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Seed Biology and Technology of *Quercus*

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F.T. Bonner and J.A. Vozzo

INTRODUCTION

The genus *Quercus*, known as oak, includes worldwide some 500 species with 58 of these species in the United States, making it this country's largest genus of native trees (Little 1979). Oak is therefore an important group of temperate-zone forest trees. In addition, oaks are significant components of many of the major forest types of the South (Burns 1983) and are the most commercially important hardwood genus. Red and white oaks together account for nearly half (46 percent) of the annual hardwood sawtimber harvested in the South (U.S. Department of Agriculture 1982). They also comprise about the same percentage of the sawtimber and growing stock occurring on commercial forest lands.

Although a majority of southern nurseries grow some oak seedlings (Monaghan 1984), both their production and customer demand are low. Some forest industries grow seedlings in their nurseries to reforest their own lands, but no widespread planting of oak seedlings occurs presently in the South. Natural regeneration, both from seeds and from sprouts, most often establishes stands. Recent successes in direct seeding (Johnson 1984; Johnson and Krinard 1985; Cunningham and Wittwer 1984; Francis and Johnson 1985) have stimulated interest in this very promising method of regeneration, which requires large amounts of acorns.

Research on the seed problems of southern oaks was conducted from 1967 to 1983 at the Forestry Sciences Laboratory, Starkville, Mississippi. This paper summarizes in two parts the results of that research, incorporating both published and some unpublished results. Part I reviews current biological knowledge of acorns. Part II recommends handling and management techniques for acorns of southern white and red oaks.[1]

Part I. Current Biological Knowledge

TAXONOMY

Taxonomically, *Quercus* belongs to one of two families in the order Fagales. Fagaceae, the beech family, has four other genera in addition to oak; *viz.*, *Fagus* beech; *Castanea*, chestnut; *Castanopsis*, chinkapin; and *Lithocarpus*, tanoak (Little 1979). Within the genus, North American oaks are further subdivided into two subgenera, *i.e.*, *Lepidobalanus* (white oaks) and *Erythrobalanus* (red and black oaks).[1] The other Fagales family, Betulaceae, is the birch family.

Oaks are dicotyledonous, monoecious, with fruit development characterized botanically as nuts. The nuts, commonly called acorns, are usually associated with an involucre forming a cup around the mature fruit. The acorn contains one seed and has a straight embryo with no endosperm. Although oak morphology is discussed later, these characters are necessary for its taxonomic determination. Engler and Prantl (1924) suggested that these characters represent a primitive evolution; however, Benson (1957) related *Quercus* to a much more sophisticated order, Rosales.

The white oaks have rounded leaf tips, smooth acorns borne on current year's twigs, and light-colored bark. Their acorns mature in the year of fertilization and are usually non-dormant. Red oaks have pointed leaf tips, hairy acorns on second-year wood, and darker bark (Schery 1958). Their acorns usually require 2 years for maturation, and many species exhibit dormancy.

[1]Throughout this paper the term "red oaks" is meant to include all red and black oaks of the subgenus *Erythrobalanus*.

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ANATOMY

Acorns develop from an entire ovary with resulting ovary walls hardening into the pericarp; by definition they are hard, one-seeded, dry, and indehiscent fruits. Other common examples of this type of fruit are chestnut and hazelnut (Holman and Robbins 1944).

Between the earliest report of *Quercus* embryo and fruit morphology by Hartig (1851) and the recent work by Mogensen (1965), the development of acorn anatomy has been investigated by relatively few researchers. For a literature review and concise descriptions of *Quercus* embryogeny, readers should refer to Brown and Mogensen (1972) and Mogensen (1973).

A diagrammatic median section of a typical mature but not yet germinated acorn is seen in figure 1. Note the relative areas of embryo axis to cotyledon before germination. Pericarp completely surrounds the entire structure and is the remnant of its ovary wall.

The comparative anatomy of dormant, stratified, and germinating oak embryos facilitates an understanding of structural changes necessary for seedling generation. Progressing from the mature dormant state through the vernalization of pretreatment leading to germination and growth, an acorn must have a concomitant development reflected by dynamic tissue changes. These anatomical maturations have been characterized using light and electron microscopy, supplemented by histochemical staining.

The following descriptions are presented in detail in Vozzo (1974), Vozzo and Young (1975), Vozzo (1978), and Vozzo (1985).

In dormancy, the acorn has a small embryo axis surrounded by well-developed cotyledons and a continuous layer of epidermis (figs. 1 and 2). During stratification (fig. 3), the embryo polarizes, then elongates into an evident shoot apex and root cap. Its shoot apex has generative rib and peripheral meristems. The cotyledons appear similar to the dormant state, but there are great physiological differences. After germination (fig. 4), the embryonic axis has fully elongated to form the hypocotyl rudiments. A well-defined transitory region separates the axis centrally into cortical and stelar zones.

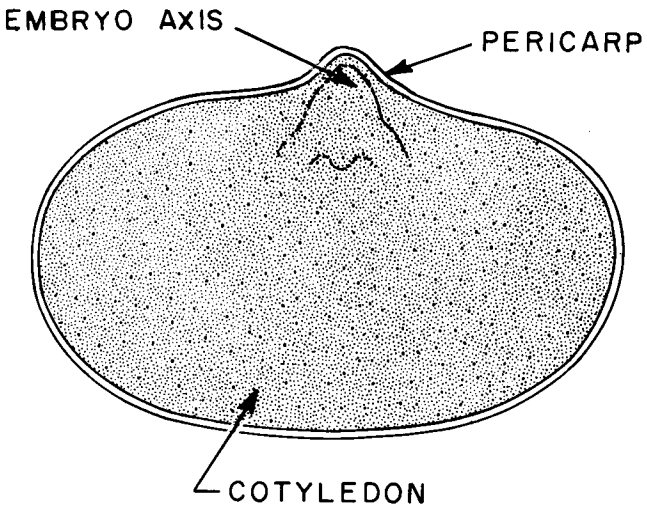


Figure 1.—*Diagrammatic median section of a typical mature acorn, showing the cotyledon, embryo axis and pericarp.*

Nucleoli in the dormant cells have a more uniformly dense structure than in a simple nucleonomatic bundle. Nucleoli are also seen with small buds attached. Nucleolar budding is absorbed in germinated embryos, but never seen in ungerminated, stratified embryo cells. Cells of germinated embryos have variable nucleolar densities with either light or dense centers as described by Srivastava and Paulson (1968).

All conditions are characterized by rough, granular endoplasmic reticula near both lipid droplets and mitochondria. Both single endoplasmic reticula and parallel stacks appear in stratified cells. No stacked reticula occur in dormant cells. The endoplasmic reticula associated with germinated cells are always distinguished by inflated terminal vesicles, which are large and appear to form vacuoles (Poux 1961). The stacked reticula seen in stratified and germinated cells confirm reports by Villiers (1971) that this is a sign of maturation.

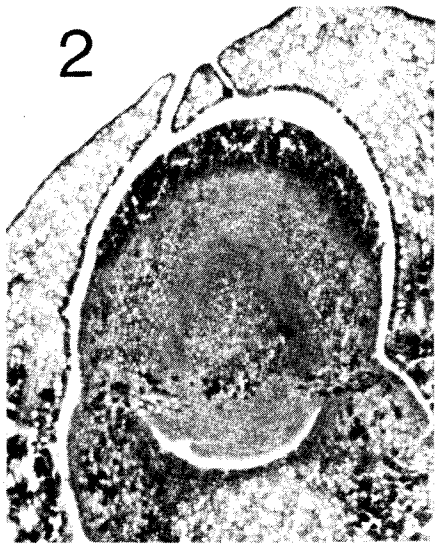
Mitochondria also reflect the changing metabolism as acorns move from dormancy to germination. Few and poorly developed mitochondria are seen in dormant embryo sections. Numerous, well-developed mitochondria are found in stratified cells, however, and as the respiratory demand increases, germinated cells show mitochondria with a nearly solid matrix.

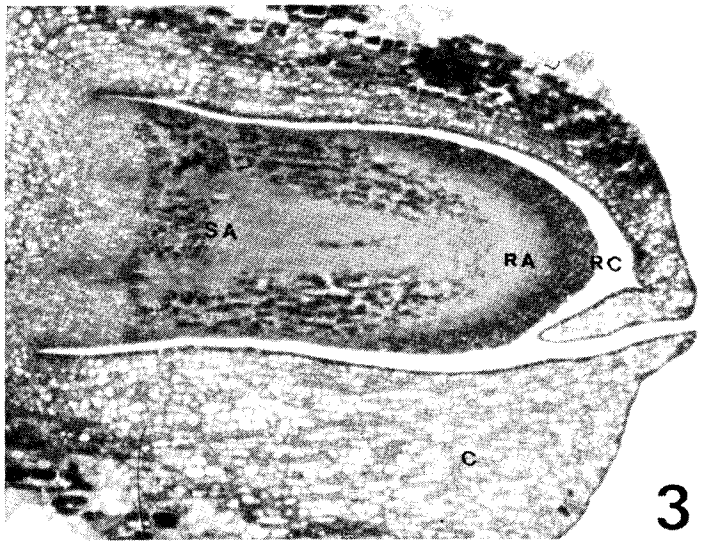
Unlike mitochondria, dictyosomes diminish in oak embryos progressing from dormant to germinated cells. Klein and Ben-Shaul (1966) attributed dictyosome absence in lima beans to metabolic demands during seed maturation, and oaks may behave in a similar fashion.

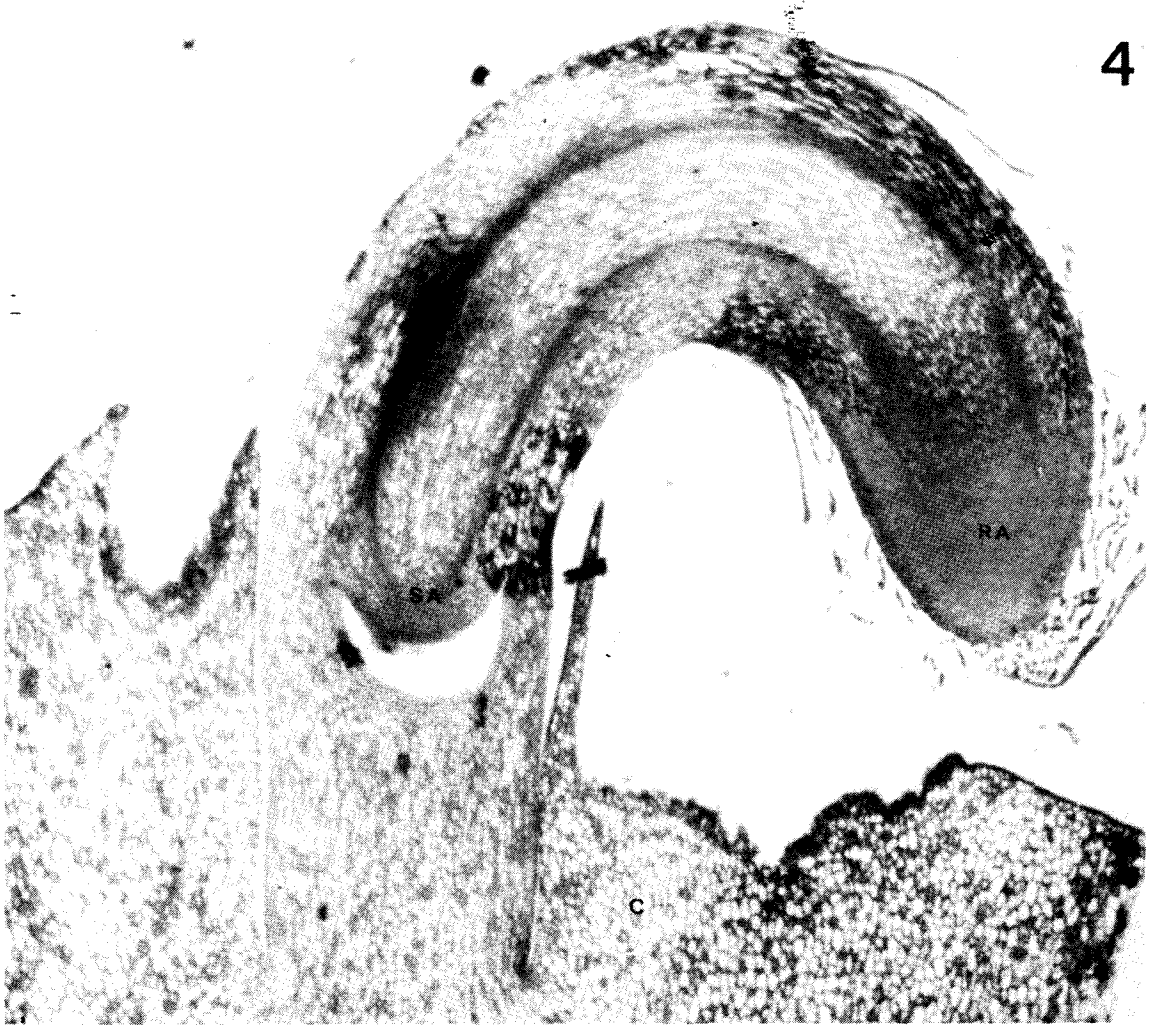
The primary cell wall remains about the same from dormancy to germination. Small dense bodies, perhaps lipid accumulations, appear adjacent to the cell wall in dormant and stratified acorns (Klein and Ben-Shaul 1966).

Another trend is the presence of microtubules. Rare in dormant cells, common in stratified cells, and less frequent again in the germinated cells, microtubules are generally associated with shaping plant cells.

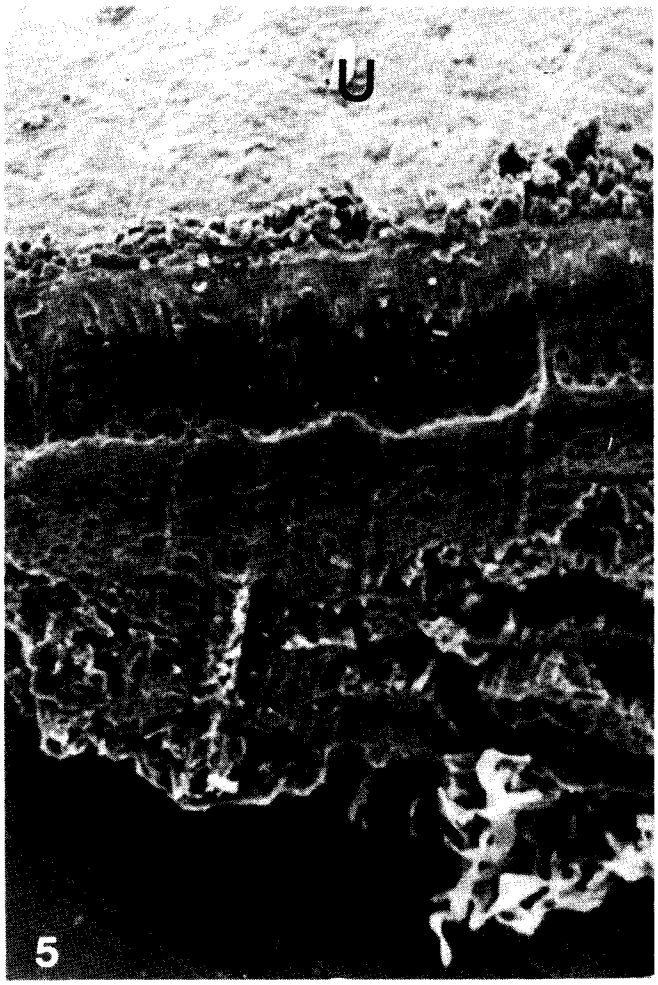
The pericarp of water oak has three distinct layers visible on careful dissection: a sclerified exocarp; a striated mesocarp; and a fragile, thin coriacous endocarp. The endocarp layer (plus adjoining seed coat), distinguished in fresh samples by its thin, brown, leathery appearance contrasted against the yellow, succulent cotyledon, usually tears away during dis-

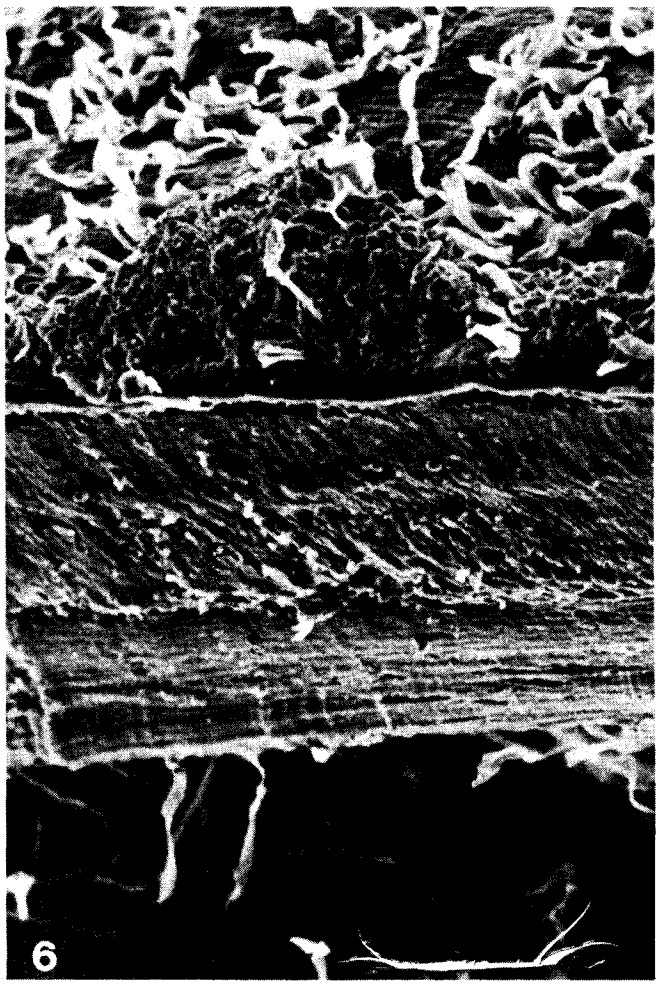






Figures 2–4.—*Median longitudinal sections of water oak embryo and cotyledon (C, cotyledon; RA, root apex; RC, root cap; SA, shoot apex). Fig. 2, dormant; Fig. 3, stratified; Fig. 4, germinated. (All figures × 32.)*





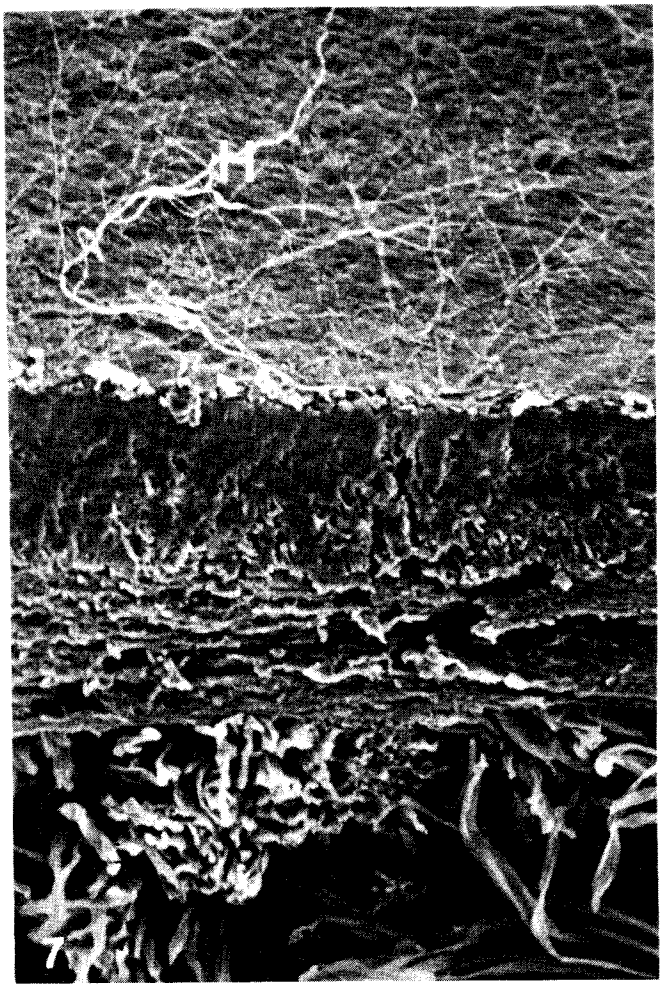
Figures 5–7.—*Pericarp sections of water oak acorns (U, unidentified spore-like structure; H, hypha-like strand). Fig. 5, dormant; Fig. 6,*

section and is difficult to see in prepared sections.

Surface textures of pericarps from dormant and germinating acorns are different. The exocarp in dormant acorns has an unobstructed, undifferentiated surface, broken occasionally by undetermined spore-like structures (fig. 5). These unidentified structures suggest the origins of hypha-like strands which appear later during the stratification process and then become commonly associated with the germinated seed. Their appearance becomes obvious during stratification (fig. 6). A profuse network of strands develops, following the contour of the exocarp surface. As germination progresses, additional strands of the network loosely envelop the outer layer of the pericarp by the time of radicle emergence from the acorn (fig. 7). These strands do not penetrate the sub-surface. A progressive decrease in density of the pericarp layers appears also to accompany stratification and germination. These changes suggest increasing permeability of the pericarp, but the pores and tubular channels are not conclusive evidence of a translocation system. However, they do represent potential pathways for gas and water exchange through the seed coat. This interesting aspect of acorn anatomy deserves further study.

METABOLISM

Concomitant with tissue differentiation and enlargement is a complex chemical transition within the embryos. Substrates, metabolites, and pooled reserves are continually in flux. Research at Starkville has focused on one species from each subgenera. *Q. nigra* (water oak) represented the subgenus *Erythrobalanus*. *Q. alba* (white oak) represented the white oak subgenus of *Lepidobalanus*. It is likely that other species within each subgenera share the same general metabolism.



stratified; Fig. 7, germinated. (All figures × 200.)

Maturation

Water Oak.—Acorns steadily increase in size and fresh weight from June through August (fig. 8). Dry weights continue increasing into September, but fresh weights do not, due to decreasing moisture contents. Acorn moisture content begins to decrease in late August, then rapidly drops throughout September to about 40 percent in October. During the rapid decline in moisture, pericarp color changes from green to dark brown or black. The loss of green color has been related to decreasing total chlorophyll content (Blanche and others 1980).

Fats and carbohydrates, the major stored energy reserves in acorns, begin to accumulate rapidly in August. Crude fat levels in water oak acorns reached 20 percent of acorn dry weight at maturity in late October (Bonner 1974a). In this time, cotyledons are nearly filled with coalesced lipid droplets, but they are seldom seen in the embryo axis (Vozzo and Young 1975). Phospholipids are localized in epidermal cells of the apical meristems and the cotyledons. Areas near nucleoli and leaf primordial zones also stain positive for phospholipids.

Soluble carbohydrates gradually increase from July to early September, then drop sharply as they are apparently converted to insoluble forms. At maturity the total carbohydrate content of water oak acorns is 26 percent, with all but 5 percent in insoluble forms (fig. 9) (Bonner 1974b). These insoluble carbohydrates, primarily starches, are much more prevalent in the shoot apex than in the root apex (Vozzo and Young 1975).

Nitrogen fractions and phosphorus, calcium, and magnesium concentrations all decline slowly during the growth and maturation periods of July to November. Although histochemical studies showed heavy staining for protein in the vascular strands leading from the cotyledons to the embryo, protein content as a percentage of acorn dry weight is low (table 1).

Little information exists on the status of growth regulators in developing acorns. Blanche (1981) found that, during maturation of water oak acorns from June to September, free abscisic acid (ABA) and indole-acetic acid (IAA) in the cotyledons increased. Both substances decreased sharply from early October to full maturity in November. Michalski (1970) reported stable levels of auxin in *Q. robur* (English oak) acorns early in maturation, then sharp decreases very similar to those noted in water oak by Blanche.

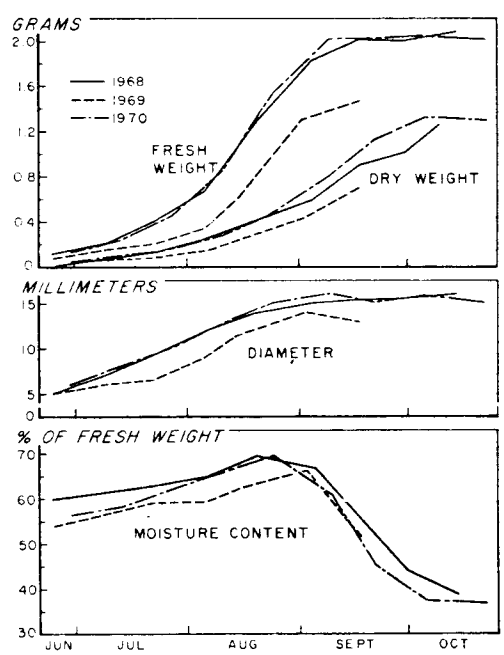


Figure 8.—*Seasonal changes in fresh weight, dry weight, diameter, and moisture content of water oak acorns. (Bonner 1974b).*

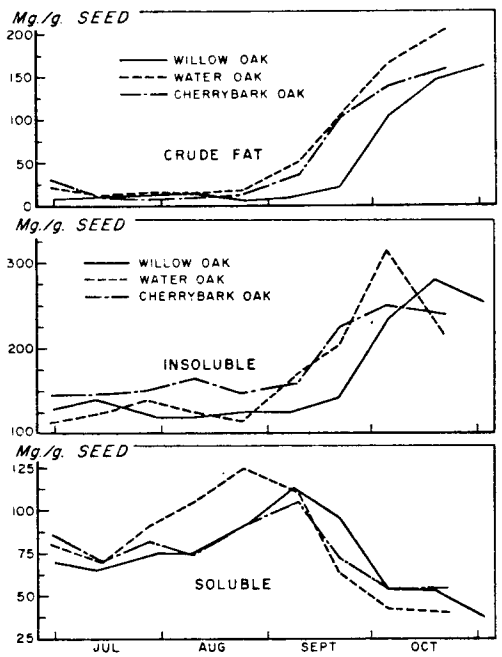


Figure 9.—*Seasonal changes in crude fat and soluble and insoluble carbohydrates in acorns of three red oaks. (Adapted from Bonner 1974b).*

Cytokinin activity decreased from a high initial level to a barely detectable level halfway through the maturity period (September), when increases in acorn diameter and length became minimal (Blanche 1981). Similar trends have been reported for cytokinins in *Q. robur* (Michalski 1974). Blanche (1981) also found inhibitory substances in aqueous extracts of pericarp tissue, which increased along with acorn development. The inhibitory substances' presence was also confirmed in pericarps of mature water oak acorns (Peterson 1983). These trends in growth regulators fit patterns reported for other seeds, and no unique function with acorn germination can be postulated.

White Oak.—Physical developmental patterns for white oak acorns near Starkville were very similar to those of red oak species. The only significant difference was moisture content at maturity. The 50 to 55 percent of fresh weight was some 10 to 15 percent greater than water oak (fig. 10). As white oak acorn moisture levels dropped, pericarp color changed from green to yellow to brown. Some acorns abscissed with a mottled green and yellow color pattern.

Carbohydrates, comprising 46 percent of acorn dry weight at maturity (table 1), are the predominant stored energy reserves in white oak acorns. Seasonal

Table 1.—*Chemical contents of whole acorns of some southern oaks*[1]

Species	Crude fat	Total carbohydrates	Total protein	P	Ca	Mg
	Percent					
Red oaks, *Erythrobalanus*						
Q. cocinea, scarlet	14.6	35.6	4.2	0.07	0.18	0.07
Q. falcata var. *falcata*, southern red	17.0	23.0	5.1	.08	.32	.14
Q. falcata var. *pagodaefolia*, cherrybark	15.8	29.5	4.0	.06	.27	.06
Q. nigra, water	20.3	25.8	3.8	.06	.32	.07
Q. nuttallii, Nuttall	13.2	46.2	4.5	.09	.04	.06
Q. palustris, pin	15.4	45.4	3.8	.08	.04	.06
Q. phellos, willow	19.6	31.2	5.9	.08	.18	.06
Q. shumardii, Shumard	9.8	29.3	3.8	.06	.27	.06
White oaks, *Lepidobalanus*						
Q. alba, white	2.9	46.6	4.6	.08	.22	.05
Q. durandii, Durand	3.8	44.9	6.2	.09	.22	.08
Q. lyrata, overcup	0.9	49.8	4.6	.12	.16	.08
Q. macrocarpa, bur	4.8	45.9	4.3	.10	.08	.06
Q. michauxii, swamp chestnut	3.3	56.1	4.1	.12	.08	.06
Q. muhlenbergii, chinkapin	6.6	34.5	4.4	.08	.18	.08
Q. stellata, post	5.2	37.9	3.8	.08	.25	.06
Q. virginiana, live	8.2	46.4	4.4	.06	.06	.07

[1]Data from Bonner (1971, 1974a).

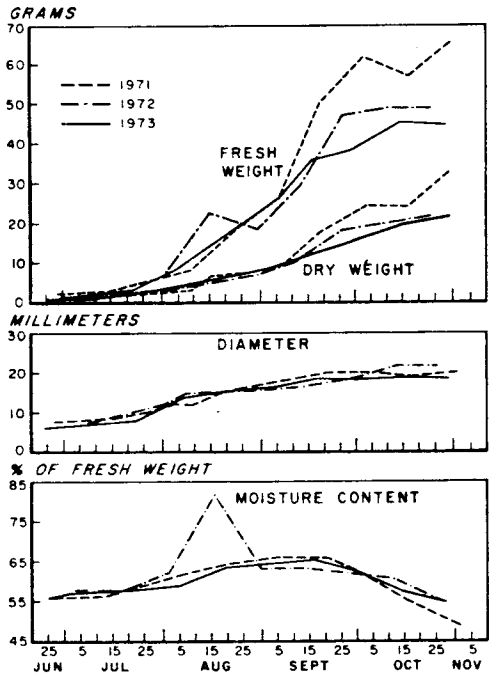


Figure 10.—*Seasonal changes in fresh weight, dry weight, diameter, and moisture content of white oak acorns. (Bonner 1976b).*

patterns during maturation were not too different from those of water oak. Soluble carbohydrates slowly declined until mid-July, peaked slightly in August, then dropped rapidly as they were converted to insoluble forms. Carbohydrates are prominant in the pericarp and root apex tissue as well as in cotyledon tissue (Vozzo 1978).

Crude fat levels reached only about 4 percent in white oak at maturity, making it a very minor component of stored acorn reserves in this subgenus. Phospholipids are concentrated in the cotyledons, embryo axis, and shoot apex. All cells with phospholipids have large dense droplets in groups of two or three, which almost fill each cell (Vozzo 1978).

As in water oak, total protein content is not high in white oak on a dry weight basis (table 1). Cytoplasmic contents in the epidermis and its adjacent lower layers have the greatest staining for proteins in cotyledons. Although there is little evidence of protein elsewhere, there are large unidentified protein storage bodies stained by chloramine-T in some cotyledon cells. However, when stained with ninhydrin, they test protein negative. Embryo axis and root apex also show the presence of protein in mature, ungerminated acorns (Vozzo 1978).

Levels of other chemical components of white oak acorns are shown in table 1.

Storage

Very little research has been conducted on metabolism of acorns during storage. Due to the naturally high moisture levels of acorns, metabolism during storage is similar to that demonstrated during stratification. During 8 months of storage at 2°C, acorns of water, Shumard, and cherrybark oaks demonstrated decreasing lipid levels and slowly increasing soluble carbohydrate levels (Clatterbuck and Bonner 1985). Insoluble carbohydrate levels increased for 3 or 4 months, then decreased. This reversal coincided with radicle emergence in the storage containers.

Respiration data in this study (Clatterbuck and Bonner 1985) indicated lipid respiration at first (RQ's 0.65-0.75) and then increasing metabolism of carbohydrates. Levels of CO_2 will increase to around 10 percent in closed storage of red oak acorns (Vozzo 1976; Tylkowski 1976). The best acorn quality was maintained at these high CO_2 levels, but no cause and effect relationship has been demonstrated. Less oxygen is consumed under these conditions (Vozzo 1986), and this may be a simple explanation of the CO_2 situation.

Similar trends in metabolism of stored reserves were found in white oak acorns (Clatterbuck and Bonner 1955), but lipid levels were much lower than in the red oaks. In storage tests of *Q. robur*, Tylkowski (1977) also reported more effective storage at high CO_2 levels than at low ones.

Germination

Water Oak.—Acorns of the subgenus *Erythrobalanus* usually exhibit delayed germination, commonly described as dormancy. As dormancy is overcome by stratification or other methods, metabolic activity increases within the acorns (Vozzo and Young 1975).

During stratification, starch is translocated from the shoot to the root of the embryo. After germination, however, starches are evenly distributed between shoot and root. Cotyledons show a continuing loss of starches during the entire process of germination. Similarly, hemicellulose is rapidly assimilated by cotyledons during germination, and its levels decrease quickly from dormant to germinated embryos.

Lipids are translocated from cotyledons to embryo axes as acorns proceed from dormancy to germination. During stratification, these droplets separate and are more commonly found as single drops in small groups in germinated embryos.

The respiratory quotient (RQ) increases as acorns pass from the dormant state (0.3) through stratification to germination (0.7). This trend indicates an increase in carbon dioxide evolution from stratified embryos. It also suggests that less carbohydrate and more lipid substrates are being used. During germination, protein location in embryos changes. Meristems in germinated embryos stain heavily near the root apex. The connecting cotyledon strands remain heavily stained for proteins even though the cotyledons begin to disintegrate.

Embryos from stratified acorns generally stain positive for proteins in the embryo axis, whereas dormant embryos are unstained. Villiers (1972) pointed out that RNA must be abundant during protein synthesis in dormant seeds. Heavy concentrations of proteins in these embryos' meristems probably indicates the abundant protein synthesis necessary for subsequent germination.

Nucleic acids are concentrated in apical stem meristems in all embryos observed. In dormant embryos, nucleic acids are diffused and extend from the shoot apex into cotyledons. In stratification, the concentration of nucleic acids increases, primarily in stem apices. Epidermis also stains positive for nucleic acids. In germinated embryos, the root apex is completely stained for localization of nucleic acids. For water oak embryos, DNA content and localization increase as germination proceeds. Although no direct relation is reported between distribution of protein and nucleic acids, similarities exist for each treatment stage: dormant, stratified, and germinating.

Water oak embryos, then, may be described as having diminished carbohydrate and lipid localization as the embryo passes through stratification to germination. Both substances are translocated from the cotyledons, and along with proteins, are moved towards the root apex as germination proceeds.

Although some red oak acorns collected in late September near Starkville germinated normally, the best germination was always obtained from acorns collected in late October and early November. These collections were made when moisture contents had fallen to 40 percent or below (fig. 8), and storage food concentrations were at peak levels for crude fat (20 percent) and total carbohydrate (26 percent) (fig. 9).

White Oak.—Since these acorns do not normally exhibit dormancy, there is no dormant condition to analyze. In fact, viviparity is common in several white oaks (*Q. virginiana*, *Q. alba*) in the Deep South, when rainy weather occurs during acorn maturation. White oaks do not store well at all, and they germinate readily at low storage temperatures (1°–3°C).

Generally there is little difference in the localization patterns of insoluble carbohydrates for either ungerminated or germinated acorns. In both conditions, the starch grains in embryos are similar in size, number, and stain intensity. However, starch grains in cotyledons of ungerminated acorns differ from those in germinated ones by having a few small starch grains one layer beneath the epidermis and by having abundant and large grains in the deeper cell layers. This pattern is reversed in germinated acorns. Undifferentiated cells of germinated acorns stain more heavily than do those of ungerminated ones in both embryonic and cotyledon cells. This pattern is also observed in the leaf primordia of germinated acorns, though the staining is less dense.

The pericarp in both conditions also shows positive histochemical staining for lignin. An interesting observation in both germinated and ungerminated embryos is that damaged tissue displays a stain reaction for lignin whereas healthy tissues do not, a development particularly manifested by phloroglucinol treatments. In one ungerminated embryo cotyledon section, cells stained heavily for lignin in an injured area. When the same cells of the surrounding damaged area were tested for starch (IKI + PAS), an intense starch concentration was observed. Individual cells that stain positive for lignin contained few starch grains, although starch grains are plentiful in the immediate area.

For germinated embryos, phospholipids are concentrated in the shoot apex and are seldom seen in cotyledons.

Nucleic acids in both ungerminated and germinated embryos are concentrated in meristems and vascular traces connecting the embryo axis to the cotyledons, and in the root cap. The localization pattern does not appear to change during germination.

Germination tests indicated that physiological maturity for acorns of white oak occurred by late October and early November in Starkville, just as for the red oaks. Maturity coincided with total carbohydrate contents of 46 percent and moisture levels of 50 to 55 percent.

General Considerations

The magnitude of change in chemical fractions is dominated by the large amount of storage tissue in the cotyledons of acorns. The cotyledons comprise 99 percent of total acorn dry weight at maturity (Vozzo 1973). Changes in nitrogen and phosphorus compounds in the meristematic regions obviously are occurring during maturation, but the macro-analyses used in our studies on total acorn tissue did not detect these changes. Comparable chemical analyses for southern oaks are summarized in table 1.

While acorns of many oak species sprout easily without stratification, white oak acorns will germinate prematurely during storage much more readily than water oak acorns. This behavior might be explained by differences in the localization of biochemical components during germination. Like *Triticum* (Evans and Berg 1971, 1972) and *Brachychiton* (West and Gunckel 1968), water oak shows a gradual movement of carbohydrate reserves from the cotyledon to the root apex of the embryo, as well as a shift in phospholipids during germination (Vozzo and Young 1975). White oak acorns, however, store carbohydrate reserves in the embryo axis prior to germination. During the germination process the acorns also utilize phospholipid reserves, since lipid concentrations subsequently shift from the cotyledons to the embryo axis (Vozzo 1978).

The progressive shift of white oak protein reserves from cotyledons to shoot and root apices during germination agrees with reports for other species, in which protein mobilization is apparently activated in the cotyledon cells by the accumulation of the enzyme endopeptidase (Harris and others 1975). White oak and water oak apparently do not differ in their utilization of nucleic acids. The localization patterns for embryonic nucleic acids of both species are similar to those reported for *Ricinus* (Sturani 1968); staining patterns for both nucleic acids and proteins agree with the results obtained for *Arachis* cotyledons and *Triticum* embryos (Marcus and Feeley 1964, 1965; Villiers 1972).

The localization patterns and distributions mentioned here are recognized as occurring with germination, but they do not necessarily trigger it. The results do indicate the nature of the changes that reserve metabolites, particularly carbohydrates, exhibit in the embryonic axis as a function of germination.

DORMANCY

As mentioned earlier, acorns of the subgenus *Lepidobalanus* (white oaks) do not have complete dormancy, at least in the South. There is epicotyl dormancy in some white oak species, notably white, chestnut, and overcup (Farmer 1977; Bonner, unpublished data). Red and black oaks (subgenus *Erythrobalanus*) exhibit variable dormancy. Not only do species differ in dormancy, but within a species differences can be due to geography or altitude. Farmer (1974), for instance, found that chilling requirement for germination of northern red oak increased with latitude and altitude of seed source.

Although seed dormancy is a natural protection for species regeneration, it must be understood and controlled for practical silvicultural management. The natural mechanism for breaking dormancy is simply the cold, wet environment in which acorns overwinter in litter on the forest floor. In forestry, we duplicate these conditions by storing fully-imbibed acorns at temperatures just above freezing for long periods of time. The reasons this treatment (stratification) overcomes dormancy are not completely clear.

A prominent contemporary concept of seed dormancy involves the interaction of plant growth regulators. These regulators may be either inhibitors or promotors and act together to influence the net result of germination or dormancy (Amen 1968). Nikolaeva (1968) has found that two plant growth regulators in particular act together: indoleacetic acid (IAA) and abscisic acid (ABA). Quantitative and qualitative analyses have been made for IAA, ABA, gibberellic acid (GA), and cytokinin in embryos of maturing and stratified water oak acorns (Blanche 1981; Hopper and Vozzo 1982). Results of both studies indicated that IAA and ABA levels increase with acorn development but decrease with acorn maturity. IAA content is always greater in the cotyledon than in the pericarp, while ABA is equally distributed between the two. Conversely, cytokinin activity decreases during maturation. Blanche (1981) found that the cytokinin activity pattern was consistent with the high rate of cell division taking place during maturation. During stratification, IAA decreased from day 10 to day 50. The inhibitor-promoter balance in the embryo axis changed between day 30 and day 50, as ABA increased and GA increased (Hopper and Vozzo 1982).

Further support for the inhibitor-promoter concept may be the reported stimulation to germinate dormant acorns by treatment with exogenous GA. Vogt (1970) and Farmer (1974) reported GA-stimulated germination of northern red oak; we have had similar results for cherrybark oak (*Q. falcata*, var. *pagodaefolia*) (Bonner 1976b).

Dormancy in *Q. rubra* has also been attributed to the pericarp by Jones and Brown (1966). They induced germination by removing or clipping the pericarp and concluded that dormancy was the result of inhibited cell expansion. Hopper and others (1985) also concluded that the pericarp was significantly involved in dormancy of *Q. rubra*, and Bonner (1968) found pericarps to inhibit germination of four red oak species. Pericarp removal from acorns had no significant effect on seedling growth of water oak, but it increased acorn germination from 10 to 55 percent (Hopper 1982).

Peterson's (1983) results with water oak showed that, although pericarp or seed coat tissues were not impediments to water uptake, the pericarp might entrap gases which could then affect water uptake. With normal water imbibition, the pericarp did expand with swelling cotyledons. After stratification at 5°C for 4 to 7 weeks and incubation at 20°/30°C, the pericarp split and radicles emerged. Mechanical strength of pericarps did not change during stratification. Peterson also found chemical inhibitors in the pericarp tissues.

Another possible factor in acorn dormancy is the interaction of seed coat (pericarp) and microorganisms. Microorganisms have been isolated in mixed culture from water oak acorns (Vozzo 1984). The conditions used for acorn stratification are also favorable for the growth of these isolated organisms—*viz.* damp, dark, chilly, and aerobic conditions. It may be more than coincidental that the duration and the growth conditions of acorns are optimal for the microorganisms as well as for the stratification process. Pre-germination treatment for water oak acorns may amount to creating conditions that allow seed coat organisms to incubate and grow over the surface while exuding sufficient enzyme activity to erode the surface layer. This action could expose the ends of translocation channels and loosen the pericarp matrix to increase water and gas passage.

The most definitive studies on acorn dormancy are those of Peterson (1983) on water oak. He concluded that delayed germination (dormancy) was the result of at least three factors: (1) Mechanical strength of the pericarp; (2) chemical inhibition by the pericarp, which was alleviated by stratification for approximately 4 weeks; and (3) slow increase in capacity to imbibe the water required for pericarp rupture. This last factor apparently did not depend on temperature within the range of 5° to 30°C, and operated during the entire stratification and germination period. Although other factors may be involved, the Peterson model of dormancy seems to explain germination behavior reported for other southern red oaks.

PREDATORS

Microorganisms.—Although the genus *Quercus* has been thoroughly studied regarding pathological problems (Hepting 1971), few references pertain to acorn pathology. Both *Q. agrifolia* and *Q. wislizenii* are subject to oak drippy-nut disease (Hildebrand and Schroth 1967). After acorn puncture by wasps, a plant exudate appears and drips. The causative organism is *Erwinia quercina*. In France, the most significant fungus for acorn infection is *Ciboria batschiana*, which causes black rot of acorns of *Q. robur* and *Q. petraea* (Dealtour and Morelet 1979).

The primary concern for acorn pathology is related to seed storage. Vozzo (1984) reported fungal observations isolated from both *Q. alba* and *Q. nigra* acorns. Axenic sub-cultures produced pure culture determinations for *Epicoccum purpurascens* and *Fusarium solani*. Although these isolations were believed to be from acorn surface contaminants, it must be noted that cracks were present in the acorn pericarps. These cracks may have allowed internal cotyledon contaminants to grow on the agar growth medium. However, cracks are quite common in both freshly collected as well as stored acorns. If certain contaminants are specific for the pericarp surface, as opposed to the cotyledon-embryo tissues, they would have potential infection capability through these commonly occurring, natural fissures in the pericarp.

One other consideration is the possibility of symbiotic or even beneficial associations between seed germination and seed contamination. Such associations have been reported for mountain rice (Probert 1981) and orchids (Rayner 1915). In the latter case, *Calluna* seeds were infected during embryogeny from the mother plant, resulting in endotrophic mycorrhizae in the subsequent seedling. The possibility of beneficial fungal associations with acorns should be considered along with possibilities previously mentioned regarding how stratification may overcome dormancy.

Insects.—*Curculio* species are major pests in *Quercus* seeds (Baker 1972; Gibson 1972; Gibson 1982). They infiltrate oak acorns by depositing eggs in chambers, and larvae can be observed feeding on cotyledon tissue at the time of acorn collection in late summer and early autumn. Since infested acorns are routinely discarded, the adults of *Curculio* are seldom seen by seedsmen. According to Baker (1972), acorns are infested by *C. sulcatulus*, *C. pardalis*, *C. orthorhynchus*, *C. longidens*, and *C. humeralis*. *Curculio* sp. were found in acorns of white oak, water oak, willow oak (*Q. phellos*), and southern red oak (*Q. falcata*) in Starkville collections, but species identifications were not made (Vozzo 1984). Gibson (1972) reported that white oak damage is caused by seven *Curculio* species. Northern red oak acorns are commonly infested by five *Curculio* species (Gibson 1982).

Melissopus latifferanus (filbertworm) is reported to feed on acorns and is responsible for severe destruction to acorn crops, particularly during poor crop years (Baker 1972; Gibson 1972). It has been observed in both southern red and water oaks at Starkville (Vozzo 1984).

The warehouse moth, *Ephestia*, a common pest of stored seed and grain, is not usually found in the wild. Considered a secondary infestation during storage, the moth was found only on water oak at Starkville (Vozzo 1984).

Valentinia species commonly feed on acorns, nuts, cones, and seeds. The larvae attack seed and prey on other insects associated with the host plant. The acorn moth, *V. glandulella*, is reported to infest acorns in the Southern United States (Baker 1972). Vozzo (1984) found this species on four oaks at Starkville, and Gibson (1982) reported it as a pest of northern red oak.

Other known insect predators for white oak are *Conotrachelus* weevils, and two gall wasps, *Cynips glandulosus* and *Callirhytis* spp. (Gibson 1972). Additional species which attack northern red oak include *Callirhytis fructosa* and three *Conotrachelus* species (Gibson 1982).

Vozzo (1984) studied the common infestation of insects and fungi in acorns of four species. Fungal isolates were recovered from the head, gut, and carcass of *Curculio* larvae. A *Penicillium* sp. was isolated from head and carcass portions, while an undetermined white fungus with sterile mycelia was isolated from the head, gut, carcass, and the whole larvae. Only two unidentified fungal spores were observed from sectioned gut of *Curculio* sp.

The scarcity of fungal spores found in insect parts isolated from acorns would not indicate that the insect is a vector or source of fungal contamination to the acorn. Limited observation, however, does not firmly establish a theory. Similarly, another question arises from the study. Did the few spores present in *Curculio* gut result from insect transmission to the acorn, or was the gut infested as a result of fungal contamination of the seed surface? Perhaps the acorn is a vector

to contaminate its associated insect population, which in turn is a secondary carrier to other organisms. Need for additional study is indicated.

Part II. Handling and Management of Acorns

COLLECTION

Acorns should be collected as soon as they are physiologically mature. In the Midsouth this condition occurs in late October to early November. Some year-to-year variation in ripening date occurs for individual trees, but this difference rarely exceeds 2 weeks. Variation in acorn ripeness is also found within a single crown. Acorns on lower branches usually ripen before those in the upper crown.

Large collections of acorn crops require careful planning. If the seed crop is very light, it may be best to postpone collection until next year. Acorn quality is frequently poor in light crop years. Also, infestation by *Curculio* sp. and other insect predators occurs in a very high proportion of the total acorns in a light crop year. When a large acorn crop is indicated, collection efforts should be intensified.

Maturity Indices

Although chemical indices of maturity are available for some species (see Part I), their use is impractical. Visual and physical characteristics of the acorns furnish simple, dependable maturity indices. Among the red oaks, the following are characteristics of a mature acorn:

1. The pericarp has lost its green color and is primarily dark brown or black.
2. The pericarp easily slips from the cup without being forced or without leaving pieces of the cup still attached to the pericarp.
3. The cup scar is "bright" in appearance. In southern red and cherrybark oaks, the cup scar may be a bright pink or orange in color. Yellow or orange is common in some other species of red oaks.
4. A cross-section of the acorn will show the following colors: high-fat species (water, willow, cherrybark)—dark yellow to orange; low-fat species (Shumard)—light yellow.

Among the white oaks, the following characteristics indicate maturity:

1. For most species, the pericarp has lost its green color and is primarily brown or black. White oak and swamp chestnut oak are sometime exceptions to this rule, since acorns from many trees are fully mature when still yellow or even a mottled yellow and green.
2. Acorns slip easily from the cups as in the red oak group. Overcup oak is an exception to this rule because its acorns retain their cups when they fall.
3. Acorn cross-sections will show the cotyledon to be firm and white to yellow in color.

Methods of Collection

The quickest and cheapest way to gather large quantities of acorns is to collect from the tops of trees felled in logging operations. When acorns are fully mature at the time of felling, they usually fall from the cups when the top hits the ground. Collectors can pull away the branches and easily pick the acorns from the ground. If acorns are not quite mature (or if the tree falls slowly), they may remain in their cups for easy picking by collectors standing on the ground.

Some ripening of immature acorns may occur while they are still attached to the logging tops. If the atmosphere is humid and the felled tops dry slowly, as many as 5 to 7 days may pass before the foliage is desiccated. When the foliage becomes fully desiccated, ripening apparently stops.

Immature acorns, unlike the seeds of some hardwood species such as sweetgum and yellow-poplar, cannot be ripened artificially after picking (Bonner 1979). Detached acorns have no external source of nutrients as the seeds of the other species have in their fruit tissues.

From standing trees, acorns can be collected by sweeping them up after they fall on the ground or by spreading dropcloths under the trees. A major caution in these two methods is to ignore the first acorns to fall; they are usually damaged by insects or birds. Although seldom, if ever, used on oaks in this country, tree shakers can easily dislodge mature acorns (Stein and others 1974).

CLEANING AND CONDITIONING

At maturity, red oak acorns contain about 40 percent moisture and white oak acorns about 50 percent (Bonner 1974b, 1976b). It is very important that excessive drying be prevented! A loss of 5-percent moisture can be tolerated, but further drying will lower acorn quality. Viability will be completely lost when moisture content drops to about 25 percent and remains there. Laboratory tests have indicated that water oak acorns will survive temporary desiccation in the 15- to 18-percent moisture range if they are rehydrated quickly (Agmata and Bonner 1985). In collection operations, however, the key to obtaining acorns of high quality is to avoid any desiccation.

On the collection day, acorns should be floated in water to remove leaves, acorn cups, insect-damaged acorns, and other trash. This step also is a major aid

in maintaining desirable acorn moisture levels. Sound, healthy acorns should sink in water. If conditions are extremely dry at collection, the acorns should be left in water 16 to 24 hours to raise moisture contents. This is critical for acorns collected from the ground because many sound acorns will float at first. Acorns collected during wet, rainy weather are usually moist enough to permit good cleaning separation without a soak period. With large batches (a bushel and larger), some stirring action is needed to give all acorns a chance to float.

After flotation, the trash should be skimmed off and the water drained away. Acorns should then be placed in cold storage, even if they are to be planted in a few days. Surface drying before storage is not necessary as long as there is not enough water present to form a pool at the bottom of the container.

Although seldom practiced, sizing of acorn lots is possible by screening or by running them over gravity separators. Round-hole screens are available for air-screen seed cleaning machines that will size the smaller acorns, such as water, willow, or cherrybark oaks. Sizing with gravity separators is not very efficient; screens should be used where sizing is desired.

The possible advantages of sizing acorns for planting has not been investigated fully. Farmer (1980) found that initial leaf areas of seedlings of northern red, chestnut, white, and bear oaks were positively correlated with acorn size. Kleinschmit and Svalba (1979) reported a strong positive correlation between acorn weight and height growth at 3 years for *Q. robur* and *Q. petraea* in Germany.

TREATMENT FOR INSECTS

When acorn collections are found to contain numerous weevil larvae, the tendency is to want to kill the insects to prevent further damage. Two methods for killing larvae (Olson 1974) present great risks for the acorns. The most common method is to immerse the acorns in hot water (120°F) for 40 minutes. Temperatures above 120°F, however, may kill the acorns. The second, and less desirable method, is to fumigate the acorns with methyl bromide, carbon disulfide, or thiamine bisulfate. Because of the acorns' high moisture contents, chemical fumigation may also harm the acorns if the treatment is not done properly.

Avoiding all treatment of acorns is the safest alternative! If proper flotation has been used to clean the seed lots, most infested acorns will have been removed. The larvae do not attack intact acorns during storage, so infestation cannot increase. Most larvae will emerge from the acorns during cold storage and die in the bottom of the container. In this way some infested acorns may be salvaged, because the larval feeding must destroy the embryo axis to prevent germination. Much feeding is found in the cotyledon tissues only, but partial damage there will not necessarily prevent germination and normal growth. Larvae emergence can be further encouraged by moving the acorns back and forth from cold (2°–5°C) to room temperature several times.

STORAGE

Seeds that cannot be stored by orthodox means (low moisture content, sub-freezing temperatures) are often called recalcitrant. Seed storage of this group, which includes all species of *Quercus*, is still the greatest challenge in seed technology. Acorns of the red oak subgenus can be stored for 3 to 5 years under special conditions, but quality decreases each year. Acorns of the southern white oaks cannot be successfully stored longer than over winter, with the curious exception of overcup oak.

Red Oak Group.—For red oaks, the best storage method is one that maintains acorn moisture content above 30 percent, allows some gas exchange with the atmosphere, and keeps the temperature near but above freezing (1° to 3°C). Under these conditions southern species—such as water, Nuttall, and cherrybark oaks—will maintain good viability (60 percent or more) for at least 3 years (Bonner 1973). Very similar methods have proven successful for northern red oak and scarlet oak (Farmer 1975; Suszka and Tylkowski 1982). Shumard and willow oak acorns do not seem to store as well.

When the moisture content of stored acorns is 30 percent or more, respiration in them is quite active. This condition creates a continual, but slow, decrease in acorn dry weight and a corresponding slow increase in moisture content, expressed as a percentage of acorn fresh weight. The cherrybark oak data in table 2 illustrate the gradual upward trend of acorn moisture during storage. For this reason, true equilibrium between internal acorn moisture and the storage atmosphere is never reached. However, approximate equilibrium moisture contents (%) have been determined for a few species (Bonner 1981), as follows:

	Storage Conditions	
	40%–55% RH, 4°–5°C	95% RH, 4°–5°C
Shumard oak	13	32
Water oak	17	29
White oak	37	50

Because starch is more hygroscopic than fat, starchy acorns (white oaks) absorb more moisture than fatty acorns (red oaks). This explains the higher moisture contents of the white oak acorns under the same conditions.

Table 2.—*Germination and moisture contents of cherrybark oak acorns stored in polyethylene bags at 3° and 8°C*[1]

Original moisture content and storage period	Germination		Final moisture content	
	3°C	8°C	3°C	8°C
	Percent			
24 percent moisture				
6 mo	80	76	27	28
18 mo	9	0	30	34
30 mo	25	24	32	33
31 percent moisture				
6 mo	99	99	34	34
18 mo	99	96	35	35
30 mo	81	71	36	36
33 percent moisture				
6 mo	100	98	34	32
18 mo	93	95	31	36
30 mo	94	34	37	40

[1]Bonner (1973).

Because of the high rate of respiration, some gas exchange with the atmosphere must be allowed. Numerous tests have shown that air-tight storage containers are lethal to acorns. Polyethylene bags with a wall thickness of 4 to 10 mils are good containers for red oaks. The material is permeable to carbon dioxide and oxygen, yet largely impermeable to moisture. Thinner polyethylene is highly permeable to moisture vapor, and acorns stored in it will dry excessively in low-humidity conditions. Polyethylene thicker than 10 mils restricts gas exchange too much. There is an apparent natural increase of CO_2 in storage containers, and better viability retention has been reported in CO_2-enriched storage atmospheres (Vozzo 1976; Tylkowski 1976). The most common practice is to store acorns in drums, cans, or boxes having a polyethylene liner bag. In this type of storage the container top should never be completely closed. Additional aeration is sometimes supplied by the weevil larvae which emerge from acorns and eat through the bottom of the polyethlyene bags.

Successful storage of northern red oak acorns for two winters in Poland has been accomplished by mixing the acorns with dry peat or pine sawdust (1:1 by volume) and storing them at −1° to −3°C in metal milk cans with tops unfastened (Suszka and Tylkowski 1982). Some acorns retained viability for five winters, but storage for more than two was not recommended. The peat or sawdust prevents desiccation. Another possible advantage of this method is that it separates all acorns within the peat and sawdust and thus avoids the spread of pathogenic fungi. This method has been tested on water and cherrybark oak acorns in our laboratory, but neither peat, sawdust, nor dry sand were as good as 4-mil polyethylene bags without media.

In Europe, acorns of *Q. sessiflora* and *Q. penduncu-lata* are often stored over one winter in pits 60 to 100 cm deep, with the top 20 cm being filled with straw or litter (Walkenhorst 1985). When radicles start emerging, the acorns are removed and sown. This system might work in the Northern United States, but mild winter temperatures in the South could prevent its use there.

Johnson (1979) reported successful 6-month storage of Nuttall oak acorns in drums of water maintained at 2° to 5°C. Tests at our laboratory gave similar results for water and cherrybark oak acorns for 5 months, but longer storage by this method (17 and 29 months) was not nearly as successful as storage in 4-mil polyethylene bags. For 5 to 6 months of storage over winter, however, this water method is satisfactory. One good feature of this method is absolute hydration of the acorns for the length of the storage period.

Another problem in acorn storage has been the tendency of acorns to germinate in the storage container. The epicotyls usually do not emerge at 2° to 5° C, but the radicles appear readily. This condition, commonly called pre-sprouting, can produce etiolated radicles 6 to 8 inches long, which are easily broken by any movement, especially planting (fig. 11). Even if the radicles are not broken, many succumb to microorganism attack during storage. We have tested a large group of chemical inhibitors to prevent pre-sprouting in storage, but no successful treatment has ever been found.

The best solution to pre-sprouting, as with weevil larvae, may be to ignore the problem. Where radicle tips have been broken in planting or killed by microorganisms, secondary root development often ensures seedling survival and even produces a multiple-root seedling which may survive outplanting better than a seedling with only one carrot-like root. A study

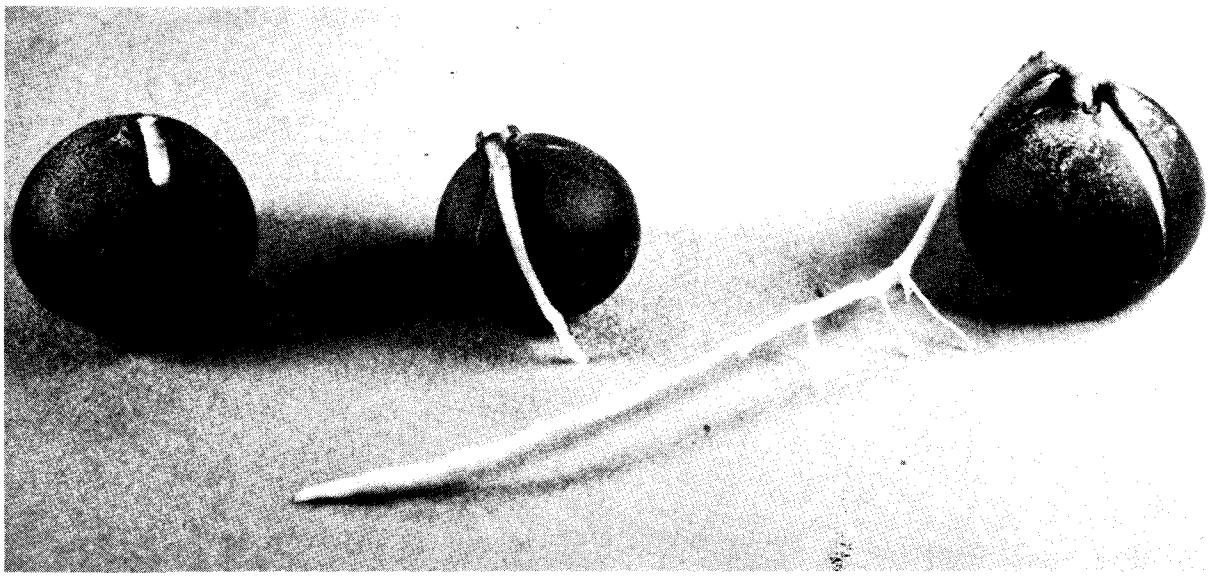


Figure 11.—*Water oak acorns that sprouted during storage at 2°C.*

with cherrybark and Shumard oaks showed that broken radicles did not adversely affect seedling production at all for these species (Bonner 1982).

Red oak species vary in degree of dormancy. Water oak is one of the most dormant, and its acorns usually germinate very little in storage. Other species, such as cherrybark or Shumard, are not very dormant, andthey exhibit excessive pre-sprouting. The relationship between dormancy and storability suggests that the more dormant species maintain viability better during storage than the less dormant species.

White Oak Group.—With only rare exceptions, acorns of the white oak group cannot be successfully stored more than 4 to 6 months (over winter). These non-dormant species sprout very readily in storage and die rapidly. The best recommendation for white oaks is to store them in the ground by planting in the fall.

If white oaks are held over winter for spring planting, the best conditions are almost the same as those recommended for red oaks: temperatures just above freezing (2°–3°C), maximum acorn moisture content (45 to 50 percent), and containers that allow gas exchange. There is good evidence, however, that cloth bags or a thinner polyethylene (1.75 mil) are better for white oak, because of the need for greater aeration (Rink and Williams 1984). The preceding recommendations for flotation, sizing, and ignoring the weevil larvae apply to white oaks.

A few exceptions to non-storability of white oak acorns are documented. Suszka and Tylkowski (1980) have demonstrated successful storage of English oak (*Q. robur*) acorns over three winters by mixing them in sawdust or peat at −1°C, as described previously for northern red oak. Storage tests with southern white oaks in our laboratory have been largely unsuccessful. The single exception has been one lot of overcup oak which retained excellent viability for 1 year (table 3). Since overcup is one species that retains its wrap-around cup when disseminated, one could speculate that the cup retards oxygen uptake by the acorns, thus slowing metabolism and prolonging viability. One lot of live oak (*Q. virginiana*) also germinated 60.7 percent after 1 year and 17.6 percent after 2 years.

Table 3.—*Viability retention of various southern white oak species stored at 2°C, high moisture content, and in polyethylene bags*

Species	Germination percent			
	Original	6 mo	1 yr	2 yrs
Q. alba—white	90+	7.0	....	...
Q. virginiana—live	96.0	...	60.7	17.6
Q. muhlenbergii—chinkapin	91.3	...	39.0	2.0
Q. michauxii—swamp chestnut	86.1	...	65.1	2.0
Q. lyrata—overcup	.....	...	95.8	...

STRATIFICATION

All acorns of the red oak group exhibit some degree of dormancy, and stratification is commonly used to enhance germination rate and uniformity in the nursery. The common stratification technique is the same as that for other tree seeds: moist storage at 2° to 5°C for several months. Current recommendations for length of stratification are summarized in table 4. For

Table 4.—*Recommended stratification lengths for southern red oaks in the Midsouth*[1]

Species	Stratification period
	------ *Weeks* ------
Q. coccinea—scarlet	4–8
Q. falcata var. *falcata*—southern red	4–8
Q. falcata var. *pagodaofolia*—cherrybark	4–6
Q. nigra—water	8–12
Q. nuttallii—Nuttall	4–8
Q. phellos—willow	4–8
Q. rubra—northern red	4–6
Q. shumardii—Shumard	8–12
Q. velutina—black	4–8

[1]Should be fully hydrated at 2°C, with some gas exchange possible.

red oak species not listed in table 4, a general recommendation is 4 to 8 weeks. Local seed sources may do better with longer or shorter periods. In general, northern sources will require longer stratification periods than sources from the southern portion of a species range. Local source tests with varied stratification periods are desirable.

If acorns are fully hydrated in cold storage, then stratification is actually taking place in this manner. In a Tennessee study with northern red oak (Farmer 1974), cold storage was sufficient to break dormancy, and additional stratification was not needed. To ensure dormancy removal, however, acorns to be stratified should be soaked again for 24 hours prior to the start of stratification. The imbibed acorns should be put in polyethylene bags (4-to-10-mil thickness), and the bags should be placed on their sides in a refrigerator at 2° or 3°C. The bags should be turned over each week or two to ensure that water does not continually pool in the bottom of the bags. If excess water is observed, it should be drained from the bags.

In direct seeding operations, better results have generally been obtained with unstratified acorns sown in winter than with stratified acorns sown in the spring (Johnson 1984). Site conditions are extremely important also; excess water on the site may force delays, which dictate the use of stratified acorns. The "stratification effect" from moist storage may be enough to stimulate germination in many cases.

For many years, nurserymen mixed acorns with moist peat or sand during stratification to help maintain seed hydration. This practice does not improve the stratification effect, however, and it results in an extra task of separating acorns from the medium at sowing time.

Many red oaks will begin germinating during the stratification period, as they do in storage. The higher the stratification temperature, the more presprouting will occur. Although this can make sowing more difficult in nurseries, it does not necessarily decrease the chances of a good seedling crop (Bonner 1982). Planting in the fall or winter without stratification is also an alternative for red oaks. Presprouting can be avoided, but uniformity of emergence in the spring will suffer. Direct seeding operations usually employ mechanical planters to put acorns 2 to 4 inches into the ground. Pre-sprouted acorns present problems, because the radicles prevent even flow of acorns through the planter. This situation is another reason why unstratified acorns planted in winter are preferred in direct seedings.

White oak acorns from southern sources are not dormant, and stratification is not normally used with these species. Further, because these acorns store so poorly, fall planting of untreated acorns is usually preferred. More northern sources of some white oaks, however, exhibit a stronger dormancy, and stratification can be beneficial to some.[2]

TESTING

Sampling

Efficient use of acorn supplies will normally require testing to determine seed quality and sometimes moisture content. Proper methods are required so that the samples truly represent the seed lots.

Small acorns can be sampled with large seed triers, but the usual procedure is to thrust the hand into the container and remove handfuls of acorns. The hand should be inserted with the fingers extended together, and closed as it is withdrawn. It is difficult to sample deeper than 40 cm with this method, so containers may have to be partially emptied to facilitate sampling. The total sample should be made up of equal portions taken from evenly distributed volumes of the total seed lot. The sample submitted to the testing facility should contain a minimum of 500 acorns per lot.

Germination Test

The usual method for evaluating seed quality is a laboratory germination test. Evaluation in a testing laboratory is preferred, of course, but rapid losses of acorn quality during shipment of samples is a major problem. If controlled temperature facilities are available, germination can be tested by the "cut and peel" method, which is very similar to techniques used for official tests (AOSA 1978). The procedure is simple:

1. Cut acorns in half (pruning shears are good for this) and discard the half with the cup scar (fig. 12).

[2]R. Tinus, personal communications.

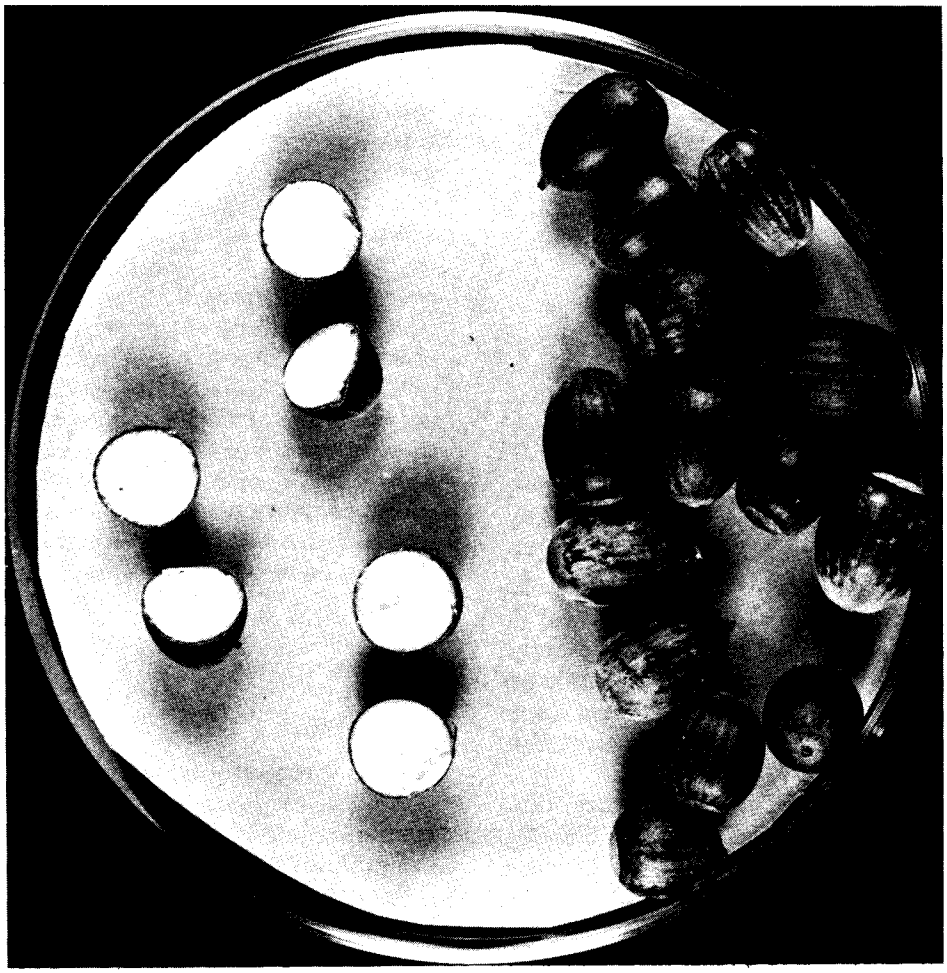


Figure 12.—*Acorns cut in half for germination testing.*

2. Peel the pericarp from the remaining half and place it on moist blotters, or similar material, cut side down.
3. Incubate at alternating temperatures of 20° (16 hours)/30°C (8 hours, with light). If only constant temperatures are available, use 25°C with 8 hours of light. Keep the test surface moist.
4. Count germination at desired intervals for up to 28 days. An acorn is scored as germinated if both radicle and shoot exhibit normal growth and morphology (fig. 13). Tests may be terminated early if germination is complete.

Although "percent of normal germination" is the primary performance measurement of the germination test, additional valuable information on acorn quality can be gained by some measure of germination rate. If germination is counted 2 or 3 times a week, expressions of rate—such as mean germination time (in days) or Peak Value (PV), where PV = maximum value of cumulative percentage divided by days of the test (Czabator 1962)—can be easily calculated. Both of these rate expressions have been shown to be significantly correlated with nursery germination of several southern oaks (Bonner 1984).

Removal of the pericarp in the "cut and peel" testing procedure essentially overcomes the dormancy in all oaks, so no stratification is needed for the test. The minimum time to complete these tests is 3 weeks, however, and time constraints may require more rapid estimates of quality.

Rapid Viability Estimates

There are three options for quick tests of viability:

1. *Cutting test*—The quickest estimate of acorn quality can be obtained by a cutting test, and one should be carried out immediately after water flotation of fresh acorns. Acorns should be cut in half to expose the embryo. Acorns with good cotyledon color and no insect damage to their embryo are usually viable. Cutting tests are much less accurate with acorns from storage, because

Figure 13.—*Normal germination in cherrybark oak. Normal growth of both radicle and shoot is evident.*

considerable decline in quality can occur without a corresponding change in cotyledon color.

2. *Radiography*—X-ray techniques can provide a quick, non-destructive test for damage or incomplete embryo development (fig. 14). Sample exposure and radiograph interpretation guides are available (Belcher and Vozzo 1979).
3. *Tetrazolium staining (TZ test)*—Tetrazolium chloride is widely accepted as a vital stain in seed science. The following procedures have been moderately successful for acorns (Bonner 1984):
   (a) Use two samples of 50 acorns each.
   (b) Cut acorns in half and discard the half without the embryo. Count and discard any rotten acorns.
   (c) Place acorn halves in a deep dish, micropyle end down, and add TZ solution until the halves are almost covered. Solution should be made with 2,3,5-triphenyl tetrazolium chloride and should have a pH of 7.0.
   (d) Cover dishes and incubate in the dark for 24 hours at 21° to 25°C. Break cotyledons apart to study stain in each acorn. A good stain is bright pink to red. Very dark red (as in blood) indicates tissue damage.
   (e) Score stain in five classes:
   I. Cotyledon and embryonic axis both stained.
   II. Axis stained, cotyledon unstained.
   III. Cotyledon stained, axis unstained.
   IV. Axis and cotyledon both unstained.
   V. Rotten or insect damage beyond recovery.

Class I is best, of course, but many acorns in Class II will also germinate. Class III acorns are very weak, and they normally will not produce plantable seedlings in nurseries. Some may germinate in greenhouses. Classes IV and V will not germinate, but in-

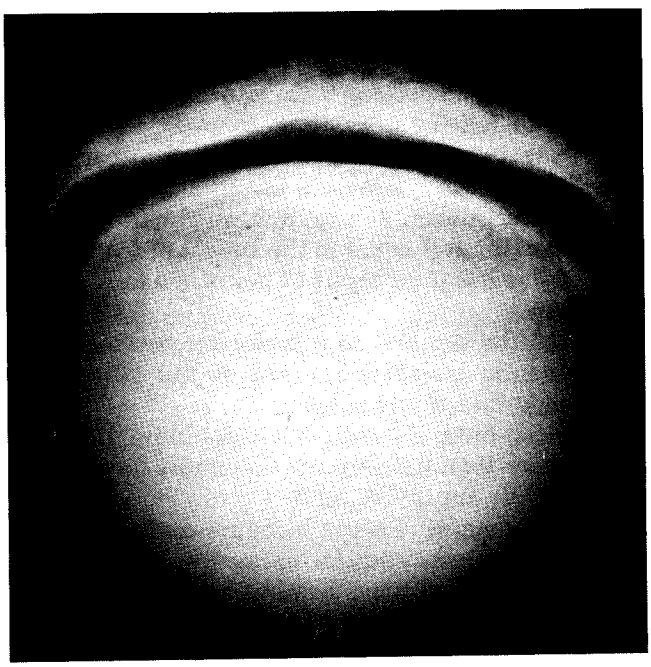


Figure 14.—*Radiograph of water oak acorn.*

clusion of the counts can show the overall quality of a lot.

Acorn characteristics of various species cause some problems in the TZ test. Water oak cotyledons fit together very tightly, thus restricting access of TZ solution to the embryonic axis. This species also has a tough testa next to the cotyledons, which restricts entrance of the solution. Similar problems occur with willow oak and Nuttall oak acorns. Scraping the testa away from the radicle area and removing more than one-half of the cotyledons might improve the condition. The more cotyledon removed, the more likely that the remaining pieces will separate when imbibed.

Cherrybark oak acorns do not present the problems described above. Their cotyledons readily separate when imbibed, and the testa on cherrybark acorns is thinner. Other red oak species that are more like cherrybark than water oak are Shumard and scarlet oaks.

Cotyledon color also influences stain interpretation. Healthy acorns with high lipid contents (water, willow, cherrybark) are orange, and the stain will show reddish-orange. Low-fat acorns (Shumard, all white oaks) have white to pale yellow cotyledons; good stains in these cases are typically pink to red.

Stain interpretation is very difficult on acorns in which the radicles are protruding through the pericarp when the tests are run. When these acorns are cut in half, the cotyledons frequently break apart and damage the axis. This condition is very common in white oak.

Moisture Content

Determinations of acorn moisture contents should be made as follows:

1. Draw two samples of about 10 acorns each.
2. Rapidly cut into halves (small acorns) or quarters (large acorns), mix, and weigh out a sample approximately equal to the weight of five acorns.
3. Dry at 103°C for about 17 hours in a forced-draft oven.
4. Cool the samples in a desiccator, reweigh, and calculate moisture content as the percentage weight loss of wet weight.

In official tests, if the duplicate determinations differ by more than 2.5 percent, they must be repeated until they do not (ISTA 1983).

Rapid estimates of acorn moisture contents can be made by drying cut samples in microwave ovens. Results may be 2 to 3 times more variable than regular oven measurements, but a single determination can be made in 5 or 6 minutes (Bonner and Turner 1980).

Detailed instructions for both types of moisture determination can be found in Bonner (1981).

Applying Test Results

Nursery practices are beyond the scope of this paper. Seedsmen should remember that, like other tree species, laboratory test results for acorns normally give higher germination values than will occur in the nursery. Local "experience factors" should always be developed for each species in each nursery. Without such information proper bed densities will be difficult to achieve. Recent recommendations of seedbed densities of 4 seedlings per square foot for cherrybark oak (Barham 1980) and white oak (Wichman and Coggeshall 1984) reflect the current preference for large oak seedlings for outplanting.

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This paper summarizes, in two parts, the results of research conducted from 1967 to 1983 on the seed problems of southern oaks, incorporating both published and unpublished results. Part I reviews current biological knowledge of acorns, and Part II recommends handling and management techniques for acorns of southern white and red oaks.