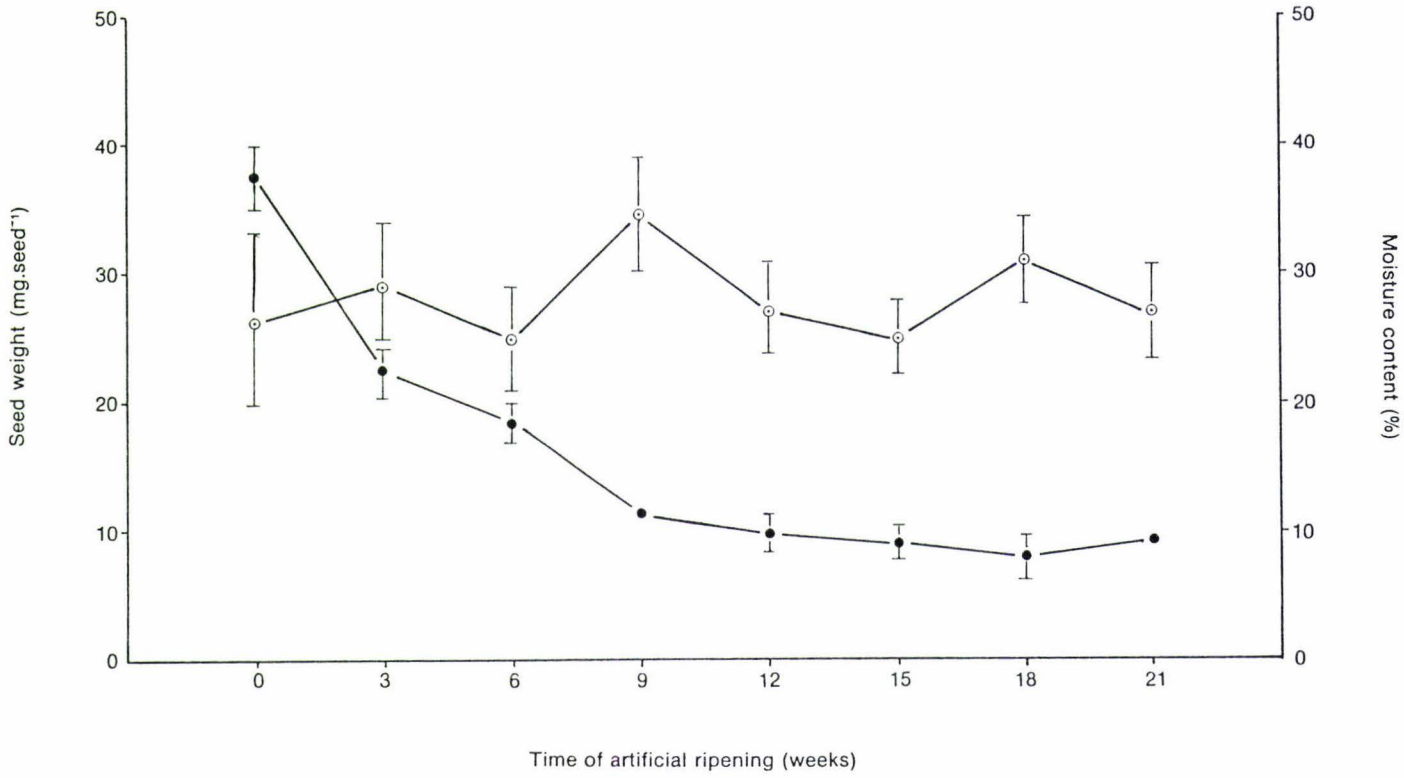


Fig. 5.7 c **Changes in the dry weight and moisture content of seeds during artificial ripening.** Cones were collected in MAY '86. Bars indicate standard errors of individual means where larger than the symbols used.

(⊙ = dry weight; • = moisture content)

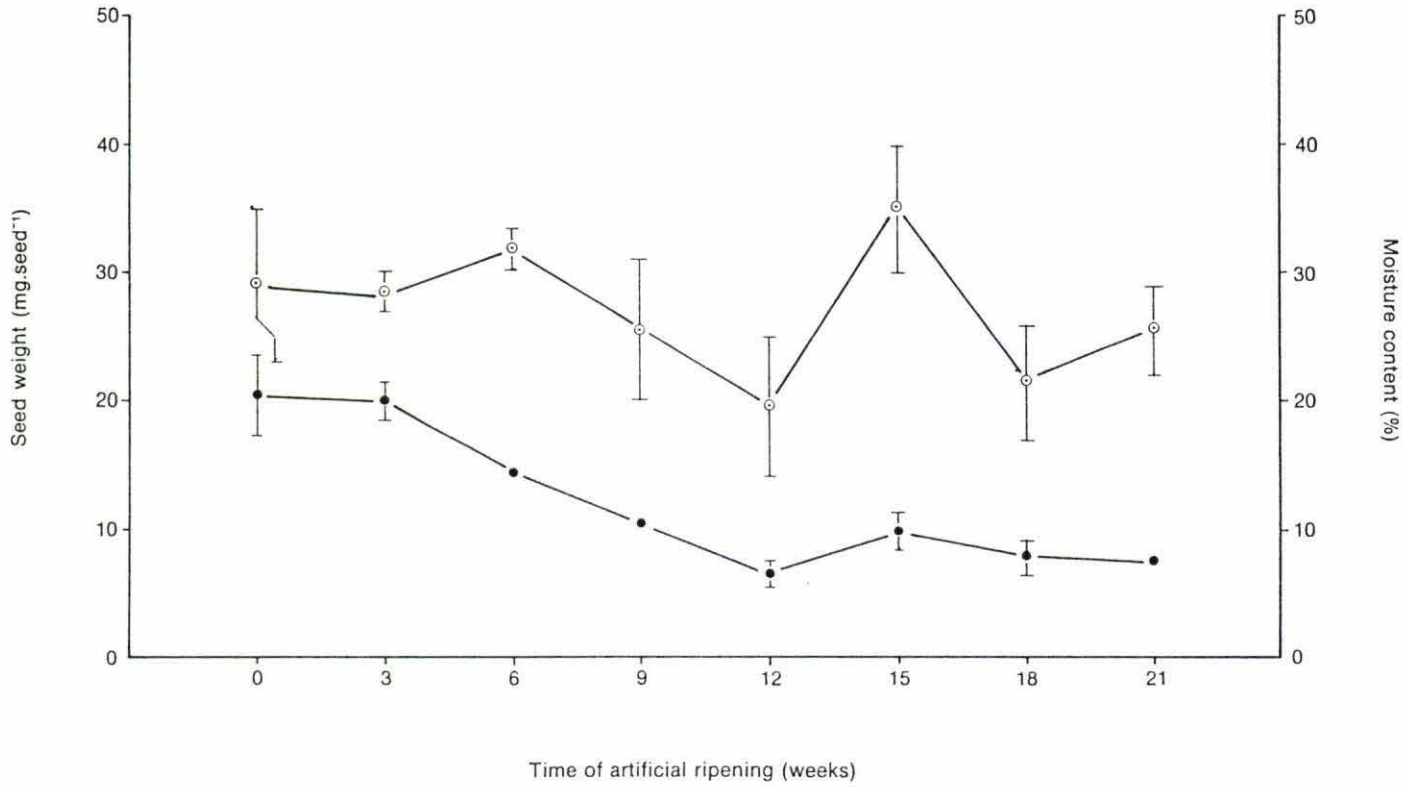


Fig. 5.7 d **Changes in the dry weight and moisture content of seeds during artificial ripening.** Cones were collected in JUNE '86. Bars indicate standard errors of individual means where larger than the symbols used.

(⊙= dry weight; • = moisture content)

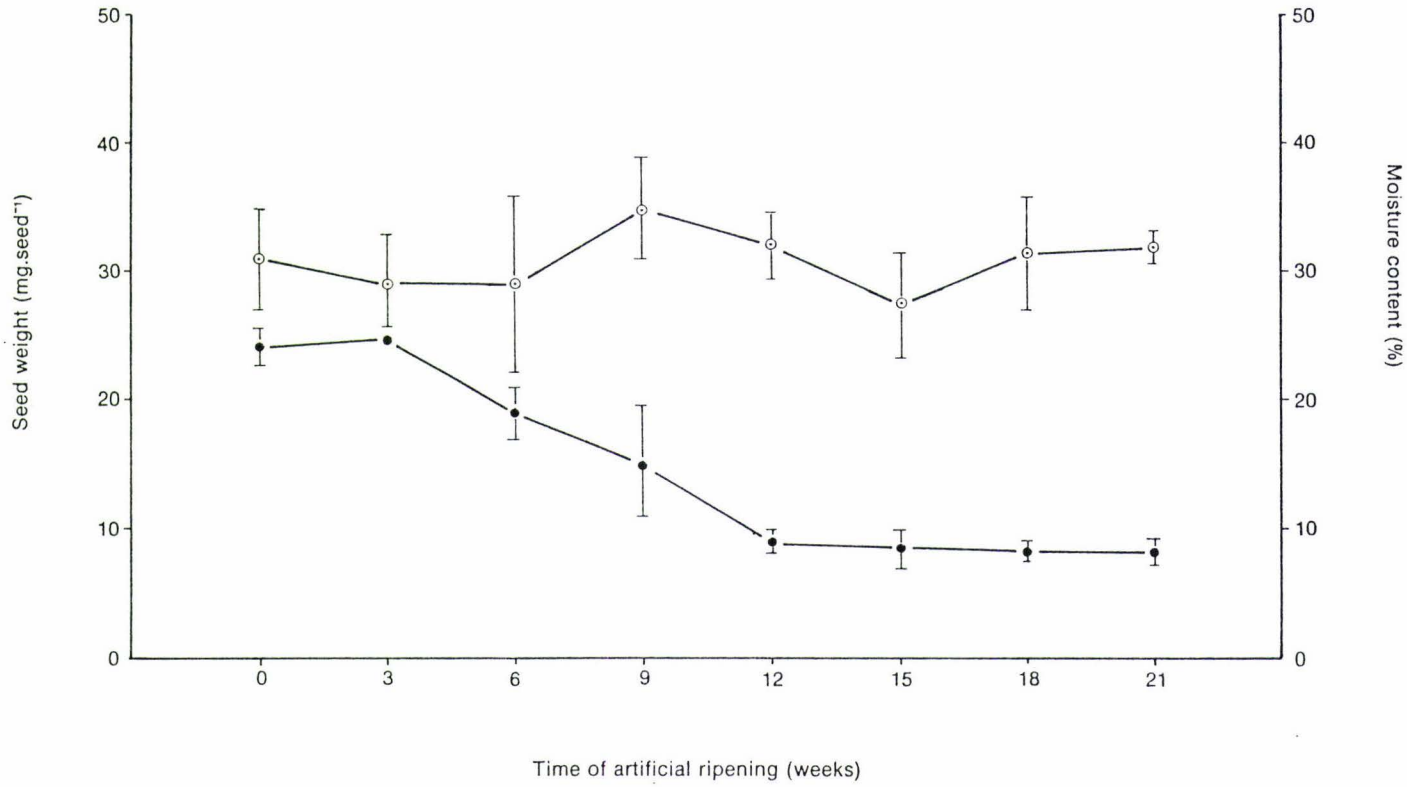


Fig. 5.7 e **Changes in the dry weight and moisture content of seeds during artificial ripening.** Cones were collected in JULY '86. Bars indicate standard errors of individual means where larger than the symbol used.

(⊙ = dry weight; • = moisture content)

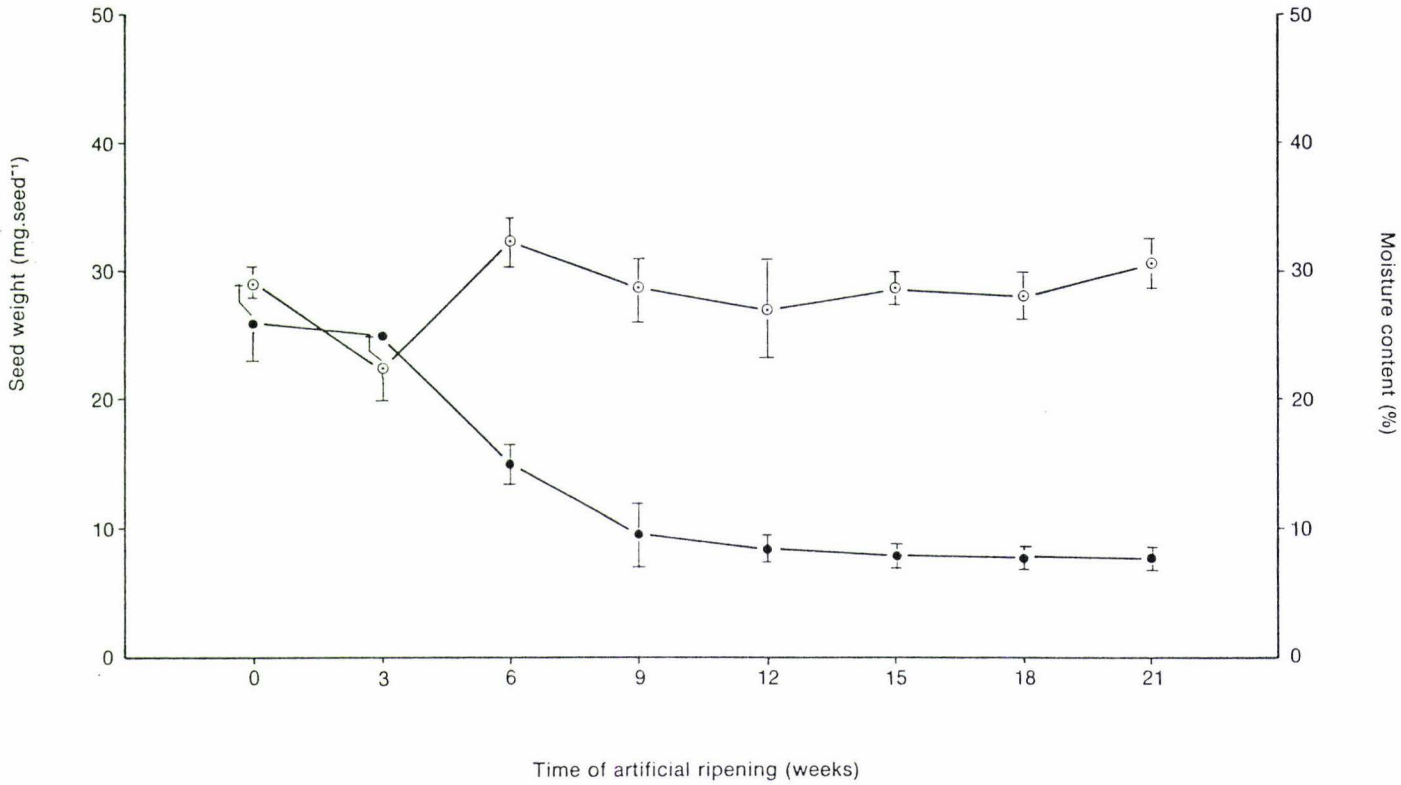


Plate 5.2 Seed coat colour changes during the second season of development. A - March; B - April; C - May; D - June; E - July and F - September.

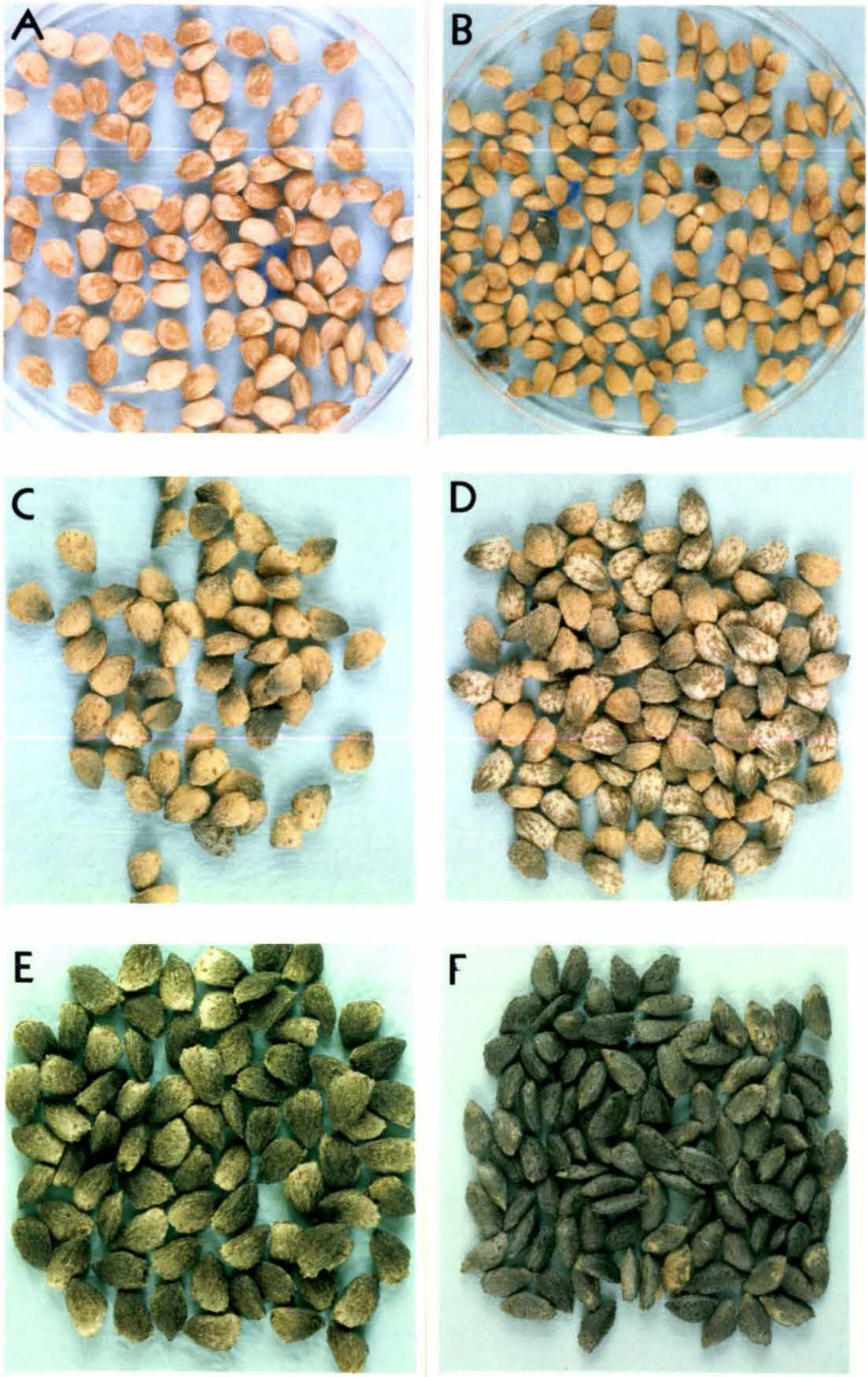

A
B
C
D
E
F

Table 5.1: The percentage of embryo-megagametophyte dry weight relative to total seed dry weight. Figures in brackets are standard errors of single means.

Sampling times	Storage period (weeks)							
	0	3	6	9	12	15	18	21
March	72.8 (±1.6)	74.5 (±1.2)	-	-	-	-	-	68.2 (±2.4)
April	77.1 (±1.8)	73.4 (±1.4)	75.6 (±2.7)	76.1 (±2.4)	74.1 (±1.4)	75.0 (±1.3)	76.7 (±2.3)	72.5 (±1.2)
May	76.7 (±1.6)	78.4 (±1.6)	80.3 (±1.4)	75.9 (±0.9)	76.6 (±0.7)	-	72.1 (±1.7)	75.9 (±0.9)
June	76.2 (±3.0)	75.5 (±2.7)	75.5 (±1.6)	77.3 (±1.3)	78.8 (±1.4)	73.4 (±1.0)	74.2 (±0.2)	73.2 (±0.2)
July	74.7 (±2.8)	75.3 (±1.1)	83.5 (±4.4)	82.1 (±5.6)	75.5 (±1.9)	76.2 (±1.1)	74.7 (±1.0)	76.0 (±0.8)
September	76.8 (±1.5)							

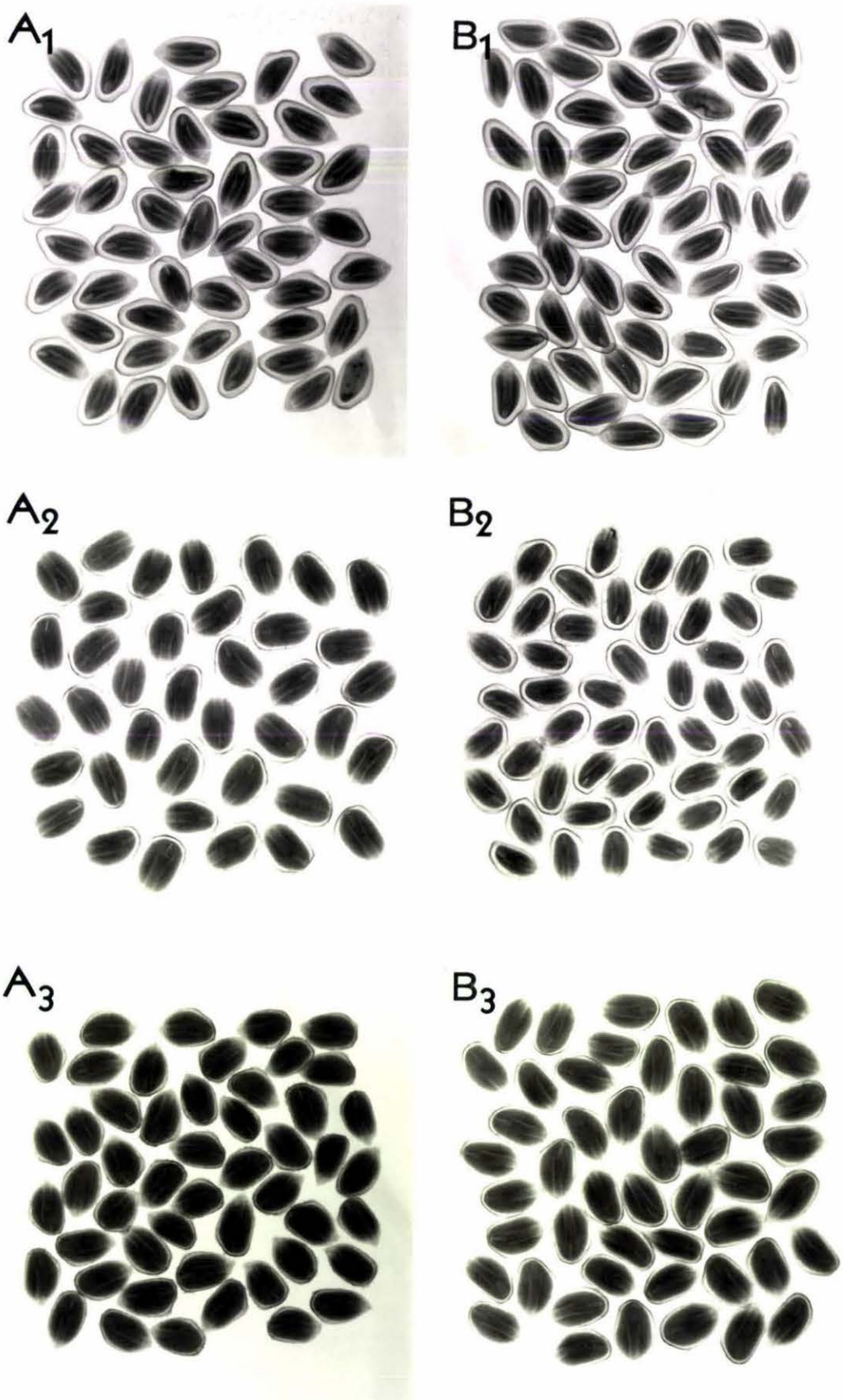
5.2.3 Anatomical development

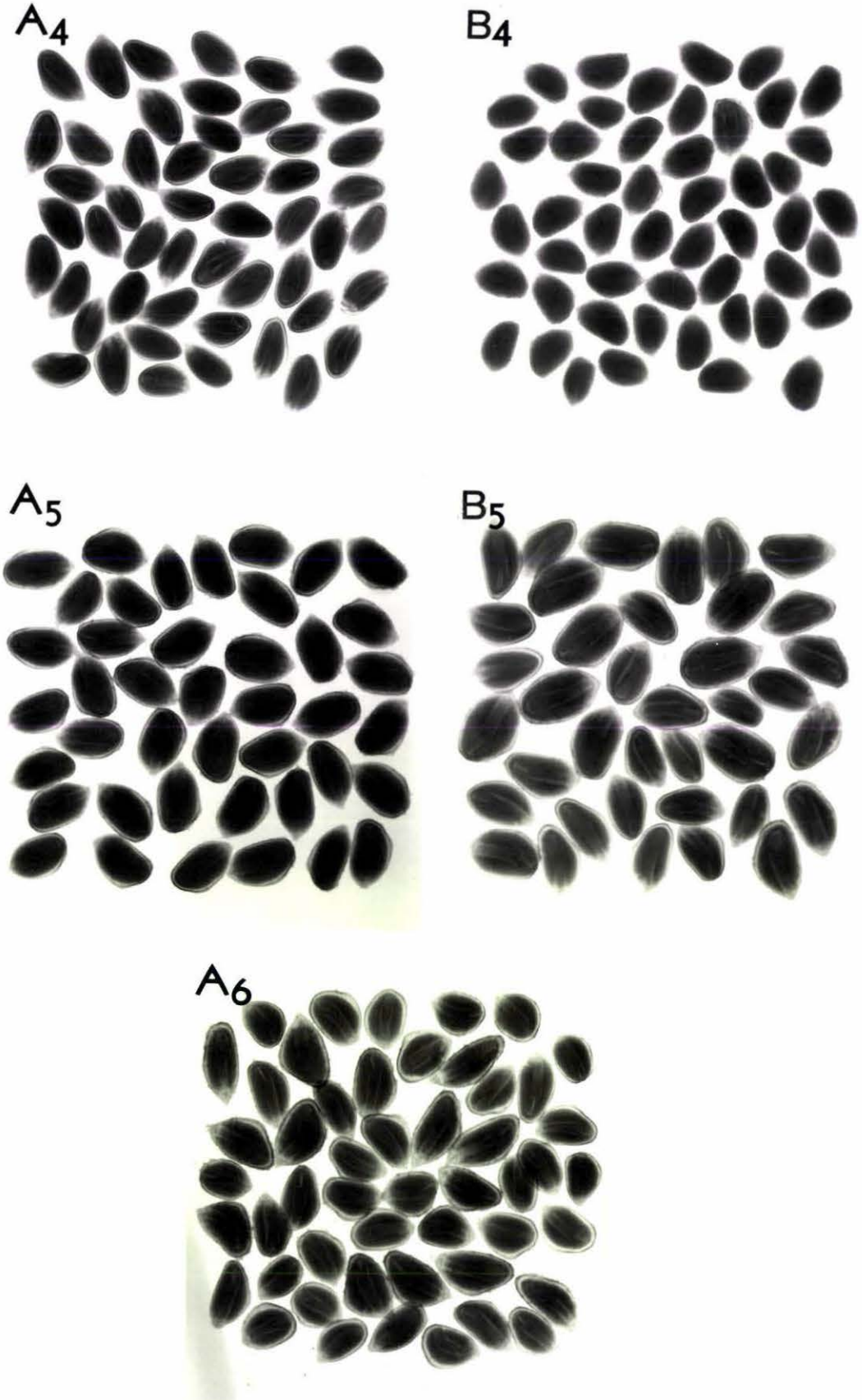
Examinations of X-ray photographs of seeds extracted from unstored-cones indicated the different level of anatomical development as regards time of collection (Plate 5.3). The seeds collected in March were already well developed in structure, but the embryo had not occupied the entire cavity and the megagametophyte tissue was still widely separated from the seed coat, clearly an indication of incomplete development. The X-ray photographs also showed that the cavity was formed for the embryo to fill.

The seeds collected in April appeared to be more well developed, with the megagametophyte tissue more firm and the space separating it with the seed coat was becoming narrower. During the later collections, viz. May, June and July, the megagametophyte was completely firm and occupied the entire seed leaving only a marginal space within the seed coat (Plate 5.3).

Artificial ripening had no obvious effect on anatomical development. As storage progresses, the X-ray examination indicated no obvious anatomical changes taking place in the seeds of any collection. In no instance did the megagametophyte tissue significantly develop to narrow the gap with the seed coat. (Plate 5.3).

Plate 5.3 A comparison of the morphological status of P. radiata seed between seeds extracted from non-stored cones ($A_1 - A_6$) and artificially ripened seeds for 21 weeks ($B_1 - B_5$). Collections were made in March (1), April (2), May (3), June (4), July (5) and September (6).


A1
B1
A2
B2
A3
B3


A4
B4
A5
B5
A6

5.2.4 Germination

Chronological changes in germination, as defined by ISTA (1985) of unstored seeds during the second season of development illustrates the relationship between time of collection and germinability. Arc sin transformed data were plotted in Figure 5.8. A gradual increase was observed during March through May. In June a significant increase to near maximum levels occurred in all three germination criteria. It is pertinent to note that the germinability of seeds collected in July and September 1986 were highly comparable to those collected in July, September and November 1985. Percent radicle emergence was greater than percent germination in the April, May and June collections. This was primarily due to the failure of the radicle to develop further as a result of radicle abnormalities, i.e. retarded radicle development. Similarly, some of the germinants failed to develop emerged cotyledons owing to lack of capacity of the embryo to do so. In the later collections, all seeds showed effectively 100% germinability.

The course of radicle emergence; germination and cotyledon emergence of artificially ripened seeds collected at different times are presented in Figure 5.9a, b and c. None of the fresh seeds collected in March germinated. However, three weeks storage increased the germination to 36%. Further storage did not improve the germination, instead it seems to have an adverse effect (Fig. 5.9a). This effect was very serious in one replicate but less in the others, as reflected by high S.E.'s. A large proportion of the seedlings failed to reach 'germinable' size due to abnormalities, primarily retarded radicle development.

A significant and promising improvement of germination was observed in seeds collected in April. Germination increased from 16% to 90% after storage for three weeks and from this point, the value remained high. Interestingly, the dramatic increase of germination took place while seed moisture content dropped from 37.6% to 22.5% (Fig. 5.7a). Further reduction of moisture content, however, affected germination only slightly.

Fig. 5.8 Chronological changes in germination capacity (arc sin transformed data) of non-stored seeds during the second season of development. Data are from non-stratified seeds. LSD is calculated at a probability level 0.05

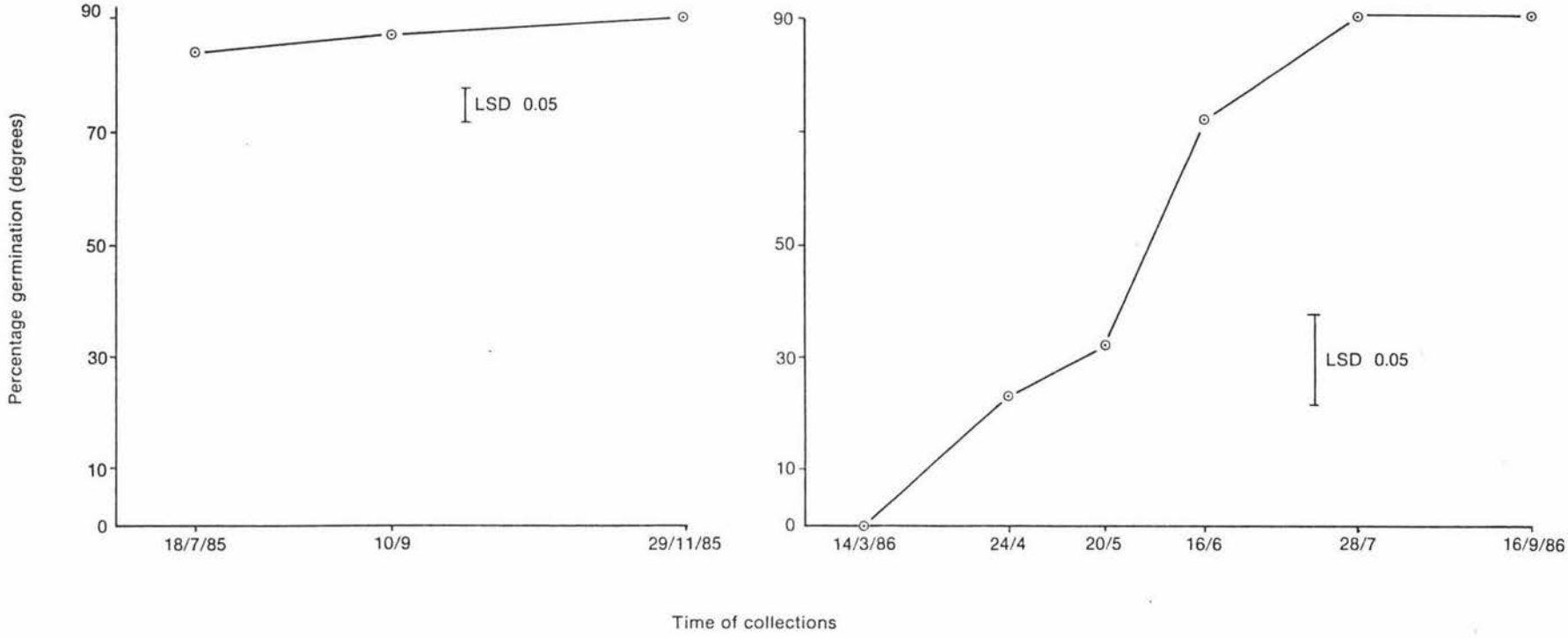


Fig. 5.9 a **The course of radicle emergence, germination and cotyledon emergence of artificially ripened seeds from cones collected in MARCH '86.** Figures indicate standard errors of individual means.

(⚀ = radicle emergence; □ = germination; ▥ = cotyledon emergence)

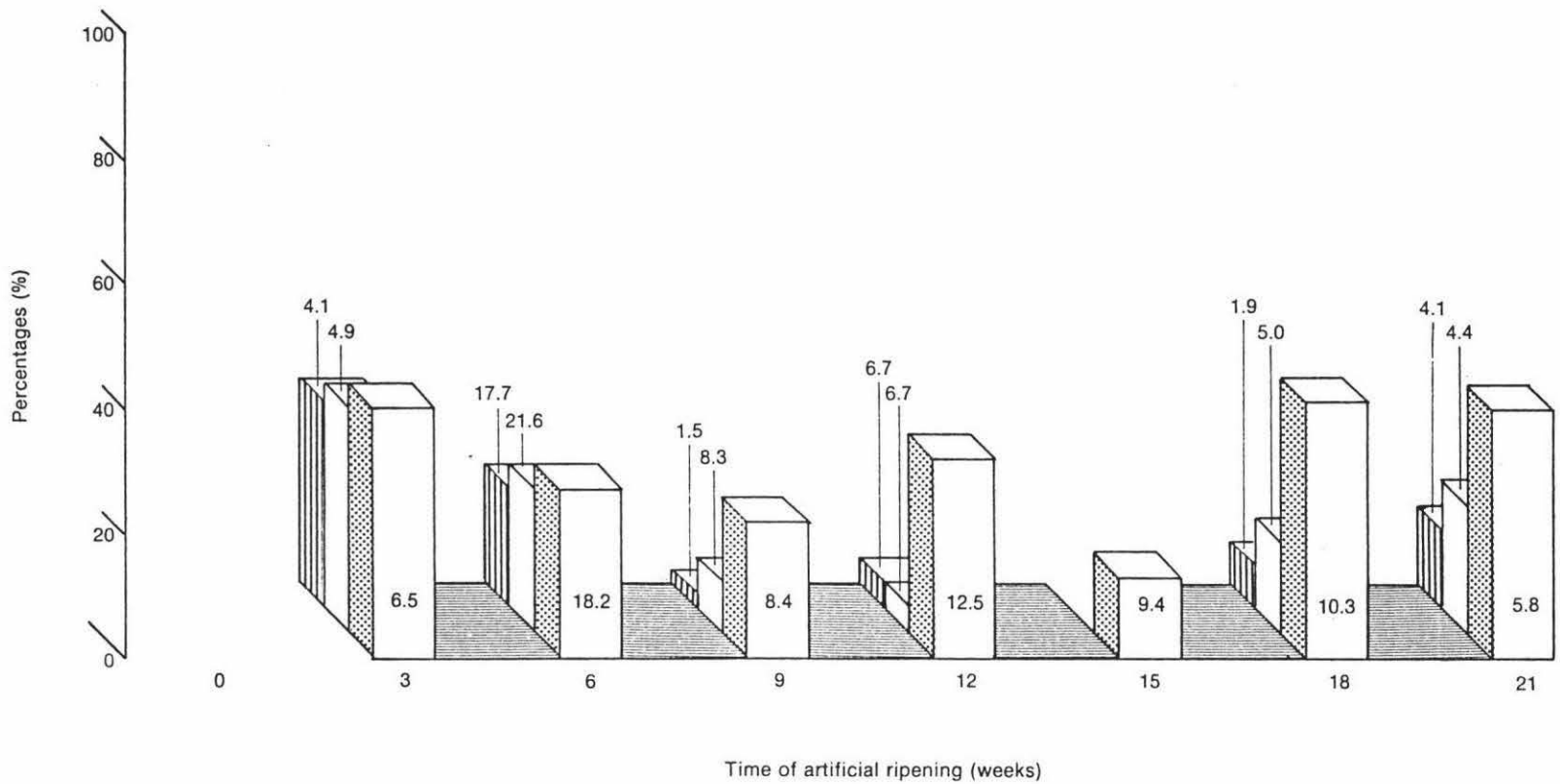


Fig. 5.9 b **The course of radicle emergence, germination and cotyledon emergence of artificially ripened seeds from cones collected in April '86.** Figures indicate standard errors of individual means.

(▩ = radicle emergence; □ = germination; ▥ = cotyledon emergence)

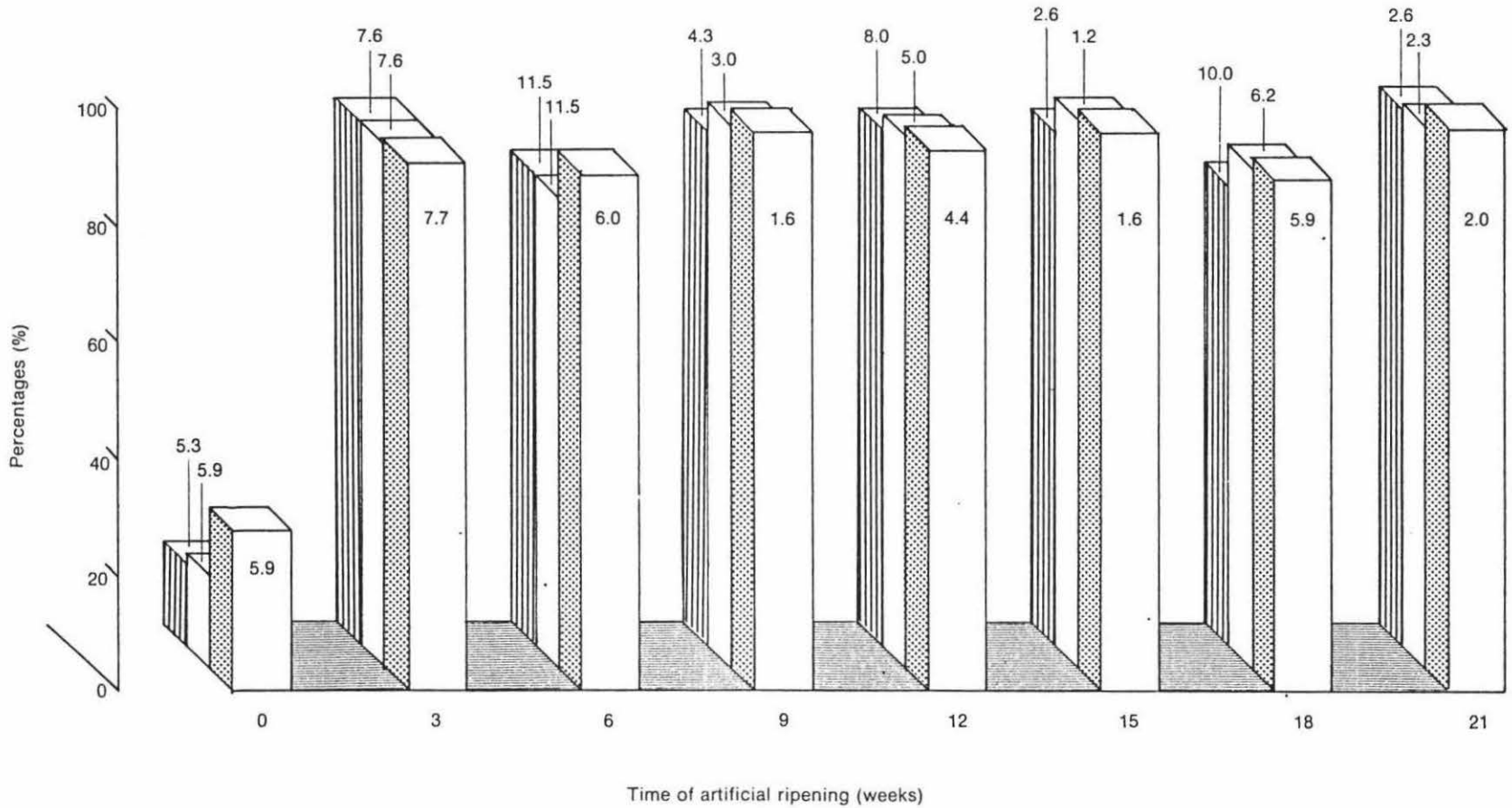
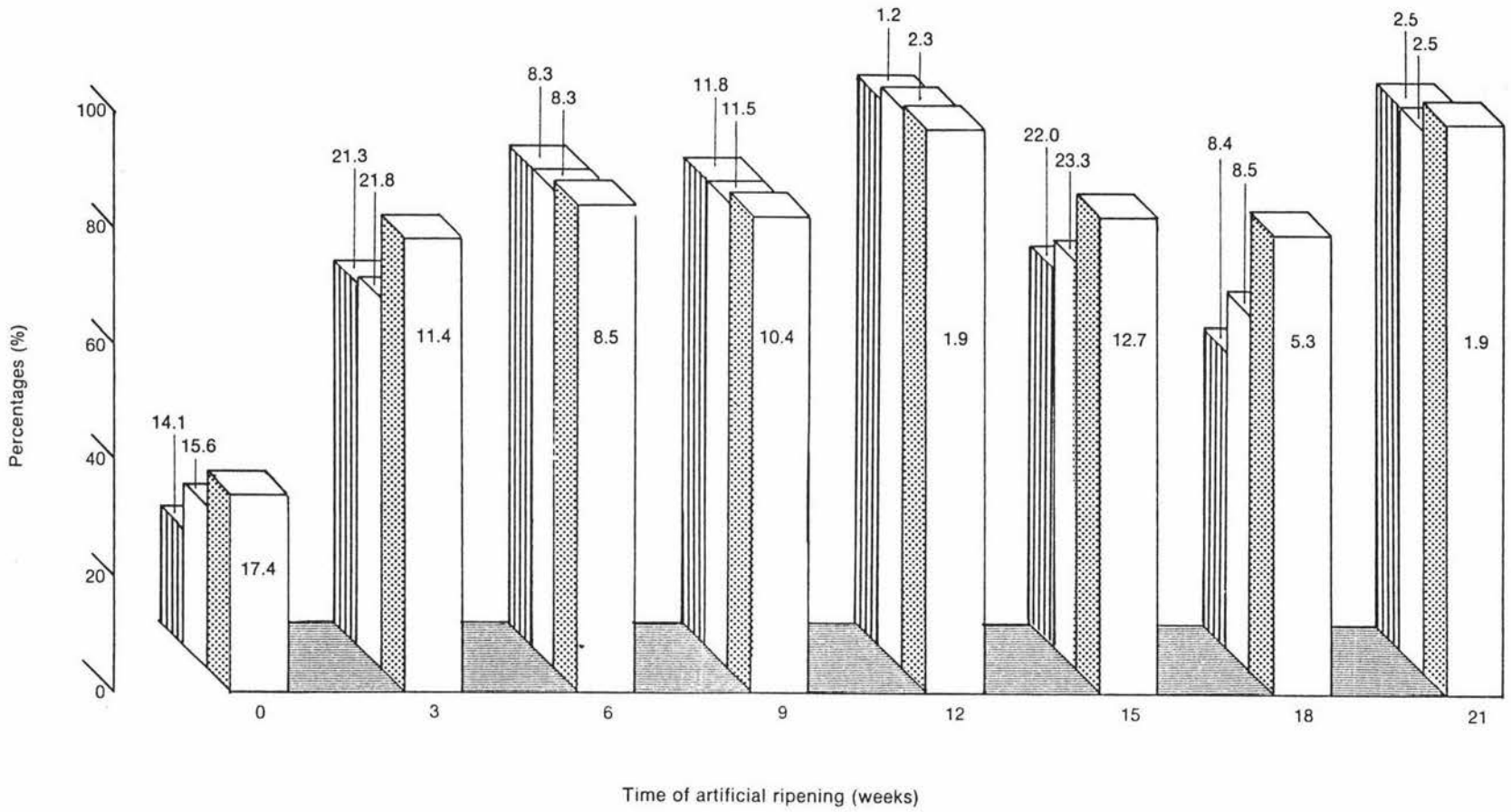


Fig. 5.9 c **The course of radicle emergence, germination and cotyledon emergence of artificially ripened seeds from cones collected in May '86.** Figures indicate standard errors of individual means.

(⊠ = radicle emergence; □ = germination; ▥ = cotyledon emergence)



A similar pattern of germination was observed on seeds collected in May, although the improvement was not as dramatic as the April sample. Unlike the April sample, the seed moisture content of the May sample was already low and decreased insignificantly after three weeks storage (from 20.5% to 20.0%). Generally, the mean values of germination were slightly lower than in the previous sample.

The initial germination of seeds collected in June and July were already 89 and 97% respectively. The storage treatment increased the germination only slightly, and in most cases it was ranging from 90-100% throughout the storage periods.

A split-plot analyses of germination data for stratified and non-stratified seeds collected in April, May and June and artificially ripened at 20°C for 6, 12 and 9 weeks, respectively, indicated that the effect of stratification was not significant (Table 5.2).

Table 5.2: Mean values of germination percentages of artificially ripened seeds from different samples: April S_6, May S_{12} and June S_9. The storage was at 20°C and extraction was done by hot-water method. (back-transformed data).

Seed lot	Stratification	
	Non-Strat.	Strat.
April S_6	93[a]	95[a]
May S_{12}	82[a]	89[a]
June S_9	100[a]	99[a]
Combined standard errors	3.35	

Means within rows followed by the same letter are not significantly different according at $p \leq 0.05$ level.

April S_6: April-collected cone artificially ripened for six weeks; May S_{12}: May-collected cone artificially ripened for twelve weeks; June S_9 June-collected cone artificially ripened for nine weeks.

5.2.5 Stratification and seed vigour

Measures of seed vigour was analysed on the basis of time to reach 50% (T50) radicle emergence, germination (as defined by I.S.T.A.) or cotyledon emergence. Figure 5.10 illustrates these results for seeds extracted from unstored cones which show improvements in vigour as seed development progresses. For all vigour measurements the May collected sample was significantly different from the other samples. This result may simply reflect another combination of clonal and cone-to-cone variation and/or climatic effects during development. Although there is a tendency for stratified seeds to have shorter T50's than non-stratified seeds, analysis of variance for each of these responses showed no significant effect of prechilling treatments. Overall mean T50 values, irrespective of stratification treatments are thus tabulated in Table 5.3 to illustrate the significant reductions in T50 with later collection dates. These results clearly show the improvement of vigour from relatively low to high levels as the seeds approach maturity.

During artificial ripening, changes in seed vigour measures also took place (Figs. 5.11a, b, c and d). In all analysed data, three weeks storage brought about a significant improvement in seed vigour at $P \leq 0.05$ level, but the advantages of storage became less pronounced as it continued or even become negative. The vigour of seeds artificially ripened for eighteen and twenty one weeks was not significantly different from those of fresh seeds in either of the two collections, April and June. Once again, there was high variation in the May sample.

Fig 5.10 Changes in vigour of non-stored seeds calculated as times to 50% radicle emergence, germination or cotyledon emergence during the second season of development in relation to time of collection and stratification. LSD is calculated at a probability level 0.05

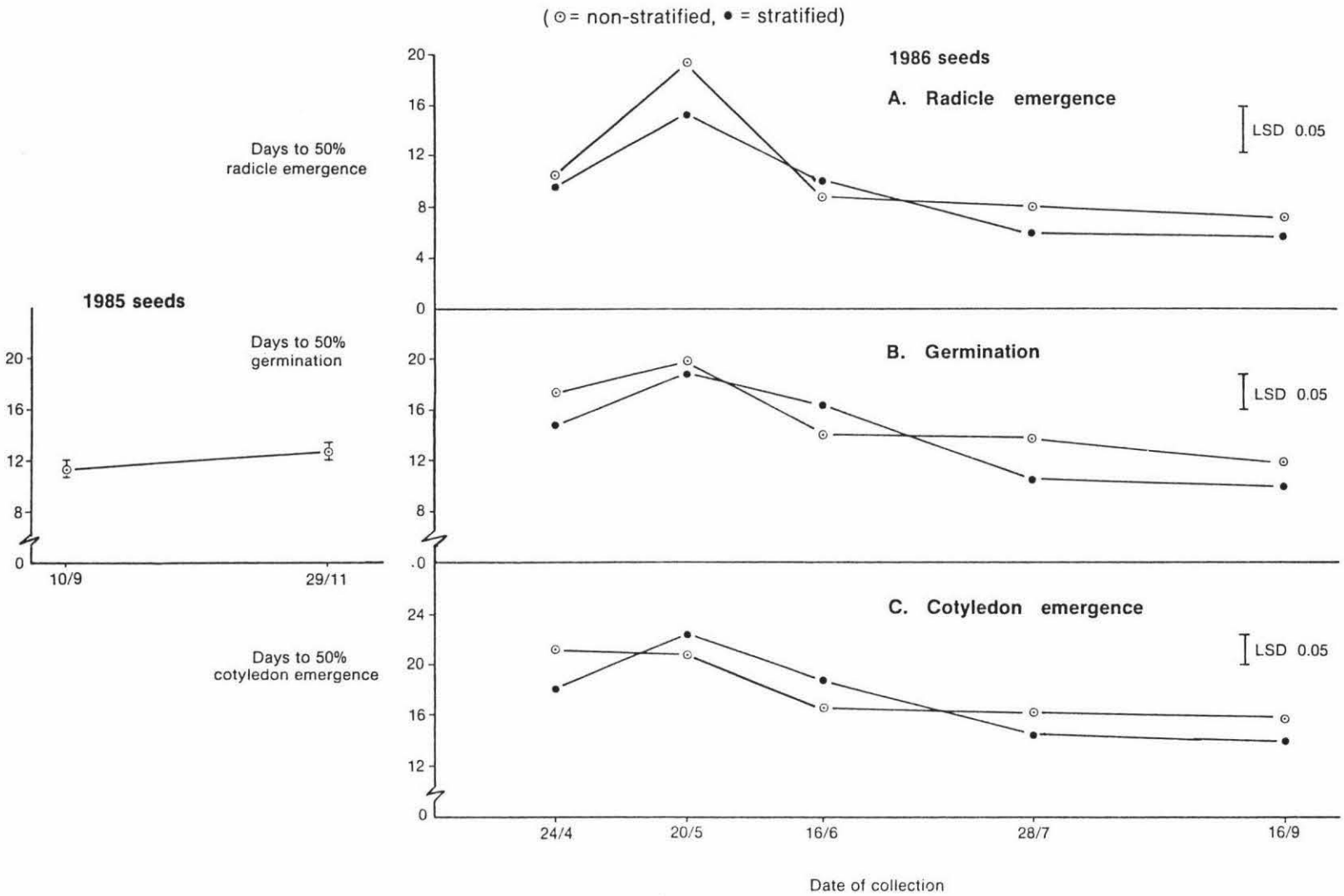


Table 5.3: Mean T_{50} values (days) of radicle emergence, germination and cotyledon emergence of seeds extracted from non-stored cones in relation to time of collection (data pooled over stratification treatments).

	Collection					LSD
	April	May	June	July	Sept	
T_{50} (radicle emergence)	10.0	17.2	9.3	6.8	6.3	3.44
T_{50} (germination)	16.0	19.3	15.2	12.2	10.9	3.04
T_{50} (cotyledon emergence)	19.8	21.5	17.6	15.2	14.6	2.60

LSD = Least significant difference at $p \leq 0.05$ level

Fig. 5.11 a **Changes in vigour of artificially ripened seeds calculated as times to 50% radicle emergence, germination or cotyledon emergence in relation to time of ripening and stratification.** The cones were collected in April '86. LSD is calculated at a probability level 0.05

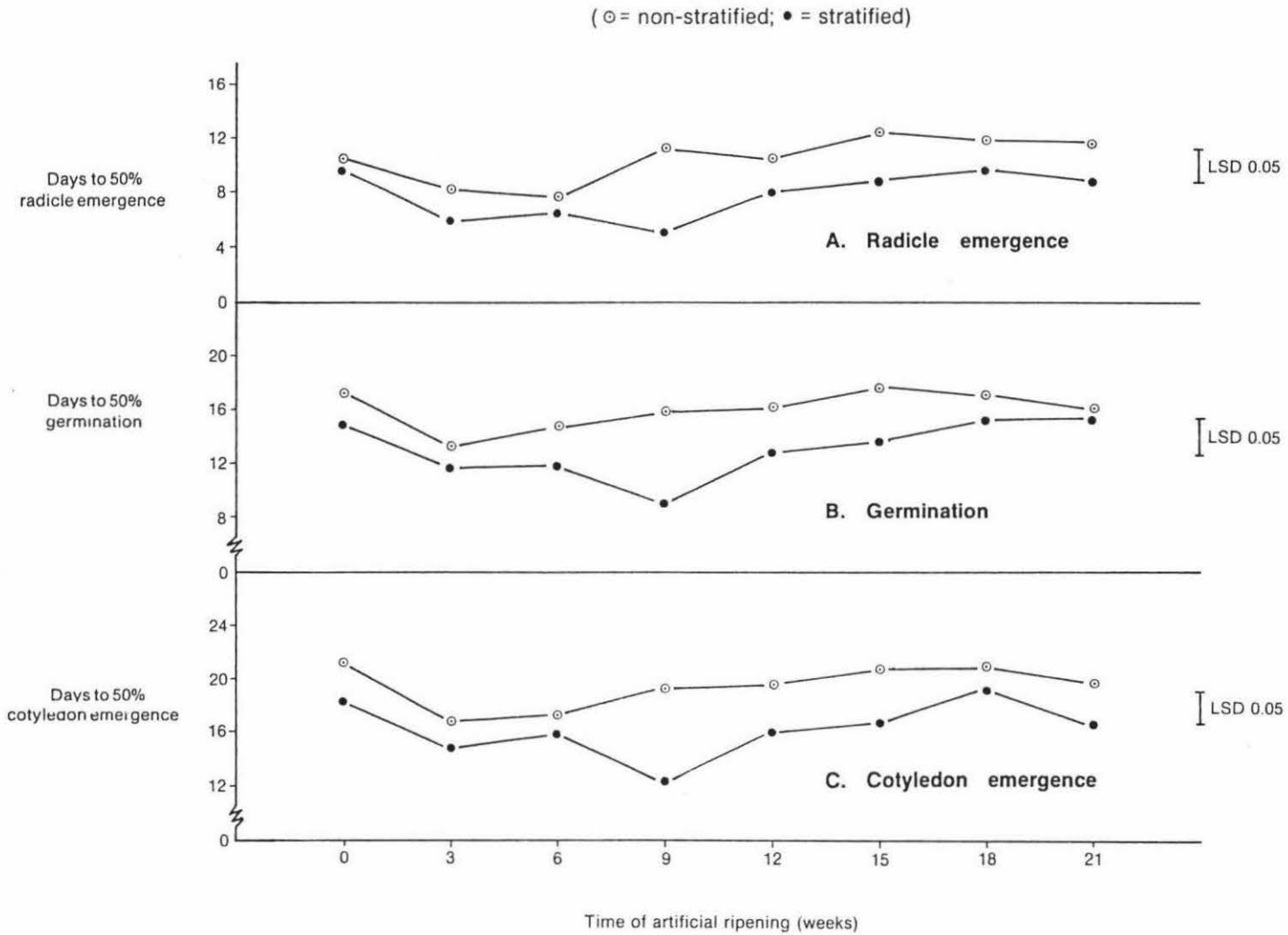


Fig. 5.11 b **Changes in vigour of artificially ripened seeds calculated as times to 50% radicle emergence, germination or cotyledon emergence in relation to time of ripening and stratification.** The cones were collected in MAY '86. LSD is calculated at a a probability level 0.05

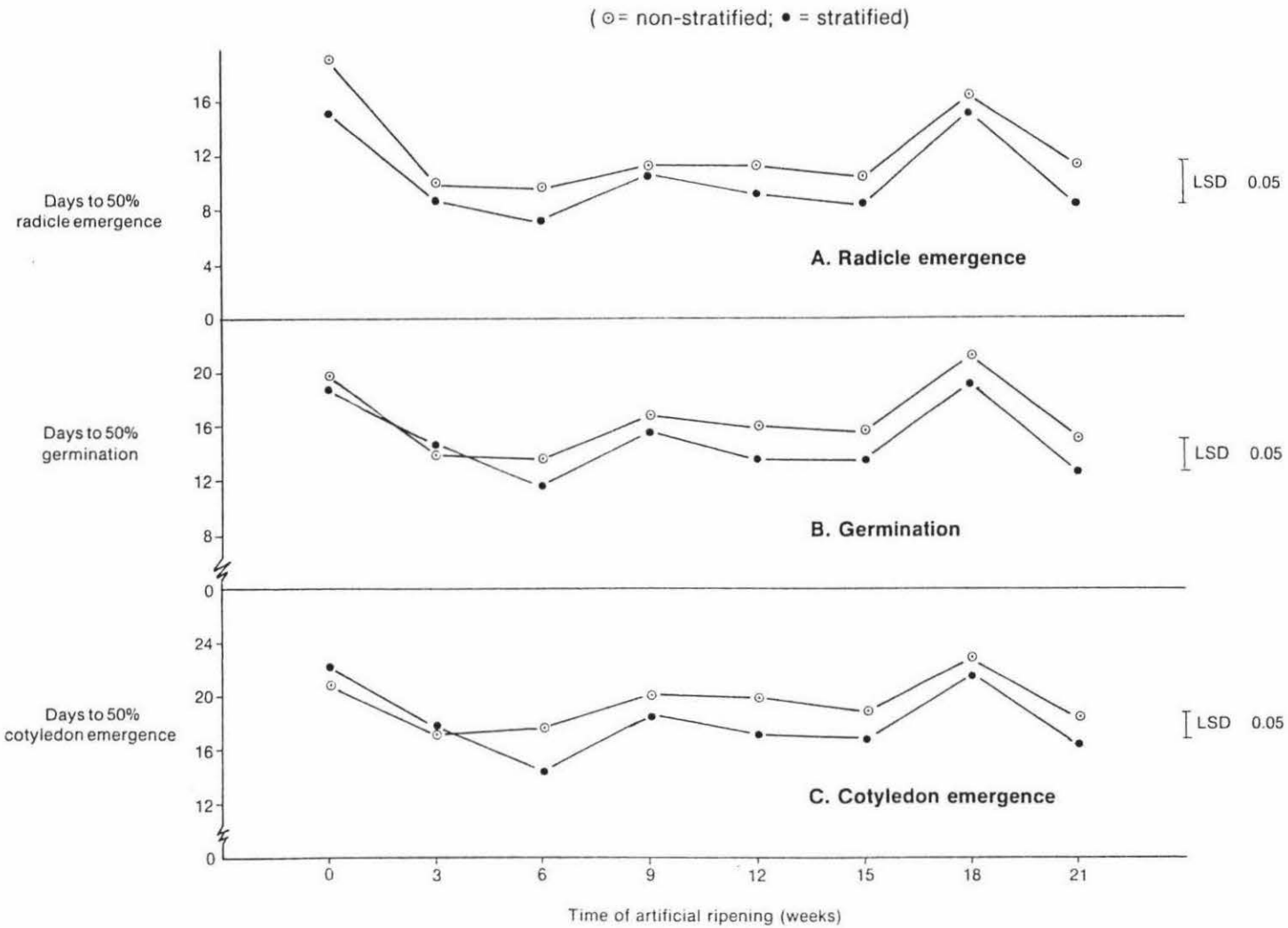


Fig. 5.11 c **Changes in vigour of artificially ripened seeds calculated as times to 50% radicle emergence, germination and cotyledon emergence in relation to time of ripening and stratification.** The cones were collected in JUNE '86. LSD is calculated at a probability level 0.05

(⊙ = non-stratified; • = stratified)

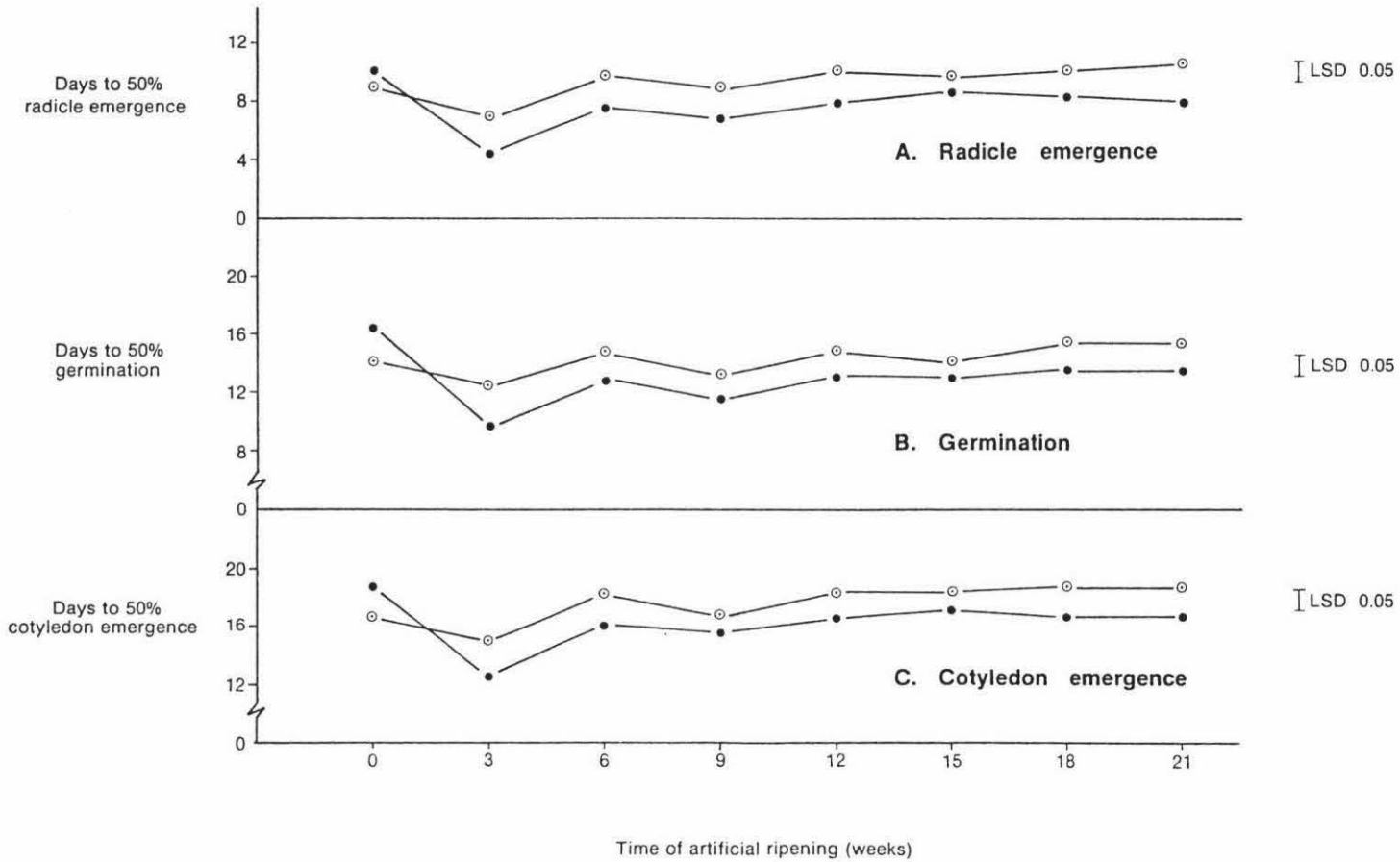
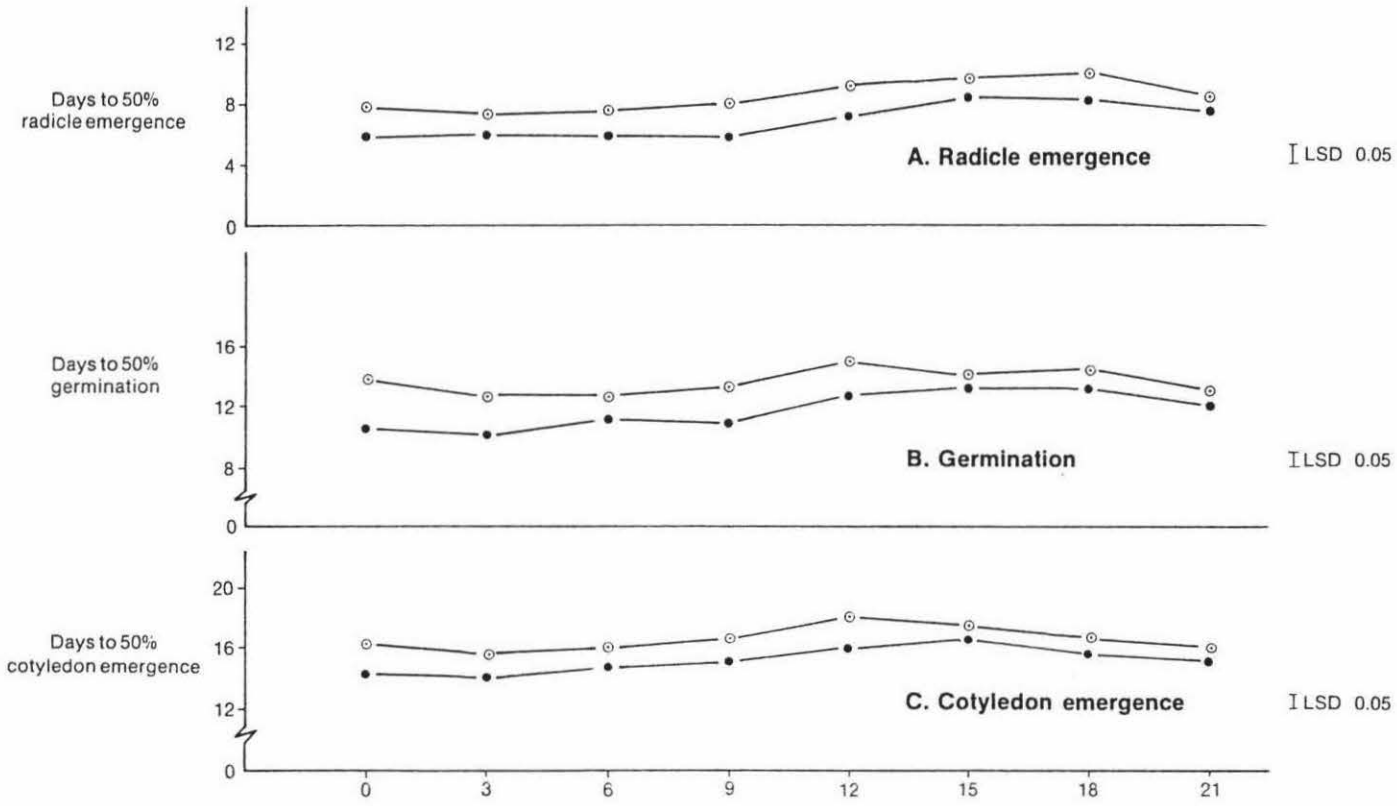


Fig. 5.11 d **Changes in vigour of artificially ripened seeds calculated as times to 50% radicle emergence, germination and cotyledon emergence in relation to time of ripening and stratification.** The cones were collected in JULY '86. LSD is calculated at a probability level 0.05

(⊙ = non-stratified; • = stratified)



Despite significant effects of both artificial ripening and stratification on seed vigour, the analyses show that there were no significant interactions between the two factors, although any interactions could have been masked by high variations within replicates. The results irrespective of stratification effects are presented on Table 5.4. Contrary to the effect of stratification on non-stored seeds, artificially ripened seeds responded very well to stratification. In all tested samples, with ploting over storage times, the statistical analyses clearly demonstrated the enhancement of seed vigour by stratification (Table 5.5).

Table 5.4: Mean T_{50} values (days) of radicle emergence, germination and cotyledon emergence of artificially ripened seeds collected in April, May, June and July in relation to storage periods (data pooled over stratification treatments).

	Storage Periods								LSD
	0	3	6	9	12	15	18	21	
A. **April**									
Radicle emergence	10.0	7.0	7.5	7.9	9.1	10.5	10.8	10.2	2.3
Germination	16.0	12.5	13.2	12.3	14.3	15.7	16.1	14.8	2.6
Cotyledon emergence	19.8	15.8	16.5	15.8	17.8	18.7	20.1	18.1	2.4
B. **May**									
Radicle emergence	17.2	9.2	8.4	10.9	10.1	9.3	15.9	9.8	3.1
Germination	19.3	14.1	12.7	16.4	14.7	14.6	20.3	14.0	2.3
Cotyledon emergence	21.5	17.3	16.1	19.3	18.4	17.8	22.3	17.3	2.1
C. **June**									
Radicle emergence	9.3	5.5	8.5	7.8	8.9	9.1	9.0	9.2	1.3
Germination	15.2	10.9	14.2	12.5	13.9	14.1	14.6	14.5	1.4
Cotyledon emergence	17.6	13.6	17.1	16.2	17.3	17.8	17.7	17.9	1.5
D. **July**									
Radicle emergence	6.8	6.7	6.7	7.0	8.4	9.9	8.6	8.1	1.4
Germination	12.3	11.4	12.1	12.1	13.9	14.6	13.7	12.8	1.1
Cotyledon emergence	15.2	14.8	15.4	16.0	17.2	17.9	16.3	15.7	1.1

LSD = least significant difference at $p \leq 0.05$ level.

Table 5.5: Mean T_{50} values (days) of radicle emergence, germination and cotyledon emergence of artificially ripened seeds collected in April, May, June and July in relation to stratification (data pooled over storage times).

	Stratification	
	Non-strat.	Strat.
A. **April**		
Radicle emergence	10.4^{b}	7.8^{a}
Germination	15.9^{b}	12.8^{a}
Cotyledon emergence	19.5^{b}	16.2^{a}
B. **May**		
Radicle emergence	12.4^{b}	10.3^{a}
Germination	16.6^{b}	15.0^{a}
Cotyledon emergence	19.4^{b}	18.1^{a}
C. **June**		
Radicle emergence	9.2^{b}	7.6^{a}
Germination	14.5^{b}	13.0^{a}
Cotyledon emergence	17.6^{b}	16.3^{a}
D. **July**		
Radicle emergence	8.4^{b}	7.1^{a}
Germination	13.7^{a}	15.4^{b}
Cotyledon emergence	16.8^{b}	15.4^{a}

Means within rows followed by the same letter are not significantly different at $p \leq 0.05$ level.

5.2.6 The effect of storage conditions and kilning extraction on germinability

Data on germinability was obtained from seeds extracted by kilning after six weeks storage onwards, since only these cones responded to this method of extraction. Table 5.6 illustrates the mean germination of seeds artificially ripened under different conditions, both extracted by kilning. The germinability was essentially parallel with the pattern of hot-water extracted seeds. Artificial ripening of March-collected samples had little improvement in the germination with storage, and prolonged storage seemed to weaken it. In the later collections, the artificially ripened samples showed a much better improvement in germination, and during storage it remained high.

Split-plot analyses of some of the data in Table 5.6 provides evidence that the artificial ripening condition had no effect on the final germination. Seeds artificially ripened at 20°C and ambient temperature were both highly germinable (Appendix 2).

Table 5.6: Changes in germination percentage of artificially ripened seeds stored under different conditions and extracted by the kilning method. Individual S.E.'s shown.

A. Non-Stratified

Storage Periods	Collection Date: March 20°C	March Ambient	April 20°C	April Ambient	May 20°C	May Ambient	June 20°C	June Ambient
0	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
6	27 (±11.8)	45 (±14.6)	93 (± 6.7)	97 (± 1.9)	96 (± 4.0)	89 (± 4.8)	-	96 (± 2.8)
9	18 (±17.7)	-	86 (± 5.3)	93 (± 3.4)	100 (± 0.0)	92 (± 2.3)	98 (± 1.4)	91 (± 5.3)
12	44 (±13.0)	-	95 (± 2.6)	96 (± 2.3)	82 (± 7.0)	85 (± 6.0)	91 (± 9.0)	91 (± 4.4)
15	3 (± 2.7)	7 (± 1.4)	64 (±32.1)	84 (± 7.1)	96 (± 4.0)	98 (± 1.4)	84 (±10.4)	95 (± 3.5)
18	-	-	93 (± 1.9)	93 (± 4.8)	98 (± 2.0)	66 (±23.4)	99 (± 1.0)	95 (± 2.7)
21	-	-	94 (± 1.1)	93 (± 1.9)	83 (±10.4)	95 (± 3.5)	-	-

continued...

B. Stratified

Storage Periods	March 20°C	March Ambient	April 20°C	April Ambient	May 20°C	May Ambient	June 20°C	June Ambient
	Collection Date							
0	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
6	36 (±12.8)	63 (±17.5)	95 (± 3.5)	87 (± 4.7)	91 (± 5.8)	79 (±18.7)	-	97 (± 1.9)
9	28 (± 2.5)	-	81 (± 3.4)	94 (± 3.5)	100 (± 0.0)	93 (± 3.5)	97 (± 2.7)	89 (± 5.9)
12	55 (±11.7)	-	99 (± 1.0)	99 (± 1.0)	89 (± 5.7)	89 (± 9.6)	98 (± 2.0)	96 (± 2.8)
15	-	-	65 (±32.7)	84 (± 8.5)	100 (± 0.0)	100 (± 0.0)	90 (±10.0)	97 (± 3.0)
18	-	-	93 (± 3.4)	96 (± 2.3)	95 (± 5.0)	65 (±18.8)	100 (± 0.0)	97 (± 3.0)
21	-	-	95 (± 1.9)	100 (± 0.0)	88 (± 8.3)	100 (± 0.0)		

5.3 The rate of respiration

The respiratory test was conducted in an attempt to investigate any differences in respiration between immature and mature seeds. Measurements were concerned with seeds during imbibition prior to germination. The results are presented in Table 5.7. The rate of oxygen uptake (QO_2) at twenty four hours after imbibition (HAI) varied depending upon the stage of maturity. Immature seeds collected in March, April and May clearly have higher QO_2 than the more developed seeds. The average respiratory quotient (RQ) ranges from 0.5 to 0.8; immature seeds tend to have lower RQs.

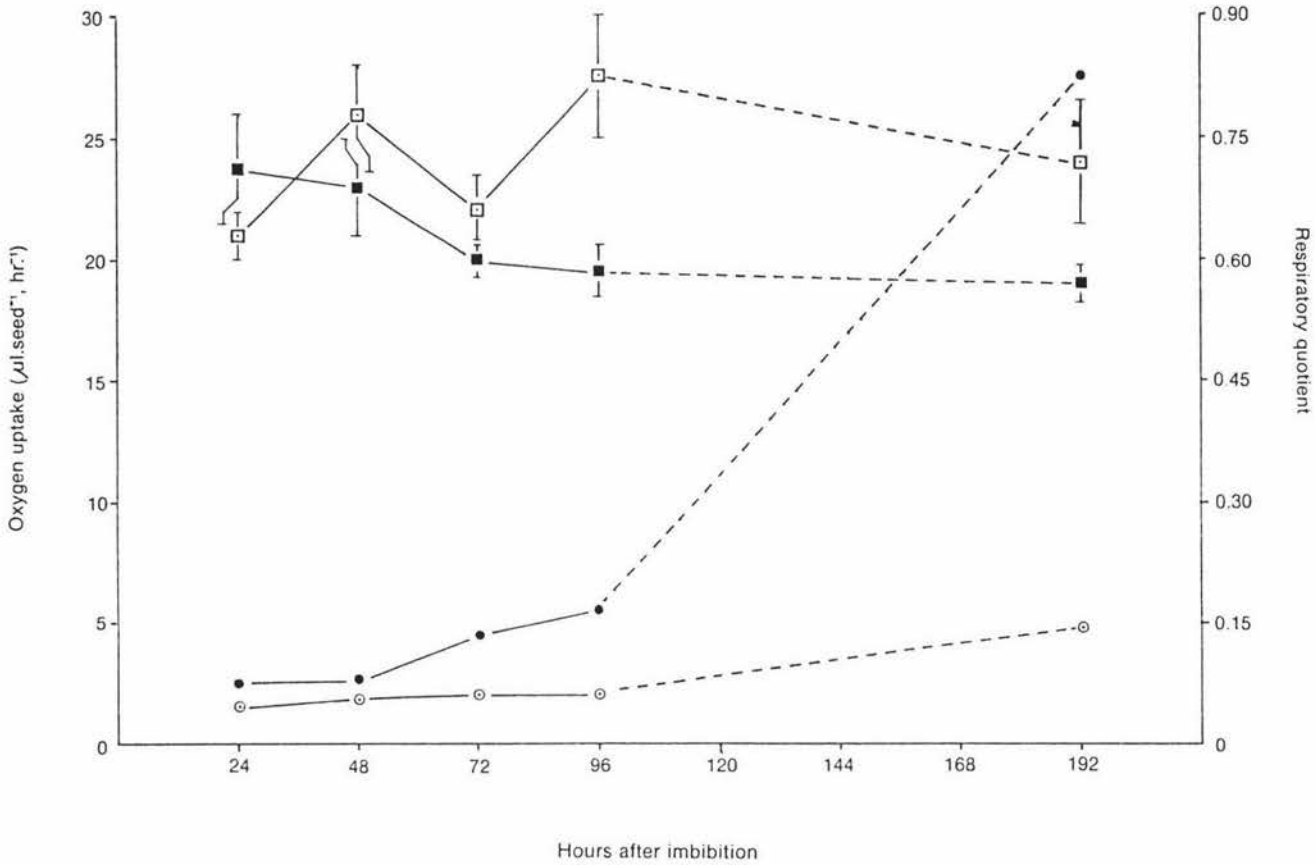
The role of seed coat in respiratory activity was studied on mature seeds harvested in November 1985. In general, the average QO_2 of intact seeds was much lower than the decoated seeds throughout the measurement (Fig. 5.12). Substantial differences were recorded at 192 HAI when 85% of the decoated seeds emerged their radicle compared to 22% of the intact seeds.

Table 5.7: Changes in oxygen uptake (QO_2) and respiratory quotient (RQ) during imbibition of seeds collected at different maturity. Figure in brackets are standard errors of single errors.

Date of collection	Hours after imbibition (HAI)							
	24		48		144		192	
	QO_2	RQ	QO_2	RQ	QO_2	RQ	QO_2	RQ
10. 9.'85	1.70 (±0.10)	0.60 (±0.01)	1.20 (±0.04)	0.60 (±0.02)	-	-	-	-
29.11.'85	1.80 (±0.03)	0.80 (±0.01)	1.90 (±0.05)	0.80 (±0.02)	-	-	4.30 (±0.08)	0.72 (±0.01)
14. 3.'86	4.18 (±0.06)	0.75 (±0.02)	-	-	-	-	-	-
24. 4.'86	3.42 (±0.01)	0.52 (±0.04)	-	-	-	-	-	-
20. 5.'86	3.29 (±0.08)	0.49 (±0.02)	1.32 (±0.03)	0.73 (±0.01)	2.43 (±0.20)	0.74 (±0.16)	-	-
28. 7.'86	1.47 (±0.03)	0.77 (±0.01)	1.65 (±0.10)	0.78 (±0.01)	3.39 (±0.20)	0.73 (±0.12)	-	-
16. 9.'86	1.83 (±0.03)	0.52 (±0.04)	2.12 (±0.14)	0.55 (±0.02)	-	-	-	-

Fig. 5.12 Changes in oxygen uptake (QO_2) and respiratory quotient (RQ) of intact and decoated seeds collected in November '86. Standard errors are shown where larger than the symbols used.

(⊙ = QO_2 intact; • = QO_2 decoated; ⊡ = RQ intact; ■ = RQ decoated)



5.4 Protein Changes During Seed Development

A preliminary study attempting to examine some biochemical, especially protein changes occuring during seed development and maturation was carried out. Five samples of tree-ripened seeds collected in April, May, June, July and September plus one sample of artificially ripened seeds for twenty one weeks collected in April were used. The results show no appreciable differences in the electrophoretic mobility or the relative intensity of the protein bands between samples. As illustrated in Plate 5.4] there are four major protein bands found in all samples. The molecular weight of each band is presented in Figure 5.13.

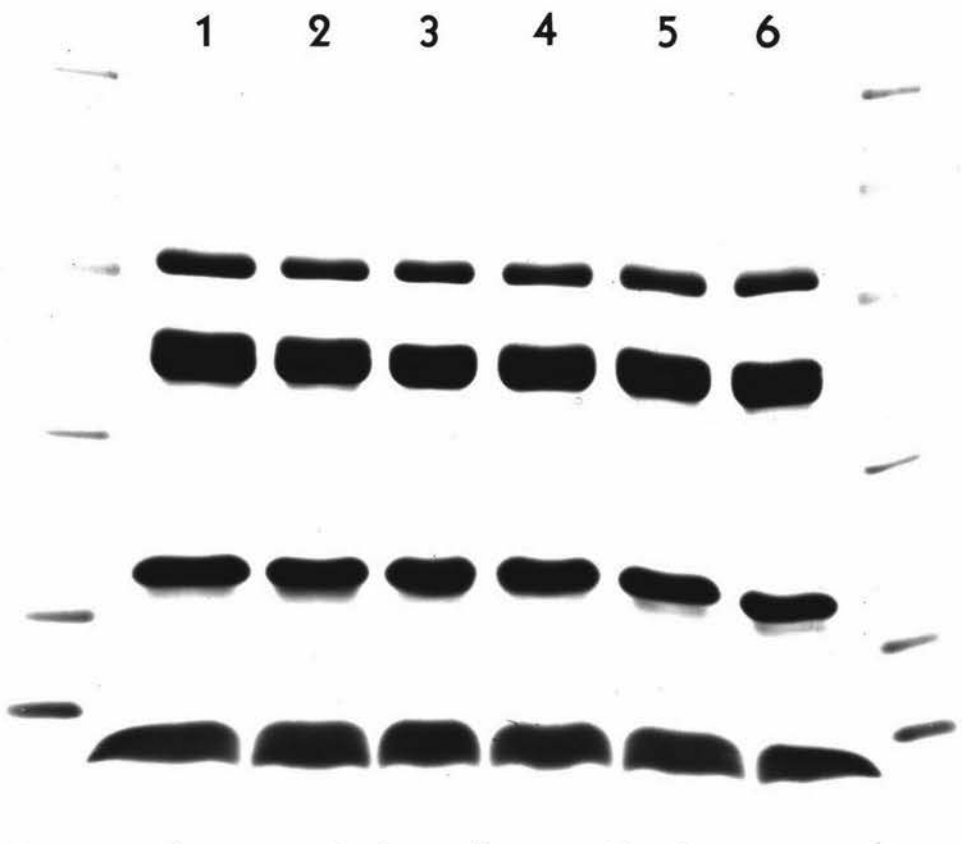
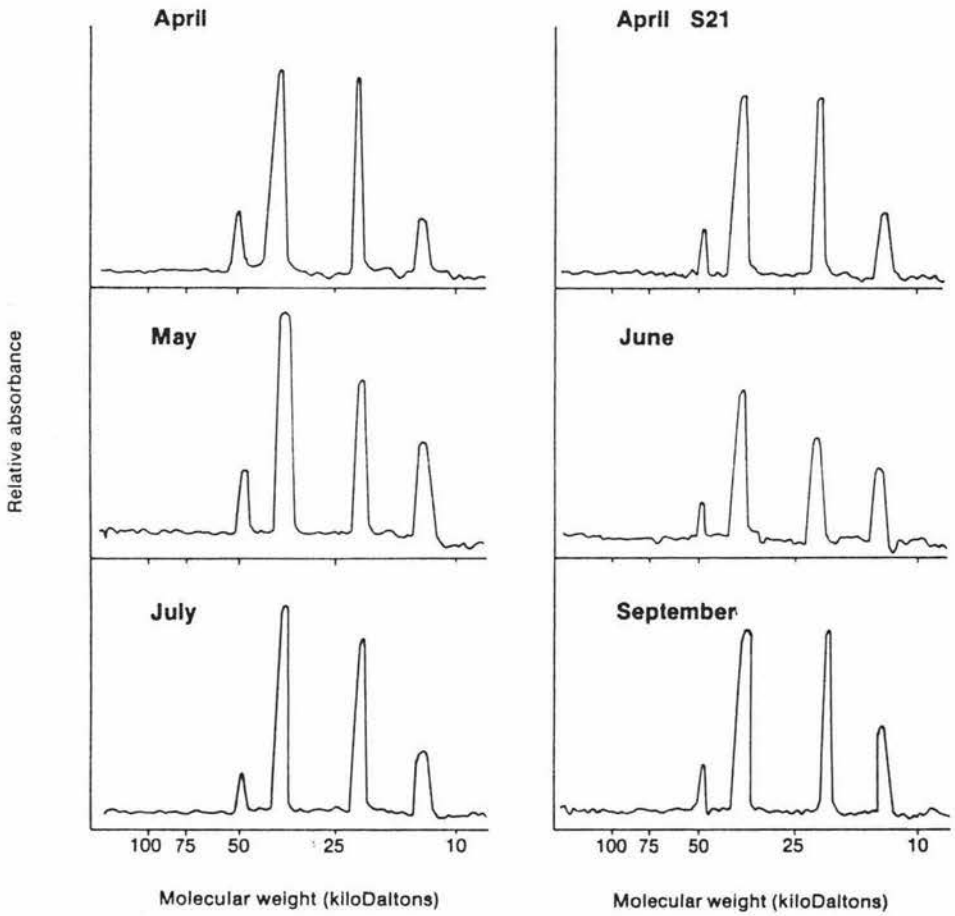


Plate 5.4 SDS-polyacrylamide gel patterns of P. radiata seed proteins extracted from non-stored cones of different maturity and from cones artificially ripened for 21 weeks (S_{21}) collected in April. 1 - April NS; 2 - April S_{21}; 3 - May NS; 4 - June NS; 5 - July NS; 6 - September NS (NS = non-stored cones).

Fig. 5.13 **Densitometer scans of *Pinus radiata* seed proteins extracted from cones of different maturity and from cones artificially ripened for 21 weeks (S21) collected in April.**



6. DISCUSSION

6.1 Introduction

The object of this chapter is to synthesize all of the results which have been presented or cited in the previous chapters. To meet this objective the discussion is broken down into two parts. In the first part, matters related to cone and seed development will be considered. This part is regarded as crucial for further discussion since information and knowledge in this particular area will have an important impact on the artificial ripening study. Accordingly, it is considered important to formulate some questions to be tackled in the discussion:

a) Does the sequence of cone development correspond with the sequence of seed development (with particular reference to the later part of the second season of development)?

b) How far can cone maturity indices be utilized to predict the state of seed development?

c) With what features of seed development can seed germinability be associated?

Information on the sequence of cone and seed development will provide an insight into the relationship between cone and seed. This will eventually be a useful tool to determine whether or not cone maturity indices can be used to indicate seed maturity. As more and more studies are revealing, the mechanism of the onset of germinability during seed development is complex: it becomes obvious that the acquisition of germination cannot be attributed to a single factor (Evans et al., 1975; King, 1976; Kermode and Bewley, 1985a, 1985b; Kermode et al., 1985a, 1985b; Finkelstein and Crouch, 1986).

The second part of this chapter will focus on artificial ripening. Some essential questions will be discussed in this part,

i.e:

a) When is the most appropriate time for cone collection to commence?

b) What is the optimal time for artificial ripening, and how does it vary according to environment?

c) Do physiological changes during artificial ripening parallel with changes during natural ripening?

d) How can artificial ripening of P. radiata seed be applied to commercial practice?

Prior to these, however, the results of the preliminary study will be discussed in the following section.

6.2 Preliminary Study

Earlier it was mentioned (section 3.3) that the starting point of this study was the brief report by Wilcox and Firth (1980) that prematurely collected seeds will undergo further maturation in the cone if the cone is stored under suitable conditions. This study attempted to investigate further some of the aspects of artificial ripening mentioned in Wilcox and Firth's report. Thus this discussion will concentrate on a few areas such as:

1. What is the level of seed maturity when collection begins in July?

2. Whether or not the artificial ripening conditions adopted for this study are optimal.

3. Whether or not the technique could be applied equally well to both OP and CP cones.

6.2.1 Seed maturity

Changes in germination during seed development have not been studied intensively in many conifer species and there is very little data on P. radiata. The present study has shown that P. radiata seeds are capable of germinating much earlier than generally thought. Freshly extracted seeds from cones collected on July 18 have a germination capacity comparable to the naturally ripened seeds collected in November. The germination capacity of OP and CP seeds was 97±0.9% and 90±0.7% respectively when the seed moisture content was relatively high, 22.4±0.01% and 21.8±0.01% for OP and CP respectively. Morphologically, the seeds were fully mature as both the embryo and the megagametophyte occupied their cavities fully. Since the germination was high, artificial ripening had little scope to improve on those figures (Fig. 4.2).

The onset of germination in developing seeds has been widely investigated in many crop species. It is found that seeds of many species are capable of germinating long before physiological maturity (maximum dry weight). For instance, Grabe (1965) reported that smooth bromegrass seeds are able to germinate when harvested only a few days after fertilization. In most species, however, early (or precocious) germination yields low-vigour seedlings (Pollock and Roos, 1972). In P. lambertiana seeds, Krugman (1966) suggested that germinability begins only weeks before final maturity. None of the seeds collected through the end of July (summer) germinated, but some of the seeds collected during the second week in August did. Good germination was found in seeds collected during September, ranging from 78 to 95%. This coincided with the natural ripening which occurred during September and October. Similar patterns of germination are found in developing Douglas-fir (Silen, 1958; Rediske, 1961), and in P. elliottii (Barnett, 1976).

Indices of cone ripeness have been investigated in an attempt to establish the relationship between cone ripeness and seed maturity, i.e. germinability. Cone characteristics such as specific gravity, moisture content and colour are widely used for

many species (Maki, 1940; Fowells, 1949; Wakeley, 1954; Franklin, 1965; Arisman, 1986). Green cones are regarded as immature, and the colour will turn brown when mature. Cones with a specific gravity below 0.9 and moisture content of less than 50% are generally mature (Edwards, 1980). Such maturity indices are particularly critical for non-serotinous cones since seed dispersal occurs only a few weeks after cone maturity.

Parameters of cone maturity discussed above appear to have little meaning for P. radiata seed. It is obvious that although the cones were completely green and the specific gravity was above 1, the seeds were highly germinable in July, and this persisted until November when the cones have turned to brown. From the viewpoint of cone harvesting, this discovery could be advantageous since collections need not then be confined to a very limited period. Earlier collection can be justified provided that the cones are properly handled. How early collection can be commenced was a matter for further investigation in the experiments covered in Chapter 5 and section 6.5.

6.2.2 The effect of artificial ripening on seed quality

The fact that the seeds collected on July 18 were highly germinable has lessened the importance of artificial ripening in improving seed germination. The results clearly showed that no appreciable increase in germination occurred during the study period (Fig. 4.2). Instead, the quality of seed progressively declined as the storage progressed. In view of the storage conditions, it is clear that the deterioration should have been inflicted by the storage temperature alone since seed moisture content was below 10%. The analysis showed that seed deterioration was magnified by high temperatures as the germination of seed artificially ripened at 30°C was significantly lower than those at 20°C. Under normal circumstances, most conifer seeds will retain full vitality for years if stored at temperatures as low as 5°C with seed moisture content reduced to below 10% (Crocker and Barton, 1953).

The expression of vigour, which was calculated as T50 of cotyledon emergence, reinforces the suggestion that deterioration is taking place during cone storage (Fig. 4.4). The seeds have become less vigorous as the storage continued. Once again the effect of temperature was very significant. Similar results on seed vigour were found in germination tests under the stress conditions of the Inventum table (Fig. 4.3). The use of the Inventum table is common in germination testing, but in this study the Inventum table was used under ambient conditions and imposed water stress on the germinating seeds. A large proportion of the seeds were able to extrude their radicles but failed to reach the standard radicle length as advocated by ISTA (1976). This happened because the way the radicles grew tended to lift them off the substratum, resulting in drying out due to insufficient humidity above the germination paper.

As to the response of pollination techniques to artificial ripening, there is no evidence to suggest that OP seeds are superior to CP seeds. Both categories had high initial germination, which declined following storage. The fact that the germinability of OP seeds was often slightly higher than CP seeds in many instances seems to reflect sampling effects, because the differences were not statistically significant. The only instance of significant differences between OP and CP seeds was found in germination tests on the Inventum table of seeds which had been artificially ripened at 20°C. In this case the germination percentage of OP seeds was significantly higher than that of CP seeds. This difference may be the result of a rare sampling effect since there are no differences according to pollination technique when seeds are artificially ripened at 30°C.

In summary, this study revealed the following aspects:

1. The acquisition of germinability during seed development takes place several months prior to natural ripening. Accordingly, to study the effect of artificial ripening on the quality of P. radiata seed, cone collections should be carried out before July.

2. High temperatures for artificial ripening, i.e. 30°C, appeared to have more potential threat to seed quality than low temperatures, i.e. 20°C. Thus, cone storage at 30°C should be omitted from future studies.

3. Both OP and CP seeds responded similarly to the treatments. Neither category could resist deterioration better than the other.

On the basis of these results the major study on artificial ripening of OP cones collected on a range of date through the second season of development in 1986 was conducted. These results, presented in Chapter 5, will be discussed in the following sections.

6.3 Cone Development

6.3.1 Fresh weight, dry weight and moisture changes

The development of cones and seeds generally spans a period of more than a year. In the case of P. radiata, it covers around 28 months starting off from female strobili initiation until seed maturation. Broadly, the development may be divided into two principle phases; the first phase begins from initiation to just before fertilization, and the second phase covers a period from fertilization to maturation (Bramlett et al., 1976). The present study investigated the latter.

Cone development exhibits a pattern of steady increase in dry weight until it reaches a maximum at which point the developmental process is taken as complete. As illustrated in Figure 5.1, cone dry weight steadily increased from March to June. (assuming the May minimum to be a sampling effect). There is, therefore, reason to suggest that cones continued to accumulate food reserves until June. As the cones accumulated reserves, dry weight increased faster than moisture loss, resulting a relatively constant fresh weight. Apart from increments of dry weight, the cones apparently

grew bigger during March to June. Data on the volumetric dimensions of cones show that the maximum size was attained in June. These results are consistent with an earlier study by Sweet and Bollmann (1971) on the seasonal development of P. radiata cones which showed a steady increase in dry weight which was parallel to the increase in size.

During the later part of the second phase of development, the cones lost moisture (Fig. 5.2). Desiccation took place throughout the study period. In P. resinosa cones, grown in central Wisconsin, Dickmann and Kozlowski (1969a) reported that the time of maximum water content (mid-June) coincides with the period when cone reaches maximum size, while dry weight is still increasing. Thereafter, percentage of moisture content began to decrease rapidly until the cones ripened. Cone maximum dry weight was reached in early August. The pattern of events observed here for P. radiata cones (Figs. 5.1; 5.2) is not the same. Moisture content decreased from March onwards, reflecting both dehydration and dry weight increment. In Pseudotsuga menziesii cones collected in Oregon, Ching and Ching (1962) found that moisture content increased until the end of July.

6.3.2 Dimensional changes

The pattern of dimensional changes was examined through interrelationships between variables such as cone moisture content, fresh and dry weight and volume. The pattern of moisture reduction does not seem to tally with the reduction of specific gravity which was determined on the weight-volume basis. As illustrated in Figure 5.2 the specific gravity increased between March and May. This apparent contradiction is attributed to the increase in seed dry weight during that period. Cone maximum dry weight was attained in June, but the specific gravity decreased during the May - June interval owing to a greater proportional increase in volume. From June onwards, cone moisture reduction was essentially parallel to the decrease in specific gravity.

Volumetric changes from tree-ripened cones suggest that from June onwards the volume size of cones progressively decreased, indicative of tissue shrinkage (Appendix 3). Dickmann and Kozlowski (1969a) observed diurnal shrinkage in the developing P.resinosa cone which was attributed to dehydration but turgor would probably be involved in this process. Harlow et al. (1964) reported that a pine cone scale consists of two zones. Dorsally, the cells were principally wood fibres extending from the cone axis; ventrally there is a band of tissue consisting of short rectangular thick-walled cells. Depending on the species, longitudinal shrinkage of this tissue varies from 10 to 36%. Shrinkage of the fibrous portion is negligible. The shrinkage of wood was generally attributed to the removal of water from the inter-microfibril zones of the cell walls and from the amorphous (noncrystalline) regions within the microfibril. The loss of free water from the cell lumens did not affect cell dimensions, however: it was the removal of bound water (below the fiber saturation point) from the normally three-layered secondary wall which initiated shrinkage.

6.3.3 Maturation

As the cones approached ripening, gradual changes in other variables also took place. The dehydration process continued as cone moisture content and specific gravity dropped to around 40% (from 50% in March) and 0.98 respectively in September. The specific gravity measured is comparable to that of equivalent cones from the previous year, which ranged between 0.96 to 0.99 (Fig. 5.2). It is important to note the relative reduction in cone moisture content, this remaining high at the end of the study period. The high moisture content when the cone has reached maximum dry weight probably corresponds with the need for mobilization of reserves to the seed. As Ching and Fang (1963) pointed out, a high moisture content within the cone scales is required for the mobilisation of nutrients to the developing seeds. Therefore cone moisture loss proceeded at a rate slower than those of the seed. The results of the present study confirms their observations (Figs 5.2 and 5.6).

Some change in colour accompanied dehydration of P. radiata cones. During the early part of the second year the cone was totally green, but changes started in September. It was observed that the intensity and rapidity of colour change varied among clones. Unlike the other parameters, i.e. dry weight, cone colour changes are not a reliable criterion in judging the stage of development. It was found in this study that the cone was still completely green in June/July when full cone dry weight had been attained.

Thus, it is perhaps possible to summarize the sequence of P. radiata cone development during the later part of the second phase with an overview concerning maturity indices. Following fertilization late in the previous calendar year rapid mobilization of metabolites essential for growth occurs and cone size increases rapidly (Sweet and Bollmann, 1971). As development proceeds, the rate of metabolism eases and during the period from March to June only a gradual increase in dry weight and size takes place. These both reach a maximum in June. As these events proceed, water has slowly moved out of the cone. However, although the cone loses moisture, the specific gravity increases because the percentage of dry weight increase is proportionally higher than the percentage of moisture loss. A change in cone colour does not happen until around September when the green cone begins to turn progressively to nutmeg brown. Accordingly, cone dry weight and cone size can be adopted as criteria suitable to determine the stage of cone development, while colour and specific gravity can be discounted.

6.4 Seed Development

6.4.1 Physiological and morphological development in relation to germinability

The development of P. radiata seed exhibited the typical features of seed development. Moisture reduction took place during the entire period of the study with rapid loss occurred between March and June. Moisture loss of the seed obviously occurs ahead

of that of the cone (Figs 5.2 and 5.6). Although the seed lost moisture, seed fresh weight remained relatively constant during that period owing to increasing dry weight.

The dependence of seeds on cones for food reserves during development has been revealed by a number of studies (Ching and Ching, 1962; Ching and Fang, 1963; Dickmann and Kozlowski, 1969b). Generally, it was concluded that the decrease in reserve materials of cones is associated with mobilization of these materials by developing seeds. Therefore there is a good reason to suggest that cones should not be detached from the parent tree before sufficient nutrients are accumulated, or otherwise the seed will be unable to complete the development process. However, when that time is reached, or when the cones are no longer dependent upon the parent plant to supply nutrients to the seeds, is not known.

With respect to changes in P. radiata cone moisture content and dry weight in this study, it can be assumed that mobilization of food reserves from cone scales to the seed had ceased by September, when the moisture content of both the cone and the seed was low. As already noted, cone dry weight itself appears to reach its maximum in June.

Morphologically, a mature pine seed contains a fully developed embryo and megagametophyte, and a hard black seed coat (Edwards, 1980). A seed is considered immature when its embryo occupies only a small portion of the embryo cavity and its megagametophyte is underdeveloped. X-ray photography provided a series of pictures of the morphological development of P. radiata seed. Apparently, embryo development is completed earlier than that of the megagametophyte. The embryo of seed collected in March and April had not occupied the whole embryo cavity and the megagametophyte left a vacant periphery within the seed coat. Further development occurred in May where the embryo had occupied almost the entire cavity and the space between megagametophyte and seed coat was narrower. From June onwards morphological development was considered as complete. Mature embryo length varied according to seed size and shape, e.g., one clone had long narrow seeds while another was characterized by short rounded seeds.

The seed coat can be useful for maturation assessment. Analyses on the percentage of embryo and megagametophyte dry weight relative to total seed dry weight indicate that the seed coat developed at the same rate as the rest of the seed, as the percentage remained constant throughout the period of observation (Table 5.1). During this time its physical appearance underwent colour changes from brown to dark brown to spotted black. As far as embryo and megagametophyte development are concerned, seed coat colour may provide a useful index of the stage of development since a large proportion of seeds collected in June onwards (and showing good germinability, Figure 5.8) had black seed coats. However, this index is rather subjective and it may vary depending upon external factors unrelated to maturity, since during the preliminary study in 1985 it was found that seed coat colour and seed size varied among different clones. Thus more information and experience will be needed. In a study on P. elliottii in Australia, Bevege (1965) found that in the seed collected on 14 January the seed coat was fully developed, though very pale in colour, but the megagametophyte filled only 70% of the seed. Experience at the University of New Brunswick with seeds of P. banksiana, P. resinosa and P. sylvestris has shown that mature full seeds are dark brown/black, whereas empty seeds remain light in colour (Arisman, 1984). Such charactertistics was also found in these P. radiata seed but again there were differences due to clonal variations.

The chronological changes in germination of P. radiata seed demonstrated the acquisition of germination capacity during the developmental process. With regard to embryo and megagametophyte development, reserves accumulation and moisture changes, the acquisition of germinability can be described as follows: None of the seeds collected in March were able to germinate coinciding with the fact that both the embryo and megagametophyte had not fully developed the seed dry weight being low and the water content being high. As the season progressed, morphological development and

mobilization of reserves continued, as did the moisture loss. Little improvement in germination was observed during April even though the embryo and the megagametophyte were more developed than in the previous sample, and the moisture content slightly lower. In May, the moisture content rapidly dropped from 38% in the previous month to 20%. However, this development had little effect on germination. By this time morphological development had almost been completed. The seed's nearly full capacity of germination was acquired in June when the germination percentage was around 90%. At this time, the embryo and the megagametophyte were fully developed, but interestingly, the seed moisture content was not significantly different from that in May. From this point no further significant changes were observed either in germination or moisture content.

The importance of the embryo and the megagametophyte development in connection with seed germinability has been put forward by several authors (Finnis, 1950; Ching and Ching, 1962; Katsuta, 1975; Edwards, 1980). Muller-Olsen et al. (1956) found a good correlation between the stage of development of these organs with germinability in Picea abies. They suggested that less developed megagametophytes produce lower germinability than well-developed ones. Seed germination was 36% when the embryo lengths was half of its cavity with well-developed tissue, and it dropped to 15% when the megagametophyte was still less developed. The germination improved when the embryo and the megagametophyte tissue developed further. Germination of 97% was found in seeds with the two organs fully developed. However, this relationship does not apply to seeds with a dormancy mechanism or after-ripening requirement. For instance, Bevege (1965) found that in P. elliottii seed low germination was obtained from fresh seeds with a fully developed embryo. Germination increased after the seeds were stored for four to six weeks.

The present study did not attempt to quantify the embryo and megagametophyte development, but the X-ray radiography suggests that during the normal development process, seed germinability of P. radiata can be correlated with the morphological development.

The rule-of-thumb will be that the more developed the embryo and the megagametophyte the better the germination.

The completion of food reserve accumulation within the seeds as marked by maximum dry weight has been associated with physiological maturity (Harrington, 1972). It is often said that seed development ceases when seeds have reached physiological maturity. Even so, full germination is not always found at physiological maturity. In many cases, maximum germination occurs prior to maximum dry weight being achieved (Adams and Rinne in legumes, 1981; Dasgupta and Bewley in cereals, 1982). On the other hand, some seeds remain ungerminable at physiological maturity owing to dormancy. The current study is unable to determine the relationship between maximum dry weight and germination acquisition. As shown in Figure 5.6 the point at which maximum seed dry weight is obtained could not be determined precisely, owing to high sampling variation. From the standpoint of dry weight increment therefore, it is difficult to ascertain when P. radiata seed is physiologically mature.

The need for respiration in germinating seed is obvious since it provides high energy compounds such as ATP which are required for the metabolic process. Thus, when net metabolic activity is related to growth, a correlation between the rate of respiration and subsequent germination can be expected. The object of this discussion is to examine the rate of respiration of imbibed seeds collected at different stages of maturity and to establish a correlation, if there is any, between respiration and germinability in P. radiata seed.

The results clearly show the difference in the rate of respiration between immature and mature P. radiata seed. The high oxygen uptake by seeds collected in March and April was observed during the first 24 hours of imbibition. As seeds matured the O_2 uptake during the same period of imbibition decreased accordingly. The correlation between respiration during this period and germinability appears to be negative. In this case the initially high O_2 uptake is not a manifestation of the metabolic status

within the seed. None of the seeds collected in March germinated. A similar situation was observed in other immature seeds collected in April and May. On the other hand more developed seeds collected in July, September and November which germinated successfully, showed low initial oxygen uptake. Removing the seed coats from mature (November 1985) seeds shows that the permeability of the seed coat to oxygen is very low in mature seeds and this is the factor limiting respiratory activity.

There was a significant difference in the oxygen uptake from the beginning of the study period which progessively increased (Fig. 5.12). In fact, the QO_2 value of intact seeds increased only 0.5 μl. seed $^{-1}$. hr^{-1} after 96 hours imbibition compared to 4 μl. seed $^{-1}$. hr^{-1} in decoated seeds during the same period. This result is consistent with earlier findings by Kozlowski and Gentile (1959) in P. strobus and by Hatano (1963) in P. densiflora and P. thumbergii seeds.

Apart from affecting the rate of respiration, removal of the seed coat also caused germination to occur more rapidly. After 192 hours imbibition, 80% of the decoated seeds had extruded their radicle compared to only 20% in the intact seeds. There are two plausible explanations for this. Firstly, the removal of seed coat has allowed increased respiration of seed tissues resulting in more rapid metabolism. Respiration, like imbibition, is considered to have a triphasic curve (Bewley and Black, 1985). One of the components of this curve is known as the lag phase which primarily involves with the preparation of the hydrated tissue for germination. During this phase respiration stabilizes or increases only slowly, hydration is completed and all pre-existing enzymes are activated. Bewley and Black (1985) suggested that removal of the testa from imbibed pea seeds diminishes the lag phase appreciably and thus results in early germination. The second possibility is that the seed coat has imposed mechanical restraint to the embryo which inhibits the rapid emergence of the radicle. Such a restraint is often considered as a factor contributing to imposed dormancy, such as in lettuce (Ikuma and Thimann, 1963; Pavlista and Haber, 1970).

The substrate for respiration during imbibition and subsequent germination comes from the food reserves of the seed. By measuring the respiratory quotient (RQ) information on the kind of substrate utilized in respiration can be obtained (Crocker and Barton, 1953; Kramer and Kozlowski, 1979). Opik (1980) suggested that a low RQ (below unity) is attributed to the conversion of lipid to carbohydrate since this conversion requires O_2 without any CO_2 evolution. The results shown on Table 5.8 and Figure 5.12 suggest that mostly lipid was oxidized during respiration. A similar result was reported by Barnett and Naylor (1970) in long leaf pine (P. palustris) in which the RQ values ranged from 0.5 to 0.9 during imbibition until germination. In slash pine (P. elliottii) the RQ increased to 1.2 during early imbibition and progressively decreased to reach 0.75 at radicle emergence.

Protein changes during tree-ripening and artificial ripening were examined by polyacrylamide gel electrophoresis (PAGE). The results clearly demonstrate that the protein complement of the seed remains unchanged. Irrespective of the treatment, i.e. tree-ripening or artificial ripening, there were four major protein bands with molecular weights of around 13.6, 21.7, 37.9 and 50.1 kiloDaltons respectively. These remain in approximately the same proportions. There was thus no evidence that appreciable amounts of new types of protein were synthesised during the second phase of development later than April, nor were there any major changes during artificial ripening.

Having gone through the discussion of seed development, it is perhaps possible to summarise the features of development which can be associated with germinability of P. radiata seeds. Undoubtedly, moisture status plays a very important role in seed development. High moisture levels signify a rapid mobilization of reserve materials when both fresh weight and dry weight increase. As the seeds enter their maturation phase reserve accumulation ceases and rapid dehydration commences. This rapid moisture loss is a signal for the cells which are synthesizing reserve materials necessary

for the maturing seeds. As Pfister (1967) reported rapid decreases in moisture content of Abies grandis seed during maturation was attributed to biochemical maturation of the seed, particularly the conversion from mobile to storage forms of food within the embryo and the megagametophyte. Similar claims were made by Rediske (1961) in Pseudotsuga menziesii and Dickmann and Kozlowski (1969b) in P. resinosa.

Under the natural development process of P. radiata seed such metabolic changes appear to have no single clearly defined indicator of acquisition of germinability. For instance, although the seed moisture content in May had dropped to a level similar to that in July/September, the germination percentage was much lower. Therefore, it is appropriate to suggest that the acquisition of germinability in P. radiata seed during development involves, apart from moisture level, several other factors. This question will be discussed further in relation to possible mechanisms of artificial ripening.

6.4.2 The stage of maturity and vigour

Initial seed quality is known to be affected by the level of maturation when the seed was collected. The seasonal changes in vigour of P. radiata seed significantly demonstrated the improvement of vigour in which mature seeds had the capacity to germinate faster than the less mature ones (Fig. 5.10). Seeds collected earlier in the season had lower vigour than those collected later. This result is in accord with the statement by Pollock and Roos (1972) that the more mature a seed is when harvested, the greater its vigour and its potential for establishment of a new seedling.

Apart from having low germination and low vigour, seeds collected in March, April and May also produced a high proportion of abnormalities. The big discrepancies between radicle emergence and germination in Figures 5.9.2a, 5.9.2b and 5.9.2c were indications of retarded radicle development. Some of the germinants also failed to reach cotyledon criterion due to the poor

condition of the seed which results in fungal contamination. This result reinforces earlier findings by Schubert (1956) that seedling abnormalities were common in immature seeds and occurred most frequently in fresh seed of western species of pine. Later, Krugman (1966) reported that immature seeds of P. lambertiana produced a lot of abnormal germinants with stunted radicles, reverse development (cotyledon emerging before the radicle), and/or persisting megagametophyte collars.

6.4.3 Indices of cone maturity in relation to seed development

Maturity indices are often integral part of the successful harvesting of conifer seed. The failure or success in pine cone harvesting can generally be attributed to the use of appropriate maturity indices. Physical indices of cone maturity such as cone colour and specific gravity have been employed as a guide to assess seed maturity. Generally, green cones are considered immature and the seeds extracted immediately after collection produce immature seeds, i.e. low germination. The results here show that cone colour changes occurred gradually, and somewhere during the transition from green to brown, the seed is already mature. In the P. radiata seeds studied here, germination improved from 0, 16, 28, 90 to 99% during the period from March to April, May, June and July respectively while the cones were still completely green. It is obvious, therefore, that the use of cone colour to assess the stage of seed maturity, i.e. germinability in P. radiata can be misleading. For the purpose of commercial harvesting, however, this finding offers flexibility in the planning and operation of cone collection.

The use of cone colour as an index of maturity in P. radiata seed is not as crucial as in most other conifer seed, owing to the serotinous nature of the cone. Unlike non-serotinous cones, P. radiata cone will remain closed for several months or even years after the colour has turned brown. Thus there is no real risk of losing germinable seeds by cone opening. On the other hand, if the mature cones remain on the tree for a considerable time the seeds will be vulnerable to deterioration since the post-November/December environment in southern-hemisphere will generally

be conducive to seed deterioration. Theoretically, seed storage commences at the time when the seeds have reached maximum dry weight. From this point, the seeds will undergo a largely irreversible process of deterioration, a process which is greatly influenced by temperature and humidity. Low temperatures and low moisture level are the two most important factors to maintain the physiological quality of seed during storage. With regards to the above account it may even be possible to commence the collection of P. radiata in autumn rather than to postpone the operation to the following summer. The time table for cone collection might conveniently be set up on a calendar basis instead of observation on cone colour changes. As a check cone specific gravity changes can be incorporated into such a calendar system since cones with specific gravity of less than 1 always contain high germinable seeds (compare Fig. 5.2 with 5.8 and 5.10). Degree-day summations could also be used as a criterion for harvesting since this can accommodate seasonal variations.

6.5 Artificial Ripening

6.5.1 The dehydration process and the onset of germinability

Maturation drying is an integral part of the development of most seeds, and development is considered to be completed when the seed has dried. Subsequent hydration of mature, non-dormant seeds leads to their germination; hence it has been suggested that this loss of water from seed tissues plays some role in the switch from developmental processes to those essential for germination (Dasgupta and Bewley, 1982; Dasgupta et al., 1982; Bewley and Black, 1985). As a matter of fact, the beneficial effect of drying upon subsequent germination of cereals and some legumes is widely known (King, 1976; Long et al., 1981; Dasgupta et al., 1982). However, seeds appear not to be tolerant of drying at all times during development. For example, Kermode and Bewley (1985a) found that seeds of castor bean undergo transitions from a desiccation - intolerant to a desiccation-tolerant state at a particular time in the course of their development.

The present study found that partial desiccation during artificial ripening was closely associated with the onset and the increase of germinability of prematurely collected P. radiata seed. Seeds collected in March failed to germinate when extracted from the cone in the hydrated state. Following artificial ripening of the cones for three weeks, 36% of the extracted seeds germinated; at the same time the moisture dropped from 44.7% to 27.8%, but further cone storage did not improve the germination eventhough seed moisture continued to decrease. Instead, the trend in germination was decreasing. It is believed that at this stage of development, the seeds had not had the capacity for maximum germination because the completion of major developmental events such as embryo differentiation and organised food reserve deposition was still underway. This is obvious from the X-ray radiography which shows incomplete morphological development.

When seeds collected in April were artificially ripened, highly significant increases in germination were observed after three weeks desiccation, from 16% in the freshly harvested seeds to 90%. This substantial increase coincided with a drop in seed moisture content from 37% to 22%. This kind of result is substantially in accord with the findings in cereals and some legume seeds. As artificial ripening progressed, desiccation continued and the moisture level reached a low of between 7-10% from nine weeks onwards. During the same time, the germination remained high ranging from 90 to 100% (Figs 5.7b and 5.9b).

The effect of desiccation on May-collected seeds, however, was not so obvious. Although there was no significant reduction in seed moisture content, the germination increased from 28% to 63% after three weeks artificial ripening. Further improvement in germination occurred as the artificial ripening continued. Compared with the April seeds, the May seeds appeared to behave more variably. These results suggests that partial desiccation may not be the only factor which apparently trigger germination. It is possible that the low moisture content observed in the May seeds needs to persist in the seed for a certain time to allow the developing seed to complete the process of switching from the

development-oriented programme to the germination-oriented programme. This assumption is plausible owing to the fact that there were high variations within the May sample compared to April, June and July seeds. Abrupt changes occurring to a population of seeds which were in the middle of the process of switching the programme may have caused this high variation. Some seeds may still be operating the development-oriented programme, some may be in the middle of the switching process, and some may have already activated the genes responsible for germination. Those in the process of switching may be the most vulnerable to the imposed desiccation.

For seeds collected in June and July, further improvement in germination cannot be expected since initial germination was already above 90% and moisture content was 20-25%.

The role of desiccation in the transition from seed development to germination was investigated by Kermode and Bewley (1985a) in Ricinus communis. They found that the onset of germination occurs in seeds at 50-55 days after pollination (DAP). This coincides with the attainment of maximum dry weight and the beginning of water loss in situ. When developing seeds were subjected to slow drying treatment, the germination of seeds at 25 DAP increased from 0 to 75% upon subsequent rehydration, and all seeds prematurely dried at 30 DAP germinated fully when rehydrated. The role of desiccation in the improvement of germination remained significant as seed maturity increases. Even up to five days prior to final natural moisture content of the seed, the germination was increased by desiccation from 77% to 100%. Since, in this case, premature desiccation induced seeds to germinate before they have completed major developmental events, it was suggested that drying (whether natural or imposed) acts to terminate developmental processes and to initiate those metabolic processes necessary to prepare the seed for germination and subsequent growth.

The germinability-moisture content relationship in P. radiata seeds appears to be less critical as maturity increases. For instance, although the moisture levels of seeds collected in June, July and September were still above 20%, their germinability was

already more than 90%. Aside from the anomaly in May, when there was low moisture content but low germination of unstored seeds, the data correspond very well with the postulates discussed above if the critical moisture content of the seeds is taken as somewhere between 20% and 25%. After three weeks artificial ripening of early-collected cones, moisture content falls to this level and seeds become germinable. Accordingly, pre-drying is essential in immature seeds, but not as seed maturity increases since seed moisture content gradually decreases to the 'required' level.

The question arising from the above discussion is how does dehydration irrevocably switch a developmental programme to a germination-oriented programme? Some progress towards answering this question has been made in cereal seeds such as wheat, and barley and in legumes, but no study in tree seeds has so far been reported. The biological importance of desiccation is generally related to the induction of hormone sensitivity and of hydrolytic enzymes essential to germination. Evans et al. (1975) found that desiccation is necessary for the development of complete sensitivity to gibberellin in barley. The aleurone layer responded extremely well following pre-drying of the seed, but the undried seeds responded only to a small degree even after very long exposure to gibberellic acid. Increased response by undried seeds can, however, be provoked by very high gibberellin concentrations but even so, enzyme synthesis and secretion never reached the levels exhibited by dried seeds. Armstrong et al. (1982) found in immature developing wheat (Triticum aestivum L.) that the insensitive aleurone layers can be induced to produce '-amylase in response to gibberellic acid by subjecting the grains to enforced desiccation prior to hormonal exposure. The change in sensitivity appeared to be affected by the water content of the tissue, contents below 25% being critical for the effect. This is exactly the level which was suggested earlier as crucial for the germination of P. radiata seed.

With Ricinus communis L. seed Kermode et al. (1985b) concluded that premature desiccation plays a role in redirecting metabolism from a developmental to a germinative mode. They found that

following premature drying at 30 or 40 DAP, the pattern of insoluble protein synthesis upon rehydration is virtually identical to that following imbibition of the mature seed.

Finkelstein and Crouch (1986) found that the switch-over mechanism from a developmental to a germinative-oriented programme is regulated both by osmotic pressure and ABA. Working on rapeseed embryo, they found that high osmoticum media prevents predesiccation stage embryos from germination and maintains cruciferin accumulation at levels comparable to embryos developing in situ. With mature dry seed embryos, the osmotic conditions used were no longer sufficient to prevent the embryos from taking up water and germinating showing that dessication had a key role in initiating the germination process.

It was beyond the scope of this study to investigate the hormonal-enzymatic responses during enforced desiccation of P. radiata seed. However, the results clearly show that partial desiccation plays a major role in the germination of prematurely collected seeds.

6.5.2 Other 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, information on the processes involved during ripening remains ellusive for many species. To a limited extent, the present study, produces an inside look into some of the process which occurred during the artificial ripening of P. radiata. It appears that artificial ripening is an imposed maturation process rather than a developmental process. No significant increases in seed dry weight were observed during storage suggesting that mobilization of nutrients from cone to the seed does not occur. As a matter of fact, the moisture level within the cone is considered too low for such metabolic process to take place. The suggestion that no major developmental process occurs during artificial

ripening is reinforced by the electrophoresis results. They clearly show that artificially-ripened April collected seed contain the same types of protein available in tree-ripened seeds collected in April, May, June, July and September. Similarly there were no changes found in the morphology of embryo or gametophyte when the seeds were examined by X-ray radiography.

Contrary to the results of the present study, Krugman (1966) found that embryos and megagametophytes of P. lambertiana from cones collected in August (late summer) increased significantly in dry weight if seeds were stored in cones at 10°C, but not if they were excised and stored in vermiculite. This result indicates a developmental process involving translocation of substances from cone scales to ripening seeds. Despite the apparent increase in seed dry weight the germination of the seeds were very low. Silen (1956) showed that the conditions of storage affects the increment of dry weight after collection. The dry weight of Douglas-fir seeds stored in damp peat moss weighed appreciably more after storage than those from corresponding lots of dry-stored cones, especially in the early collection. He concluded that storage in damp peat moss is suitable for Douglas-fir since it yields better germinable seeds than in dry storage.

Vigour has been used for many species as one of the criteria to determine seed quality. Unlike germination percentage, vigour provides more meaningful information concerning the physiological status of a seed or seedlot. It is defined as the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence (Perry, 1978). Vigour represents more than just the capacity to germinate, but also the capacity to germinate faster and to survive under severe environmental conditions. It is a general consensus that mature seeds have higher vigour than less mature ones (Pollock and Roos, 1972).

This is confirmed in the present study where measures of seed germination and seedling growth rate have been used as vigour indices. Seeds collected in April not only had low germination but

also low vigour. For instance, the T50 values of radicle emergence was 10.45 (±0.5) days. Artificial ripening brought about a significant increase in vigour, the time to reach 50% radicle emergence decreasing to 7.30 (±0.2) days after three weeks storage. This is comparable with vigour improvements resulting from tree ripening, viz. 7.03 (±0.3) days, for September harvested seeds.

However, as storage of prematurely harvested cones was prolonged, especially for nine weeks or more, seed vigour decreased until eventually it reached the initial level of freshly harvested seed. The deterioration observed during artificial ripening is probably favoured by the storage temperature, i.e. 20°C. A similar problem was also observed in the 1985 study in which severe reduction in germination and vigour were found in seeds which had been artificially ripened at higher temperatures (30°C). Therefore, if high vigour is to be maintained, artificial ripening should be conducted at temperatures not exceeding 20°C over limited period, i.e. six weeks and the extracted seeds will best be stored under suitably low temperatures. Note that only after six weeks storage the seed can be extracted by kilning method (refer to 6.5.4). It is also important to mention that this deterioration in vigour may not be a serious problem in the nursery as clearly shown by the high germination percentage, even after twenty-one weeks of artificial ripening. Moreover, once the seedling becomes photosynthetic it will have the capacity to growth vigorously.

It may be argued that the deterioration process could have been caused by the hot-water extraction method. Earlier findings in the preliminary study showed that the germinability of hot water-extracted seed was significantly lower than those of kilning-extracted (see 3.5). Contrary to the earlier findings, the present study shows that the T50 value in germination of hot water-extracted seeds is not significantly different from those of kilning-extracted seeds (Appendix 4). The fact that the pattern of vigour shows a consistent reduction in all samples clearly shows that the extraction method does not mask the effect of deterioration but it is possible that the hot water could exaggerate the effect of storage.

The artificial ripening conditions did not affect seed germination; both 20°C and ambient storage produced highly germinable seeds, supporting earlier suggestions that maturation, i.e. desiccation and not development, is taking place during artificial ripening. Wilcox and Firth (1980) indicated that cone storage at room temperature is suitable for artificial ripening of P. radiata seeds collected in July. In contrast, Fielding (1964) working with the same species suggested that 10°C storage can bring an increase in the germination of seed prematurely harvested in June. It was revealed in the present study that the seeds used in those two studies could have had high germination before they underwent treatments, thus eliminating the effect of storage temperatures.

6.5.3 The effect of stratification on germination and vigour

The ISTA Rules (1966) prescribed stratification of seven days at 3-5°C prior to a germination test of P. radiata seed. However, this requirement has since been dropped from the ISTA Rules 1976 and 1986. Nevertheless, it was considered necessary to adopt stratification in the germination test to check whether or not it will affect final germination. The results provide evidence that the final germination of tree-ripening seeds is not affected by stratification. However, qualitative observation suggests that stratification appeared to affect uniformity of germination.

It was mentioned earlier (6.5.2) that prematurely harvested seeds deteriorated during prolonged artificial ripening. This deterioration was clearly shown by the vigour results, a situation which is commonly observed in deteriorating seed (Delouche and Baskin, 1973). When the effect of stratification was analysed, the percentage germination of stratified and non-stratified seeds was not significantly different. However, the expression of vigour calculated as time to 50% germination (T50) showed that the stratified seeds were able to germinate faster than the non-

stratified ones, and this applied to all seedling criteria irrespective of collection time (Table 5.5). There is evidence therefore, to suggest that stratification partially offsets the deterioration that had been going on during artificial ripening through a mechanism of allowing the seeds to fully rehydrate while preventing them from germinating. The nature of this process is not known but reference to other studies suggests that biochemical effects and repair mechanisms may be involved during stratification.

In a study on Douglas-fir seed, De Matos Malavasi et al. (1986) found that stratification improved the energy status (as measured by available ATP) of seeds from both the embryo and the megagametophyte. ATP increased by thirteen times in the embryo and by six times in the megagametophyte. The relationship between energy status and germination was pointed out by Ching (1982) who noted that energy status of seeds during formation, storage and germination is closely associated with seed vigour in terms of the speed of germination. Generally, the higher the energy status, the more and faster the growth. An increase in the levels of gibberellin-like substances in seeds during stratification has been observed in a number of species (Webb et al., 1973 in Acer saccharum; Paul et al., 1973 in P. taeda). Similarly elevated levels of cytokinin activity may occur during stratification in A. saccharum (van Stadden et al., 1972). More recently, Taylor and Wareing (1979) reported in Douglas-fir and sugar pine that the level of endogenous levels of gibberellins in seeds increase during stratification. It was suggested that high level of gibberellin activity is necessary to promote rapid germination under favourable conditions.

The other possible explanation to this matter is repair and replacement during imbibition. Villiers and Edgcumbe (1975) showed that maintaining seeds imbibed without allowing germination reverse the trend of increasingly rapid deterioration which occurred when the moisture content was increased in equilibrium with a range of atmospheric humidities. Sub-cellular damage occurs at a rate dependent on temperature and moisture level in seeds which are not

fully hydrated. In this dry condition normal metabolism does not occur and so the damage accumulates until the cells become so disorganized that they are unable to germinate. In fully hydrated seeds the damage still occurs but repair and replacement take place as it occurs, thus preventing cummulative damage.

It appears that the effect of stratification treatment on the artificially ripened P. radiata seed is similar to the effect of low-temperature pre-sowing treatment on the germination of artificially aged tomato seeds reported by Coolbear et al. (1984). The low temperature pre-sowing treatment significantly increased rates of germination, but had no effect on the final germination; implying that factors affecting germination rate and germinability act independently.

Apart from the biochemical and repair mechanisms discussed above, it is also possible that stratification could simply be a way of increasing the effective germination time especially since stratified seeds will already be hydrated at the start of the germination trial. Unfortunately, no information can be obtained regarding the lowest temperature limit suitable for germination of P. radiata seed. The differences in the T_{50} values of the three germination categories between non-stratified and stratified seeds ranged from 1.3 days in late collected seeds to 3.3 days in early collected seeds (Table 5.5) suggest that it is unlikely that the significant differences observed due to the treatment of early collected seeds are merely a manifestation of differences in hydration between stratified and non-stratified seeds at the start of the germination test.

The fact that the effect of stratification in artificially ripened P. radiata seed is largely on the rate and uniformity of germination rather than on the final percentage suggests that events closely related to the promotion of rapid germination may be the predominant events occuring during stratification. As a result individual seeds which are in a situation of capitalizing on this condition will be able to synchronize their germination with the rest of the seeds.

6.5.4 Practical implications

This study produces a body of evidence that prematurely harvested Pinus radiata seed can be brought to good germinability after a period of storage. It is obvious that for such imposed maturity to proceed the seeds must have reached a stage of physiological and morphological maturity at which the seeds are no longer dependent upon the tree for nutrients and are able to generate their germination-oriented programmes. The study found that some non-germinable seeds collected mid-March can be induced to germinate after three weeks artificial ripening. However, lack of physiological and morphological maturity prevented a large proportion of the seeds from germinating. By April 24, artificial ripening clearly showed a highly significant increase in germination to a level comparable to the naturally mature seeds. At this stage of development, time of storage becomes less critical for the germinability, but remains a deciding factor for vigour since further storage reduces the vigour significantly.

From the standpoint of commercial application, artificial ripening of immature seeds collected on April 24 for three weeks is impractical because cones do not respond to the kiln-extraction method. If this technique is to be used successfully, the cones need to be stored for six weeks. It may be argued that time of storage cannot be used as the sole measure of when artificial ripening should be completed. In this case cone specific gravity can be a useful guide for seed maturity since cones with specific gravity of less than 1.00 always contain successfully artificially ripened seeds.

The method of cone storage used in this study, in individual bags, would not be suitable for a commercial operation. Other types of cone storage which provide immediate aeration can be adopted. Such good aeration is very important to prevent temperature build up and mould development because the cones are wet. In practice, cones may be spread in trays, or on a storage floor.

7. CONCLUSION

7.1 General Conclusion

The emphasis of this study has been on exploring three major areas, namely the sequence of cone development and the acquisition of germinability during seed development, the effect of partial desiccation on germination, and the practical implications of artificial ripening of P. radiata seed. Apart from the finding that artificial ripening has the potential to be successfully adopted into the current seed-production practice the highlights of this study include a demonstration that, as parameters of cone maturity, cone moisture content and specific gravity appear to have no distinctive patterns and, secondly, that seed moisture level is crucially important in the onset of and improvement of germinability.

Cone moisture content begins to decrease not later than April while nutrient accumulation is still underway and the cone is still enlarging. Both maximum dry weight and volume are attained in June, a situation when development is normally taken as being complete. Specific gravity increases while the cone loses its moisture owing to a greater proportional increase in dry weight than in volume. As the cone reaches maximum dry weight, cone tissues begin to shrink and specific gravity decreases accordingly. The colour changes progressively from deep green to nutmeg brown and finally to honey brown begining in September and continuing until November/December.

The use of cone maturity indices to assess germinability of P. radiata seed appears to be less critical than in many other pine species. This is due to the fact that seed development and the acquisition of germinability proceed at a rate much faster than the maturation of the cone itself. Thus, while the cone is still in the transition from development to maturity, the seeds are already capable of full germination. In other words a cone with a green colour and high specific gravity does not necessarily contain poorly germinable seeds.

Once the embryo and the megagametophyte have reached sufficient size, the onset of germination capacity during seed development appears to be closely associated with the level of moisture within the seed which will allow the switch on of the germination metabolism. On the other hand, the germination performance is affected by the level of embryo and megagametophyte development. None of the seeds are germinable in March as the moisture content is still around 45%. The germination capacity progressively increases as the embryo and the megagametophyte develop further and the moisture content decreases. Surprisingly, when the moisture content dropped to approximately 20% in May and the seeds are morphologically more developed, the germination did not improve substantially. In contrast, full germination was observed in June onwards when the moisture content ranged from 20% to 25%. Therefore, it is possible that other factors besides moisture status are involved in initiating germination. The hypothesis is that the 'required' low moisture content needs to persist in the seed for a certain period of time to allow the developing seed to complete the process of switching from the development-oriented programme to the germination-oriented programme within the seed.

Artificial ripening of P. radiata seed is a maturation process rather than a developmental one owing to the fact that no further development of embryo and megagametophyte of the early collected seeds is observed and seed dry weight remains relatively unchanged. Furthermore, electrophoretic analysis shows that the main protein complement of the seeds remains proportionally the same irrespective of time of collections and artificial ripening during the period from April to September. The actual maturation process, during which artificial ripening improves germination substantially, is observed in April- and May-collected seeds. Thenceforth, artificial ripening is simply a way to dehydrate the cone so that seed extraction can be carried out successfully by the kilning method. As in tree-ripening, the key feature during artificial ripening seems to be desiccation of the seeds to a level which enables them to substantially improve their germination capacity. This dehydration is particularly crucial for seeds with

high moisture level, i.e. above 30%. As the seeds become more mature, the moisture content declines accordingly and dehydration become less critical for germination. A moisture content in the range of 20-25% is possibly the critical level the seeds must have before they are able to switch to the germination programme successfully. However, full germinability can be accomplished only when the embryo and the megagametophyte have reached a stage of development whereby they can provide sufficient food reserves and other biochemical elements necessary for germination. Consequently, it is important that cones should not be detached from the parent tree too prematurely.

The practical implications of this study are potentially good. For instance, the necessity to commence cone harvesting when cone colour turns to brown is no longer a prerequisite. Indeed, collection in autumn/winter, when cones are still completely green, is justifiable. It is important, however, that these cones are allowed to dehydrate for at least six weeks or until the specific gravity drops below 1.00 so as to make the cones extractable by standard kilning methods. On the other hand, seeds should not be left in the cone for more than nine weeks or else they may deteriorate owing to unfavourable storage conditions. In commercial operations, the cones may be spread over the floor of controlled room to artificially ripen with a maximum temperatures of 20°C or left at ambient conditions, providing the temperature is not allowed to get too high.

7.2 Scope for Further Investigation

During the development process seeds are vulnerable to any extreme climatic conditions. It is therefore important to take into account the climatic and geographical variation if generalisation is to be made. The result of this study provides some evidence that the 1985 and the 1986 study are comparable. Nevertheless, it remains important to carry out a longer-term study to ensure that seasonal variations in development are clearly understood so that the correct decisions about harvesting cones for artificial ripening can be made. Since this study has provided

some fundamental information, a further study could be done on a pilot scale incorporating orchard locations and clonal variations. One of the problems which may be encountered is that none of the cone maturity indices are applicable to assess the stage of seed development and a calendar basis is not an objective criterion. This might be resolved by using degree-day summations as a criterion for cone harvesting.

This study has demonstrated that the acquisition of germination capacity is closely associated with the level of the embryo and the megagametophyte development. The more they develop the better their germination performance. The onset of germination, however, is attributed to the level of moisture within the seed but this level of moisture may have to exist for a certain period of time. These revelations offer an interesting avenue for further investigations. Fundamental questions, such as the nature of the relationship between desiccation and germination are still unknown. With reference to cereals and legumes seeds, desiccation on developing P. radiata seeds may affect hormonal and enzymatic activities responsible for germination. This is certainly worth pursuing in an attempt to obtain more information on the mechanism of germination in conifer seeds.

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Appendix 1:

Composition of protein Standards for SDS-PAGE of low molecular weight

Protein	Molecular weight (Daltons)
Lysozyme	14,400
Soybean trypsin inhibitor	21,500
Carbonic anhydrase	31,000
Ovalbumin	45,000
Bovine serum albumin	66,200
Phosphorylase B	92,500

Source: Salmond Smith Biolab Ltd.

Appendix 2:

ANOVA of the effect of artificial ripening conditions (20°C vs ambient) on germinability of seeds from cones collected at different maturity and artificially ripened for different period of time (see 5.2.6).

Source of variation	df	SS	MS	F
Unit				
Seed sample	2	966.85	483.42	5.45*
Error	9	798.82	88.76	
Total	11	1765.67		
Sub Unit				
Ripening temperature treatment	1	14.88	14.88	< 1 ns
Interaction	2	209.84	104.92	< 1 ns
Error	9	1114.83	123.87	
Total	23	3105.22		

ns = not significant

* = significant at 5% level

Appendix 3:

Volumetric changes (cm^3) of tree-ripened cone harvested at different time. Figure in brackets are standard errors of single means (see 6.3.2).

March	April	May	June	July	September
217.5 (±26.6)	226.0 (±30.3)	228.0 (±38.6)	266.7 (±20.8)	207.0 (±23.2)	188.7 (±37.9)

Appendix 4:

ANOVA of the effect of extraction methods on germinability of seeds from cones collected at different maturity and artificially ripened at 20°C for different period of time (see 6.5.2).

Source of variation	df	SS	MS	F
Unit				
Seed sample	3	38.14	12.71	4.32*
Error	12	35.33	2.94	
Total	15	73.47		
Sub Unit				
Extraction treatment	1	5.28	5.28	2.27^{ns}
Interaction	3	16.94	5.65	2.42^{ns}
Error	12	27.94	2.33	
Total	31	123.64		

ns = not significant

* = significant at 5% level

Appendix 5:

Composition of various solutions being used in electrophoresis.

1.	Sample buffer	
	Stacking (0.5M Tris-HCL) buffer (pH 6.8)	1.25 ml
	SDS (10%)	2.00 ml
	Mercaptoethanol	0.50 ml
	Glycerol	1.00 ml
	Bromophenol blue	0.50 ml
	Distilled water	4.65 ml
2.	Extracting solution	
	8M Urea with 1% SDS and 10% mercaptoethanol	
3.	Sealing gel (12.5% Acrylamide)	
	Acrylamide/Bis solution (30 g Acrylamide to 0.8 g Bis made up to 100 ml	4.15 ml
	Resolving gel buffer (3M Tris to pH 8.8 with 1M HCL and brought to 100 ml with water)	1.25 ml
	SDS (10%)	0.10 ml
	Water	4.00 ml
	1.5% Ammonium persulphate (freshly prepared)	0.50 ml
	TEMED	15.00 μl
4.	Resolving gel (10% SDS-PAGE gel)	
	Acrylamide /Bis solution (30 g Acrylamide to 0.8 g Bis made up to 100 ml)	10.00 ml
	Resolving gel buffer	3.75 ml
	SDS 10%	0.30 ml
	Water	14.45 ml
	1.5% Ammonium persulphate (freshly prepared)	1.50 ml
	TEMED	15.00 μl

5.	Stacking gel	
	Acrylamide /Bis solution (30 g Acrylamide to 0.8 g Bis made up to 100 ml)	2.50 ml
	Stacking gel buffer (0.5M Tris adjusted to pH 6.8 with 1M HCL and made up to 100 ml with water)	5.00 ml
	1.5% Ammonium persulphate (freshly prepared)	1.00 ml
	TEMED	15.00 μl
6.	Running buffer	
	Tris	30.30 g
	Glycine	144.00 g
	SDS	10.00 g
	Made up to 1000 ml with H_2O	
7.	Coomassie blue stain solution	
	Coomassie blue	1.20 g
	Distilled water	500.00 ml
	Methanol	500.00 ml
	Glacial acetic acid	200.00 ml
8.	Destaining solution	
	Distilled water	600.00 ml
	Methanol	300.00 ml
	Glacial acetic acid	100.00 ml

Appendix 6:

Colour charts according to the British Colour Council. 1 - B.C.C. 174; 2 = B.C.C. 233; 3 - B.C.C. 204; 4 - B.C.C. 168; 5 - B.C.C. 67 (see 3.4)