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THE PHYSIOLOGY AND BIOCHEMISTRY OF DESICCATING WHITE OAK AND CHERRYBARK OAK ACORNS

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Abstract—The recalcitrant behavior of white oak (*Quercus alba* L.) and cherrybark oak (*Q. pagoda* Raf.) acorns was examined in terms of effects of moisture content on seed longevity, viability, and biochemistry. Acorns of both species were fully hydrated and then subjected to drying under ambient conditions of temperature and relative humidity. Acorns were sampled regularly for moisture determination (gravimetric analysis), germination, and Fourier transform infrared spectroscopic analysis. Invertase activity was also determined in the desiccating white oak acorns. Transmission/reflectance spectra were collected on dry and rehydrated samples of cotyledon and embryonic axis tissue. Germination was highly dependent on initial acorn moisture content, and there were significant changes in lipid profiles of desiccating embryonic axis and cotyledon tissues during short-term drying. A strong absorbance near 1740/cm in cherrybark cotyledon tissue indicated a high concentration of ester carbonyl groups (storage lipids). Additionally, in acorns of both species, evidence of reversible shifts between gel and liquid crystalline phases were found in membranes of axes and cotyledons. Gas chromatographic analyses of desiccating white oak acorns revealed significantly greater sucrose concentrations in embryonic axes than in cotyledons; also, sucrose concentrations dropped dramatically with onset of root extension. It is possible that elevated sucrose concentrations afford protection against cell collapse and cell-wall membrane damage during moisture stress.

INTRODUCTION

Desiccation-resistant seeds can be dried without damage to moisture content (MC) of $\leq$ 12 percent and stored for long periods of time, while recalcitrant, or desiccation-sensitive, seeds cannot (Roberts 1973). This susceptibility makes any period of storage for some recalcitrant seeds very short, while others, such as water oak (*Quercus nigra* L.), can survive for 3 years under proper storage conditions (Connor and Bonner 1999, Connor and Sowa 2001). Hypotheses to explain the physiological basis of recalcitrance include (1) changes in membrane and storage lipids (Flood and Sinclair 1981, Pierce and Abdel Samad 1980); (2) physical disruption of seed membranes (Seewaldt and others 1981, Simon 1974); (3) changes in seed proteins and carbohydrates (Bochicchio and others 1997, Finch-Savage and others 1994, Greggains and others 2000); (4) changes in the water properties of desiccating seeds (Farrant and others 1985, 1988); and (5) aberrant metabolic processes during hydrated storage and as water is lost (Pammenter and others 1994). To date, the storage of some recalcitrant seeds is still an insurmountable problem.

MATERIALS AND METHODS

We collected white oak acorns (*Q. alba* L.) locally in Starkville, MS, within 2 days of shedding. Cherrybark oak (*Q. pagoda* Raf.) acorns were purchased from a commercial supplier. Acorns were soaked overnight in tap water prior to the experiments to insure full hydration. We conducted short-term desiccation experiments over 2 consecutive years. We spread acorns in a single layer on a lab bench to dry them and, at regular intervals, took subsamples and performed five analyses.

Germination Tests

We randomly selected and germinated 100 acorns as 2 replications of 50 seeds each. After imbibing in tap water overnight, acorns were cut in half. The half with the cup scar was discarded (Bonner and Vozzo 1987), and the pericarp was removed from the remaining half. Acorns were placed cut side down on moist Kimpak®. White oak acorns were germinated under a diurnal cycle of 20 °C for 16 hours in the dark and 30 °C for 8 hours with light, and cherrybark acorns were germinated under greenhouse conditions. Germination was tallied each week for up to 4 weeks. An acorn was scored as germinated if both the radicle and shoot had emerged and exhibited normal morphology and growth.

Moisture Content

We determined MCs of whole acorns using four to five replicates of three white oak acorns and four cherrybark oak acorns each. The randomly selected acorns were cut into quarters and dried in aluminum cans at 103$\pm$2 °C for 17$\pm$1 hours in a mechanical convection oven. We calculated seed MC on fresh-weight basis (Roberts 1973).

Carbohydrates

We dissected white oak embryonic axes with immediately adjacent cotyledon tissue from the acorns; tissues were frozen in liquid nitrogen and lyophilized. The freeze-dried cotyledons were ground in a Wiley® mill using a 20-mesh screen, while embryonic axis tissue was ground by hand with a mortar and pestle. A 0.3- to 0.5-g dry tissue sample was used for each carbohydrate extraction. The tissue sample was placed in 10 ml of an 80-percent ethanol solution and heated in a 75 °C water bath for 1 hour. The sample was then filtered, rinsed with more of the ethanol solution, and evaporated to dryness. The evaporation flask

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was rinsed with 10 ml of distilled water, and the water plus contents stirred with 1 g of Amberlite® MB-3 resin for 1 hour. This sample was then filtered, rinsed, and freeze-dried overnight. Phenyl-β-D-glucopyranoside was added as an internal standard. The dried sample was dissolved in 1 ml of trimethylsilylimidazole, heated in a 75 °C water bath for 30 minutes, blown to dryness, and then redissolved in 1 ml chloroform and stored until analysis. We performed analyses on a HP® 5890 gas chromatograph (GC) using a Supelco® SPB-5 capillary column (30 m by 0.25 mm ID by 0.25 film thickness). We made three to five extractions from each cotyledon sample and one to two from the embryonic axis sample, depending on amount of tissue available. We identified carbohydrates by comparing them with standards of pure sugars prepared in the same manner and injected onto the GC.

Invertase Analyses

We extracted invertase from cryostored cotyledon and embryo samples. After thawing and then remaining in a hydrated atmosphere for 15 minutes at room temperature, tissues were homogenized with a mortar and pestle in a small amount of an extraction buffer consisting of 200 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, 2-percent v/v glycerol, and 1-percent w/v insoluble PVP (Sung and others 1993, Xu and others 1989). Homogenate was placed in a test tube with a total of 1:5 or 1:3 (g fresh weight/ml) of the extraction buffer and incubated at room temperature for 15 minutes. The sample was then passed through one layer of Miracloth, fractioned with 70-percent $(NH_4)_2SO_4$, and centrifuged at 18,000 g for 20 minutes at 4 °C. The resulting pellet was resuspended in 5 ml of a resuspension buffer containing 10 mM Hepes/NaOH (pH 7.5), 2 mM DTT and 2 mM Mg acetate, and then desalted by passing through a Sephadex G-25 column. We assayed samples for acid and neutral invertase activity at pH 5.0 and 7.0, respectively, using a Milton Roy Spectronic 501 spectrophotometer set at 340 nm.

Fourier Transform Infrared (FT-IR) Spectroscopy

We placed thin slices of both white oak and cherrybark oak acorn cotyledon tissue and squashes of three to five embryonic axes between CaF_2 windows of a demountable transmission cell. A minimum of duplicate samples was analyzed. For each spectrum, 512 scans at 2/cm resolution were collected and averaged on a Nicolet 20 DXB spectrometer using a liquid nitrogen-cooled MCT-A detector. Single-beam spectra were ratioed against an open-beam background to yield transmission spectra. Attenuated total reflectance spectra were similarly recorded on thin slices of white oak embryonic axes and cotyledon tissue that were layered onto a 45° ZnSe crystal in an ARK sampling device (SpectraTech, Inc.). Instrument collection parameters were the same as for the transmission experiment. However, absorbance is the equivalent to log 1/R, where R = reflectance.

RESULTS AND DISCUSSION

Cherrybark oak acorn viability was sensitive to MC (fig. 1). If we allowed acorns to desiccate, germination declined rapidly in these recalcitrant seeds. Over the course of the experiments, MC in cherrybark oak dropped from 31.6 to 11.0 percent. Once MC was < 17 percent (day 4), viability declined rapidly. The MC of embryonic axes was always higher than that of the cotyledons.

White oak acorns, among the most recalcitrant of the Fagaceae, had a much higher initial MC than did cherrybark

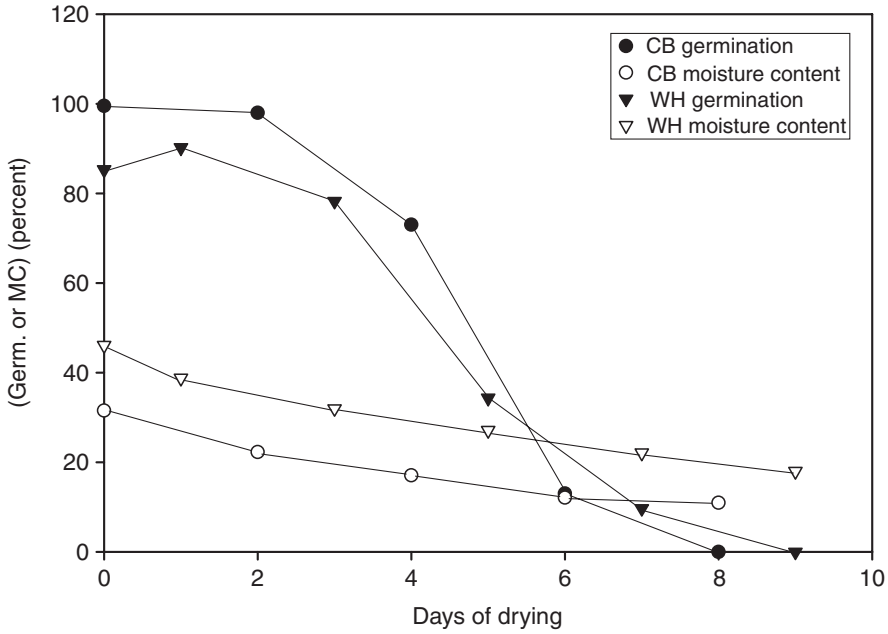

Figure 1—Germination and moisture content (MC) of white oak (WH) and cherrybark oak (CB) acorns desiccated over 8 to 10 days.

acorns, averaging 46 percent (fig. 1). MC dropped to 18 percent during the course of the experiments. Once MC reached 27 percent, viability was < 50 percent. Like cherrybark oak, the embryonic axes always had a higher MC than did the cotyledons.

We found phase changes in the membrane lipids in the embryonic axis and cotyledon membranes of both cherrybark oak and white oak, exhibited by peak shifts of the symmetric and asymmetric $-CH_2$ vibrations near 2850 and 2910/ cm (figs. 2 and 3). The transformation from liquid crystalline to gel phase can disrupt transport through membranes. The phase changes were reversible upon rehydration of the acorns up through day 5 of desiccation; after that time, reversibility was only partial, suggesting that damage to the membranes was permanent. Changes in membrane lipids in as few as 2 to 3 days of drying suggested that the physiological deterioration of acorns begins relatively soon after they are shed.

Cherrybark oak acorns have a high amount of storage lipids, evidenced by an ester carbonyl peak near 1740/cm (fig. 4). Unlike membrane lipids, the peak frequency of these lipids did not shift or change as acorns deteriorated, indicating little or no mobilization of lipids and transport from the cotyledons to the axes.

Unlike cherrybark oak, white oak acorns did not have a high amount of storage lipids, relying instead upon carbohydrates. The most prevalent carbohydrate in the acorns was sucrose, the amount of which fluctuated constantly as acorns desiccated (fig. 5). This fluctuation indicated that the metabolism of acorns was still very active despite the reduced MC and deteriorating state of the acorns. The

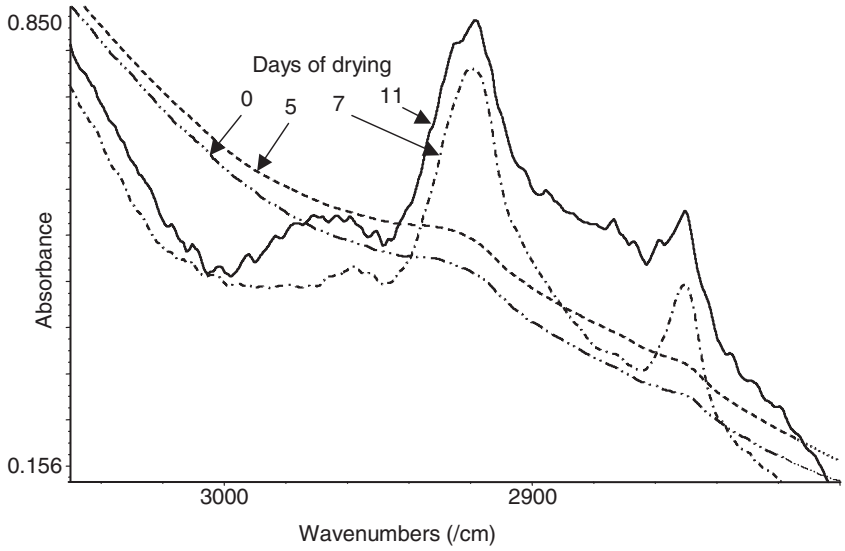

Figure 2—Membrane lipid vibrations in fresh (day 0) white oak embryonic axes and in those dried 5, 7, and 11 days. Peak frequencies occurred near 2850 and 2910/cm.

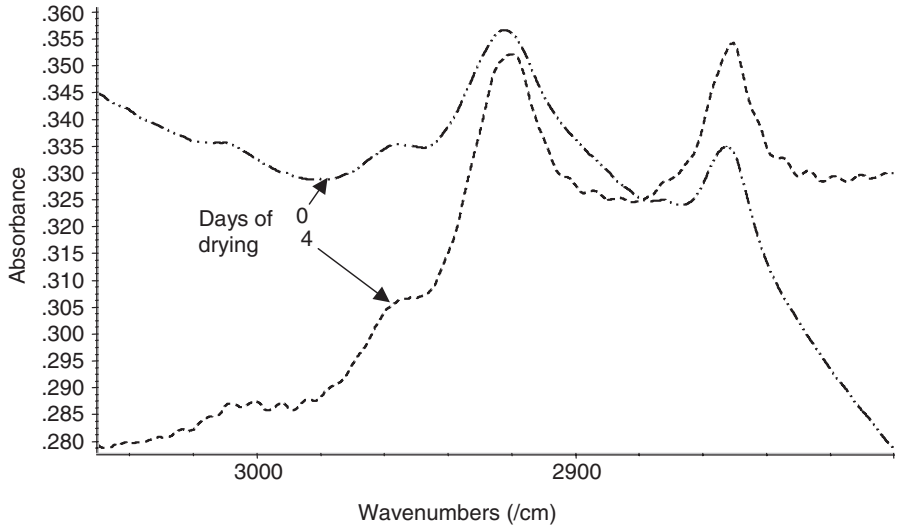

Figure 3—Membrane lipid vibrations in fresh (day 0) cherrybark oak embryonic axes and in those dried 4 days. Peak frequencies occurred near 2850 and 2910/cm.

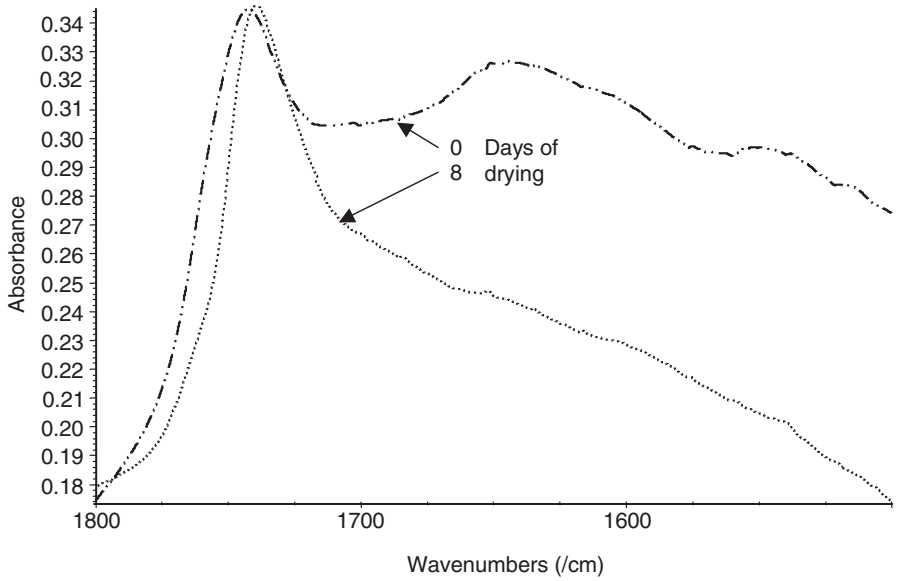

Figure 4—Storage lipid vibrations in fresh (day 0) cherrybark oak embryonic axes and in those dried 8 days. Peak height of the ester carbonyl vibration occurred near 1740/cm.

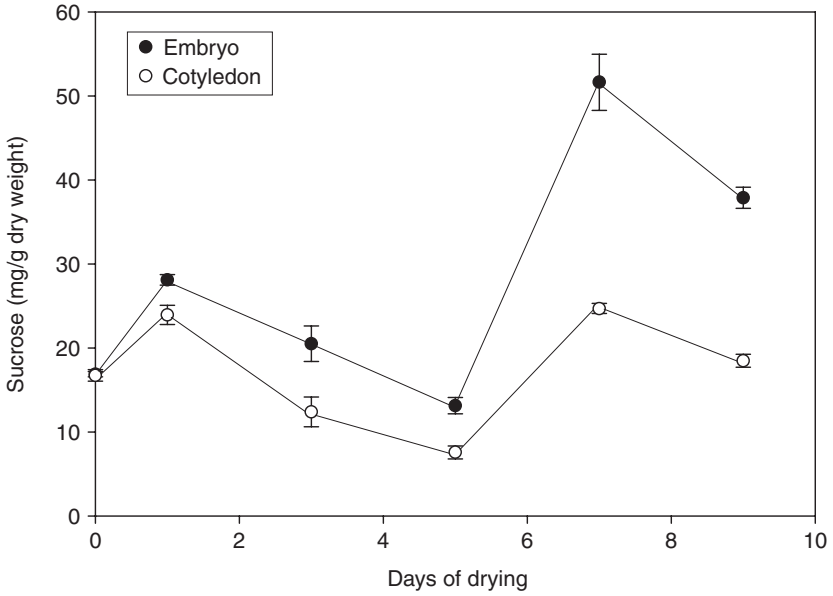

Figure 5-—Sucrose content (mg/g dry weight; ± one standard error) in desiccating white oak acorn embryonic axes and cotyledons.

fluctuating amounts of invertase found in the white oak acorns (table 1) enhanced this observation. Drops in MC did not terminate sucrose metabolism; there were significant changes in the sucrose content in both the embryonic axes and the cotyledons. The sucrose increase as tissue dried, while obviously not functioning to preserve seed viability, might function secondarily to protect membranes from desiccation damage and cells from collapse. The sucrose FT-IR peak at 1045/cm (fig. 6) confirmed the GC analyses.

In practical terms, these results advise against allowing acorns to remain too long exposed to the elements before collecting. The frequently hot, dry conditions during acorn fall in the South may lead to serious viability losses. In the laboratory, under ideal conditions, white oak acorn viability dropped from 90.1 to 78.3 percent after only 3 days of drying. Cherrybark oak acorn viability dropped from 98.0 to 73.0 percent after 4 days. It is doubtful that germination would be as high in the field; it is also possible that seedlings grown from stressed acorns might exhibit reduced growth and vigor.

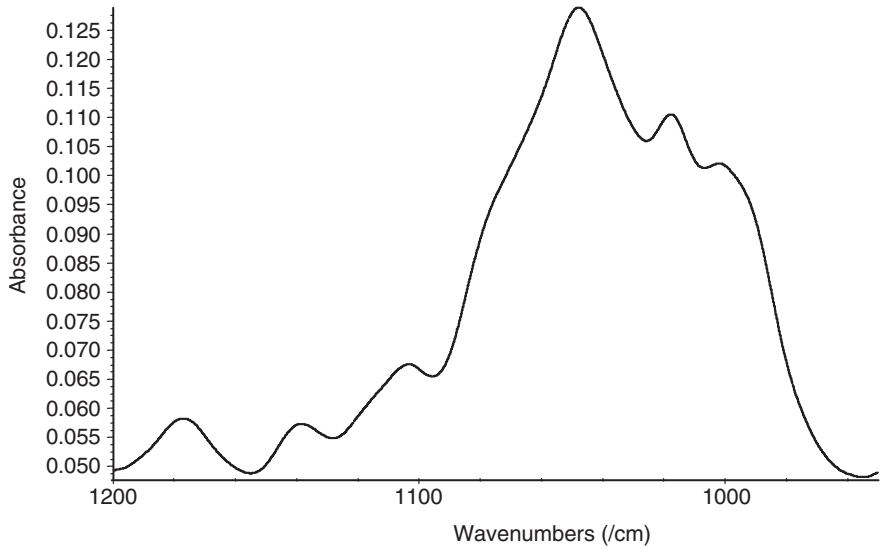

Figure 6—Attenuated total reflectance spectrum of carbohydrates in white oak acorn embryonic axes.

Table 1—Invertase activity in white oak acorn cotyledons

Drying	Invertase activity[a]	Hydrolyzing potential[b]
days		
0	847	289.7
1	397	135.8
3	709	242.5
5	492	168.3
7	327	111.8
9	870	297.5

[a] Units of invertase in 1 g of fresh cotyledon tissue; where one unit will hydrolyze 1 µmole of sucrose to invert sugar per minute at 55 °C.
[b] µmoles of sucrose that can be converted to invert sugar by the invertase present in 1 g of fresh tissue.

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Description: Ninety-two papers and thirty-six poster summaries address a range of issues affecting southern forests. Papers are grouped in 15 sessions that include wildlife ecology; fire ecology; natural pine management; forest health; growth and yield; upland hardwoods - natural regeneration; hardwood intermediate treatments; longleaf pine; pine plantation silviculture; site amelioration and productivity; pine nutrition; pine planting, stocking, spacing; ecophysiology; bottomland hardwoods - natural regeneration; and bottomland hardwoods—artificial regeneration.