Tree Seed Components

A conifer seed has three main components: the seed coat, embryo, and megagametophyte. These components are shown dissected from a longitudinal section of a Douglas-fir seed in FIGURE 3. The embryo or **zygote** is the result of the fertilization of the egg within an ovule by the sperm within a pollen grain. It has all the necessary structures and information to produce a tree. The **megagametophyte** serves as the food reserve for the embryo until it is able to **photosynthesize**. The seed coat protects the inner structures from damage, but can also restrict oxygen uptake, gas exchange, water uptake, or radicle emergence due to its anatomical structure.

An illustration of a typical conifer seed with the seed wing attached is presented in FIGURE 4 (page 6). The three seed coat layers are exaggerated in size to allow them to be differentiated. Throughout the text and in performing cutting tests this figure will be a useful reference in identifying the anatomical features of conifer tree seed. The thinner, pointed micropylar end of the seed is associated with the site of radicle emergence while the wider, more rounded **chalazal** end is associated with the cotyledons.

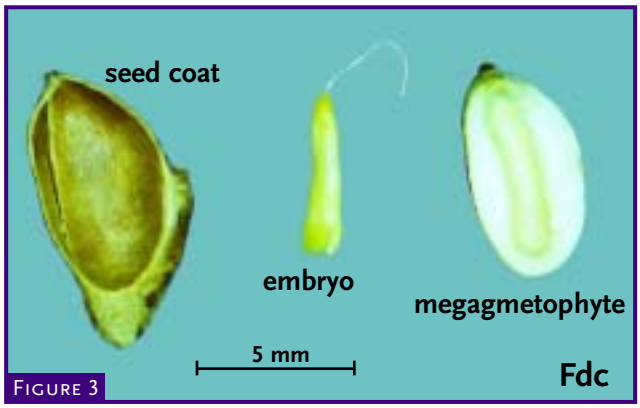

FIGURE 3

The seed coat, embryo, and megagametophyte dissected from a coastal Douglas-fir seed.

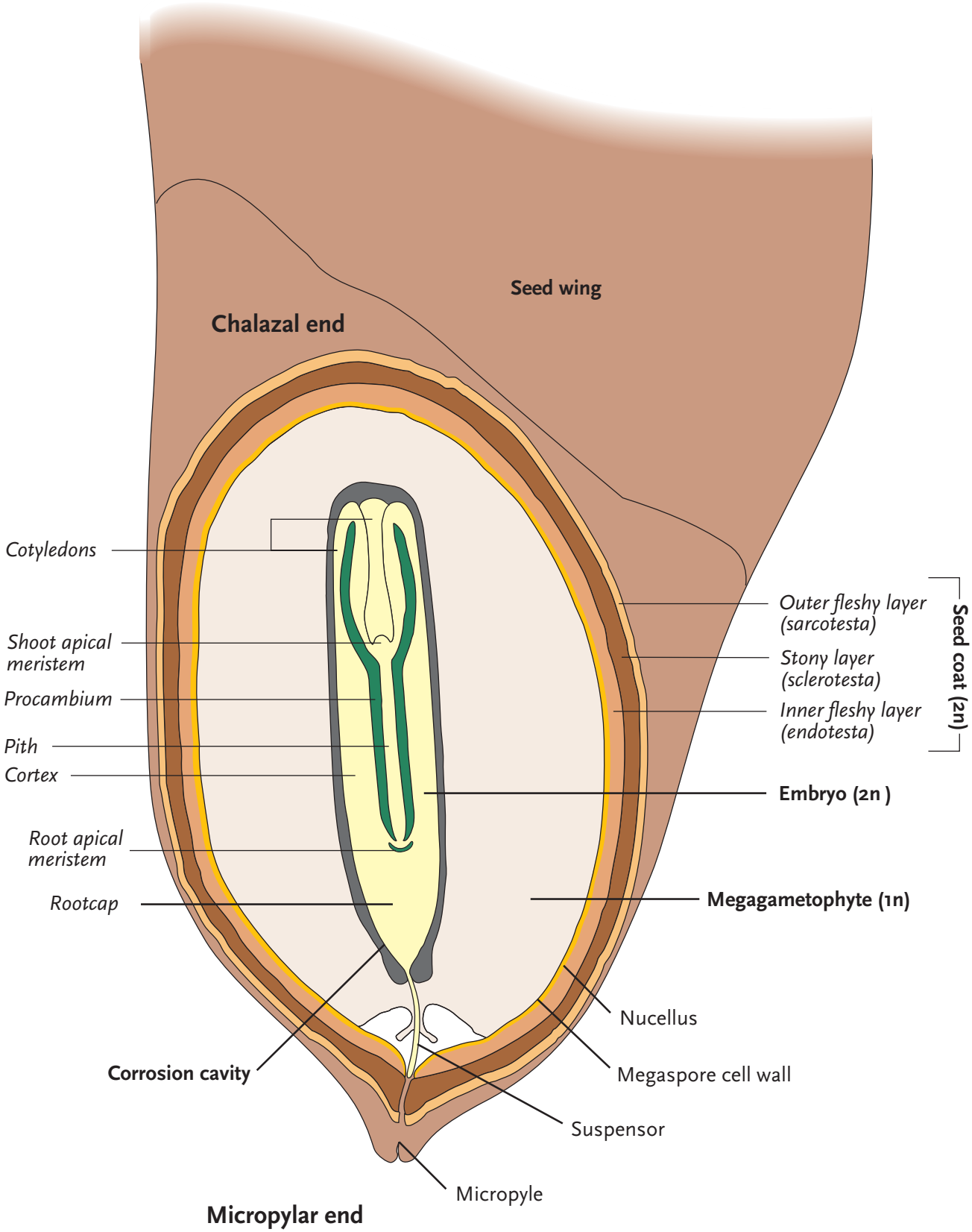

FIGURE 4

The anatomical details of a generalized conifer seed in longitudinal section. Chromosome complements for tree seed components indicated by 2n=diploid and 1n=haploid. A removable copy of this figure is located at the end of this volume.

Seed Coat

The seed coat is the readily observable feature of the seed and protects the inner tissues from insects, fungi, adverse conditions and mechanical damage. It is derived entirely from the mother tree, is **diploid** and appears similar for seed collected from a single tree. When seed from many trees are combined into a seedlot the seed coat appears quite variable (Figure 5). Seed coats vary in terms of colour, shape, texture, and the presence of resin vesicles. These attributes, along with seed size, form the basis for species identification using seed. The variability in seed coat morphology between species is illustrated with lodgepole pine and western redcedar in Figure 6. In lodgepole pine, the seed is quite dark, almost black, with characteristic ridges. No resin vesicles are present and the entire seed wing is absent. In contrast, western redcedar has a very light-coloured seed coat, resin vesicles, and a persistent seed wing.

Seed coat colour is related to the presence and distribution of **tannins** within the cells of the seed coat. The tannins accumulate during seed development giving rise to the change from light to dark seed coats. Not all seed coats are dark and within a seedlot one may see light-coloured seed, dark seed, and intermediate or mottled seed as shown for ponderosa pine (Figure 5). Variability in seed coat morphology can also be caused by abrasion of the outer tannin containing parenchyma cells, exposing the lighter coloured middle seed coat layer. Even in the very dark seed coat of lodgepole pine (Figure 6), you can see some light-coloured cells that do not contain tannins. Seed coat colour has been implicated in differences in germination[46] and in susceptibility to damping-off fungi[35], but the results are not conclusive.

Figure 5

Variability in seed coat morphology from a single seedlot of ponderosa pine.

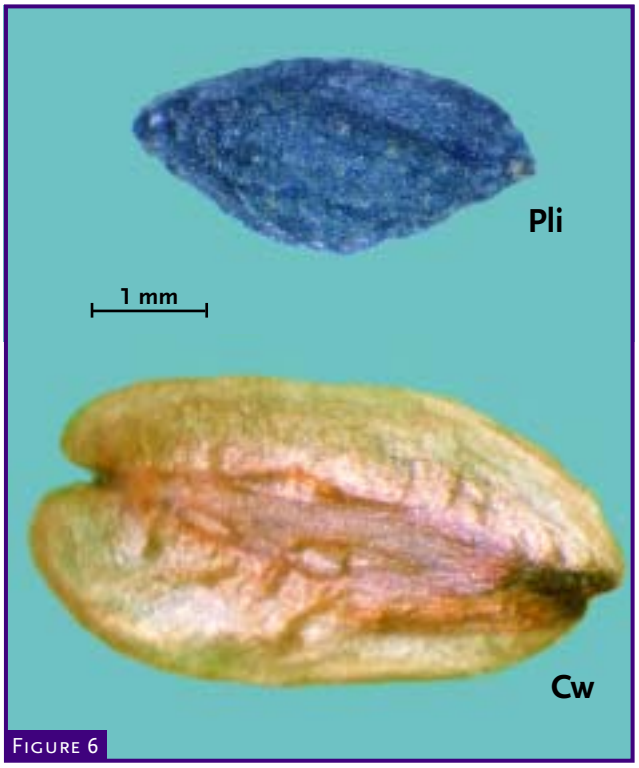

Figure 6

The seed coat morphology of lodgepole pine and western redcedar.

The colour or appearance of the seed coat is also influenced by the presence or absence of surface moisture. In Figure 7 (page 8), imbibed seed of interior spruce illustrate the difference between surface dry and surface moist seed. The dark-coloured seed have excess moisture on the seed coat while the lighter coloured seed have been surface dried to remove this moisture. The removal of excess surface moisture is important to enable the seed to be efficiently sown by mechanical sowing machines. Seed with excess moisture tend to clump together causing problems in sowing. To achieve uniform drying and to avoid removing moisture from within the seed, it is important to incorporate some movement or rotation of the

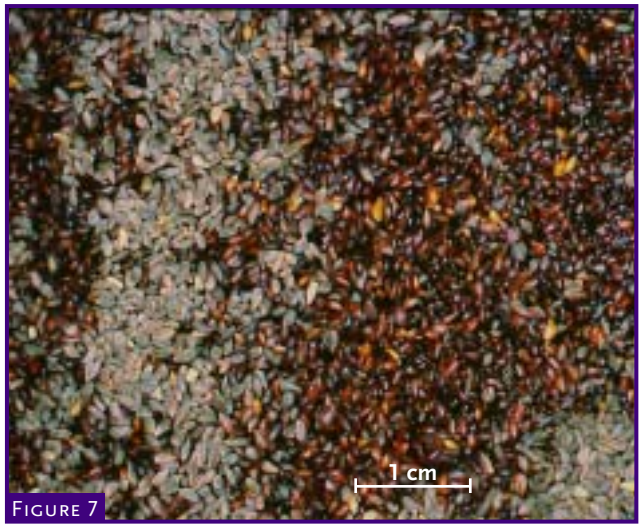

FIGURE 7

A spruce sowing request showing imbibed seed with excess surface moisture (darker) and seed that has reached a surface dry condition (lighter).

seed during drying. This is especially important in seed having a depth greater than a few cm to avoid drying only those seed in contact with the ambient air.

The seed coat of conifers is generally regarded as having three distinct layers: outer layer (sarcotesta), middle layer (sclerotesta), and inner layer (endotesta) (FIGURE 4, page 6, and FIGURE 8). The layers differentiate from the light-coloured integument or ovule wall at the time of pollination and may provide protection for the developing embryo. At seed maturity, the outer layer consists of unspecialized, tannin-filled parenchyma cells; the middle layer is generally thickest and consists mainly of specialized support cells with characteristic holes (simple **pits**) that allow for the passage of water

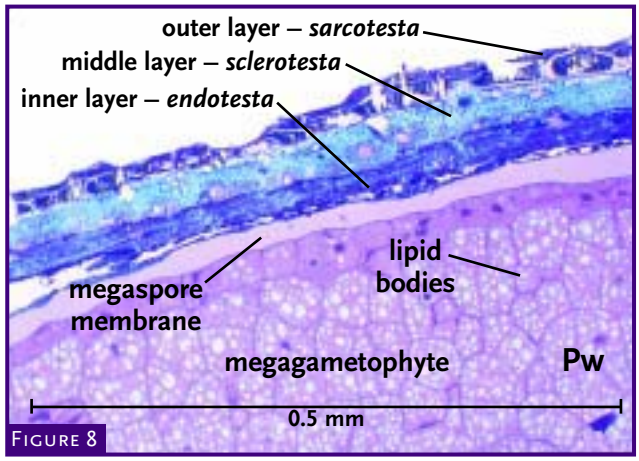

FIGURE 8

A magnified microtome section of the surface layers in western white pine.

between cells (FIGURE 8); and the inner layer consists of unspecialized cells without tannin deposits[39]. In FIGURE 8 note how the outer layer is thin, irregular, and appears broken in areas. The outer and middle layers are generally thicker at the tips of the seed, the micropylar and chalazal ends. The middle layer is often referred to as the 'stony layer' because of the predominance of thick-walled sclerenchyma cells that may slow imbibition or radicle emergence.

Most conifers do not possess **vascularized** seed coats, although many **gymnosperms** and **angiosperms** do. In coastal Douglas-fir, lack of a vascularized seed coat can be explained by the separation of the ovule from the ovuliferous scale at the time of fertilization. The precursors for storage products and development are already present within the ovule at fertilization, but their makeup will change during seed development. Initially the substances are primarily soluble, mobile substances that are converted into the less soluble starches, **lipids**, and proteins[32]. It is possible that diffusion of moisture will occur from cones to seed during development. The living cone still plays an important part in seed development by providing the proper microclimate and protection for seed maturation. Consequently, it should not be assumed that cones can be collected prematurely because a direct contact does not exist between the cones and seed.

It is suggested that growth inhibitors are present in the seed coats of conifers. One study with *Pinus pinea* L. concluded that inhibitors are present and that they are water-soluble. One inhibitor may be **abscisic acid** (ABA)[24]. It is probable that other genera contain similar inhibitors, but little research has been conducted in this area. Repeated washing of the seed removes the inhibitor and relieves dormancy[8]. This is a good reason to exchange water or perform running water soaks during seed imbibition. Another reason for exchanging water is to reduce the amount of fungal inoculum present on the seed coat.

True firs (Abies spp.), hemlocks, and western redcedar have resin vesicles in their seed coats. These vesicles are surrounded by **epithelial cells**

that produce and secrete the resin into the vesicle. Resin vesicles form early in seed development in the middle or outer layer of the seed coat and are usually more abundant on the lower surface of the seed (formerly in contact with the ovuliferous scale). Resin vesicles in western redcedar are narrow, linear, and only slightly raised while those of the hemlocks are elliptical and prominently raised above the seed coat. In Figure 9 several seed of western hemlock are shown illustrating the prominent resin vesicles that originate from the middle seed coat layer. All but the top seed have had the outer seed coat layer removed. Its resin vesicles are hidden from view although their outlines are apparent. This seed morphology, with all three seed coat layers present, is the preferred product after seed processing to reduce resin vesicle damage.

The role of resin vesicles is not clear although they have been implicated in: i) preventing germination in the fall; ii) protecting the embryo from excessive drying; and iii) playing a role in seed coat dormancy. A reduction of germination through damage to these structures has been reported[17,21]. Seed with resin vesicles must be handled with extreme care to avoid a reduction in quality.

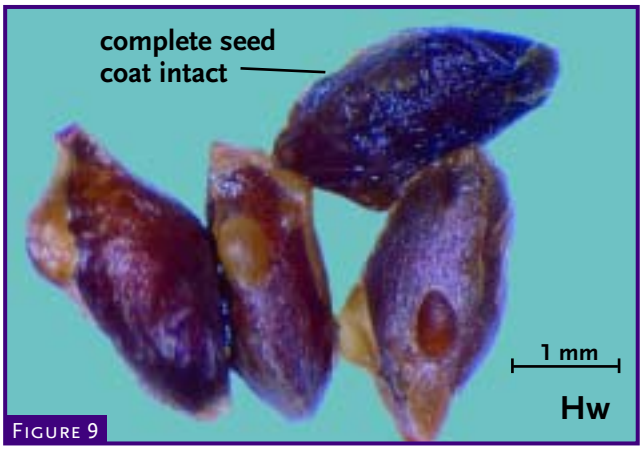

Figure 9

Seed of western hemlock displaying prominent resin vesicles. All seed, except the upper right, have had the outer seed coat layer removed.

Megagametophyte

The megagametophyte surrounds the embryo, providing protection and nourishment for initial growth. It originates from the mother tree and is **haploid**. In the developing seed, cells in the central portion of the megagametophyte, containing primarily starch[32], disintegrate to form the **corrosion cavity** into which the embryo develops. The megagametophyte cells are large, thin-walled, and spherical in shape (Figure 8). In Douglas-fir, the dry weight composition of the megagametophyte is 60% lipids, 16% proteins, and 2% sugars[32]. Lipids are an efficient means of energy storage due to the larger number of carbon-hydrogen bonds that release a greater amount of energy when oxidized than other organic compounds[42]. Although proteins are less abundant they are important as a source of amino acids (nitrogen-rich molecules) during germination. The lipids and proteins are found within specialized bodies (lipid and protein bodies) that are uniformly distributed throughout the megagametophyte[32]. Protein bodies also contain spherical structures, globoids, which store minerals for germination[34]. The lipid bodies can be seen as the white droplets in the cells of the megagametophyte in Figure 8.

The colour, condition, and texture of the megagametophyte varies with moisture content. In the 'dry' state (< 10% moisture content) the megagametophyte appears creamy-yellowish in colour and gaps usually exist between the megagametophyte, seed coat, and embryo. The structures appear shrunken. This is due to the natural dehydration that occurs during seed maturation. In the imbibed seed (≈30–40% moisture content) the megagametophyte is white and appears to occupy the entire space between the embryo and seed coat. The megagametophyte appears homogeneous and is often described as being shiny or crystalline due to the reflection of moisture in imbibed seed. It is the imbibed megagametophyte that deserves the common analogy of the 'firm meat of the coconut.'

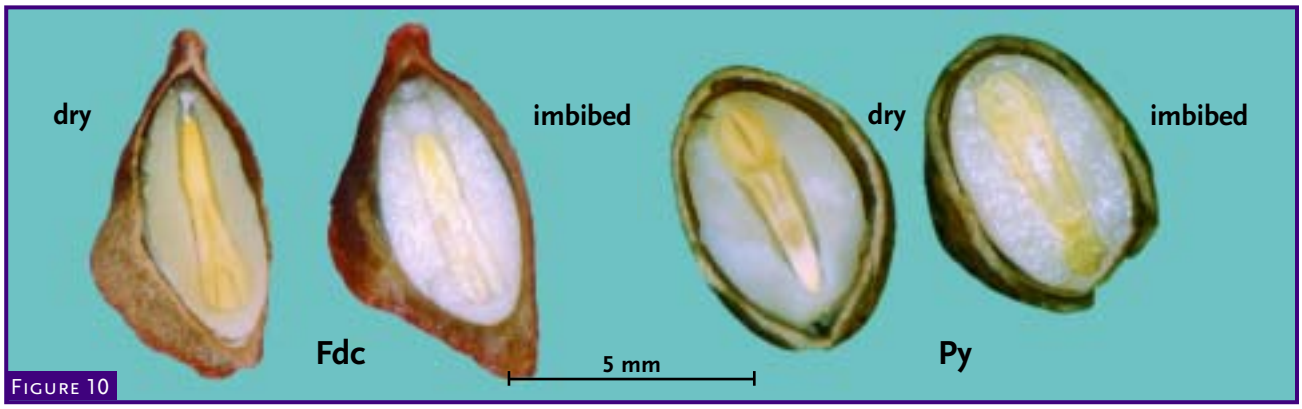

FIGURE 10

Longitudinal sections of dry and imbibed seed of Douglas-fir and ponderosa pine.

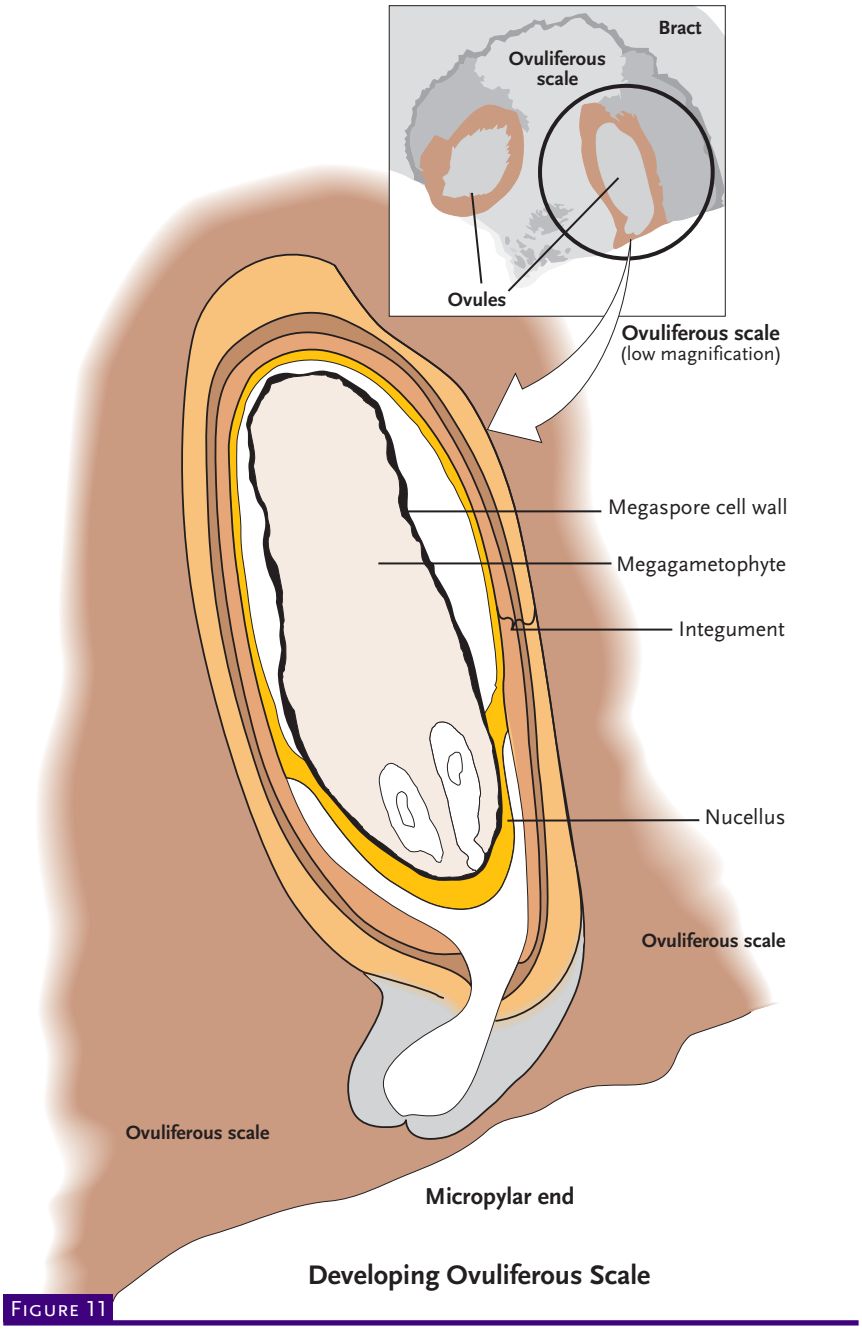

FIGURE 11

Longitudinal sections of a developing ovule at time of pollination.

In FIGURE 10 dry and imbibed seed of Douglas-fir and ponderosa pine are compared to illustrate the differences in morphology due solely to moisture content. Note the spaces around the megagametophyte and darker coloration of structures in the 'dry' seed. In the imbibed seed, the megagametophyte is white and appears shiny due to the reflection of moisture. In addition to causing changes in morphology, the addition of water also initiates many biochemical processes in the seed, the initial stages of seed germination.

Note the thicker seed coat in ponderosa pine and the incomplete dewinging of coastal Douglas-fir.

Although not strictly part of the megagametophyte, two other structures are evident; the **nucellus** and **megaspore cell wall**. The nucellus is the inner tissue of the ovule in which the megagametophyte develops. The nucellus becomes compressed during seed development (compare FIGURE 4, page 6, and FIGURE 11) and can be found as a papery covering outside the megaspore cell wall (FIGURE 12)

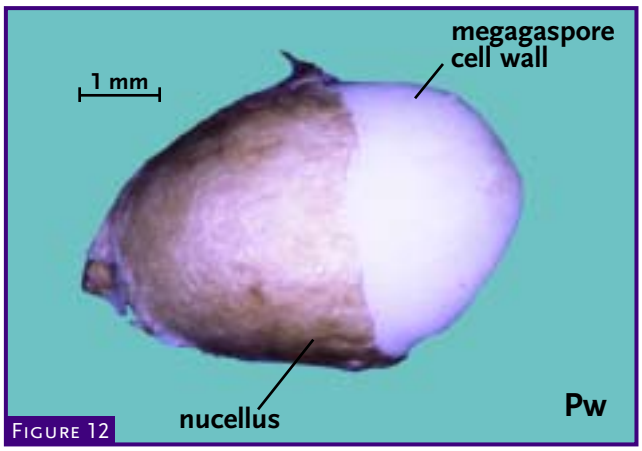

FIGURE 12

A western white pine seed with the seed coat removed revealing the nucellus and megagametophyte.

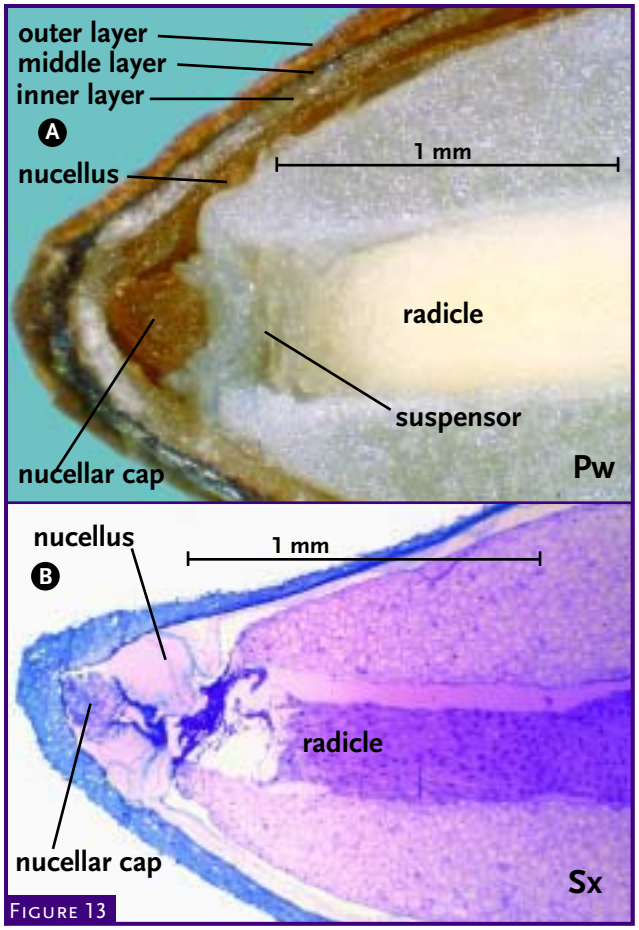

FIGURE 13

The anatomical detail of the micropylar end of (A) an imbibed western white pine seed from a longitudinal razor-cut section and (B) a 'dry' interior spruce seed from a longitudinal microtome section.

or reduced to a mass of tissue near the micropylar end of the seed, termed the nucellar cap (FIGURE 13).

The megaspore cell wall surrounds the megagametophyte. It is a lipid-rich, multilayered tissue that is more difficult to view with the unaided eye. This tissue is analogous to the pollen wall in the male gametophyte. In studies on Scots pine and Norway spruce, the megaspore cell walls were clearly shown to restrict water uptake[46,47].

The megaspore cell wall and nucellus are often compressed and difficult to distinguish in mature seed but distinct in the ovule at the time of pollination (FIGURE 11). Under high magnification the nucellar cap or 'plug' found in many species is clearly visible at the micropylar end of the seed (FIGURE 13). Allen and Owens[1] describe this plug as an infolding of the megaspore cell wall and hardening of the nucellus at the micropylar end forming a distinct brown tip where the micropyle and nucellus meet. This plug can be an impediment to radicle emergence and can sometimes act as a collar around the emerging germinant, restricting growth. Although these tissues do not usually play an active role in germination, they are readily observable and should not be mistaken for deteriorated seed contents or abnormal growth.

Embryo

After the fertilization of an ovule, the embryo develops to maturity by a complex series of stages known as **embryogeny**. All of the rudimentary structures and information necessary to produce a mature tree are contained in the embryo. The embryo is the only seed component containing genetic information from both parent trees. The mature embryo is comprised of cotyledons, a shoot apical meristem, **hypocotyl**, radicle, root apical meristem, and rootcap (FIGURE 4, page 6). In FIGURE 14 a dissected, germinating embryo, illustrates the green cotyledons that will function in photosynthesis. The red on the radicle is the expression of **anthocyanins**.

In a cut seed of yellow pine (FIGURE 15) the **procambium** pith, shoot and root apical meristems, and the rootcap can be seen. The rootcap accounts for approximately one-third of the embryo in this seed. Remnants of the nucellus are obvious at the periphery of the megagametophyte where it has been torn during dissection. This photo also shows the embryo as being bright yellow. Although this yellow colour is not typical, when compared to other illustrated cut seed, it shows the variability of embryo colour.

The finger-like projections at the chalazal end of the embryo are the cotyledons, or 'seed leaves.' Conifers have a variable number of cotyledons ranging from two (western redcedar) to up to 12 or more (ponderosa pine). Cotyledons are arranged in an inverted 'umbrella-like' arrangement above the shoot apical meristem within the seed. The general cross-sectional shape of a cotyledon is triangular, but the specific shape depends on the number of cotyledons present as all cotyledons, regardless of number, will share the same 360° circle. If you slice through the cotyledons to obtain a cross-section, you can see a circle that is divided equally into the individual cotyledons (FIGURE 16).

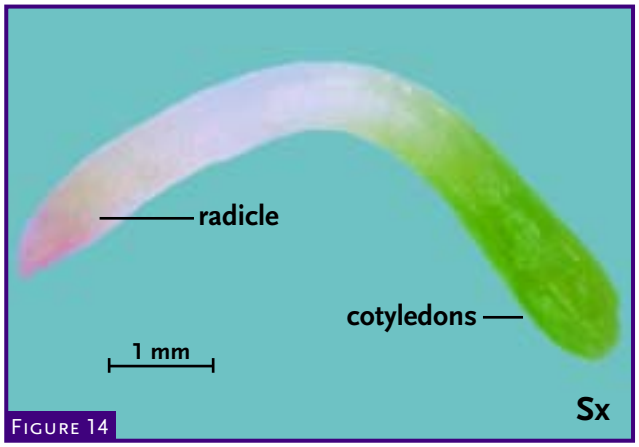

FIGURE 14

A dissected embryo from a germinating interior spruce seed.

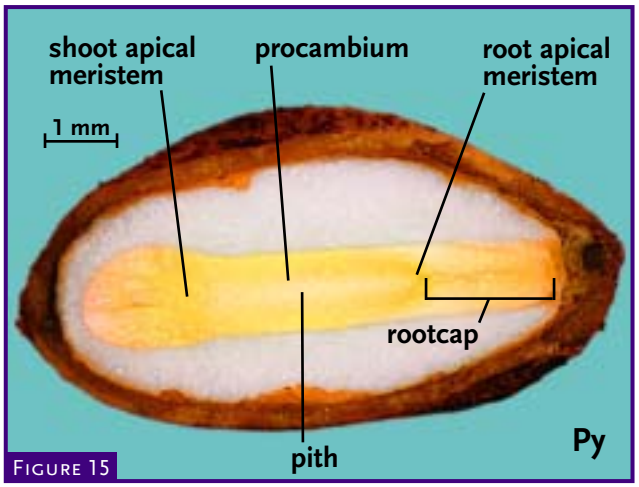

FIGURE 15

A longitudinal section of a ponderosa pine seed.

If only two cotyledons are present they will be shaped as half-moons (180°), if four are present, they will appears as quarters of a pie (90°), and if 12 are present, they will each have a 30° angle as their upper surface. The cotyledons consist of an epidermis, cortex, and provascular tissues.

At the base of the cotyledons is the shoot apical meristem. This is a small, highly organized dome of inactive cells which are activated upon germination and will initiate all subsequent above-ground structures. Below the apical meristem is the embryonic stem or hypocotyl. With a good dissection, distinct tissues can be seen within the

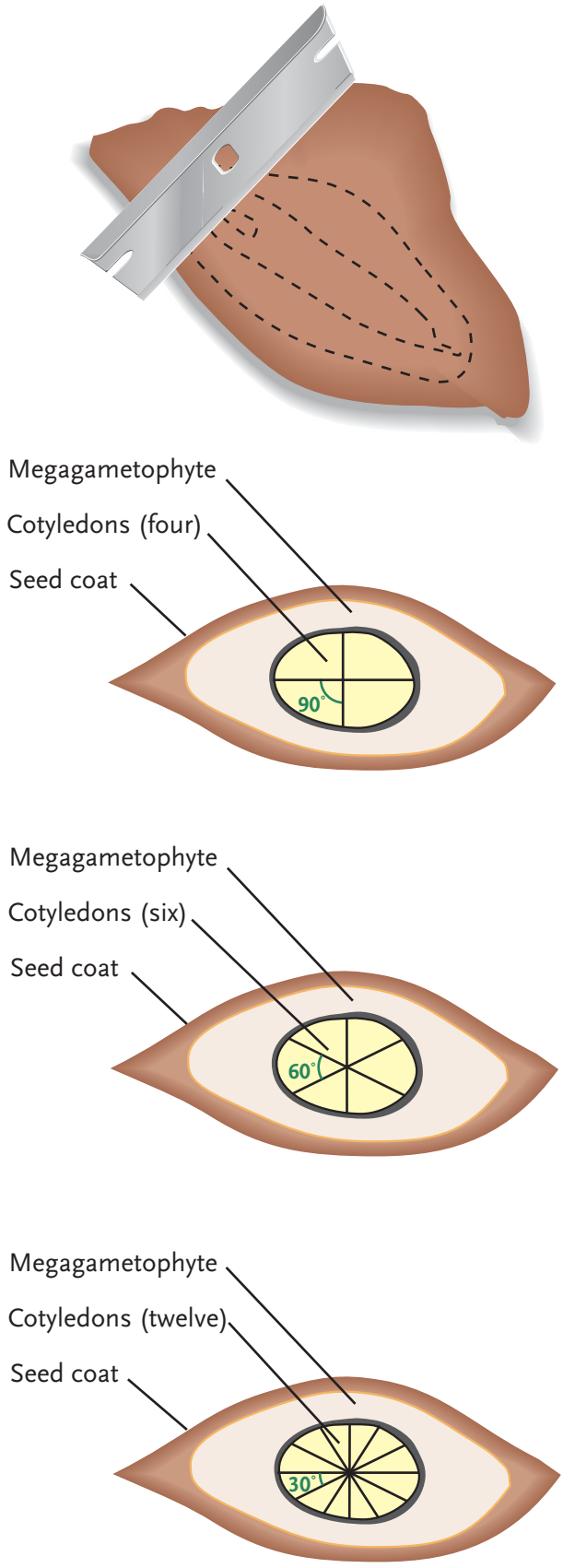

FIGURE 16

A cross sectional; illustration of the origin of cotyledon shape.

hypocotyl (FIGURE 4, page 6). Good dissections are a function of technique, but also species. Hypocotyl details are readily observed in ponderosa pine or Douglas-fir, but very difficult to see in hemlock or larch. The innermost tissue of the hypocotyl is the pith which is mainly composed of loosely arranged parenchyma cells. The procambium surrounding the pith is a meristematic region that contains the first elements of the xylem and phloem[44]. Details of the type of vascular elements present in the dormant embryo are extensively reviewed by Berlyn[7]. Each cotyledon contains a provascular strand to allow for transport of water and sugar. These strands join to form the procambium slightly below the shoot apical meristem.

Outside the procambium of the hypocotyl is the cortex, which is composed of parenchyma cells that characteristically have fluid filled membranes (**vacuoles**). The epidermis is the outermost layer of cells in the hypocotyl and cotyledons that provides protection and reduces water loss. Compared to the cortex, the epidermal cells are quite compact without intercellular spaces. There is no obvious transition zone between the shoot and root in the embryo. Although the radicle does not contain pith it may exist in the primary root for up to 120 days[7]. The vascular structures of the root are similar to the hypocotyl. The root apical meristem is recognized as a spherical group of cells found about 1/2 to 2/3 of the way down the embryo toward the root tip. The remainder of the embryo consists of a large rootcap that protects the root apical meristem and aids penetration into the soil. The rootcap consists of parenchyma cells with abundant accumulated starch. The root does not have an epidermis[9]. The thread-like structure at the base of the embryo is the suspensor (FIGURES 3, 4 pages 5, 6), which acts like an umbilical cord to the very young embryo. The suspensor also aids in pushing the early embryo into the corrosion cavity through cell elongation.

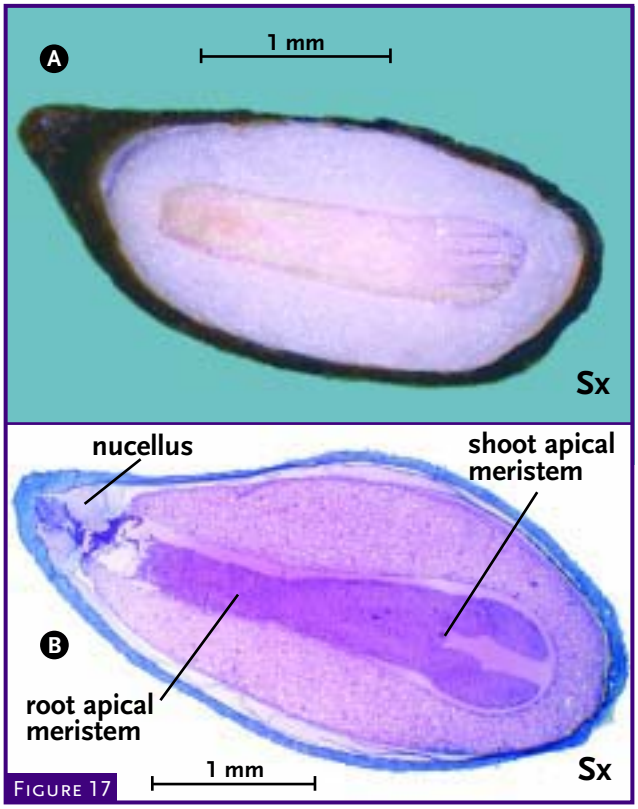

FIGURE 17

Longitudinal section of an interior spruce seed from (A) an imbibed razor-cut section and (B) a 'dry' microtome section.

In FIGURE 17, a comparison of a razor-cut longitudinal section and a microtome-cut longitudinal section of a spruce seed is presented. FIGURE 17A is a fully imbibed seed with the embryo, megagametophyte, and seed coat clearly visible. The seed is not cut exactly in half in this example and the procambium and rootcap are not visible. A proper cut would dissect through the micropylar end of the seed coat and give a better view of the embryo. If a cut is not perfect it may still yield enough information to predict the probable fate of the seed. FIGURE 17B shows a dry seed in which one can clearly see the shrinkage around the embryo and megagametophyte. The number of cotyledons visible is reduced to just two with the thin microtome section passing through one cell layer. The shoot apical meristem lies between these two cotyledons, and the root apical meristem can be identified by the clustering of more densely staining cells. Other seed structures like the megaspore cell wall, nucellar cap, and compressed suspensor can be seen in this photograph.