Update on Oak Seed Quality Research: Hardwood Recalcitrant Seeds

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Abstract: In 2 experiments, acorns of cherrybark oak (*Quercus pagoda* Raf.) and water oak (*Q. nigra* L.) were stored at 2 temperatures and 2 moisture contents for 3 years, and acorns of white oak (*Q. alba* L.) and cherrybark oak were desiccated over a span of up to 11 days and examined for physiological and biochemical changes. We found that after 2 years of storage, only cherrybark and water oak acorns that had been stored fully hydrated retained high viability. In addition, those stored at –2 °C (28 °F) were more viable than those stored at 4 °C (39 °F). In the desiccation study, we found rapid decreases in acorn viability, accompanied by changes in the lipid, protein, and carbohydrate fractions. Changes were seen in membrane lipids and proteins in as few as 3 to 4 days of drying, suggesting that the physiological deterioration of these acorns begins relatively soon after shedding. We suggest that all precautions against moisture loss be taken during collection and storage, especially if acorns are not used immediately.

Keywords: acorns, biochemistry, FT-IR, gas chromatography, oaks, recalcitrant seeds, storage

Introduction

Low temperature storage of hardwood seeds has been studied for the last 30 years, yet the biochemical and physiological causes of recalcitrance in tree seeds remain unknown. While orthodox seeds can be dried without damage to moisture contents of less than 12% (fresh weight basis) and stored at low temperatures for long periods of time, recalcitrant seeds cannot (Roberts 1973). Some temperate tree genera with recalcitrant seeds are *Castanea*, *Aesculus*, and some *Acer* and *Quercus*. Sensitive to moisture loss and metabolically active, the acorns of the white oak subgenus Lepidobalanus germinate soon after seed fall and cannot be stored for more than a few months (Rink and Williams 1984). However, acorns of the red oak subgenus Erythrobalanus can be stored for greater than 1 year, although viability loss may be high (Bonner and Vozzo 1987; Connor and Bonner 1999). Hypotheses to explain the physiological basis of seed recalcitrance include: 1) changes in membrane and storage lipids (Pierce and Abdel Samad 1980; Flood and Sinclair 1981); 2) physical disruption of seed membranes (Seewaldt and others 1981); 3) changes in seed proteins and carbohydrates (Finch-Savage and others 1994a,b; Bochicchio and others 1997; Greggains and others 2000); 4) changes in water properties of desiccating seeds (Farrant and others 1985); and 5) aberrant metabolic processes during hydrated storage and as water is lost (Pammenter and others 1994). While the latter hypothesis explains the cause of recalcitrance and is accepted by many researchers, it still does not quantify or define the physiological and biochemical processes responsible. The information given in this paper summarizes the projects underway at the Center for Bottomland Hardwoods Research. The objectives of our current research are: 1) to study the seed storage requirements of various species of hardwoods with recalcitrant seeds; and 2) to examine the effects of storage and desiccation on the biochemistry of recalcitrant seeds.

Materials and Methods

General

Acorns of cherrybark oak (*Quercus pagoda* Raf.), water oak (*Q. nigra* L.), and white oak (*Q. alba* L.) were collected and soaked overnight in tap water to ensure full hydration. Floaters were discarded. Moisture contents of these fresh acorns were determined by drying 2 to 4 samples at 105 °C (221 °F) for 16 to 17 hours. For germination tests, acorns were cut in half

horizontally and the seed coat was removed from the half containing the embryo. The acorn was then placed cut-side-down on moist Kimpak and kept under an alternating temperature regime of 20 °C (68 °F) for 16 hours in the dark and 30 °C (86 °F) for 8 hours with light (Bonner and Vozzo 1987). Counts were conducted weekly for 4 weeks.

Storage Studies

Experiment 1—Cherrybark and water oak acorns were collected in 1999 and 2000. Half of the acorns were stored fully hydrated and the other half dried on a lab bench for 48 hours and then stored. Lots consisted of 110 to 120 acorns in 4-mil polyethylene bags stored at either 4 °C (39 °F) in a Lab-Line Ambi-Hi-Low Chamber or at –2 °C (28 °F) in a modified chest freezer. Original percent germinations and moisture content were determined and then tested at yearly intervals. Acorns were germinated as 2 replications of 50 seeds per sampling period and were soaked overnight in tapwater prior to germination testing.

Experiment 2—We used Fourier transform infrared spectroscopy (FT-IR) and gas chromatography (GC) to examine the biochemical changes taking place in desiccating acorns of white oak (*Q. alba* L.) (Connor and Sowa, forthcoming) and cherrybark oak (Sowa and Connor 2003). Collected acorns were soaked in tapwater overnight and then spread on blotter paper in a single layer on a lab bench to dry for up to 11 days. Moisture contents and viability were determined for each experiment performed. Seed samples were collected at regular intervals over the course of the 11-day drying period. At each FT-IR sampling period, transmission spectra were recorded on thin slices of cotyledon and embryonic axes squashes that were placed between CaF_2 windows of a demountable transmission cell. Duplicate samples were analyzed on each sampling day. Additionally, acorns that had been dried were rehydrated overnight and scanned the following day. For each spectrum, 512 scans at 2 per cm resolution were collected. GC analyses were performed on carbohydrate extractions of white oak only. Samples were extracted as detailed in Connor and Sowa (forthcoming) and analyzed on a HP 5890 GC using a Supelco SPB-5 capillary column.

Results

Experiment 1—Water oak acorn moisture was 30.5% (fresh weight basis) for fully hydrated acorns and 25.6% for those that had dried 48 hours prior to storage. Drying reduced initial viability by 9% (Figure 1A). After 1 year in storage, temperature of storage was significant but seed moisture content was not; seeds stored at –2 °C had a higher viability than those stored at 4 °C. However, after 2 years in storage, the results were reversed; acorn moisture content was significant and temperature of storage was not. Acorns that had been dried prior to storage had lower viability than those stored fully hydrated.

Cherrybark oak acorns had an initial moisture content of 29.6% for fresh acorns and 19.9% for those that had been dried before storage. Drying had little effect on initial viability, reducing it by only 2% (Figure 1B). However, unlike the water oak experiment, temperature of storage was not a significant factor. Only acorns stored fully hydrated retained high viability. In both water oak and cherrybark oak, moisture contents did not change during the course of the experiment.

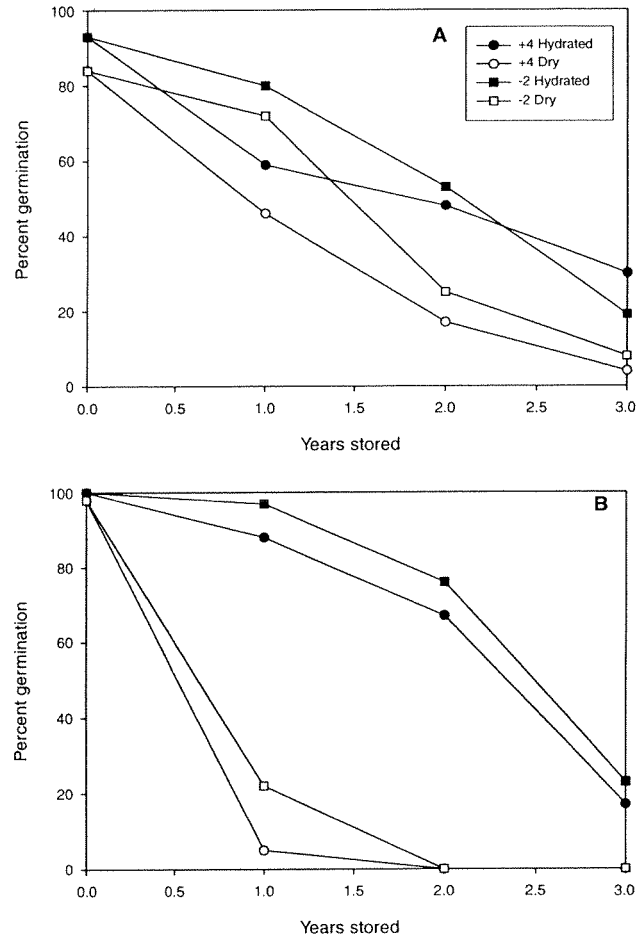

Figure 1—Viability of water oak (A) and cherrybark oak (B) acorns stored at two moisture contents (hydrated or dry) and two temperatures (4 °C [39 °F] or –2 °C [28 °F]) for 3 years.

Experiment 2—Viability declined rapidly in desiccating seeds of both species (Figure 2) and was sensitive to seed moisture content levels of 25 to 30% in white oak and approximately 17% in cherrybark oak. Seed moisture dropped to about 10% after 7 days of drying in white oak and to 12% after 6 days of drying in cherrybark oak.

Phase changes were seen in the membrane lipids of the embryonic axes and cotyledon tissue of both species (Figures 3 and 4). These were exhibited by peak shifts of the symmetric and asymmetric $-CH_2$ vibrations near 2850 and 2910 per cm. As viability was lost, the peaks did not return to their original frequency/bandwidth when rehydrated, indicating that the membrane lipid phase remained less fluid than in the fresh acorns. The fact that changes were seen in membrane lipids in as few as 3 to 4 days of drying suggests that the physiological deterioration of these acorns begins relatively soon after shedding. It was also possible to detect

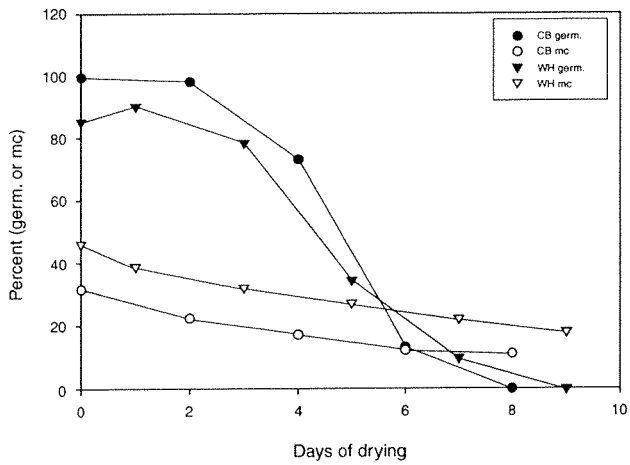

Figure 2—The effects of desiccation on the viability (germ) and moisture content (mc) of cherrybark oak (CB) and white oak (WH) acorns.

differential drying within the acorns (Figure 5). Narrow peak widths and lower peak frequencies indicate dry tissues, while broad peaks indicate hydrated tissues.

Changes in the protein secondary structure, indicated by shifts in the frequency and bandwidth of the amide I and II vibrations near 1650 and 1550 per cm, were also found in drying acorns (Figure 6). FT-IR analyses showed that the loss of protein secondary structure was irreversible; rehydrating the acorns did not restore them to the fresh condition.

GC analyses indicated that while stachyose and raffinose were absent from white oak acorns, there was an abundance of sucrose in both the embryonic axes and cotyledon tissue (Figure 7). Changes in sucrose concentration were not significant until 5 days of drying; then sucrose concentration increased by 3X in the cotyledons and almost 4X in the embryonic axes.

Discussion

In experiment 1, cherrybark and water oak acorns retained high viability for 2 years if stored fully hydrated. While the drying period of 48 hours before storage had little effect on initial viability—reducing germination in water oak acorns by 9% and that of cherrybark oak acorns by only 2%—the damage was evident after 1 year in storage. Water oak acorns stored for 1 year at 4 °C and cherrybark acorns stored for 1 year at either temperature had significant losses in viability if dried before storing. The result of a seemingly small drop in moisture content on long-term storage viability emphasizes the importance of proper seed collection and handling procedures. High moisture content must be maintained in recalcitrant seeds in order to retain high seed viability. We strongly suggest that all precautions against moisture loss be taken during collection and storage, especially if acorns are not used immediately. Otherwise, severe losses in seed quality can occur. Orchard managers and seed companies must place emphasis on

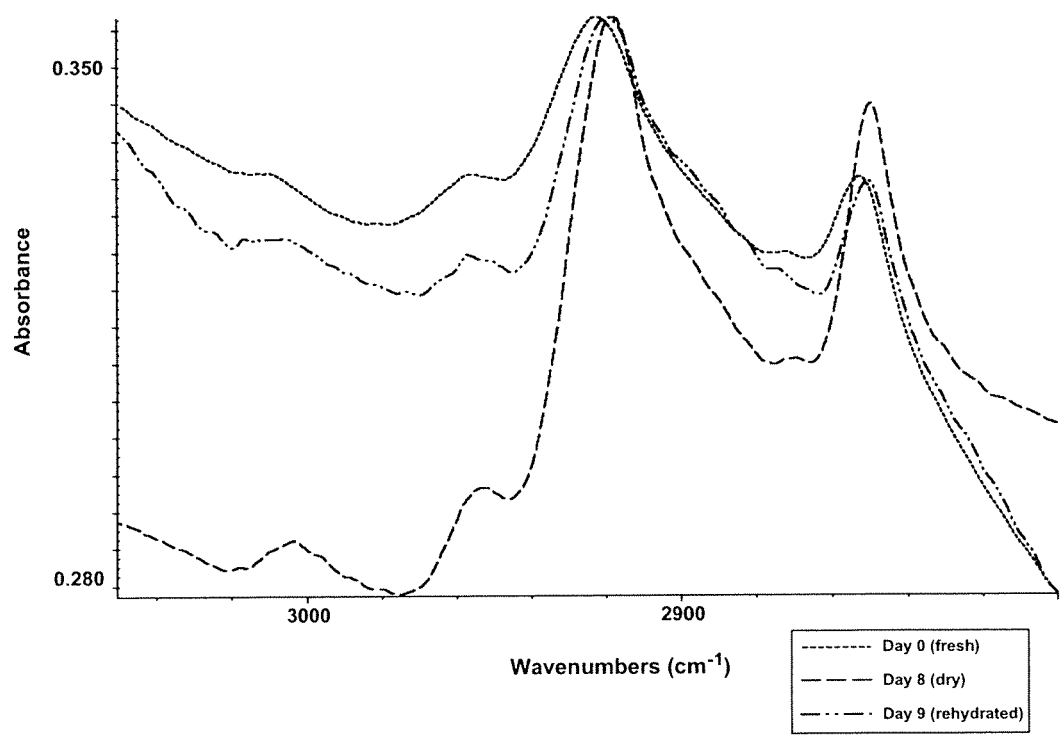

Figure 3—Membrane lipid vibrations in cherrybark oak embryonic axes at day 0 (fresh), day 8 (dry), and day 9 (rehydrated). Peak frequencies are at 2852.2, 2849.7, and 2850.3 per cm.

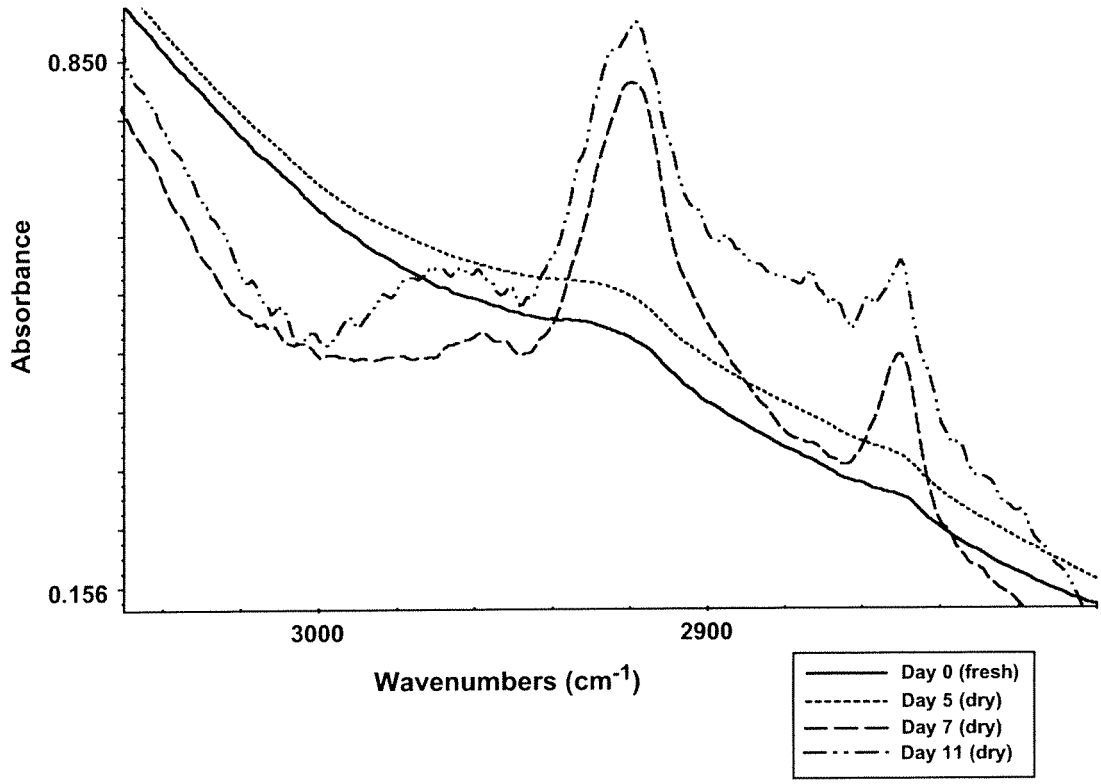

Figure 4—Transmission IR spectra of lipid membranes of rehydrated white oak embryonic axes. Membrane lipid vibrations in fresh (day 0) axes and those dried 5, 7, and 11 days.

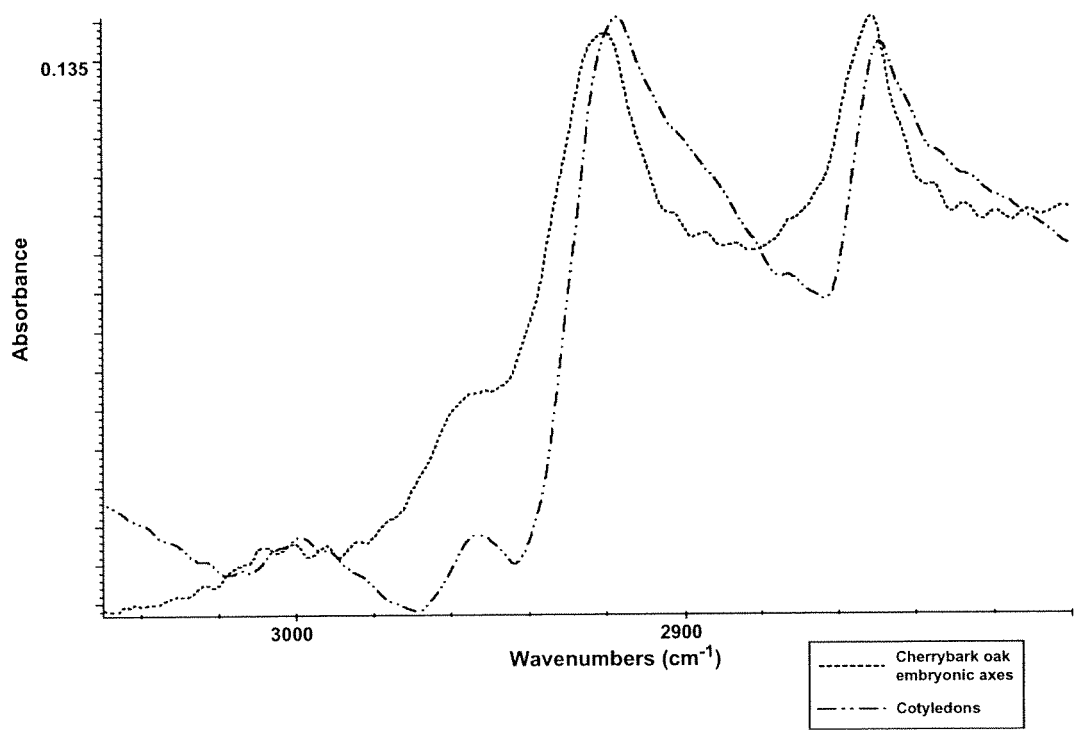

Figure 5—Membrane lipid vibrations in day 4 (dry) cherrybark oak embryonic axes and cotyledons. Narrow peaks in the cotyledons emphasize differential drying.

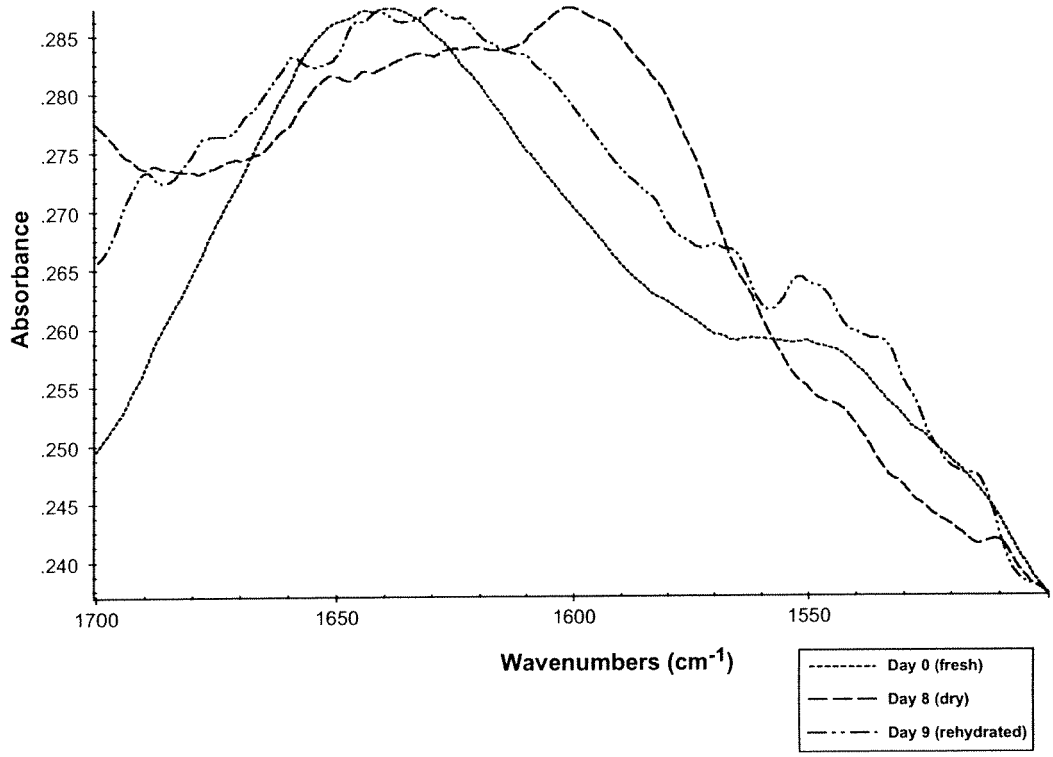

Figure 6—Amide I and II vibrations representing protein secondary structure in fresh (day 0), day 8 (dry), and day 9 (rehydrated) cherrybark oak embryonic axes.

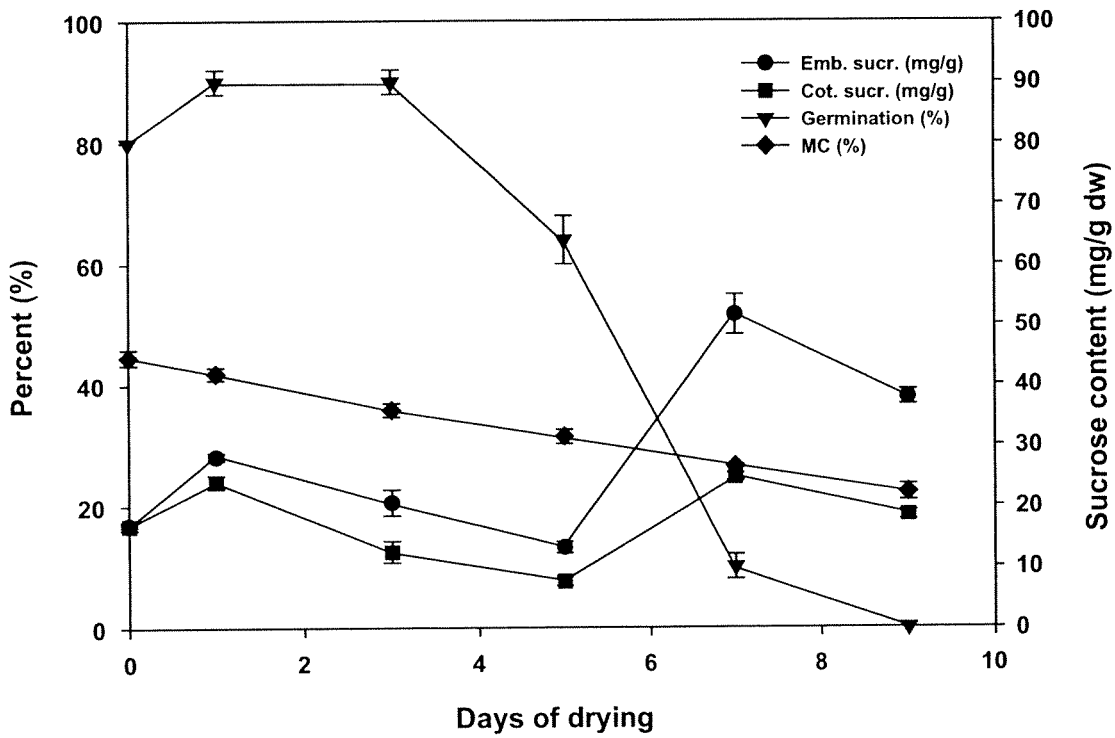

Figure 7—Germination, moisture content (mc), and sucrose content (sucr) in desiccating white oak embryonic axes (emb) and cotyledons (cot). Data from gas chromatography experiments.

careful handling of acorns during the collection process. The sooner acorns can be collected after dropping from the tree, and placed under refrigeration, the higher the probability of successful long-term (1-year) storage. In years with high temperatures and low rainfall during acorn drop, collection in the field must be prompt to ensure that acorn moisture content does not fall below the critical level or that acorns are not put under too much moisture stress before they reach a storage facility. Red oak acorns, as a whole, generally survive for 1 year in storage. Cherrybark oak acorns that dried for only 2 days in the laboratory had less than 25% viability after 1 year of storage under ideal conditions, and were dead after 2 years; those stored at high moisture contents retained at least 67% viability.

Results of experiment 2 emphasized the effects of short-term drying on viability. In cherrybark oak acorns, an extremely rapid decline in germinability occurred between days 4 and 6; the transition point occurred somewhere near a seed moisture content of 17%. We found similar effects of desiccation in white oak acorns. In the FT-IR and GC studies, changes were taking place in the storage and membrane lipids, in the protein secondary structure, and in the carbohydrates during the desiccation process. Membrane lipids changed phase from liquid crystalline to gel upon drying and did not recover when acorns were rehydrated by soaking in water. This structural change ultimately results in acorn mortality, since cell contents can pass indiscriminately through the leaky membranes. Despite the fact that white oak embryonic axes maintain a fairly high moisture content through the desiccation experiment, damage ultimately becomes irreparable. In all species studied, structural damage was seen first in the cotyledon tissue. In addition, the increasing sucrose content in the white oak embryonic axes, coupled with declining acorn viability, suggests that the sucrose is no longer being used for growth and development and/or that starch is being broken down by enzymatic activity in the deteriorating seeds.

Storing seeds fully hydrated has many drawbacks. Seeds continue to respire and can germinate even when stored under low temperatures. Insects remain active, and fungal damage can also occur. Thus, the probability of seed deterioration during storage increases. As our experiments have shown, the acorns of cherrybark oak, water oak, and white oak will decline under hydrated storage. They are metabolically active and can lose viability when stored for longer than 2 years. If subjected to desiccation before storage, however, the situation becomes even more untenable. Shifts in membrane lipids from the liquid-crystalline to the gel phase, changes in protein secondary structure, and cessation of growth and development all occur in desiccating acorns. Thus, while far from ideal, storing acorns of cherrybark oak, water oak, and white oak fully hydrated is at this time the best possible option.

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