# THE REPRODUCTIVE BIOLOGY OF INDONESIAN DAMMAR

# (*AGATHIS DAMMARA* (LAMBERT) RICH.)

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**THE REPRODUCTIVE BIOLOGY OF INDONESIAN DAMMAR (*AGATHIS DAMMARA* (LAMBERT) RICH.)**

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We accept this thesis as conforming
to the required standard

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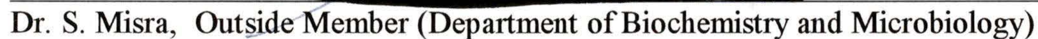

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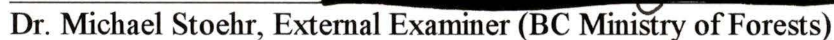

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## ABSTRACT

This project utilized a planted monoculture stand to study the reproductive biology of Indonesian Dammar (*Agathis dammara* (Lambert) Rich.). The reproductive cycle, pollination, seed production and embryology were studied. Seed cones and pollen cones were tagged, measured and observed monthly or twice monthly to determine the cycle and sequence of cone and seed development. Fixed and sectioned specimens were used to study the developments from pollination to seed maturity using light microscopy.

Pollination occurs almost at any time of the year but the peak periods occur two to three times a year, from February through March, August through September and October through November. It took 5 to 6 months to develop from the pollen mother cell to mature pollen. Pollen is non saccate. Pollen tubes enter the nucellus and branch. Pollen tubes contain several prothallial cells and a body cell. The body cell forms two male gametes. More than one pollen tube is common in an ovule.

The Dammar reproductive cycle is similar to that of temperate *Agathis*. It took 15 to 18 mo from pollination to seed maturity. Cone abortion is over 30% and seed abortion over 60%. Ovule development is similar to other species within the Araucariaceae. Up to 10 archegonia per megagametophyte are found, but 4 to 6 are more common. Mature archegonia, proembryo and early embryo stages are frequently found in one megagametophyte. Proembryogeny and early embryogeny in Dammar are similar to that in other members of the Araucariaceae with the presence of cap cells, central cells and

suspensor. Simple polyembryony was found before massive embryo stage, whereas cleavage polyembryony did not occur in Dammar.

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Chapter 1

# INTRODUCTION

*Agathis dammara* (Lambert) Rich., locally called Damar or Dammar, is a well known conifer whose economic importance has long been recognized in Indonesia. Damar, with some other *Agathis* species that grow naturally in the country rank among the 260 commercially important Indonesian timber species. Dammar grows up to 45.5 m tall and 5.5 m in girth (de Laubenfels 1979). The local name is derived from its valuable resin product called dammar (Suryamihardja 1979). In world trading, *Agathis* is also called kauri or Indonesian kauri (Soerianegara and Lemmens 1993), derived from the maori name for *A. australis* in New Zealand. Most species of *Agathis* and some *Araucaria* and *Podocarpus* species, native to the southern hemisphere are distinctive because of their broad leaves that present a striking contrast to the usual foliage of conifers (Vasilieva 1972, Page 1980, Gifford and Foster 1989).

Dammar grows naturally on the islands of Moluccas (de Veer 1954, de Laubenfels 1979, 1978, 1988), but has been planted in Java since in the middle of the nineteenth century (de Veer 1954). Other *Agathis* species native to Indonesia grow on the islands of Sumatra, Borneo, Celebes and in West New Guinea. The first Dammar seeds planted in West Java were imported from Ambon, a small island of the Moluccan archipelago. Since Dammar grew well, it was widely planted in West, Central and East Java.

*Agathis* and *Araucaria* belong to the Araucariaceae. The name is from the Greek 'Agathis' meaning a ball of thread, referring to the globose seed cone (Dallimore and Jackson 1966). Opinions differ regarding the number of species of *Agathis*. Dallimore and Jackson (1966) described 21. Later, Whitmore (1977, 1980) confirmed only 13 and considered some to be synonyms. A recent study based on cuticular morphology of fossils suggests living 21 species of *Agathis* (Stockey and Taylor 1981, Stockey 1982, 1994, Stockey and Atkinson 1993). *Agathis dammara* grown in Java has long been called

*Agathis loranthifolia* (de Veer 1954, Dallimore and Jackson 1966, Suryamihardja 1979, Whitmore 1980, Bruijnzeel and Wiersum 1985). This botanical name was used by Meijer Drees (Dallimore and Jackson 1966) and was first introduced by Salisbury (de Laubenfels 1978, Whitmore 1980). *Agathis loranthifolia* is considered to be a synonym of *A. dammara* or to be a geographical sub-species (provenance) (Whitmore 1977, 1980, de Laubenfels 1979, Soerianegara and Lemmens 1993). De Laubenfels (1972, 1978, 1979) and de Laubenfels and Silba (1987) have described *Agathis* from Mollucas, Borneo and some South Pacific islands in more detail.

The live crown of *Agathis* is conical and comprised of a central, orthotropic shoot with whorled first order branches terminated by lateral, plagiotropic shoots and branchlets to the fifth order (Nikles 1973). *Agathis* is monoecious, but there is marked dichogamy (a temporal separation of pollen and seed cone development) on any individual trees (Nikles 1973, Whitmore 1977), however, self- and cross pollination are still possible. Many references to dioecious *Agathis* probably arise from such marked dichogamy, though it may also be because young trees often produce seed cones several years before pollen cones (Whitmore 1977).

Seed cones or megasporangiate strobili in *Agathis* are globose or broadly ovoid, compact and symmetrical. Seed cone scales are tightly packed with only a thickened and flanged external edge exposed in the unopened cone (de Laubenfels 1978) or they may be fan-shaped, with a thickened margin overlapping a portion of the scales (Dallimore and Jackson 1966). The scales abscise and cones disintegrate at maturity (Eames 1913, Nikles 1973, Dallimore and Jackson 1966, Suryamihardja 1979, Whitmore 1980). In *Agathis* the ovule arises from the adaxial surface of the fused bract scale. At maturity, a single seed is slightly embedded in the adaxial surface of the scale with usually a single wing developed on one side (Whitmore 1980) as in *A. australis* (Nikles 1973).

A process may occasionally develop into a second wing (Dallimore and Jackson 1966). *Agathis* has only two cotyledons whereas most *Araucaria* species have four (Nikles 1973). The pollen cone is a simple strobilus containing many microsporophylls. The pollen cone is stiff, dense and cylindrical and born solitary on a stalk in a leaf axil. Each microsporophyll bears several microsporangia (Gifford and Foster 1989).

Only very limited studies have been done on the reproductive biology of tropical *Agathis*. Most available data are very old and lack details. De Veer (1954) briefly mentioned flowering phenology of some Dammar trees growing along roadsides in West Java (Suryamihardja 1979). The reproductive biology of *Agathis* was first studied in detail by Eames (1913) and briefly reviewed by Whitmore (1977) and Ecroyd (1982). Recently, Owens *et al.* (1995a, b, c) carried out a complete study on the reproductive biology of New Zealand *A. australis*. The purpose of this study is to observe the reproductive biology of Dammar with the emphasis on the phenology of the reproductive cycle, pollination, embryo and seed development.

# Chapter 2

# LITERATURE REVIEW

## 2.1. Floral initiation and the reproductive cycle

Tree species vary enormously in the time of floral initiation and most tropical and subtropical species have a very short period between floral initiation and anthesis. However, in tropical and subtropical areas, flowering is often seasonal, affected by seasonal rainfall and temperature changes.

In most temperate tree species, floral initiation occurs in spring or summer prior to winter dormancy (Sedgley and Griffin 1989). In tropical hardwoods, which have not been intensively studied, phenology appears to vary considerably in time of floral initiation and may or may not be related to the seasonal changes. Information regarding flowering and floral initiation in tropical trees is extremely limited.

The time of first flowering in Dammar is about 15 years of age, but viable seeds usually are not obtained before the trees are about 25 years of age (de Veer 1954). A planted stand of *Agathis* in Java flowers throughout the year, but bears mature cones mainly from February through April and again from August through October. The period of seed cone maturity varies from one place to another. In some areas, seed cones mature in March and April (West Java), May (West and Central Java) or June through August (East Java) (Whitmore 1977). In West Java, seeds for plantation are frequently collected from July through September but this may slightly change depending on previous climatic condition. There is no information about Dammar seed cone maturity in Borneo, Celebes, Mollucas and New Guinea.

Unlike angiosperms, where the time between ovule initiation and seed maturity may be only a month or so, in conifers the same process may take several months or a year or more. Owens and Blake (1985) and Owens (1991) described three primary types of reproductive cycle that occur in most temperate conifers. The first common type is a two year reproductive cycle in which floral initiation takes place in the first year and pollination, fertilization, embryo development and seed maturity and release occur in the following year. This type of cycle is found in most members of the Pinaceae (except *Pinus)*. The second type is the three-year reproductive cycle in which floral initiation occurs the first year and pollination, fertilization and partial embryo and seed development occur in the second year but the seed matures and is released in the third year. This type of cycle is found in *Chamaecyparis nootkatensis* and some species of *Juniperus*. In the third type of reproductive cycle, pollination occurs in the second year and fertilization, embryo development and seed maturation occur in the third year. This type occurs in most species of *Pinus*, *Dacrydium*, *Podocarpus*, *Saxegothaea*, *Sciadopitys*, *Sequoia* and *Cephalotaxus*.

Members of the Araucariaceae were reported earlier to have a two year reproductive cycle (Nikles 1973, Singh 1978). Later, they were considered to have three year cycle similar to *Pinus* (Owens and Blake 1985). Temperate *Araucaria cunninghamii* in New Zealand (Nikles 1973) has a three year reproductive cycle in which fertilization occurs about 23 months after seed cone buds emerge. About four months after fertilization, seed cones shed their seeds. Species that grow in similar regions, such as *Agathis australis* (Ecroyd 1982, Owens *et al.* 1995a, b, c) undergo floral initiation before winter dormancy, followed by pollination in the following spring. Fertilization occurs about one year after pollination followed by embryo and seed development. Seed matures about 17 to 18 months after pollination. In this cycle, there are two distinct dormant periods, one between cone initiation and pollination and one between pollination and fertilization.

Information regarding the reproductive cycle in most tropical conifers is still extremely limited compared to those growing in temperate regions. *Pinus merkusii* and *Pinus kesiya* growing in northern Thailand are reported to have a period from pollination to seed maturity of about 12.5 months and 23 months, respectively (Sirikul 1993). To date, there is still no available data regarding the cycle of tropical *Agathis* or *Araucaria.* Based on climatic differences, reproductive biology of tropical *Agathis* might be morphologically similar to that of temperate *Agathis* but may posses a shorter reproductive cycle due to the absence of distinct dormant periods.

### 2.2. Pollen cones and pollen

Conifer pollen cones bear many microsporophylls. Each microsporophyll bears two microsporangia on its abaxial surface in the Pinaceae or more in the Cupressaceae and Taxodiaceae (Owens and Blake 1985). Species of *Araucaria* and *Agathis* may have thirteen to fifteen microsporangia per microsporophyll (Gifford and Foster 1989). Each microsporangium contains sporogenous tissue which later undergoes mitosis to form pollen mother cells. Each pollen mother cell undergoes meiosis to form a tetrad of haploid microspores and each microspore develops into a mature pollen grain (Singh 1978).

Pollen in families such as the Cupressaceae and Taxodiaceae have no prothallial cells, whereas two prothallial cells occur in the Pinaceae. The number of cells in the pollen at pollination differs from species to species. In some members of the Cupressaceae and Taxodiaceae, pollination occurs at the 1-cell stage or as 2-cell pollen containing tube and generative cells. In many members of the Pinaceae, pollination occurs at the 4-cell stage in which it contains two primary prothallial cells, a tube and a generative cell or the 5-cell stage after the generative cell divides to form the stalk and body cells (Owens and Blake 1985). Members of the Podocarpaceae and Araucariaceae have multicelled pollen at shedding similar to those in the Pinaceae, but have a larger number of prothallial cells resulting from division of the primary prothallial cells (Singh 1978, Ecroyd 1982).

Mature pollen of *Araucaria brasiliensis* contains 15-25 prothallial cells, a tube nucleus and a body cell (Burlingame 1913). Mature pollen of *A. australis* contains several prothallial cells, a generative cell and a tube nucleus (Ecroyd 1982). The tube cell contains large starch grain and numerous small lipid bodies (Owens *et al.* 1995b).

**2.3. Pollination mechanism**

The pollination mechanisms in conifers have been described by Chamberlain (1935), Singh (1978) and Owens and Blake (1985). Intensive studies have been made in *Pinus taeda* (Greenwood 1986), *Picea sitchensis* (Owens and Blake 1984), *P. engelmanii* (Owens *et al.* 1987), *Tsuga heterophylla* and *T. mertensiana* (Owens and Molder 1984d) and *Picea glauca x engelmanii* (Runions 1995). In these species, pollination mechanisms can be divided into three major groups. First is through the production of a pollination drop that is secreted at the time of cone receptivity and capture and carries pollen into the ovule. This mechanism is found in the Cupressaceae, Taxodiaceae, Taxaceae, Podocarpacease, Cephalotaxaceae and most species of Pinaceae. A second mechanism lacks pollination drops. Here, pollen is engulfed by modified integument tips as occurs in *Pseudotsuga* and *Larix* (Owens and Blake 1985). The third mechanism also lacks pollination drops and occurs in a few species of Pinaceae (*Tsuga*), Taxodiaceae (*Saxegothaea*) and in the Araucariaceae. This mechanism is referred to as protosiphonogamy (Thomson 1907) and siphonogamy (Singh 1978). Pollen lands away from the micropyle, anywhere on the surface of the bracts or scales (Nikles 1973, Whitemore 1977) and germinates *in situ*. After germination, the pollen tubes (like fungal hyphae) grow over the surface of the scale or bract toward the micropyle (Nikles 1973, Singh 1978). This mechanism is often called the *Agathis-Araucaria* type (Singh 1978). In *Araucaria* pollen lodges on the upper parts of the scale or between separated neighboring scales as in *A. cunninghamii* (Nikles 1973, Haines *et al.* 1984) but often at a considerable distance from the ovule, as in *A. braziliensis* (Burlingame 1913). A similar pattern occurs

in *Agathis*. Here, the pollen lands on the fused bract scale, germinates and the pollen tube forms (Singh 1978, Ecroyd 1982, Owens *et al.* 1995a).

The nucellus in *Agathis* projects beyond the micropyle and a pollen chamber is absent (Singh 1978). In *A. australis* (Eames 1913, Owens *et al.*1995a), pollination occurs before the differentiation of the integument and archesporial tissue in the ovule. This is in contrast to that commonly found in other conifers where there is sufficient differentiation to form a micropyle to receive the pollen (Eames 1913) or the presence of a pollen chamber as occurs in some members of the Pinaceae. Owens *et al.* (1995a) describes the presence of a nucellar flap that extends beyond the micropyle in *A. australis.* This nucellar flap is visible after pollination and has a role in the pollination mechanism.

It was suggested that a chemotropic substance produced by the nucellus causes the pollen tube to grow to the nucellus (Singh 1978). Pollen tubes grow through the nucellus and then toward the archegonia (Eames 1913). In *A. australis*, the tubes often branch before they reach the archegonia (Owens *et al.* 1995a). Pollen tubes may grow from one archegonium to another (Eames 1913, Ecroyd 1982).

## 2.4. Megagametophyte development

The megagametophyte in gymnosperms develops from a functional megaspore that has undergone several free nuclear divisions and cell wall formation (Singh 1978). There are many free nuclei embedded in a parietal layer of cytoplasm leaving a large central vacuole (Singh 1978, Gifford and Foster 1989). The number of free nuclei may vary among species and depend on the size of the megagametophyte, but it is usually constant within a species (Gifford and Foster 1989).

Chamberlain (1935) and Singh (1978) describe the stages of free nuclear division. At early stages, the divisions are generally synchronous. After several mitotic divisions, anticlinal walls appear and the megagametophyte begins to appear like a honeycomb. Each honeycomb cavity is a tube-like cell called an alveolus (Chamberlain 1935) that grows toward the centre of the megagametophyte. The alveoli (except those functioning as archegonial initials) undergo several periclinal divisions followed by cell-wall formation. In *A. australis,* cell walls form centripetally producing elongate or pyramidal-shaped primary prothallial cells. This is followed by periclinal then anticlinal divisions. The division and elongation of these cells finally fills the large central vacuole of the megagametophyte (Owens *et al* 1995a).

The stage of megagametophyte development at which the archegonial initials begin to form varies among species. In *Taxus*, *Torreya* and some other conifers, archegonial initials do not begin to form until the megagametophyte is completely cellular, whereas in others, they are initiated during cell wall formation (Gifford and Foster 1989). In *A. australis*, archegonial initials appear soon after cell-wall formation begins (Owens *et al.* 1995a).

Archegonial development in *Agathis*, or *Araucaria*, has been described by Singh (1978), Ecroyd (1982) and Owens *et al.* (1995a). The archegonial initial which is a superficial cell differentiates into a large elongate vacuolate cell with a large nucleus. The enlarged initial divides unequally forming a primary neck cell which in turn divides to form several neck cells. The central cell enlarges and its nucleus usually remains close to the neck of the archegonium. The central cell nucleus then divides forming a ventral canal nucleus which is not separated by a wall from the large egg cell and an egg nucleus. The ventral canal nucleus has been reported to disappear soon after formation (Ecroyd 1982). However, Owens *et al.* (1995b) found that it is present but its location in the egg cell is variable. The egg nucleus enlarges and in some genera the mature egg nucleus is surrounded by a perinuclear zone as in *A. australis* (Owens *et al.* 1995a) and some

members of the Pinaceae (Owens and Blake 1985). At maturity, the egg cell of some genera contains many small and large inclusions. The latter form from proplastids enlarging and engulfing portions of egg-cell cytoplasm including many organelles (Singh 1978, Gifford and Foster 1989). Large inclusions are absent in *A. australis* (Owens *et al.* 1995a, b).

Total number of archegonia per megagametophyte varies among species and may depend on the size of the megagametophyte. The number of archegonia in Pinaceae ranges from two to six (Wilson and Burley 1983, Gifford and Foster 1989). Species in the Cupressaceae and Taxodiaceae have an archegonial complex in which the number of archegonia vary from five to about 100 (Singh 1978, Wilson and Burley 1983). In Araucariaceae, there may be many archegonia in a single megagametophyte (Singh 1978), *A. cunninghamii* has 3-5 archegonia (Nikles 1973). The number ranges from 8-15 in *A. australis* (Singh 1978, Ecroyd 1982, Owens *et al.* 1995a) and 3-12 in *A. robusta* (Kaur and Bhatnagar 1984). A recent study of *A. australis* report the average number of archegonia as 4.5 and no archegonial complexes were found (Owens *et al.* 1995a).

**2.5. Fertilization**

Fertilization occurs when one male gamete fuses with the egg nucleus. The pathway through which the pollen tube reaches and enters the archegonium may vary among species. Pollen tubes grow directly to the archegonial chamber in species in which the archegonia are not deep seated, as in *Pseudotsuga menziesii*. There the pollen tube penetrates between neck cells and releases the male gametes. After penetration the adjacent prothallial and the neck cells degenerate (Owens and Morris 1991a). In some species, the neck cells degenerate prior to pollen-tube penetration, as in some members of the Pinaceae and in *Podocarpus* and *Cephalotaxus* (Singh 1978). Contents of the pollen tube then pass through the ventral canal cell as in *P. glauca* (Owens and Molder 1984), *T. heterophylla* and *T. mertensiana* (Owens and Molder 1975). In *A. australis*, Kaur and

Bhatnagar (1984) and Owens *et al.* (1995b) describe the mechanism in more detail. In this species, the pollen tube enters the archegonium through the neck cells (Owens *et al.* 1995b) or occasionally adjacent jacket cells (Eames 1913). Both or only one male gamete may be released into the egg along with some male cytoplasm (Eames 1913, Owens *et al.* 1995b). The second male gamete may stay in the upper portion of the archegonium or both male gametes sometimes make contact with the egg nucleus (Kaur and Bhatnagar, 1984). In *Pinus*, both male gametes enter the egg cytoplasm, but the second male gamete, some cytoplasm, the tube and often stalk nuclei usually remain in the micropylar end of the archegonium (Singh 1978).

The incorporation of varying proportions of male and female cytoplasm into the neocytoplasm gives rise to various forms of cytoplasmic inheritance in conifers. A detailed review on this subject for both angiosperms and gymnosperms is presented in Mogensen (1996). In gymnosperms, conifers are a major group that have been studied extensively. Plastids in this group are predominantly paternally inherited, whereas mitochondria are either paternally or maternally inherited. This is in contrast to the majority of angiosperms in which both plastids and mitochondria are generally maternally inherited. In *Agathis,* the only genus in the Araucariaceae that has been studied extensively, both plastids and mitochondria are paternally inherited (Owens *et al.* 1995b). This is based on a light microscope study in *Agathis robusta* (Kaur and Bhatnagar 1984) and an ultrastructural study in *A. australis* (Owens *et al.* 1995b).

### 2.6. Proembryo

Terminology used in embryogeny of conifers has been extensively described by Chamberlain (1934) and Singh (1978). In the Araucariaceae, terminology has also been described by Eames (1913), Haines and Prakash (1980) and Kaur and Bhatnagar (1984). The terminology for *A. australis* has been reinterpreted and updated by Owens *et al.* (1995c). Singh (1978) divides embryo development in gymnosperm into three stages.

The proembryo stage includes free nuclear division and cell wall formation until the embryo pushes out the archegonium. The early embryo stage starts after the embryo pushes out the archegonium until meristematic tissues (i.e. root meristem) forms. The late embryo includes all remaining stages until embryo development is complete.

Fusion between male and egg nuclei result in the formation of a zygote that is enclosed by a new cytoplasm, the neocytoplasm. The zygote undergoes free nuclear division resulting in a two then four nucleate proembryo (Singh 1978). At this time in most conifers, the nuclei migrate to the chalazal end of the archegonium. At migration the number of free nuclei may vary among species. In *A. australis*, the migration is slight (Owens *et al.* 1995b), but in members of the Pinaceae, the migration is obvious.

Cell-wall formation occurs at the 8-cell proembryo in the Pinaceae, 16 cell in *Cephalotaxus*, 32-cell in *Podocarpus* and 64-cell in *Agathis* (Roy Choudhury 1962, Singh 1978) and *Araucaria* (Haines and Prakash 1980, Haines 1983a). In the Pinaceae, cell-wall formation results in two tiers of four cells each that are clearly defined into primary upper tier containing four cells and primary suspensor tier containing four cells (Singh 1978). The two primary tiers then divide and form a 16 cell proembryo in most species. The division of the primary upper tier results in the embryonal tier and suspensor tier and the division of the primary suspensor tier forms a dysfunctional suspensor tier or rosette tier and the open tier as occurs in *Pinus* and *Picea* (Owens and Molder 1984a, b). Later development is characterized by the elongation of the suspensor tier causing the tip of proembryo to be pushed out of the archegonium.

The above features differ in members of Araucariaceae due to the number of free nuclei that forms before the suspensor elongates and the presence of cap cells. Three tiers are recognized in the proembryo of *Agathis* (Singh 1978, Owens *et al.* 1995c) and *Araucaria* (Singh 1978, Haines and Prakash 1980, Haines 1983) and some Podocarp-aceae. The distal tier consists of a large apical cell and an adjacent ring of smaller cells.

Later these cells develop into a cap consisting of an elongated layer of cells in the apex of the proembryo. The second tier consists of large subapical cells and a surrounding ring of cells. The subapical cell divides forming the central cells or the embryonal group (Owens *et al.* 1995c). These central cells were called the embryo proper by Eames (1913) and Chamberlain (1934). The surrounding ring of cells divide and elongate forming lateral cap cells (Owens *et al.* 1995c). The third tier consist of cells that are commonly small, less regularly arranged, variable in number and elongate. The latter tier forms the primary suspensors.

## 2.7. Early, late and mature embryo

The elongation of the primary suspensor tier causes the proembryo to be pushed out of the archegonial jacket. Singh (1978) described the formation and variation of the suspensors in conifers. The terminology used may not fit some genera. However, in general the initial suspensor elongation results from the elongation of the primary suspensor of the proembryo and subsequent elongation is from secondary suspensors derived from other cells in the proembryo and early embryo. There are two types of secondary suspensors as described in Singh (1978). The embryo tier may divide transversely to form embryonal suspensors which elongate. The derivatives may also divide and elongate. Another secondary suspensor system consists of embryonal tubes made up of a group of unequally elongating cells derived from the proximal end of the embryonal mass. The elongation of this suspensor system pushes the embryonal mass into the centre of megagametophyte. During early embryo development the suspensor system becomes coiled and parts of them collapse at the mid- to late-embryo stages.

A detailed description of suspensor system has been given for *Araucaria* (Singh 1978, Haines and Prakash 1980, Haines 1983) and for *Agathis* (Singh 1978, Owens 1995c). Both genera are similar in most respects. In *A. australis* as described in Owens *et al.* (1995c), the primary suspensor was derived from the third tier of the proembryo. As the central cells further divide and form a massive embryo, cells at the base of the massive embryo divide and form a secondary suspensor, the embryonal tubes that elongate.

The cap cells that form in the proembryo show certain functions in the growth of the embryo. One of them is related to the apparent digestion of food material from the surrounding gametophytic cells (Eames 1913, Singh 1978), whereas other functions suggested are giving protection to the meristematic embryonal group and secreting the enzymes to form the corrosion cavity (Owens *et al.* 1995c). The role of cap cells in preventing cleavage polyembryony as suggested in *Araucaria* by Haines and Prakash (1980) is doubted in *A. australis* (Owens *et al.* 1995c). Cap cells degenerate as the embryo develops into the massive embryo.

Development of the embryonal mass involves the formation of two distinct proximal and distal regions. The distal region arises from cell division in all planes and the proximal region arises primarily from transverse division of cells. Cells in the distal region contribute to the formation of hypocotyl, shoot apex and cotyledons, whereas those in the proximal region form the root cap (Singh 1978). The root cap differentiates at a later stage forming two recognizable regions as found in *Pinus, Larix*, *Pseudotsuga*, *Welwitschia* and *Ephedra* (Singh 1978) as well as *Araucaria* (Haines 1983b) and *A. australis* (Owens *et al.* 1995c). The central region forms the column and the peripheral region forms the pericolumn (Singh 1978). Cotyledons arise from the shoulder of the young embryo leaving a central mitotically inactive shoot apical meristem (apex) between. Terminology used in late embryogeny and mature embryo of *P. menziesii* (Allen 1946, 1947, Allen and Owens 1972) is considered applicable for conifer late embryogeny (Owens *et al.* 1995c). In this terminology, cells in the distal region form the stele

promeristem that forms the hypocotyl-shoot axis, shoot apex and cotyledons, whereas cells in the proximal region form the generative root meristem and rib meristem that later form root apex, column, pericolumn of the root cap. This terminology is used in the following chapters, unless otherwise indicated.

In the Araucariaceae, longitudinal sections of mature embryos clearly show a shoot apex, cotyledons, root meristem, provascular system, column and calyptro-periblem (Haines 1983b, Taylor and Taylor 1993). The column and pericolumn of the root cap are relatively long and the cotyledons are short, but variation may exist in some genera. The number of cotyledons varies. There are two in *Agathis,* two or four in *Araucaria*, three in *Taxus* and two to several in some members of the Pinaceae (Singh 1978).

Fertilization of more than one archegonium is common in conifers and results in multiple zygotes (simple or archegonial polyembryony). However, only a small percentage of mature conifer seed show more than one embryo (Singh 1978). Multiple embryos may also rise from the cleavage or separation of each file or embryonal unit in the early embryo and each may grow separately. Various patterns of cleavage are described by Singh (1978) but this type of polyembryony was not found in *Agathis* (Singh 1978, Owens *et al.* 1995c).

### 2.8. Seed development

#### 2.8.1. Seed coat

There is considerable development in the ovule before and after fertilization. There is compression of the nucellus, reduction in the amount of megagametophyte in relation to the developing embryo and the differentiation and maturation of the seed coat (Singh 1978). The time at which the seed coat starts to differentiate differs. Differentiation begins before fertilization in *Pseudotsuga* (Allen and Owens 1972), *P. glauca* (Owens and Molder 1984), *T. heterophylla* and *T. mertensiana* (Owens and Molder 1984b) or about

the time of fertilization in *Abies* (Owens and Molder 1985). In *Araucaria*, seed coat differentiation begins at meiosis of the megaspore mother cell (Haines 1983).

In the Araucariaceae, the seed coat is derived from the integument (Singh 1978, Haines 1983), similar to other conifer species. The initial change during seed coat differentiation is an increase in the number of cell layers in the integument, followed by the deposition of tannins in some cells. Three layers differentiate in most conifer seeds (Allen and Owens 1972, Singh 1978): an outer parenchymatous sarcotesta, a middle sclerenchymatous sclerotesta and an inner thin-walled endotesta.

### 2.8.2. Seed wing

The presence of seed wings is characteristic of some conifers and they play a role in seed dispersal. In the Pinaceae the wing develops from the ovuliferous scale and begins to differentiate at about the time of pollination (Allen and Owens 1972, Singh 1978, Owens and Molder 1984a, b, c, 1985). In members of the Cupressaceae and Taxodiaceae wings arise from lateral extension of the integument (Owens and Molder 1984b). In Araucariaceae wings reportedly arise from the entire scale (Singh 1978). Structural studies of *Araucaria* (Haines 1983b) show the wing to arise from the outward growth of the meristematic edge of the scale at the time of meiosis.

### 2.8.3. Storage products

The seed storage tissue in conifers is the megagametophyte. The storage products accumulate during embryo development and nourish the developing embryo and young seedling upon germination (Allen and Owens 1972, Singh 1978). The composition of the storage products may vary, but lipids and protein are most abundant in the megagametophyte of *P. menziesii* (Owens *et al.* 1992). Starch is at least one of the storage products found in some members of *Araucaria* (Haines 1983b).

**2.9. Seed efficiency**

There are several factors, both internal and environmental, that influence seed production. Detailed discussions of this subject have been written for a few conifers (Owens and Blake 1985, Owens 1991, Owens 1995). Abortion may occur at any stage of development. Extreme environmental conditions may cause the failure at pollination, hence no fertilization. A large number of unpollinated and unfertilized ovules may lead to the abortion of seed cones in species of pine. In other situations the cones continue to develop but produce a low number of filled seeds (Owens 1995). Other factors such as animals and birds may also reduce cone and seed production.

Seed production is often measured by seed efficiency (Owens *et al.* 1991), the proportion of filled seeds compared to the seed potential. A filled seed is a fertilized, fully developed ovule having all seed components. Seed potential is the total number of ovules in a seed cone that can possibly be fertilized and develop. This potential is greatly influenced by factors such as genetics, size of cone and condition of the tree. The number of ovules that develop into filled seeds is also influenced by many factors, both genetic and environmental, including pollination and fertilization success, ovule and embryo abortion, insects and diseases (Owens 1995).

Chapter 3

# MATERIALS AND METHODS

## 3.1. Materials

Materials used for this study were collected in a planted *Agathis* forest located in Sukabumi, Gunung Walat Educational Forest of Bogor Agricultural University, about 160 km south of Jakarta, West Java, Indonesia. The site lies between 6 to 7° S latitude and 106 to 107° E longitude with an altitude of 400 to 1200 m above sea level. The average monthly rainfall recorded from 1992 to 1994 in May through September (dry season) ranges from 71 to 172 mm and in October through April (wet season) from 210 to 315 mm.

The stand is 40 to 60 years of age and planted as a monoculture. It has experienced several thinnings both natural and artificial, with the present spacing from 3 to 6 m. About 120 trees are reproductive with a likelihood of cross pollination. On every collection date, specimens were collected from two trees randomly selected from these 120 trees. Seed cone buds, seed cones and pollen cones were collected by climbing the trees and cutting small branches. Specimens were placed in a cooler and sprinkled with water. The cooler was brought directly to Forest Tree Seed Technology Center in Bogor, about 60 km away. There, the specimens were dissected and fixed on the same day or the following day.

## 3.2. Methods

### 3.2.1. First-year collection

The first collections were carried out from January 1993 through May 1995. These collections were made based on the knowledge of temperate reproductive phenology in which each stage of the reproductive material is found only in a certain season. Four types of specimens were collected; pollen cones, seed-cone buds, first-year

seed cones (post pollination but pre-fertilization), and second-year (post-fertilization) seed cones. Three to four seed-cone buds and seed cones were collected from two randomly selected trees on each collection date. Collections were made one to four times per month. Later, after sectioning and staining of some specimens, it was found that this method failed to determine the duration of each stage of ovule development and hence could not provide sufficient information to determine the reproductive phenology. Therefore, a modification in collection methods was made to accommodate this in the second year.

### 3.2.2. Second-year collection

Collections were made from June 1995 through May 1996. These collections were similar to the first-year except for the seed cones. The terms first-year and second-year seed cone were not used in these collections. Rather several seed cones of different size were collected on each collection date. Each size of seed cone, presumably reflecting a different developmental stage, was used as a reference point.

To determine the phenology and time required to develop from one stage to another, several seed-cone buds and seed cones of different sizes on each of several trees were tagged and numbered. Their diameter was measured monthly or twice monthly. Sixteen cone buds from four trees, 41 post-pollination seed cones from five trees and four developing or nearly mature seed cones from three trees were selected for this purpose. The cone diameter from the first stage (cone bud) to the following stage (post-pollination), and from post-pollination to cone maturity was used to draw a continuous line that shows all the developmental stages and their relation to the time required to pass from one stage to another. The size of separate receptive cones (at pollination) was also used as a reference point. Diameter and length of nine collected, fixed and intact receptive cones were also measured to determine the size of receptive seed cones. Length of dissected cone scales was also measured.

A similar approach was applied to the pollen cones. Eighteen early emergent pollen cones from four trees were measured monthly or twice monthly until pollen was shed. Pollen cones 8 to 9 mm in diameter were used as a reference point to determine the time required from that stage to the size when pollen is mature and shed.

Stages of reproductive development in each ovule obtained from each size of seed cone was observed using light microscopy. Ovules from the first-year collections were treated in the same manner. Ovule length from both first- and second-year collection were measured to determine their relationship to the cone diameter. Each stage of development was then related to ovule length and cone diameter. Data obtained from monthly or twice monthly measurements of seed cone diameter were then plotted as reference point to determine the time from one stage to another. Finally, the general phenology was determined using these data and developmental observation.

### 3.2.3. Dissection

#### 3.2.3.1. Cone buds

Seed-cone buds were sliced longitudinally along opposite sides, parallel to the cone axis leaving a central portion about 1 to 2 mm thick. The central portions were fixed and some were embedded, sectioned and stained. Several cone buds per collection were used for the structural study.

#### 3.2.3.2. Ovule primordium and cone scales

At pollination (receptive cone), 4 to 6 cone scales were collected from each of several receptive seed cones. The number of receptive cones collected varied from none to two on every collection date. Some scales were sliced longitudinally on either side of the single median ovule primordium leaving a central portion 1 to 2 mm thick bearing the ovule. Some entire scales were fixed.

### 3.2.3.3. Pre- and post-fertilization ovules

Up to ten ovules from several seed cones of various sizes (Section 3.2.1) and diameter (Section 3.2.2) were collected. Cones were cut longitudinally into two parts and scales bearing an ovule were removed. Ovules were sliced down both side (as above) leaving only the central 1 to 2 mm portion. Some intact ovules were fixed.

### 3.2.3.4. Pollen cones

To observe the general structure of pollen grains, pollen cones of various sizes were collected on each collection date. Cones were sliced on opposite sides leaving a central 1 to 2 mm portion which was fixed. Number of microsporophylls per pollen cone was counted from large and small pollen cones. Microsporangia were observed and counted on microsporophylls removed from mature pollen cones.

### 3.2.4. Fixation, dehydration, embedding, softening, sectioning and staining

Dissected and whole specimens were immediately put in scintillation vials containing formalin-acetic acid-alcohol (FAA) (Berlyn and Miksche 1977), aspirated using a low vacuum of about 15 to 20 pps for 1 to 2 minutes (Owens *et al* 1991) to enhance fixation then stored at room temperature. This process was carried out in the lab of Forest Tree Seed Technology Centre in Bogor. All fixed specimens were then brought to the Forest Biology Centre, University of Victoria for further processing.

Specimens to be sectioned were dehydrated through a tertiary-butyl-alcohol series (Johansen 1940) then embedded in TissuePrep2. Prior to sectioning, embedded specimens were softened 4 to 7 days in Gifford's solution (Gifford 1950) and serially sectioned at 6 to 7 μm using a rotary microtome. The sections were stained using Iron-Hemotoxylin-Safranin (Berlyn and Miksche 1976) and covered with cover slip using Entellan (Merck D76). The slides were then dried at 37° C for about 2 w. The study of development and

phenology was carried out using these specimens and some dissected, fixed and fresh specimens using dissecting and compound light microscopes.

### 3.2.5. Pollen flight

To observe the availability of pollen (pollen cloud) during the cycle, two modified wind vanes were used as pollen monitors. The vanes rotated on vertical rods so they always faced in the wind. The vanes were attached to the lower branches of the conical tree crown. The trees selected for wind vane attachment were of average height (30 to 40 m) in the stands, had typical crown form and were located in the middle of the stand where most specimens were collected. The distance between the two trees was approximately 50 m.

A microscope slide coated with petroleum jelly was attached to the front of each vane. On the middle of the slide, a permanent 1 cm ink-square was drawn prior to their attachment. The slides were changed every 3 to 7 days. The number of pollen trapped on that 1 cm square area of the slides was counted using a compound microscope. Final data from November 1993 through November 1994 were tabulated for each month period.

### 3.2.6. Pollen diameter measurement

Several mature (brown) pollen cones from several trees in the stand were collected. The cones were dried indoors and outdoors by spreading them on the surface of paper. Several days later, the pollen grains were shed. Extraneous materials were removed by filtering through a fine mesh screen. The grains were further dried in an air conditioned room at 20 to 25$^{o}$ C for 1 w and then put in vials and stored in a refrigerator at 4 to 5$^{o}$ C then brought to Victoria. Moisture content was not measured.

In Victoria, some of the grains were used to measure pollen diameter. They were dusted onto a microscope slide and then observed using a compound microscope equipped with a grid in the eyepiece. A hundred pollen grains were measured using a 10 power objective and another a hundred pollen grains using 40 power objective.

### 3.2.7. Cone abortion and seed efficiency

The number of flagged cones (Section 3.2.2) that aborted during measurement was monitored and tabulated. The causes of the abortion could not be determined.

To determine seed production per seed cone, 370 mature seed cones were collected from November 1993 through November 1994. Each cone was left 2 to 3 days at room temperature or until the scales abscised. The number of empty and filled seeds were counted separately for each cone. The seed efficiency was calculated as the percentage of filled seed. Seed potential is the total number of both filled and empty seeds.

Chapter 4

# OBSERVATIONS AND RESULTS

## 4.1. Pollen and seed cone

Pollen cones are borne solitary or in pairs in axils of leaves. They may be in a cluster on short shoots (Fig. 1). The time at which they are initiated was not determined, however observations from 1993 through 1995 show both pollen cones and seed cones emerged at any time throughout the year. Pollen cones are distinguishable quite early. The cone is cylindrical, oblong to oval and usually mature when it is about 1 cm in diameter but variable in length. The longest mature pollen cones observed were 4 to 5 cm long. Each pollen cone bears 150 to 200 peltate microsporophylls (Fig. 2) depending on cone size and each microsporophyll bears seven microsporongia on the abaxial surface (Fig. 3).

Seed cones arise from a terminal or axillary bud and are raised on a stalk of a variable length (Fig. 4). Axillary shoots may form one or a few leaf primordia then form a terminal seed cone bud (Fig. 4). Seed cones of various size, from buds to mature seed cones were normally found every month. The seed cone bud is round or subglobose, 7 to 8 mm in diameter and covered by several bud scales (Fig. 4). Seed-cone buds are only distinguishable from vegetative buds after dissection. At pollination, the seed cone is oval or elliptic, 1 to 2 cm in diameter and 1.5 to 2.5 cm in length. Cone scales are spirally arranged and bend outward allowing pollen to pass between (Fig. 5). The scales then thicken sealing these spaces. Post-pollination seed cones remain elliptic or oval until later development. Developing seed cones have compact scales and are globose or subglobose (Fig. 6). Cone scales abscise at cone maturity when cones are 9 to 12 cm in diameter. A paper-like seed wing develops on one side of the ovule as the ovule develops (Fig. 7). Wings developing on both sides were rarely found. The mature seed cone is dark green

and the inner part of the scales and seeds are brown at maturity. The surface of the seed is sculptured with rough lines (Fig. 8).

Observations carried out from June 1995 through May 1996 show a large number of cone abortions or the failure of cones to develop further (Appendix 1). In 370 mature seed cones, the average number of ovules was 98 with an average of only about 36 (37 %) filled seeds. The average number of filled seeds collected monthly did not show a trend relating to wet or dry seasons (Figs. 9, 10).

### 4.2. Reproductive cycle

Observations of ovules throughout development are summarized in Appendix 2. Pollination occurs 3 to 4 mo after seed-cone buds become identifiable. At this stage the nucellus and archesporial cells are not completely developed and the integument is not differentiated. Sporogenous cells and meiosis of the megaspore mother cell appear soon after, followed by the formation of the functional megaspore. The functional megaspore develops rapidly followed by several free nuclear division then a period of cell-wall formation that is completed 10 to 12 mo after pollination. At the same time, pollen tubes also grow and even branch in the nucellus and around the developing megagametophyte.

There is rapid cone and ovule growth during cell wall formation. Megagametophyte maturation and fertilization takes place 1 to 2 mo after cell-wall formation begins. Ovules elongate and enlarge during free-nuclear division and more rapidly during cell-wall formation through megagametophyte maturation, whereas cone diameter remains about the same (4 to 5 cm). Fertilization occurs 13 to 16 mo after pollination or about 17 mo after seed-cone buds become identifiable. This is followed by proembryo and embryo development. There is also rapid seed-cone growth from the proembryo to the late embryo stages. Slow cone growth occurs from the late embryo to the mature embryo stages and takes 2 to 3 mo. Embryos mature 15 to 18 mo after pollination or 20 to 24 mo after seed cones become identifiable.

Fig. 1. Cluster of pollen cones at various stages of development. 0.35x

Fig. 2. Longitudinal section of a developing pollen cone showing microsporpophylls (M) and microsporangia (arrow). 1.2x

Fig. 3. A microsporophyll removed from a nearly mature pollen cone showing seven microsporangia (arrow) on the abaxial surface. 10x

Fig. 4. Seed cone buds borne on axillary shoots(A) with leaf base (arrowhead). 0.80x

Fig. 5. Receptive seed cone. 1.6x

Fig. 6. Globose nearly mature seed cone. 1.5x

Fig. 7. Ovules (OV) with papery wings (W) developing on one side. 2.5x

Fig. 8. Mature seed showing surface of seed coat, chalazal end (CE), micropylar end (ME), remaining portion of wing (W) and rudimentary wings (arrow). 6.0x

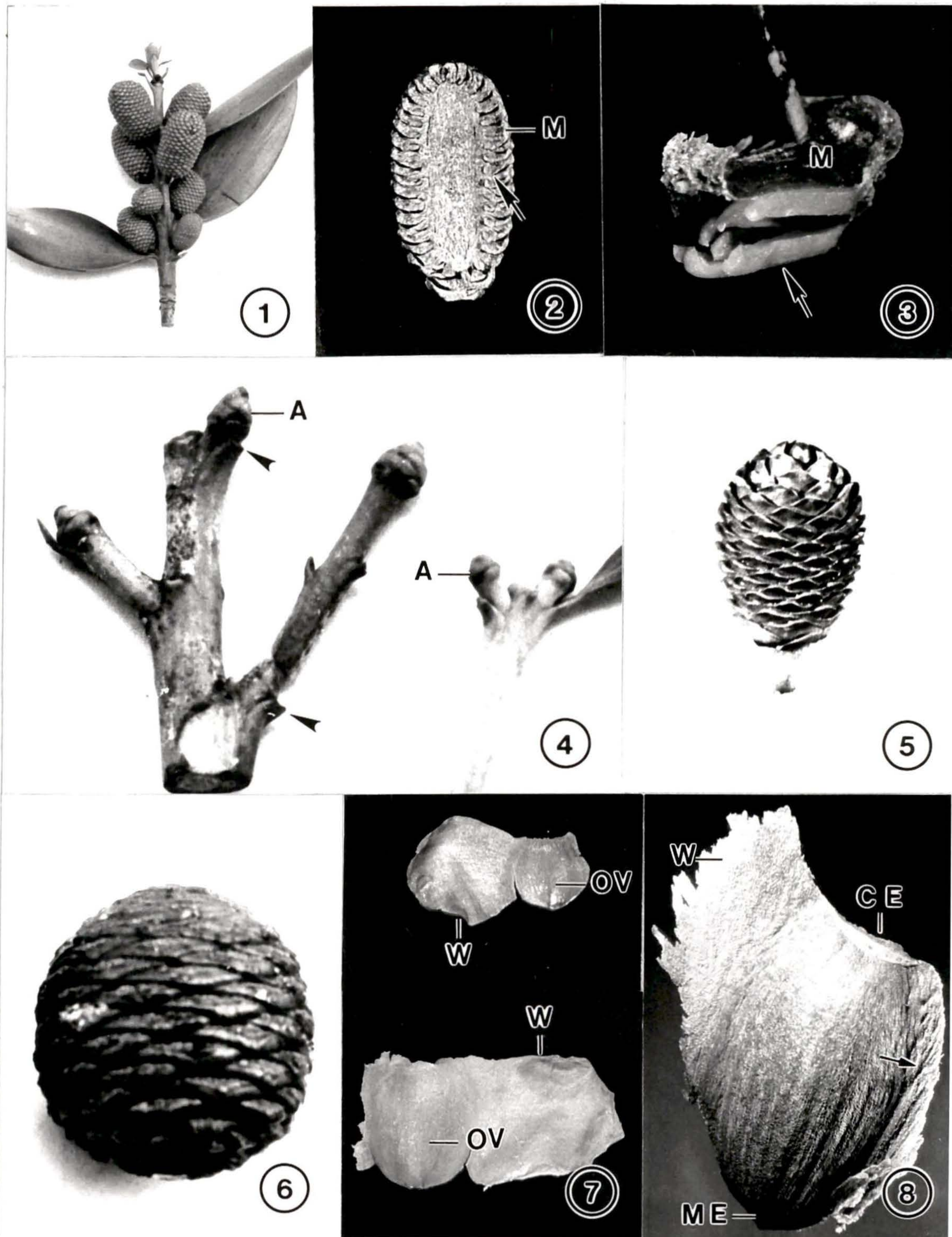
M
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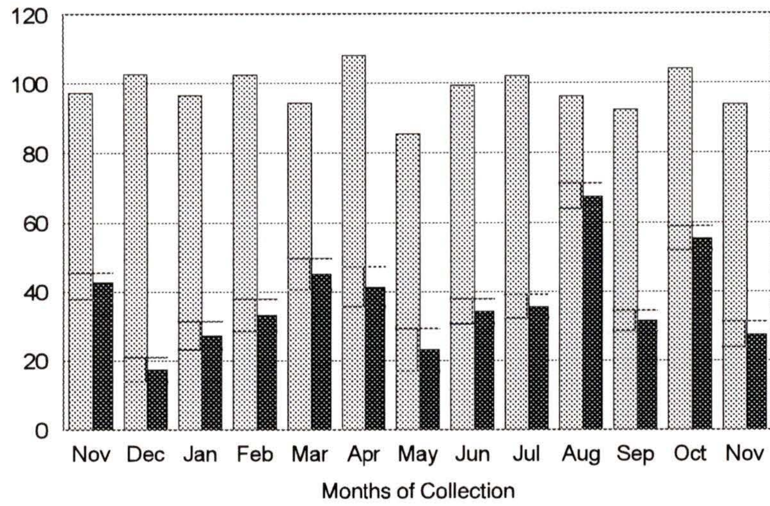
Fig. 9. The average seed potential (▒) and seed efficiency (▓) collected monthly from November 1994 through November 1995.

Fig. 10. Average monthly rainfall (mm) for 1992 through 1994 (-⊟-) and monthly rainfall for November 1992 through November 1993 (▓) and November 1993 through November 1994 (▒) recorded 5 to 10 km away. (Source: Litbang Pertanian Tanaman Pangan, Ministry of Agriculture, Sukabumi, West Java)

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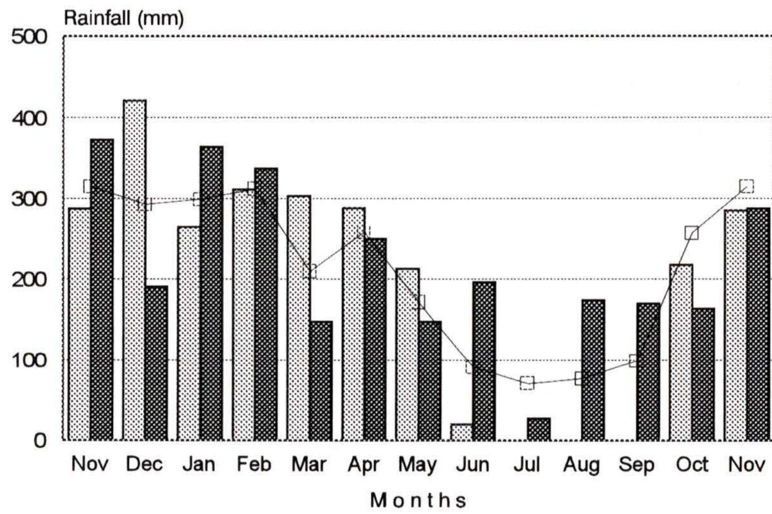



10

Figs. 11-15. Longitudinal section of microsporangia

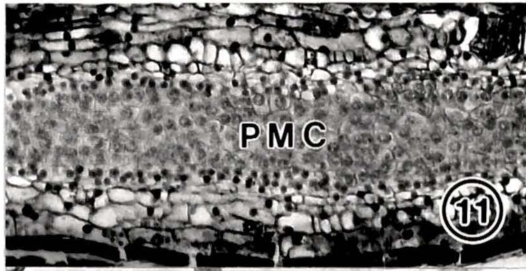
Fig. 11. Pollen mother cells (PMC). 100x

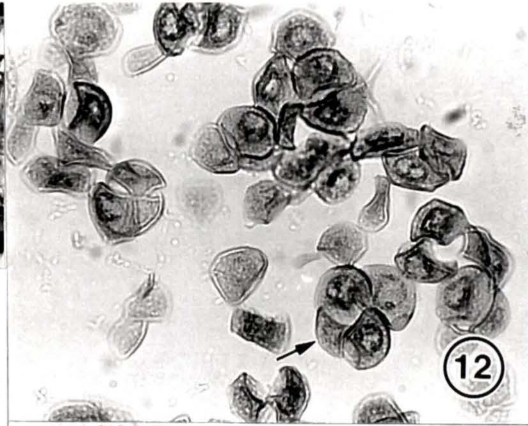
Fig. 12. Tetrad of microspore (arrow). 350x

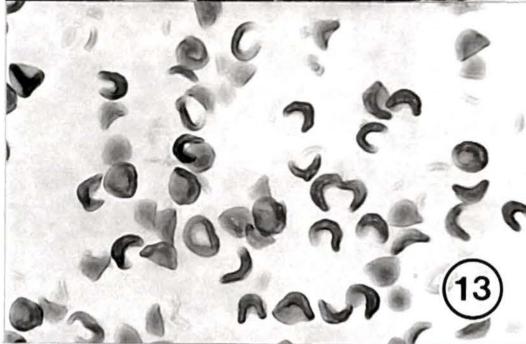
Fig. 13. Angular microspores after the dissolution of pollen-mother-cell wall. 150x

Fig. 14. Developing pollen. 125x

Fig. 15. Sections of a mature pollen grain showing ektexine (EKT), endexine (EN) and intine (IN) of pollen wall. 980x


PMC
11


12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14

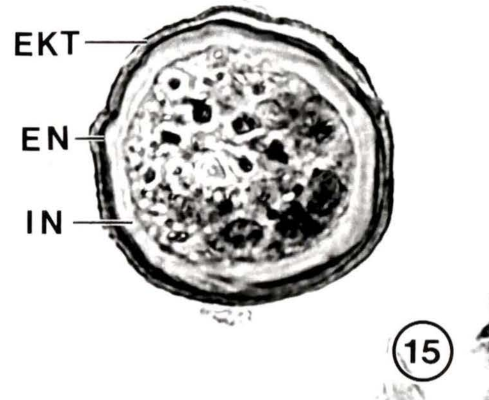
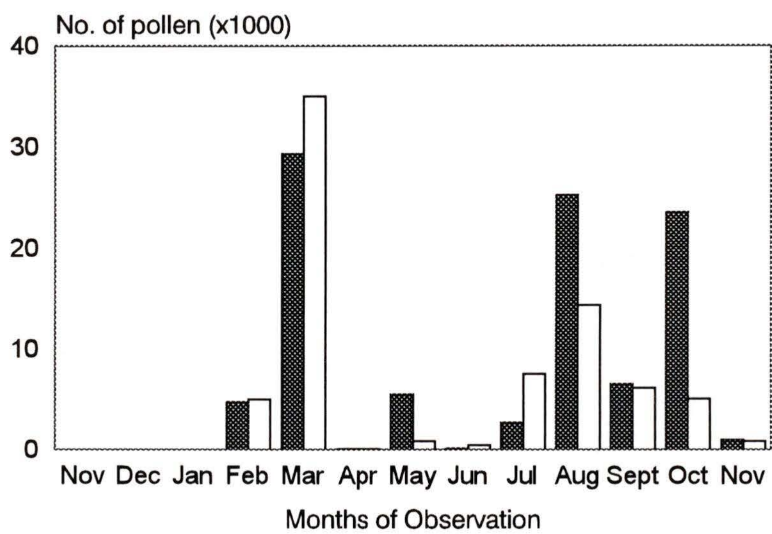

EKT
EN
IN
15

Fig. 16. Observation on pollen cloud from November 1993 through November 1994 using two wind veins A (□) and B (▩) as pollen monitors. Number of pollen trapped per month in 1 $cm^2$ is plotted on the y-axis (x 1000). Distance between the two monitors is 50 m.




16

Fig. 17. Receptive seed cone showing the separated scales (arrowhead). White dots are resin granules (arrow). No pollen is visible. 5.0x

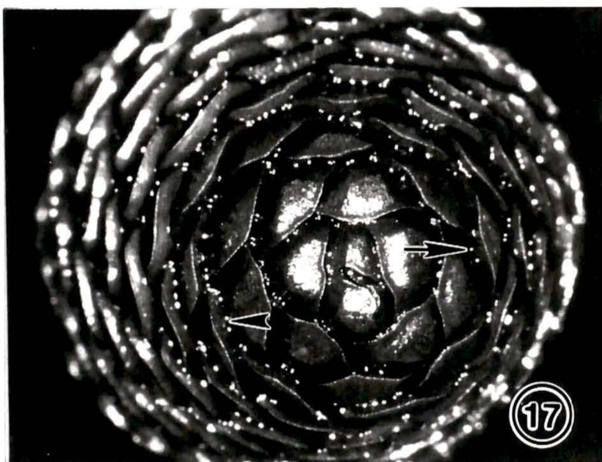
Fig. 18. A seed cone scale at receptivity showing an ovule primordium (OP). 20x

Fig. 19. Longitudinal section of an ovule primordium at receptivity. 115x

Fig. 20. Longitudinal section of an ovule showing integument (I), nucellar flap (NF), pollen tube (arrowhead) and free nuclear megagametophyte (*). 48x

Fig. 21. An ovule tip showing pollen tubes (arrow) and integument (I). 80x

Fig. 22. Longitudinal section of ovules showing pollen tubes containing male gametes (G) in the body-cell cytoplasm (BC) and a prothalial cell (PC). 278x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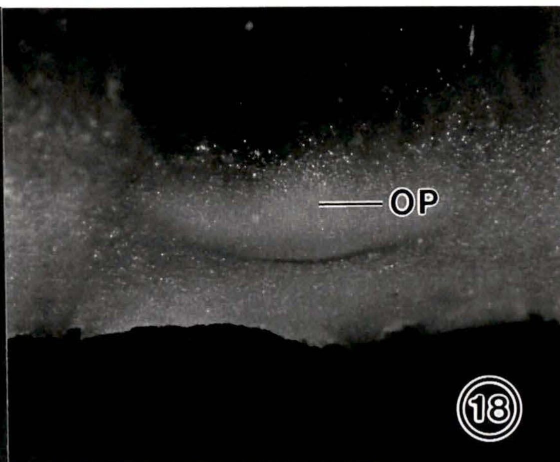
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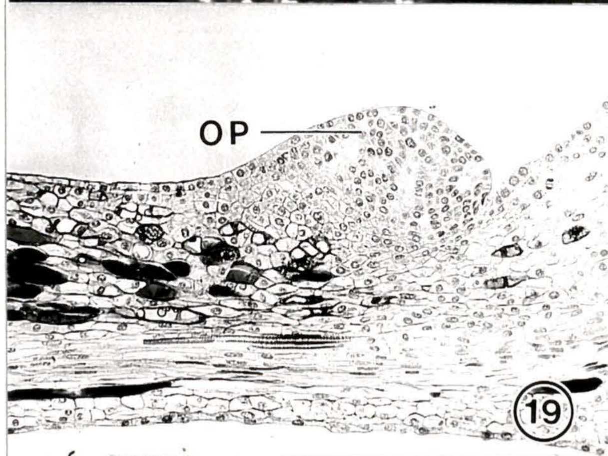
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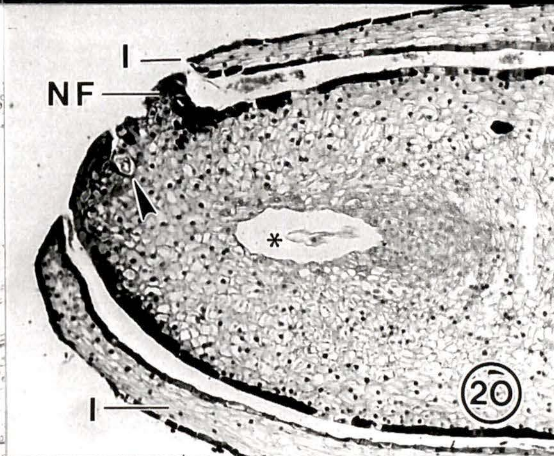
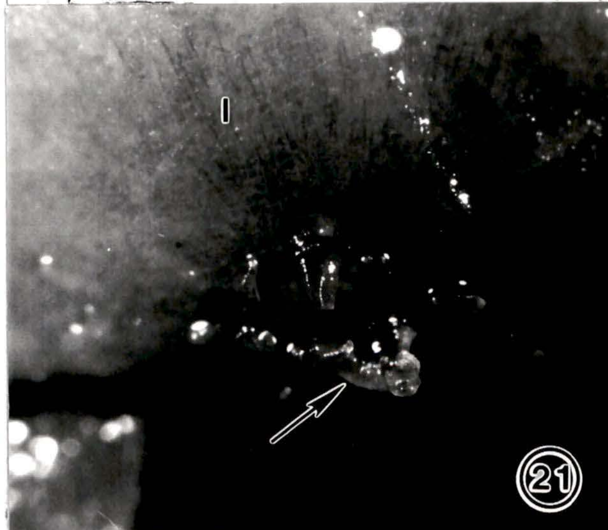
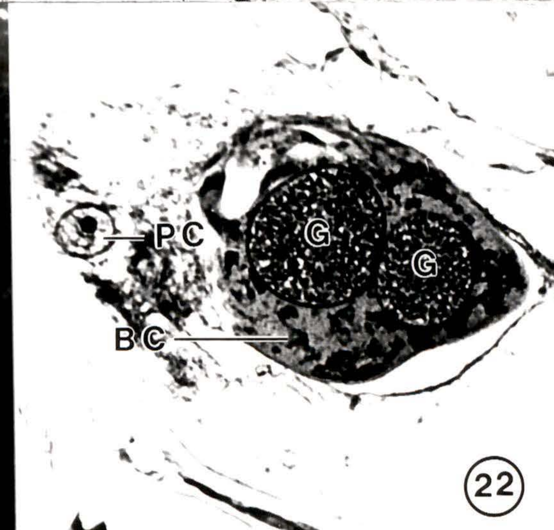
19


I
NF
*
I
20


I
21


PC
G
G
BC
22

### 4.3. Pollen development

Pollen cones 8 to 9 mm in diameter show pollen mother cells (Fig. 11). This is followed by meiosis and formation of tetrads of microspores (Fig. 12) in the thecal fluid. The pollen mother-cell wall disintegrates and the microspores expand (Fig. 13). The number of pollen produced in each microsporangium was not counted. At maturity, the pollen is smooth, nearly round with no sacci (Figs. 14, 15). The exine is yellow or reddish-yellow. Pollen diameter ranges from 20.16 to 50.40 μm with an average of 41.80 μm. It took 5 to 6 mo from the pollen mother cell stage to pollen shed.

### 4.4. Pollination and the pollination mechanism

Pollen was shed during most months in the stand but there were two or three peak pollination periods (Fig. 16). From two pollen monitors used, there was slight variation in time at which the most pollen was shed. The highest number of pollen trapped in monitor A occurred from February through March, July through September and in October. The highest number of pollen trapped in monitor B occurred from February through March and a long period from July through October. The number of receptive cones at pollination was not counted. Monthly rainfall recorded 5 to 10 km away from the location showed no particular trend in relation to pollen shed (Fig. 10).

Seed cone receptivity occurs 3 to 4 mo after the seed-cone bud become identifiable. Seed cones enlarge rapidly at pollination. Receptive seed cones are oval or elliptic and 1 to 2 cm in diameter and 1.5 to 2.5 cm in length. Scales are about 4.5 mm long and widen and thicken toward the tip and bend slightly upward. Scales separate at pollination (Fig. 17). On the proximal adaxial surface of the scale, a broad ovule primordium is initiated (Fig. 18). At this time the integument, archesporial cells and nucellus are not differentiated (Fig. 19). About 1 to 2 mo after pollination, the ovule primordia enlarge and become easily detached from the scale. A seed wing extends from one side of the ovule on the surface of the scale. After this time, the integument becomes

visible and the nucellar tip projects slightly beyond the integument tip. A nucellar flap (Fig. 20) appears when ovules are at the early free nuclear stage.

About 3 to 4 mo after pollination, ovules develop to the early to mid free nuclear stage and pollen tubes had grown into the nucellar tip (Figs 20, 21). From the nucellar tip, the tubes grow deeper into the nucellus where they branch and grow to and around the megagametophyte causing most of the middle parts of the megagametophyte to be indented. The tubes are also found growing on the surface of integument, penetrating the integument and growing between the integument and nucellus. From there the tubes penetrate the nucellus and grow toward the megagametophyte. More than one pollen tube is frequently found in an ovule. Pollen tubes contain several prothallial cells, cytoplasm and a tube nucleus. Two round male gametes (Fig. 22) were frequently found in the middle of the body-cell cytoplasm.

### 4.5. Pre-fertilization ovule

The integument did not cover and enclose most of the nucellus until 1 to 2 mo after pollination, when the ovule was about 2.5 mm long. Sporogenous cells appear near the micropylar half of the nucellus. The megaspore-mother cell forms in the micropylar portion of the sporogenous cells. It enlarges and forms a large central vacuole. Meiosis was not observed. About 4 to 5 mo after pollination the functional megaspore had undergone free nuclear division and its central vacuole enlarged. Several free nuclei form in the parietal layer of cytoplasm just inside the megaspore wall (Fig. 23). At early free nuclear division the ovule is about 3 mm long and enlarges to about 4 mm at late free nuclear division (Fig. 24). It took 4 to 5 mo to complete free nuclear division. Cell-wall formation began (Fig. 25) followed by the enlargement and elongation of the ovule to about 4.5 mm in length. During cell-wall formation to the mature megagametophyte, the ovule enlarges to 6 mm in length.

About 10 to 12 mo after pollination, after cell-wall formation, archegonial initials and young archegonia at the central cell stage were observed. Archegonial initials have a single nucleus and are enclosed by a single layer of archegonial jacket cells (Fig. 26). The archegonial initials enlarge and divide to form a primary neck cell and central cell nucleus that remained close to the primary neck cell in the distal part of the archegonium (Figs. 27, 28). Later, the central cell divides forming an egg-cell nucleus (Fig. 29) and a ventral canal nucleus (Figs. 30, 31) usually found in the periphery of the egg near the neck cells. At maturity, the egg nucleus is found near the centre of the archegonium. A perinuclear zone forms around the egg nucleus in the mature archegonium. Both stain darkly. Large and small vacuoles were widely distributed in the egg cell outside the perinuclear zone (Fig. 29).

Observations of over 40 megagametophytes showed that archegonia were mostly found in the distal half of the megagametophyte. Most ovules have more than one archegonium. Up to 10 archegonia were found in the megagametophytes, and 4 to 6 archegonia are common. The actual number of archegonia per megagametophyte was underestimated because some peripheral sections were not mounted. Archegonia are variable in shape and position from shallow to deeply seated in the megagametophyte at the base of a depression, the archegonial chamber. In some megagametophytes, 4 to 8 archegonia were arranged parallel to the longitudinal axis of the ovule in the distal part of the megagametophyte. Each archegonium has a separate archegonial jacket, however, two archegonia that partially shared an archegonial jacket are occasionally found.

Figs. 23-31. Longitudinal sections of ovule showing stages of megagametophyte development.

Fig. 23. Sporogenous tissue (ST) and early free nuclear division of megagametophyte (arrowhead). 107x

Fig. 24. Late free nuclear division showing a large central vacuole (V), parietal layer of cytoplasm (PL), pollen tube (*) containing body cell (BC) and prothallial cell (PC) inside tube cytoplasm. 107x

Fig. 25. Early cell wall formation at micropylar end of megagametophyte (MG). 107x

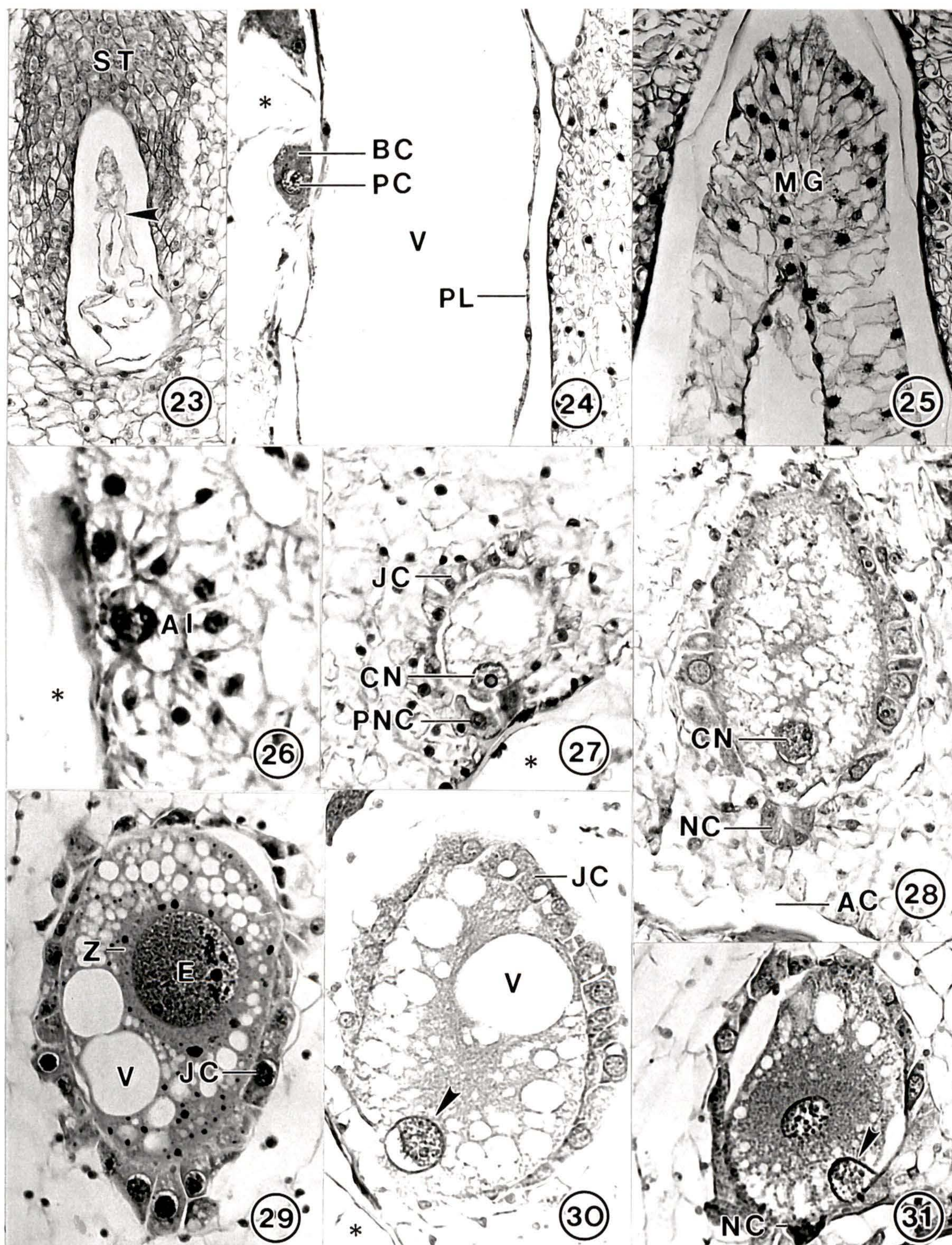
Fig. 26. Megagametophyte showing an archegonial initial (AI), pollen tube (*). 356x

Figs. 27, 28. Median longitudinal section of archegonia showing jacket cells (JC), central cell nucleus (CN), primary neck cell (PNC), pollen tube (*), neck cells (NC) and archegonial chamber (AC). 167x, 186x

Fig. 29. Mature archegonium cut obliquely showing egg nucleus (E), perinuclear zone (Z), vacuoles (V) and archegonial jacket. 209x

Fig. 30. Mature archegonium showing ventral canal nucleus (arrowhead) in egg cytoplasm, large vacuole and jacket cell. 211x

Fig. 31. Mature archegonium showing egg cell, ventral canal nucleus (arrowhead) and degenerated neck cells (NC) at fertilization. 187x


ST
23
*
BC
PC
V
PL
24
MG
25
*
AI
26
JC
CN
PNC
*
27
CN
NC
AC
28
Z
E
V
JC
29
JC
V
*
30
NC
31

Figs. 32-39. Longitudinal sections of ovules showing stages of proembryo and early embryo development.

Fig. 32. Four nucleate proembryo in darkly stained neocytoplasm (N), pollen tube (*) and megagametophyte (MG). Egg cytoplasm (arrowhead) and neck cells (arrow) are degenerating. 128x

Fig. 33. Eight nucleate proembryo in darkly stained neocytoplasm. Egg cytoplasm and jacket cells (arrow) are degenerating. 209x

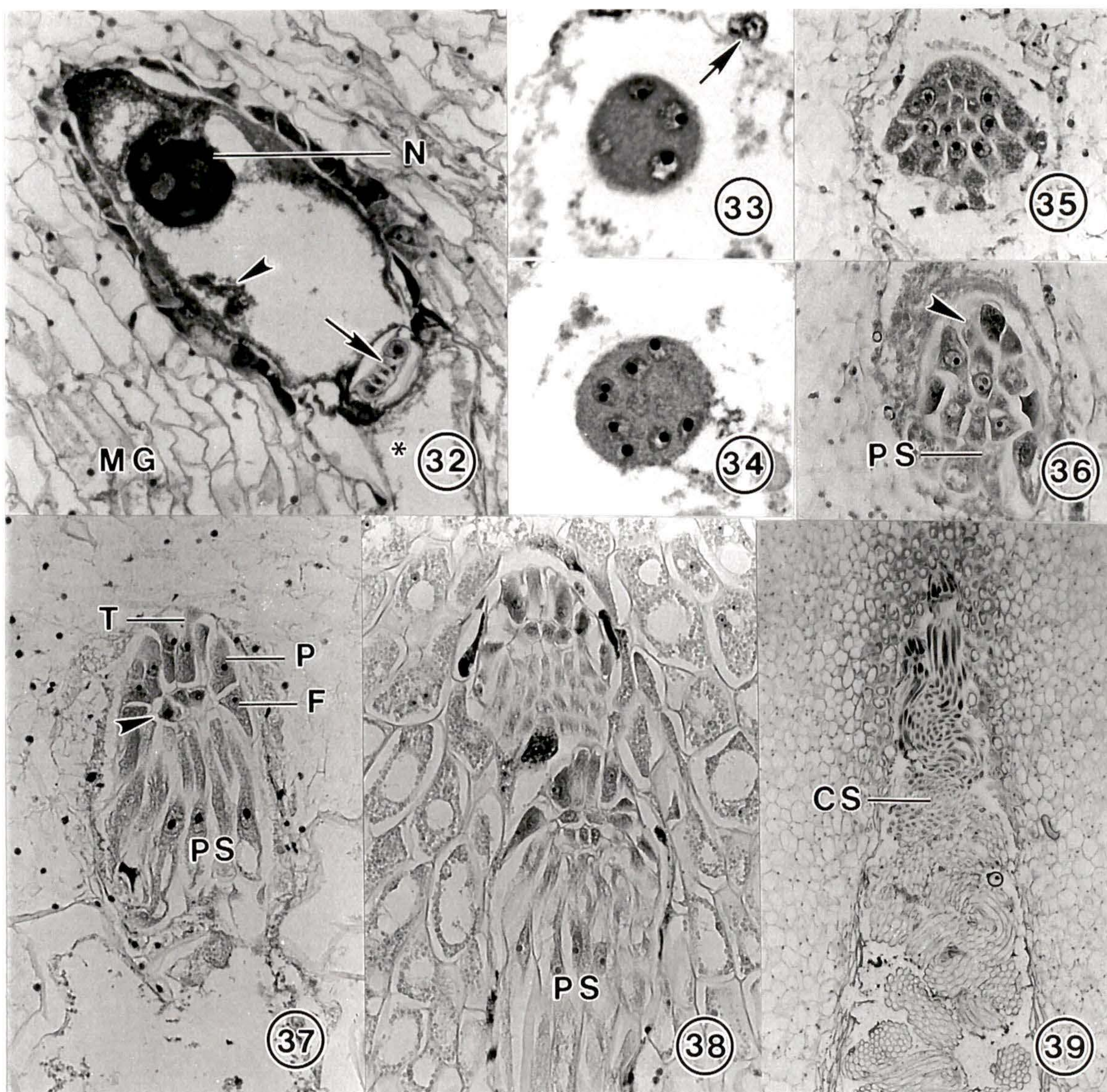
Fig. 34. Sixteen nucleate proembryo in darkly stained neocytoplasm. Egg cytoplasm is degenerating. 209x

Figs. 35, 36. Proembryo at about the 64-cell stage before the primary suspensor (PS) cells start to elongate showing cap cells (arrowhead). 122x, 110x

Fig. 37. Late proembryo stage inside the archegonial jacket showing terminal cap cells (T), peripheral cap cells (P), flank cells (F), central cells (arrowhead) and elongating primary suspensor cells (PS). 95x

Fig. 38. Two early embryos in the middle of the megagametophyte with cap cells, central cells and elongating primary suspensor cells. 121x

Fig. 39. Two early embryos showing coiled suspensor system (CS). 30x


N
MG
*
32
33
34
35
PS
36
T
P
F
PS
37
PS
38
CS
39

### 4.6. Fertilization

Fertilization occurred 13 to 16 mo after pollination. Pollen tubes grow to the developing archegonia when archegonia mature, then penetrate the archegonia through the degenerated neck cells (Fig. 31). Penetration through jacket cells was not found. Most neck cells remain intact before and after the penetration (Fig. 32) leaving some debris perhaps contributed from both male and female organelles. Both male gametes and the body cell cytoplasm enter the archegonium, however, the two male gametes do not make contact with the egg nucleus. One male gamete was occasionally found inside the archegonium near the proembryo. Fusion of male gamete with the egg nucleus was not observed. The neocytoplasm enclosing the zygote stains darkly.

### 4.7. Proembryo

Immediately following fertilization, the first two mitoses rapidly occurs forming a two nucleate and then a four nucleate proembryo and the archegonial jacket begins to degenerate (Fig. 32). Degeneration continues during subsequent free nuclear divisions. Eight free nucleate (Fig. 33) and 16-free nucleate proembryos (Fig. 34) are found near the distal portion of the archegonium. The nuclei are irregularly distributed in the neocytoplasm. Each nucleus contains a dark nucleolus. The remaining egg cytoplasm is present but degenerates as the proembryo develops. Proembryo cell-wall formation begins after the 32-free nucleate proembryo stage resulting in a round to conical group of isodiametric cells. This was observed in the 64-cell proembryo (Figs. 35, 36). The proximal cells of the proembryo elongate forming primary suspensors. Several cells in the central part of the proembryo remain isodiametric and form the central cells. Cells at the distal end (terminal cap cells) and several cells surrounding the central cells (peripheral cap cells and flank cells) form cap cells that later elongate. Suspensor cell elongation forces the central cells and cap cells through the archegonial jacket and into the megagametophyte (Figs. 37, 38).

## 4.8. Early, late and mature embryo

The primary suspensor elongates forcing the embryo toward the center of the megagametophyte. The cells of the megagametophyte tissue degenerate forming a corrosion cavity in advance of the embryo. As the primary suspensors continue to elongate, the cap cells and central cells move further toward the middle of the megagametophyte and the proximal parts of the suspensor start to coil. The primary suspensor becomes very long with several coils. Cap and central cells stain slightly darker than other early embryo cells. Megagametophyte cells adjacent to the corrosion cavity contain abundant starch (Fig. 39).

Two or three early embryos were commonly seen in a megagametophyte. Early embryos growing side by side were usually about equal in size (Figs. 38, 39). Variable stages including fertilization, proembryo and early embryo stages were frequently found in the same ovule.

As the early embryo elongates into the megagametophyte the cap cells start to degenerate and the central cells continue to divide forming an embryonal mass. The proximal cells of the embryonal mass divide forming embryonal tubes (Figs. 40, 41). Cytoplasm of the embryonal mass stains darkly, whereas of embryonal tubes do not. Embryonal tubes start to degenerate leaving some debris as the massive embryo continues to grow (Figs. 41, 42).

The massive embryo undergoes cell division and distal and proximal regions differentiate (Fig. 42). The distal region gives rise to the two cotyledon primordia , the apical meristem and stele promeristem and the proximal region forms the generative root meristem and rib meristem (Figs. 43-45). The stele promeristem extends from the embryo apex to the root initials and is surrounded by a poorly defined cortex promeristem. The stele and cortex promeristems enlarge and form the embryonic stele and the embryonic cortex. A rib meristem, lying between the root initials and the

suspensor system, adds peripherally to the embryonic cortex and to the suspensor (Figs. 45, 46). The generative root meristem and rib meristem further differentiate into a central core (column) and the peripheral tissue (pericolumn). Division of cells in the central core and the peripheral tissue gives rise to the root cap (Fig. 46). The root apical meristem is situated between the embryonic stele and the column. The cotyledons elongate several millimeters beyond the apex which remains mitotically inactive. In mature seeds, the two cotyledons occupy about 1/5 of the total embryo length and there is little space between the mature embryo and the surrounding megagametophyte (Figs. 46, 47).

### 4.9. Storage products and the seed coat

Storage products start to accumulate soon after the embryos push out of the archegonia. Megagametophyte cells surrounding the corrosion cavity, especially those close to the cap and central cells have more starch and stain more darkly than cells further away from the corrosion cavity. At maturity, megagametophyte cells are more darkly stained than that of embryo. Starch is found in both embryo and megagametophyte. Other storage products were not determined.

At the meiosis of megaspore mother cell, the ovule integument began to differentiate into the seed coat consisting of inner, middle and outer layers. The inner and outer layers of the integument are thin and darkly stained, whereas the middle layer is thick but lightly stained. The thickness of the seed coat remains similar during early free nuclear division (Fig. 20) and at fertilization ovule (Fig. 49). Observation on fixed mature seeds (Fig. 48) and sectioned seed coats (Fig. 50) show both inner (endotesta) and outer (sarcotesta) layers are dark brown and the middle (sclerotesta) layer is light brown.

Sarcotesta has rough lines on the surface (Figs. 8, 50). Both seed coat and the remaining nucellus are thin (Fig. 48).

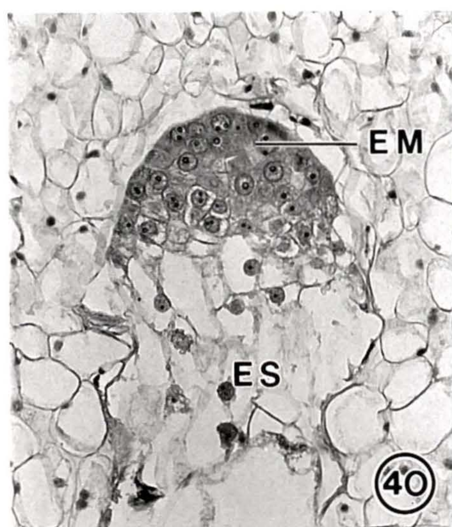
Figs. 40-43. Longitudinal sections of ovules showing early embryo stages.

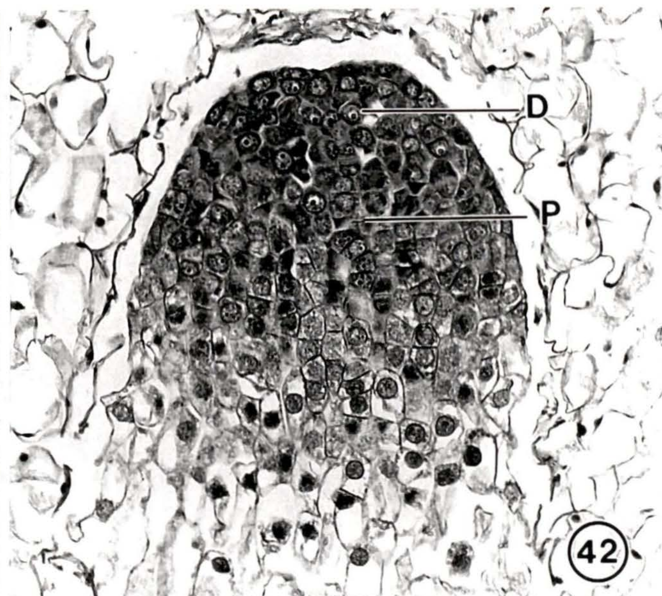
Fig. 40. Early embryo showing embryonal mass (EM)and embryonal suspensor (ES) in megagametophyte (MG). 140x

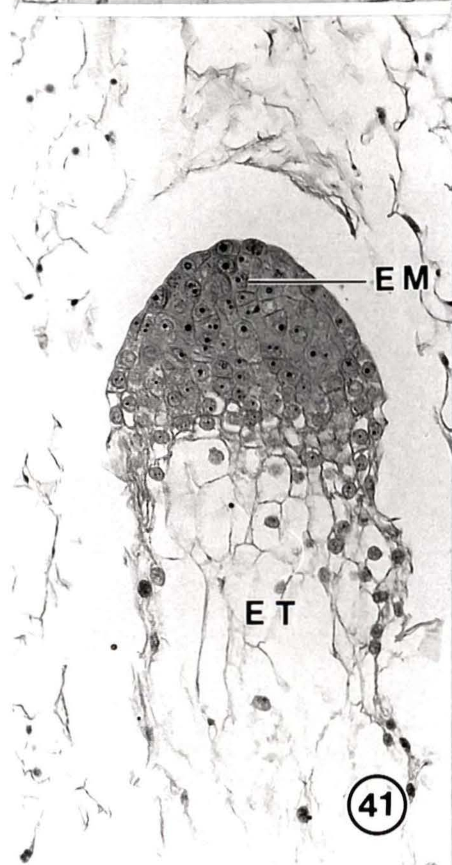
Fig. 41. Early embryo showing embryonal mass and embryonal tubes (ET). 146x

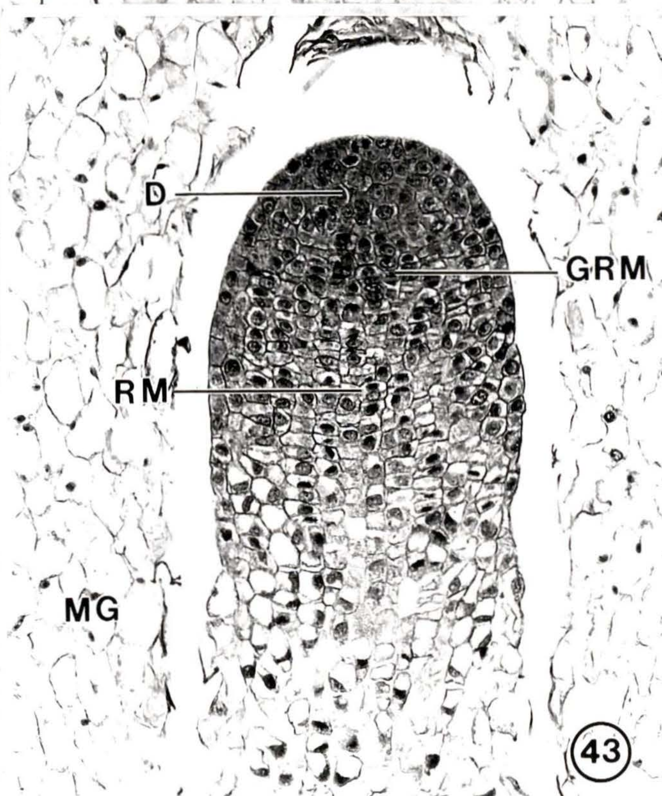
Fig. 42. Early embryo showing the origin of the distal (D) and proximal regions (P). 130x

Fig. 43. Early embryo at the time of differentiation of distal region (D), the generative root meristem (GRM) and rib meristem (RM). 112x


EM
ES
40


D
P
42


EM
ET
41


D
GRM
RM
MG
43

Figs. 44-46. Median longitudinal sections of late embryos

Fig. 44. Late embryo showing stele promeristem (SP) and cotyledonary primordia (CP). 44x

Fig. 45. Late embryo showing cotyledonary primordia, shoot apex (arrowhead) and root apex (RA). 58x

Fig. 46. Late embryo showing elongated cotyledons (C), shoot apex (arrowhead), root apex, column (COL), pericolumn (PC) and root cap (RC). 37x

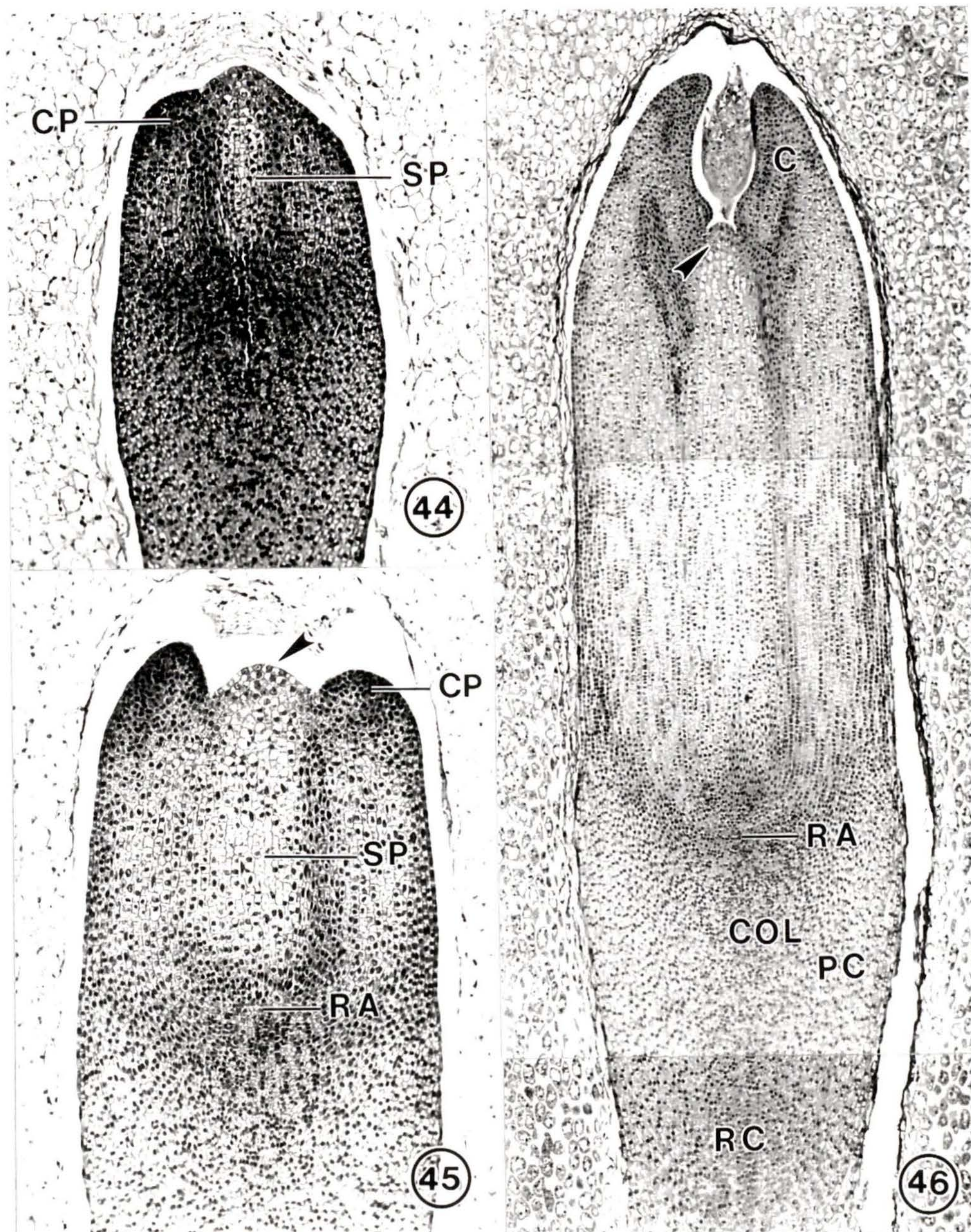
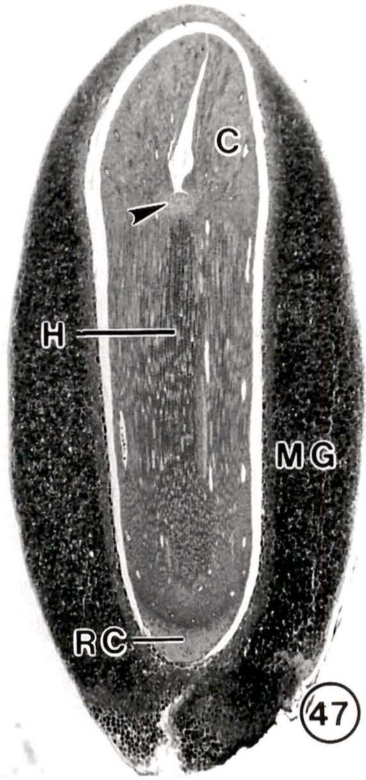

CP
SP
44
CP
SP
RA
45
C
RA
COL
PC
RC
46

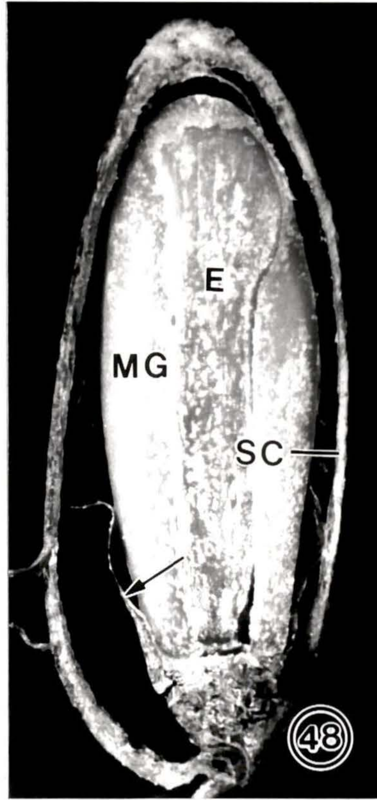
Fig. 47. Median longitudinal section of a mature seed with seed coat removed showing megagametophyte (MG) and mature embryo with cotyledons (C), apex (arrowhead), hypocotyl shoot axis (H) and root cap (RC). 10x

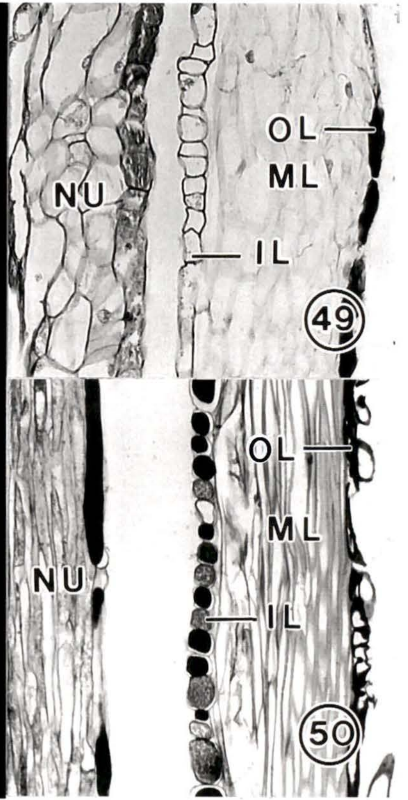
Fig. 48. Dissected mature seed showing seed coat (SC), embryo (E), megagametophyte (MG) and nucellus (arrow). 8x

Fig. 49. Sections of the integument at fertilization showing future inner (IL), middle (ML), outer layers (OL) of seed coat and the nucellus (NU). 322x

Fig. 50. Sections of the mature seed coat showing inner layer or endotesta, middle layer or sclerotesta, outer layer or sarcotesta and the nucellus. 108x


C
H
MG
RC
47


E
MG
SC
48


OL
ML
NU
IL
49
OL
ML
NU
IL
50

# Chapter 5

# DISCUSSION

## 5.1. Reproductive cycle

Dammar grown in Java was found to initiate cones at any time throughout the year. As a result seed cones and pollen cones at various stages and sizes were found at every collection. Continuous Dammar cone initiation is similar to what has been reported by de Veer (1954), but it is in contrast to temperate Araucariaceae and other temperate conifers in which floral initiation occurs only at a certain season (Owens and Blake 1985). The variation and frequency of flowering among individual trees, branches or twigs is not known. An earlier report mentioned that marked dichogamy (asynchrony) occurs in Dammar (de Veer 1954). Variation in time of seed cone maturity between West, Central and East Java (de Veer 1954, Suryamihardja 1979) was reported to be a response to the local annual environmental conditions (de Veer, 1954). That may in part result in continued cone initiation.

Dammar has a two year reproductive cycle. Despite the absence of distinct cold periods resulting in dormancy, it took about 3 to 4 mo from identifiable seed cone buds until pollination and another 15 to 18 mo from pollination to seed maturity, or a total of 20 to 24 mo for the entire cycle. The time from cone bud differentiation until identifiable seed-cone buds was not determined, but the total cycle from bud differentiation to seed cone maturity would be more than two calendar years. The presence of dry and wet seasons may affect the physiological processes and may influence the phenology of ovule and cone development. Low temperature usually slows most physiological processes in temperate plants and dry periods may cause dormancy in some tropical trees. Dammar reproduction is similar to that of temperate *A. australis* (Owens *et al.* 1995a) and other members of the Araucariaceae (Nikles 1973, Singh 1978, Haines and Prakash 1980) except for *A. cunninghamii* which is considered to have a three-year reproductive cycle,

taking about 23 months from seed-cone bud to fertilization. The only other tropical conifers whose reproductive cycles have been described are *Pinus merkusii* that takes 12.5 month and *P. kesiya* that takes 23 months from pollination to seed maturity in northern Thailand (Sirikul 1993). However, in these species the cycles are more predictable than in Dammar because they have one period of cone initiation and of pollination every year.

**5.2. Pollen cone and pollen structure**

Dammar pollen cones are simple strobili, cylindrical and stout with approximately 150-200 spirally arranged peltate microsporophylls depending on cone size. The cone is born solitary or in clusters, terminal, sub-terminal or axillary in the leaf axils. They are green and easily recognizable at emergence. Each microsporophyll bears on the abaxial surface seven cylindrical microsporangia, each with a narrow tip. They grow from the abaxial surface of the microsporophyll toward the cone axis. Some aspects of the pollen cones and microsporophylls are similar to that in other members of the genus (Whitmore 1980). The number of microsporangia in *Agathis* and *Araucaria* was mentioned in earlier literature. Gifford and Foster (1989) reported 13 to 15 and Page (1990) 4 to 15 microsporangia in *Agathis* and *Araucaria*. Eames (1913) reported 8 to 12 in *A. australis* and Burlingame (1913) 10 to 15 in *A. brasiliensis* where he estimated 500 to 1000 pollen grains were produced in each microsporangium.

Pollen mother cells are round with no intercellular spaces. After meiosis and the breakage of mother cell wall, each microspore expands forming an angular microspore. The angular microspores enlarge and form more or less round microspores. The microspores are finely sculptured and appear equal in size. They are suspended in thecal fluid until the pollen nearly matures. The fluid may play a role during the deposition of substances from the degenerating plasmodial tapetal cells onto the pollen wall as in other conifers (Singh 1978). Observations of fixed mature microsporangia revealed yellowish mature pollen grains with little space in between. Dammar pollen development and

structure are similar to *Agathis* as described by Singh (1978) and the brief descriptions given of the pollen wall in *A. australis* (Owens *et al.* 1995b) and in *A. brasiliensis* (Burlingame 1913). Even though Burlingame (1913) described development in more detail, he did not mentioned the angular stage in *A. brasiliensis.* Mature Dammar pollen showed structures in the peripheral portion of the pollen which appeared to be the prothallial, stalk and body cells as described by Singh (1978) in *Agathis* and by Burlingame (1913) in *Araucaria brasiliensis.* Internal structure was not easily recognized in paraffin sections. Burlingame (1913) reported 15 to 25 prothallial cells in *A. brasiliensis*, a recognizable tube nucleus and a single body cell and many large starch grains. A stalk cell in araucarian pollen is reported to be very evanescent (short lived) and often difficult to distinguish from the tube cytoplasm (Burlingame (1913), whereas Singh (1978) believed it to be absent. Most Pinaceae have two prothallial cells, but there may be fewer in *Tsuga heterophylla* (Colangeli 199). They are absent in the Taxaceae, Cupressaceae and Taxodiaceae (Owens and Blake 1985).

Paraffin sections of Dammar pollen show the wall to consist of a clear thick intine, dark endexine and lightly stained ektexine. Mature pollen is large, nonsaccate, spheroidal and finely sculptured. The shape however may slightly change from fresh to the dried condition. Dried pollen is about 42 μm in diameter, slightly smaller than that of *A. australis* (46μm) (Pocknall 1981).

### 5.3. Pollination and the pollination mechanism

Pollination occurs year-around with two to three peak periods. Monitoring from two different locations in the collection site showed variation in the pollen flight among location, but similar seasonal variation throughout the stand. Pollen flight in November through January was the lowest and concurrent with heavy rainfall. June through August was dry, but a relatively low amount of pollen was shed, although this increased in

August. This suggests the amount of pollen released is more affected by the number of cone produced rather than the rainfall.

Several factors may influence the amount of pollen shed. The degree-day heat sum as noted by Segley and Griffin (1989) influences pollen cone development which has been well demonstrated in temperate regions. There is no synchronous production of pollen cone and seed cones in this species (de Veer 1954) as there is in most temperate conifers (El-Kassaby 1995). The low amount of pollen available during seed cone receptivity can lead to low pollination success, which in turn, results in low fertilization. Unpollinated or poorly pollinated ovules abort in some conifer species (Owens 1995). Environmental conditions, such as heavy rain, may also cause low pollination success.

The pollination mechanism in Dammar is characterized by the absence of a specialized integument to capture pollen. The pollen lodges anywhere on the scale and may rest from days to weeks before it germinates and pollen tubes grow. This is similar to other Araucariaceae (Eames 1913, Singh 1978), *Tsuga heterophylla* in the Pinaceae (Colangeli 1989) and a few other conifer species (Singh 1978). In most conifers pollen is taken into the micropyle where it germinates. In some species the nucellus produces a pollination drops which functions to collect pollen. The pollen lands on the integument tip or on the surface of the pollination drops (Owens and Blake 1985) and then is withdrawn with the drop into the micropyle.

The germination of pollen on the scale was not observed, but pollen tubes were found on and in the nucellar tip, on the surface of the integument, between the integument and the nucellus, and even penetrating the integument at the micropylar or chalazal ends of the ovule. This was also reported in *A. australis* (Eames 1913) but pollen tubes only on the nucellus were observed by Owens *et al.* (1995a). The nucellar tip that projects out of the broad micropyle weeks to months after pollination may be involved in contacting the pollen tubes or providing a surface on which pollen tubes may grow. The nucellar flap, first mentioned in *A. australis* (Owens *et al.* 1995a) was thought to play a role in pollen

tube growth but not in capturing pollen. Eames (1913) noted that pollen germinated near the ovule then the tubes passed toward the micropyle. Thomson (1907) and Singh (1978) referred to this as protosiphonogamic and siphonogamic, repectively.

In the nucellus the tubes branch and grow around the megagametophyte causing the central parts of the megagametophyte to become indented. The tubes grow toward the tips of young archegonia without penetrating them. More than two pollen tubes per ovule were common at every collection date indicating a certain degree of pollination success.

In the nucellus the tubes grow from one archegonium to another and in some cases toward the chalazal end of the megagametophyte. This may be related to the scattered positioning of archegonia which are mostly deeply seated in the megagametophyte. Eames (1913) and Ecroyd (1982) suggest that such growth creates the pathway for male gametes prior to the penetration of the mature archegonium. The growth of the tubes has also been associated with the presence of unidentified chemotropic substances, especially in the nucellus (Singh 1978).

In the nucellus, pollen tubes contain several prothallial cells in the tube cell cytoplasm and a body cell that forms two, frequently round male gametes, surrounded by darkly stained body-cell cytoplasm.

### 5.4. Seed cone and seed

Seed cone buds are covered by bud scales. They are globose or subglobose similar to that of developing and nearly mature cones. However, the cones become oval or elliptical at pollination and remain approximately this shape until several months after pollination. Later, scale and ovule enlargement causes cones to increase more in width than length changing the shape to globose or subglobose. At maturity, the seed cones are large, dark green. The inner portion of the mature scale and seeds are brown. The scales

abscise as the cone matures and dries. The seed, which becomes loosely attached several weeks to months after pollination, falls off as the scale abscises.

Dammar, like other *Agathis* species, has a large seed cone with completely fused bracts and ovuliferous scales that are more or less spirally arranged. The term bract, ovuliferous scale, bract scale or cone scale have been used (Eames 1913, Burlingame 1913, Soerianegara and Lemmens 1993, Owens *et al.* 1995a), especially in taxonomic descriptions (de Laubenfels 1978, Whitmore 1980). The bract and scale appear to be totally fused in *Agathis* (Eames 1913), therefore the simplest term 'scale' is commonly used. In *Araucaria* they are partially fused and free at the tip (Eames 1913, Burlingame 1913, Singh 1978, Gifford and Foster 1989, Page 1990) but scale is still used. The abscission of the scale at maturity occurs in all Araucariaceae (Whitmore 1977, Page 1990) as in the more familiar *Abies* of the Pinaceae (Owens and Molder 1984, Page 1990). In Dammar the color of the seed cone and inner part of the scales and the seeds are used as indicators of maturity in the field (Suryamihardja 1979). Mature cones are usually put in a room at ambient temperature and after two or three days the scales abscise. A single seed with attached papery wing falls from the scale.

### 5.5. Pre-fertilization ovule

The development of the megagametophyte in Dammar is similar to other members of the Araucariaceae, but may differ in the number of free nuclear divisions. Archegonia are variable in size, shape and are unevenly spaced in the megagametophyte. Each archegonial initial has a large nucleus and is surrounded by several cells which divide forming the archegonial jacket. At the central cell stage, the nucleus is found near the neck cells and small and large vacuoles are distributed throughout the cell. The primary neck cell divides and forms several neck cells which are clearly observed even after penetration of the pollen tube. In the mature archegonium, the egg nucleus is surrounded by a perinuclear zone and is either in the centre or the chalazal half of the archegonium.

Vacuoles are distributed around the perinuclear zone to the periphery of the egg cytoplasm. A ventral canal nucleus is usually found in the periphery of the micropylar half of the archegonium some distance from the neck cells. This is similar to that of *A. australis* (Eames 1913, Owens *et al.* 1995a), the only other *Agathis* species that has been described.

The number of archegonia is variable in Dammar as well as many other species of the Araucariaceae. The number in Dammar is 4 to 6, with up to 10. The total number of archegonia per megagametophyte is probably more because the peripheral portions of the megagametophytes are not sectioned. Eames (1913) noted from 3 to 25 in *A. australis*, but Owens *et al.* (1995a) reported about the same number as in Dammar. The variation in number of archegonia may be affected by the size of megagametophyte. Gifford and Foster (1989) noted the number of free nuclear divisions may affect the size of megagametophyte, which in turn, may influence the number of archegonia that forms, but this number is normally constant within the species.

An archegonial complex is absent, but a group of several archegonia may be arranged in the proximal half of the megagametophyte, as noted by Eames (1913) in *A. australis* and described in Singh (1978). However, they have separate archegonial jackets and are lanceolate or oval in shape. Two archegonia which partially shared an archegonial jackets were found, as in *A. australis* (Owens *et al.* 1995a).

### 5.6. Fertilization

The pollen tubes penetrate the neck cells which remain intact until several mitoses of the proembryo or in some cases they break away or degenerate soon after penetration. The body-cell cytoplasm and male gametes move toward the pollen tube tip, then are released into the archegonium. One male gamete fuses with the egg nucleus as in other *Agathis* species (Kaur and Bhatnagar 1984, Owens *et al.* 1995b). The second male gamete was occasionally found intact inside the archegonium until the 8-free nucleate

proembryo stage. The body-cell cytoplasm that contains the paternal organelles is incorporated into the neocytoplasm. On rare occasions portions of the male cytoplasm were left outside the archegonium. Male cytoplasm is incorporated into the neocytoplasm, which in turn, contributes to the cytoplasmic inheritance of this species. In *A. robusta* (Kaur and Bhatnagar 1984) neocytoplasm is paternal in origin, similar to that in *A. australis* (Owens *et al.* 1995b).

### 5.7. Proembryo and embryo development

Proembryo and embryo development in Dammar are similar to other members of the Araucariaceae. After five to six proembryo free nuclear divisions cell-wall formation occurs. The proembryo forms distal, central and proximal cells which, in turn, develops into three distinct structures: a protective cap, central cells (embryo proper) and primary suspensors, respectively. These are called three tiers in the terminology used by Eames (1913) and Owens *et al.* (1995c) for *A. australis*. The primary upper tier and primary embryonal tier found in most conifer families, as described in Singh (1978), are not applicable since the number of cells and the differentiation of cells in the proembryo in the Araucariaceae are different. The distal tier consists of a large apical cell and an adjacent ring of cells. These elongate to form the embryo cap which degenerates during the early embryo stage. The subapical cell and surrounding ring of cells form the second tier which is analogous to the embryo proper of Eames (1913) and Chamberlain (1935). The division of the subapical cell forms central cells of Owens *et al.* (1995c) and the outer ring of cells form the lateral cap cells. The third tier consisting of the small, irregularly arranged cells forms the primary suspensor. Cap cells and primary suspensor cells elongate. Before the elongation was prominent the proembryo appeared to be conical and was located at the distal end of the archegonium. The megagametophyte cells near the tip of the cap cells appear to degenerate as the central cells divide. Eames (1913) first proposed that cap cells function as the source of food for the inner cells of the growing

embryo. Later Haines and Prakash (1980) described other possible functions in *Araucaria*, such as embryo protection from abrasion and crushing, enzyme secretion to form the corrosion cavity and prevention of cleavage polyembryony. The studies of Owens *et al.* (1995c) on *A. australis* support the first two functions but not the last proposed by Haines and Prakash (1980).

As the cap cells degenerate, the division of central cells forms a massive embryo. The development of the massive embryo to the late embryo is characterized by the differentiation of distal and proximal regions of the embryo. This appears to be similar in most conifer species, but may have different terminology and the terminology introduced by Allen (1946) is used here. The distal region forms the two cotyledon primordia, the apical meristem and the stele promeristem and the proximal region forms the generative root meristem and rib meristem. The generative root meristem and rib meristem form the root apical meristem, a central core (column) and the peripheral portion (pericolumn) that together formed a root cap. The root apical meristem located between the column and stele promeristem, is visible in the club-shape embryo and the mature embryo. The stele promeristem elongates as the embryo grows, as do the cotyledons. A provascular cylinder appears in the peripheral portion of the stele. All components of the embryo except the apex enlarge and elongate filling almost half of the mature seed. Cotyledons occupy about 20% of the total length of the embryo and hypocotyl and root cap occupy about 80%. The megagametophyte is almost equal in volume to the embryo, whereas there is little nucellus remaining. There are two cotyledons in Dammar as in other *Agathis* species, whereas the number is variable in *Araucaria* depending on species (Haines 1983b).

**5.8. Seed coat development and storage products**

The differentiation of the seed coat from the integument begins at meiosis of the megaspore mother cell as in the *Araucaria* (Haines 1983b). The integument remains in the same state until fertilization and early embryogeny, but elongates as the ovule enlarges. The inner layer of the integument is composed of one layer of darkly stained cells similar to the outer layer. The middle layer is composed of a thick layer of lightly stained large cells periclinally oriented to the ovule surface. In the mature seed these layers form the three layers of the seed coat: the inner (endotesta), middle (sclerotesta) and outer (sarcotesta) layers. The thickness of these three layers remains as in previous stages, but the cells in the middle layer become very elongate. Sectioned material shows the inner layer is darkly stained, presumably containing phenolic compound. The outer layer also stains darkly but has a different appearance to that of the inner layer. The outer layer has rough lines on the surface. The Dammar seed coat is different from four species of *Araucaria* described by Haines (1983b) in term of the number of cells composing the inner and outer layers. The cells in the inner layer of *A. cunninghamii*, *A. heterophylla* and *A. hunsteinii* form two lateral large-celled areas (zones), but they do not in *A. bidwillii* (Haines 1983b). The middle (sclerostesta) layer in Dammar is more or less similar to that in *Araucaria*, especially in *Araucaria hunsteinii*. Haines (1983b) noted that there is an unusual distribution of sclerenchyma in some Araucarian seed coats, therefore he suggested the term sclerotesta as used in most gymnosperm seed coat may not be applicable to the seed coat in the Araucariaceae

The seed coat and seed wing play an important role in the fate of the seed during dispersal, storage and germination (Allen and Owens 1972, Singh 1978, Haines 1983b). The structure and the chemical components of the seed coat may protect the seed from many types of predation. The seed coat in Dammar is relatively thin and presents no obstacles in seed germination. Germination usually takes place within 16 days after

sowing and no pretreatment is required (Komar 1992). The wing, as in many other winged seeds, aids in seed dispersal.

Seed storage products are important in seed development and germination. Storage product accumulation began during early embryo development. Most megagametophyte cells near the tip of the embryo were darkly stained and filled with starch. The accumulation of starch continued in both megagametophyte and embryonic cells. At maturity, starch was uniformly distributed in the embryo and megagametophytic cells. Megagametophyte cells stain more darkly and contain other storage products that were not observed in this study. These have been identified as lipids and protein in other conifer seeds (Owens *et al.* 1992). In *A. australis* the storage products are primarily lipids and protein with little starch present (Owens *et al.* 1996). Earlier, Haines (1983b) mentioned that starch is predominant in *Araucaria*, but he did not test for lipids and proteins.

**5.9. Seed production and seed efficiency**

Cone abortion in Dammar exceeds 30%. However, the mechanism and the physiological causes of the abortion are not known. Ovule abortion, presumably due to the low pollination and fertilization success, may cause cone abortion as well as the presence of the extreme environmental conditions and predation. Animals, particularly squirrels, are frequently found in the collection site and cause some cone loss. Owens and Blake (1985) and Owens (1995) suggest several possible causes of low seed production in temperate conifers but there is limited information available for tropical conifers. Low pollination and fertilization success, nutrient competition and extreme environmental conditions contribute to abortion (Owens and Blake 1985, Owens 1995). A lack of pollination that has mostly been demonstrated in angiosperms also occurs in conifers, such as in *A. australis* (Owens *et al.* 1995a), *Thuja* (Owens and Molder 1984) and some seed

orchard trees (El-Kassaby 1995). Poorly pollinated ovules cause cone abortion in some pines (Owens 1995) but this has not been demonstrated in *Agathis*.

Abortion of the megagametophyte was evident in Dammar, but the percentage of abortion and the stage at which it occurs was not determined. This abortion contributes to the high proportion of empty seed which is over 60% (seed efficiency of less than 40%) of the seed potential. However, this is more or less similar to that found in *A. australis* (25 to 35 % of sound seed) (Ecroyd 1982). Megagametophyte abortion also occurs in some members of the Pinaceae and Cupressaceae (Owens and Blake 1985) and usually lead to ovule and possibly cone abortion. In the Pinaceae and Cupressaceae, the abortion occurs at or shortly after the meiosis of megaspore (Owens and Blake 1985). In some conifers, the megagametophyte abortion occurs without preventing further development of seed cone (Owens and Blake 1985, Owens 1995) but results in a high proportion of empty seeds. The pollination may not be the problem in Dammar since pollen tubes were found in the ovule at most collection date, but high proportion of self-pollination may occur which generally results in post-zygotic embryo and ovule abortion in conifers.

Chapter 6

# SUMMARY AND CONCLUSIONS

## 6.1. Reproductive cycle

1. Floral initiation in Dammar appears to occur at any time throughout the year.
2. Dammar has a 2-year reproductive cycle. It takes 13 to 16 mo from pollination to fertilization, 15 to 18 mo from pollination to seed maturity and 20 to 24 mo from identifiable cone buds to seed maturity.

## 6.2. Pollen

1. The pollen cone consists of 150 to 200 peltate microsporophylls, each bearing seven microsporangia.
2. Pollen is yellow, non saccate and $\pm$ 42μm in diameter when dry. The body cell forms two male gametes in the pollen tubes as they grow through the nucellus. The pollen and pollen tubes also contain several prothallial cells.
3. It takes 5 to 6 mo from the pollen-mother cell stage to the mature pollen.

## 6.3. Pollination

1. Dammar has two or three periods of peak pollination but some pollen may be shed at all times of the year. The pollen lands between the scales and pollen tubes form 1 to 2 mo after pollination. Pollen tubes grow on the surface of the integument, between the integument and nucellus and nucellar tip. They may first penetrate the integument and then the nucellus. The tube branches before reaching the archegonium. More than one pollen tube per ovule is common.

### 6.4. Ovule and megagametophyte

1. At pollination, ovule primordia are not differentiated into integument and archesporial cells.
2. Each megagametophyte contains one to 10 archegonia, but four to six archegonia per megagametophyte are more common.

### 6.5. Fertilization

1. At fertilization the pollen tube penetrates the neck cells. One male gamete fuses with egg nucleus and the second male gamete may remain in the pollen tube or inside the archegonium.

### 6.6. Embryology

1. Proembryo and early embryos have cap cells, central cells (embryo proper) and primary suspensors similar to other members of the Araucariaceae.
2. More than one proembryo or early embryo is common in the same ovule.
3. Simple polyembryony occurs but cleavage polyembryony does not.

### 6.7. Cone and seed abortion

1. Cone and seed abortion are major constraints in Dammar seed production. Cone abortion at various stage reaches over 30%. Seed abortion (empty seeds) reaches over 60% of seed potential. Asynchrony between pollination and seed cone receptivity, low amount of pollen during most of the year, self-pollination, predation and environmental conditions are possible causes of the abortion.

## LITERATURE CITED

Allen GS and JN Owens. 1972. The life history of Douglas-fir. Canada Forest Service. Environment Canada.

Allen GS. 1946. Embryology and the development of the apical meristem of *Pseudotsuga menziesii*. I. Fertilization and early embryogeny. American Journal of Botany. 33: 666-677

Allen GS. 1947. Embryology and the development of the apical meristem of *Pseudotsuga menziesii*. II. Late embryogeny. American Journal of Botany. 34: 73-80.

Berlyn GP and JP Miksche. 1976. Botanical microtechnique and cytochemistry. The Iowa State University. Press, Ames, Iowa.

Bruijnzeel LA and KF Wiersum. 1985. A nutrient balance sheet for *Agathis dammara* Warb. plantation forest under various management conditions in central Java, Indonesia. Forest Ecology and Management. 10:195-208

Burlingame LL. 1913. The morphology of *Araucaria brasiliensis*. I. The staminate cone and male gametophyte. Botanical Gazette. Vol. 55, No. 2.

Chamberlain CJ. 1935. Gymnosperms: structure and evolution. University of Chicago Press, Chicago.

Colangeli AM. 1989. Pollination and pollen and seed development in Western Hemlock. Ph.D. Thesis, Department of Biology, University of Victoria.

Dallimore W and B Jackson. 1966. A handbook of coniferae and ginkgoaceae. (4th ed.) Edward Arnolds Publication Ltd. page: 92-105

De Laubenfels DJ and J Silba. 1987. The *Agathis* of Espirito Santo (Araucariaceae, New Hebrides). Phytologia 61(7): 448-452

De Laubenfels DJ. 1972. Flore de la Nouvelle-Caledonie et dependances. No. 4. Gymnospermes. Musee Nationale d'Histoire Naturelle. Paris. 167pp.

De Laubenfels DJ. 1978. The mollucan dammars (*Agathis*, Araucariaceae). Blumea 24: 499-504

De Laubenfels DJ. 1979. The species of *Agathis* (Araucariaceae) of Borneo. Blumea 25: 531-541

De Laubenfels DJ. 1988. Coniferae: Araucariaceae. 2. *Agathis*. Page 429- 442. In CGGJ van Steenis (Ed). Flora Malesiana.

De Veer EJA. 1954. Artificial regeneration of *Agathis loranthifolia* Salisb. General Papers for 4th World Forestry, India.

Eames AJ. 1913. The morphology of *Agathis australis*. Annals of Botany 27(105): 1-38

Ecroyd CE. 1982. Biological flora of New Zealand. 8. *Agathis australis* (D.Don). Lindl. (Araucariaceae) Kauri. New Zealand Journal of Botany. 20: 17-36

El-Kassaby Y. 1995. Evaluation of the tree-improvement delivery system: factors affecting genetic potential. Tree Physiology. 15: 545-550

Gifford EM 1950. Softening refractory plant material embedded in paraffin. Staining Technology 25:161-162.

Gifford EM and AS Foster. 1989. Morphology and evolution of vascular plants. 3rd Edition. WH Freeman and Co., NY., USA. page: 401-451

Greenwood MS. 1986. Gene exchange in loblolly pine: the relation between pollination, female receptivity and pollen availability. American Journal of Botany. 73(10): 1443-1451

Haines RJ and N Prakash. 1980. Proembryo development and suspensor elongation in *Araucaria* Juss. Australian Journal of Botany. 28: 511-522

Haines RJ 1983a. Embryo development and anatomy in *Araucaria* Juss. Australian Journal of Botany. 31(2): 125-140

Haines RJ 1983b. Seed development in *Araucaria* Juss. Australian Journal of Botany. 31(3): 255-267

Haines RJ, N Prakash and DG Nikles. 1984. Pollination in *Araucaria* Juss. Australian Journal of Botany. 32(6): 583-594

Johansen DA. 1940. Plant microtechnique. McGraw-Hill, New York.

Kaur D and SP Bhatnagar. 1984. Fertilization and formation of neocytoplasm in *Agathis robusta*. Phytomorphology. 34: 1-4

Komar TE. 1995. The effect of storage temperature and moisture content on the viability of *Agathis loranthifolia* Salisb. Fellowship Report. ASEAN Tree Seed Centre, Thailand.

Meijer Drees E. 1940. The genus *Agathis* in Malaysia. Bull. Jard. Bot. Buitenzorg Ser. 3(26): 455-474

Mogensen HL. 1996. The hows and whys of cytoplasmic inheritance in seed plants. American Journal of Botany. 83(3) 383-404

Nikles DG. 1973. Biology and genetic improvement of *Araucaria cunninghamii* Ait. in Queensland, Australia. Proceeding Joint IUFRO Workshop. Gainesville, Fla. 2: 304-334

Owens JN and M Molder. 1975. Pollination, female gametophyte and embryo and seed development in yellow cedar (*Chamaecyparis nootkatensis*). Canadian Journal of Botany. 53: 186-199

Owens JN and M Molder. 1984a. The reproductive cycle of lodgepole pine. British Columbia Ministry of Forest, Information Service Branch, Victoria.

Owens JN and M Molder. 1984b. The reproductive cycle of Interior spruce. British Columbia Ministry of Forest, Information Service Branch, Victoria.

Owens JN and M Molder. 1984c. The reproductive cycle of western red cedar and yellow cedar. British Columbia Ministry of Forest, Information Service Branch, Victoria.

Owens JN and M Molder. 1984d. The reproductive cycle of western and mountain hemlock. British Columbia Ministry of Forest, Information Service Branch, Victoria.

Owens JN and M Molder. 1985. The reproductive cycle of the true firs. British Columbia Ministry of Forest, Information Service Branch, Victoria.

Owens JN and MD Blake. 1985. Forest tree seed production. Canadian Forestry Service, Government of Canada.

Owens JN and S Simpson 1986. Pollen from conifers native to British Columbia. Canadian Journal of Forest Research 16(5): 955-967

Owens JN., SJ Simpson and GE Caron. 1987. The pollination mechanism in Engelman spruce (*Picea engelmani*). Canadian Journal of Botany 65:1439-1450

Owens JN. 1991. Flowering and seed set. In Physiology of Trees (Ed). AS Raghavendra. John Willey & Sons, Inc. page: 247-271

Owens JN and SJ Morris. 1991. Cytological basis for cytoplasmic inheritance in *Pseudotsuga menziesii*. II. Fertilization and proembryo development. American Journal of Botany 78: 1515-1527

Owens JN, P Sornsathapornkul and S Tangmitcharoen. 1991. Manual studying flowering and seed ontogeny in tropical forest trees. ASEAN-Canada Forest Tree Seed Centre, Thailand.

Owens JN, SJ Morris and S Misra. 1992. The ultrastructural , histochemical, and biochemical development of the post-fertilization megagametophyte and the zygotic embryo of *Pseudotsuga mensiezii*. Canadian Journal of Forest Research. 23: 816-827

Owens JN. 1995. Constraints to seed production: temperate and tropical trees. Tree Physiology 15: 477-484

Owens JN, GD Catalano, SJ Morris and AT Kristie. 1995a. Reproductive biology of *Agathis australis*. I. Pollination and prefertilization development. International Journal of Plant Science. 156(3): 275-269

Owens JN, GD Catalano, SJ Morris and AT Kristie. 1995b. Reproductive Biology of *Agathis australis*. II. Male gametes, fertilization and cytoplasmic inheritance. International Journal of Plant Science. 156(4): 404-416

Owens JN, GD Catalano, SJ Morris and AT Kristie. 1995c. Reproductive Biology of *Agathis australis*. III. Proembryogeny and early embryogeny. International Journal of Plant Science. 156(6): 793-806

Page CN. 1980. Leaf micromorphology in *Agathis* and its taxonomic implication. Plant Systematic and Evolution. 135: 71-79

Page CN. 1990. Araucariaceae. In Families and genera of vascular plants. Springer-Verlag, Berlin Heidelberg, Germany. page: 294-299

Pocknall DT. 1981. Pollen morphology of the New Zealand species of *Libocedrus* Endlicher (Cupressaceae) and *Agathis* Salisbury (Araucariaceae). New Zealand Journal of Botany. 19: 267-272

Roy Choudhury C. 1962. The embryogeny of conifers: a review. Phytomorphology 12: 313-338

Runions CJ, GL Catalano and JN Owens. 1995. Pollination mechanism of seed orchard Interior Spruce. Canadian Journal of Forest Research. 25: 1434-1444

Sedgley M and AR Griffin. 1989. Sexual reproduction of tree crops. Academic Press. 378pp

Singh H. 1978. Embryology of gymnosperms. Gebruder Borntraeger, Berlin.

Sirikul W. 1993. Seed production of Pines in Thailand. Proceeding International Symposium on genetic conservation and production of tropical forest tree seed. ASEAN-Canada Forest Tree Seed Centre, Thailand.

Soerianegara I and RHMI Lemmens (Ed.). 1993. Timber trees: Major Commercial Timbers. PROSEA. Pudoc Scientific Publication, Wageningen. page: 78-83

Stockey RA and TN Taylor. 1981. Scanning Electron microscopy of epidermal patterns and cuticular structure in the genus *Agathis*. Scanning Electron Microscope 3: 207-212

Stockey RA. 1982. The Araucariaceae: an evolutionary perspective. Review Palaeobotany and Palynology. 37: 133-154

Stockey RA and IJ Atkinson. 1993. Cuticle micromorphology of *Agathis* Salisbury. International Journal of Plant Science. 154 (1): 187-225

Stockey RA. 1994. Mesozoic Araucariaceae: morphology and systematic relationship. Journal of Plant Research. 107: 493-502

Suryamihardja S. 1979. Seeds characteristics of *Agathis loranthifolia,* Salisb. The Malayan Forester. 42(3): 214-220

Taylor TN and EL Taylor. 1993. Araucariaceae. In The biology and evolution of fossil plants. Prentice Hall. Engelwood, New Jersey. page: 699-702.

Thomson RB. 1907. The Araucariaceae, a 'protosiphonogamic' method of fertilization. Science, N.S. 25: page 271

Vasilieva GV. 1972. Materials on the comparative anatomy of leaf in species of *Agathis* Salisb. (Araucariaceae). Botanical Gazette. 57: 108-118

Whitmore TC. 1977. A First Look of *Agathis*. Tropical forestry Paper No. 11. Department of Forestry, Commonwealth Forestry Institute, University of Oxford. 54pp.

Whitmore TC. 1980. A monograph of *Agathis*. Plant Systematic and Evolution. 135: 41-69

Wilson MF and N Burley. 1983. Mate choice in plants. Princeton, N. J.

Appendix 1. Number of aborted cones

| Reproductive Materials | Jun | Jul | Aug | Sep | 1995 Oct | Nov | Dec | Jan | Feb | 1996 Mar | Apr | May |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cone buds (16)[1] | | | | | | | | | | | | |
| Dia. (mm) | 7.4 | 7.4 | 7.5 | 10.3 | 18.8 | 25.7 | 28.1 | 28.3 | 29.5 | 30 | 31.3 | 32.6 |
| No. aborted | o | o | 1 | 1 | 2 | 2 | 3 | 6 | 7 | 7 | 7 | 7 |
| % aborted | o | o | 6.2 | 6.2 | 12.5 | 12.5 | 18.7 | 37.5 | 43.7 | 43.7 | 43.7 | 43.7 |
| Post-poll. cone (41) | | | | | | | | | | | | |
| Dia. (mm) | 33.5 | 33.6 | 34.2 | 34.7 | 39.4 | 44.6 | 51.7 | 59.1 | 62.1 | 63.4 | 64.9 | 67.1 |
| No. aborted | o | o | o | o | 2 | 4 | 6 | 7 | 9 | 12 | 12 | mature |
| % aborted | o | o | o | o | 4.9 | 9.8 | 14.6 | 17.1 | 21.9 | 29.3 | 29.3 | mature |
| Dev. cone (4)[2] | | | | | | | | | | | | |
| Dia. (mm) | 65 | 67.5 | 68.1 | 66.5 | 67(m) | seed shed | | | | | | |
| No. aborted | o | o | o | 2 | 4 | (mature) | | | | | | |
| %aborted | o | o | o | o | o | mature | | | | | | |
| Pollen cone (18) | | | | | | | | | | | | |
| Dia (mm) | 8.9 | 9.3 | 9.7 | 10.6 | 13.6 | mature | | | | | | |
| No. aborted | o | o | o | o | o | pollen shed | | | | | | |

Note:

1 : Number in parentheses indicates the number of tagged and regularly measured cones.

2 : Developing and nearly mature cones are considered as complementary data to the seed cone buds and post-pollination seed cones, but not treated and included in the proportion of cone abortion as appear in the text.

Appendix 2. Stages of development and reproductive cycle

| Months[1] after cone bud | Months[2] after poll-ination | Cone diameter (cm) | Ovule length (mm) | Average ovule length (mm) | Stages of development |
|---|---|---|---|---|---|
| | | <2.0 | - | - | Pre-pollination |
| 0 | 0 | 2.0-2.3 | - | - | Pollination |
| 5 | 1-2 | 2.4-2.6 | 2.5-3.0 | 2.8 | ST-MMC |
| | 2-3 | 2.7-2.9 | 2.8-3.2 | 3.0 | MMC- FM |
| | 4-10 | 3.4-3.6 | 3.5-4.0 | 3.74 | mid FND |
| 15 | 10-11 | 3.7-3.9 | 3.5-4.8 | 4.17 | late FND-CWF |
| 16 | 11-12 | 4.0-4.3 | 3.8-6.2 | 4.76 | late FND-CWF |
| 17 | 13-16 | 4.4-4.6 | 3.8-8.0 | 6.36 | mature MG-fertilization |
| 17 | 13-16 | 4.7-4.9 | 6.4-8.4 | 7.50 | Proembryo |
| 17 | 13-16 | 5.0-5.3 | 6.4-10.1 | 7.90 | Early embryo |
| 17 | 13-16 | 5.4-5.6 | 8.5-10.1 | 9.16 | Mid embryo |
| 18 | 14-17 | 5.7-5.9 | 10.5-11.0 | 10.75 | Late embryo |
| 18 | 14-17 | 6.0-6.9 | 7.4-13.5 | 10.63 | Seed maturation |
| | | 6.4-6.6 | 9.9-11.9 | 11.28 | |
| | | 6.7-6.9 | 10.7-13.5 | 11.77 | |
| 19 | 15-16 | 7.0-7.3 | 11.6-13.5 | 12.66 | Seed maturation |
| | | 7.4-7.6 | 13.0-13.6 | 13.23 | |
| | | 7.7-7.9 | 13.0-13.7 | 13.45 | Seed shed |
| | 16-17 | 8.0-8.3 | 14.4-15.0 | 14.7 | Seed shed |
| 22-24 | 17-18 | >8.4 | >14.1 | >14.1 | |

Note:

1 : Number of months from identifiable cone buds to the consecutive stages of ovule development.

2 : Number of months from pollination (receptive seed cones) to the consecutive stages of ovule development.

ST : sporogenous tissue, MMC : megaspore mother cell, FM : functional megaspore, FND : free nuclear division, CWF : cell wall formation of megagametophyte (MG)

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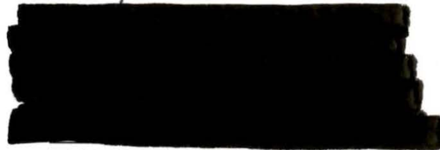

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