

From Forest Nursery Notes, Summer 2009

171. Acorn storage: can you really fool Mother Nature? Connor, K. USDA Forest Service, Rocky Mountain Research Station, Proceedings RMRS-P-58. p.108-113. National Proceedings: Forest and Conservation Nursery Association - 2008. 2009.

Acorn Storage: Can You Really Fool Mother Nature?

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Connor, K. 2009. Acorn storage: Can you really fool Mother Nature? In: Dumroese, R.K.; Riley, L.E., tech. coords. 2009. National Proceedings: Forest and Conservation Nursery Associations—2008. Proc. RMRS-P-58. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 108–113. Online: http://www.fs.fed.us/rm/pubs/rmrs_p058.html.

Abstract: Moisture levels in acorns before storage are critical. Two years after being dried before storage, water oak (*Quercus nigra*) acorns had 17% to 25% germination, while cherrybark oak (*Q. pagoda*) acorns were dead. Acorns stored fully hydrated fared far better after 2 years in storage, with germination ranging from 48% to 53% in water oak acorns, and from 67% to 76% in cherrybark oak acorns. Survival of acorns in the field was also dependent on moisture. The moderating effects of high relative humidity and rainfall throughout the collection period of the second experiment led to higher viability of white oak acorns left in the field for up to 15 days. We also observed a higher sucrose concentration in desiccating white oak acorns. While this increase may serve to initially protect cellular membranes in the acorn tissues, the mechanism is obviously not successful in preserving viability, which dropped rapidly after day 5 of the experiment.

Keywords: acorn storage, moisture content, insect damage, sucrose content

Introduction

Orthodox seeds are easily stored. When dried to moisture contents (MCs) of 12% or less, they become metabolically quiescent and can be stored at temperatures ranging from 4 to 18 °C (39 to 64 °F) or cryostored in liquid nitrogen at –196 °C (–321 °F) (Roberts 1973). However, some seeds are desiccation-sensitive, or recalcitrant. These seeds are sensitive to moisture loss and/or to low temperatures, and must be stored fully hydrated (Roberts 1973; Ellis and others 1990). In this state, they are not only metabolically active, but are also subject to deterioration through fungal and insect attacks. These are the seeds that cannot be stored for long periods of time and that pose great difficulties for seed buyers and sellers.

Attempts to improve storage longevity of recalcitrant seeds have produced mixed results. Generally, enhanced storage is the result of technical manipulation. For instance, Withers and King (1980) used 2-step freezing to enhance storage of cell cultures. Some researchers have successfully preserved embryonic axes and somatic and zygotic embryos (Shibli and Al-Juboory 2000; Towill and Bajaj 2002; Fang and others 2004). Dereuddre and others (1991) and Shibli and Al-Juboory (2000) used encapsulation-dehydration and Mycock and others (1995) used rapid cooling in storing somatic embryos. Flash-drying (Berjak and others 1989; Wesley-Smith and others 1992) and vitrification (Touchell and others 2002) have also been utilized. Other researchers have cryopreserved shoot tips, buds, and apical meristems (Towill and others 2004). In general, these are labor-intensive processes, and primarily used to store germplasm of valuable agricultural species, fruit trees, or economically important forest tree species.

While such studies hold promise for future advancements and provide avenues of preservation for threatened germplasm, our studies focused on storing entire seeds rather than seed tissues. Here, we examined seed storage at different temperatures and MCs and how our laboratory desiccation experiments compared to field longevity. The objectives of these studies were: (1) to study storage of cherrybark oak (*Quercus pagoda*) and water oak (*Q. nigra*) acorns at two temperatures and two MCs; (2) to determine changes in MC and germination in acorns left exposed to natural conditions and track the percentage of collected acorns that had insect damage through the shedding season; and (3) to give a brief overview of changes that occurred in the sucrose content of drying white oak (*Q. alba*) acorns.

Methods

Experiment 1

Acorns of cherrybark oak and water oak were collected and soaked overnight in tap water to ensure full hydration. Floaters were discarded. Half of the acorns were stored fully hydrated and the others half dried on a lab bench for 48 hours prior to storage. Acorns were stored in 4-mil polyethylene bags in lots of 110 to 120 acorns. Storage was at either 4 °C (39 °F) in a Lab-Line Ambi-Hi-Low Chamber (Lab-Line Instruments Incorporated, Melrose Park, IL) or at -2 °C (28 °F) in a modified chest freezer. Original germination percentages and MCs were determined and then tested at yearly intervals for up to 3 years. For germination tests, acorns were cut in half horizontally and the seed coat removed from the half containing the embryo. These sections were placed, cut side down, on moist Kimpac® and put in a Stultz germinator (Stultz Scientific Engineering Corporation, Springfield, IL) set at 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). Acorns were germinated as two replications of 50 seeds per sampling period and were soaked overnight in tap water prior to germination testing. Counts were conducted weekly for 4 weeks. MCs of these fresh acorns were determined by drying two to four samples at 105 °C (221 °F) for 16 to 17 hours (ISTA 1993).

Experiment 2

Two large, open-grown white oak trees, less than 100 m (330 ft) apart, were selected for this study at Starkville, MS. Acorn collections began when the daily number of acorns shed reached at least 500, and ended when acorn fall dropped below 500. Collected acorns were marked with Uni-Paint® PX-21 Opaque oil-base paint-marking pens (Mitsubishi Pencil Company, Tokyo, Japan), a different color or color combination for each day of collection. Remaining acorns were raked aside so that only freshly shed acorns were collected each day. Marked acorns were placed under the canopy of a non-oak tree. On the last day of collection, all marked acorns were brought to the lab. Germination tests and MC determinations were performed as in experiment 1. Germination was tallied weekly for up to 4 weeks on 6 replications of 50 acorns from each day's collection. The number of acorns that germinated while acorns were still in the field was also recorded. MC was measured using five replications of three acorns each. Insect damage was recorded for each acorn cut open and used in the above two measurements and for all other acorns collected. Damage was recorded as either present or absent. Minimum and maximum temperatures, relative humidity, and rainfall data were obtained from the Mississippi State University weather site for the length of the collection period.

Experiment 3

We collected 2,000 white oak acorns, soaked them overnight in tap water, and spread them evenly on four trays lined with blotter paper. Acorns were divided into two subsets, wet and dry. The wet subset consisted of two trays of acorns that were covered with Kimpac®, kept at room temperature, and

moistened throughout the 9-day experiment. The remaining two trays of acorns that were kept at room temperature and allowed to dehydrate were the dry subset. At intervals of 0, 1, 3, 5, 7, and 9 days, acorns were randomly selected from each subset and tested for germination, MC (as in experiment 1), and sucrose content via gas chromatography.

Sugars were extracted for the gas chromatography analyses as follows: at each sampling time, embryonic axes of white oak with immediately adjacent cotyledon tissue were dissected from the surrounding cotyledon tissue of 50 wet and 50 dry acorns; tissues were immediately frozen in liquid nitrogen and freeze-dried. The freeze-dried cotyledons were finely 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 (0.3 oz) of an 80% ethanol solution and heated in a water bath at 75 °C (167 °F) for 1 hour. The sample was then filtered, rinsed with more of the ethanol solution, and rotoevaporated to dryness. The evaporation flask was rinsed with more of the ethanol solution and rotoevaporated to dryness. The evaporation flask was rinsed with 10 ml (0.3 oz) 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. The dried sample was dissolved in 1 ml of trimethylsilylimidazole, heated in a water bath at 75 °C (167 °F) for 30 minutes, blown to dryness, and then redissolved in 1 ml chloroform and stored until analysis. Analyses were performed on an HP® 5890 gas chromatograph using a Supelco® SPB-5 capillary column (30 m x 0.25 mm ID x 0.25 film thickness). A minimum of three extractions were made from each cotyledon sample, and one or more from the embryonic axis sample, depending on amount of tissue available. Sucrose was identified by comparing it with a standard of the pure sugar prepared in a similar manner and injected onto the gas chromatograph.

Results

Experiment 1

Initial MC for water oak acorns was 30.5% and, after drying for 48 hours on the lab bench, 25.6%. Germination of the fresh acorns was 93%, while germination of acorns that were dried for 48 hours dropped to 84 % (fig. 1A). After 1 year of storage, we found that the temperature at which acorns were stored was significant, but acorn MC was not. Seeds stored at -2 °C (28 °F) had higher viability than those stored at 4 °C (39 °F). However, after 2 years of storage, the situation was reversed; MC of the stored acorns was significant, not temperature of storage. Acorns that had been dried prior to storage had lower viability than those that had not been dried; this pattern continued into year 3. Germination of dried acorns dropped to 25% or less by year 2, while acorns that were fully hydrated before storage germinated at about 50%. By year 3, hydrated acorns were still maintaining about 25% germination, while those dried before storage were dead.

Drying reduced the MC of cherrybark oak acorns by about 10%, from 29.6% to 19.9%, but had little effect on initial acorn viability, reducing it only 2%, from 100% to 98% (fig. 1B). Unlike water oak, temperature of storage was not significant.

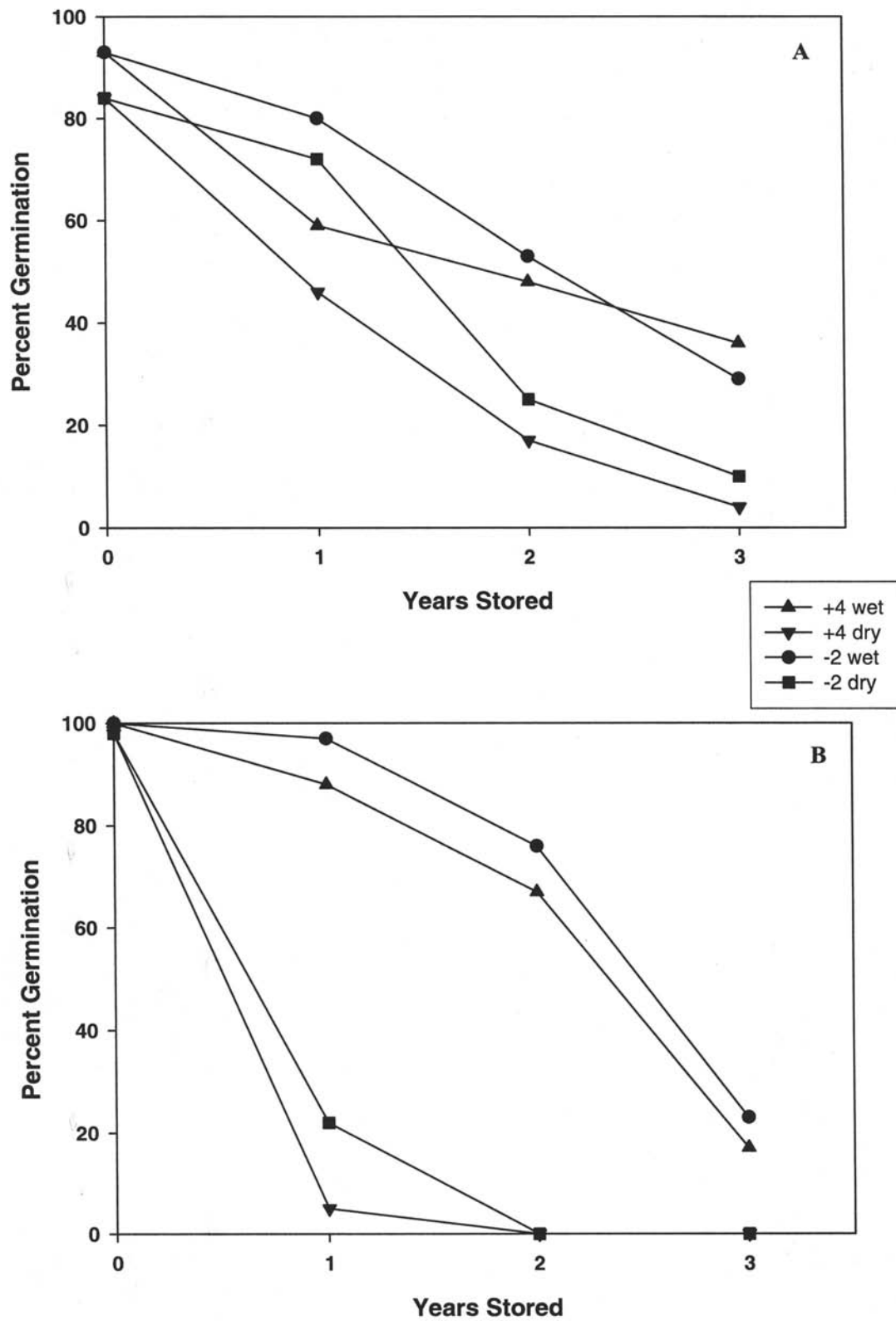


Figure 1. Germination percentage of water oak (A) and cherrybark oak (B) acorns stored at two different temperatures and two different moisture contents for 3 years.

MC, however, was highly significant, and acorns stored fully hydrated for 1 year retained viability over 88%, while germination of cherrybark oak acorns dried before storage dropped to 22% or less. After 2 years, germination of stored hydrated acorns remained a respectable 67% and 76% for acorns stored at 4 and 2 °C (39 and 36 °F), respectively.

Experiment 2

Acorn fall on Tree 1 began 7 days earlier than on Tree 2; the experiment on Tree 1 ran from 12 October through 26 October; the experiment on Tree 2 ran from 19 October through 1 November. Unlike the laboratory experiments, germination tests on acorns left in the field gave variable results, and no distinct pattern of decreasing viability was observed. Although some acorns remained in the field for 11 days, acorn MCs remained relatively high, never dropping below 37% on Tree 1 and 35% on Tree 2. In our laboratory experiments, white oak acorns dried to 22% MC in only 9 days.

The moderating effects of the rainfall that occurred throughout the experiment helped keep MC high in the acorns from both trees. Each tree’s collection period had 8 days where some precipitation occurred. The higher

germination percentage results from the field-collected acorns are probably a result of this higher MC.

We believe that the random declines in acorn viability we observed are more a reflection of problems in the germination cabinets than of physiological changes. The number of acorns being tested filled every tray in the four germination cabinets used for this experiment. We experienced some difficulty with moisture accumulation on the trays and resulting mold growth. It is our opinion that germination would have been uniformly high if the moisture wicking problems could have been controlled and mold growth reduced.

Although the two trees were separated by no more than 100 m (330 ft), the insect damage on Tree 1 was significantly higher than that on Tree 2 (fig. 2). On average, 66% of the acorns collected from Tree 1 in the first 4 days of the experiment were damaged, and this percentage did not drop below 40% throughout the experiment. Damage was much lower on Tree 2, averaging 19% over the first 4 days. Damage on this tree also peaked early in the experiment, supporting the claim that damaged acorns are the first to fall. Because we did not begin collecting acorns until 500 per day were available, damage may very well have been even higher in the early drop.

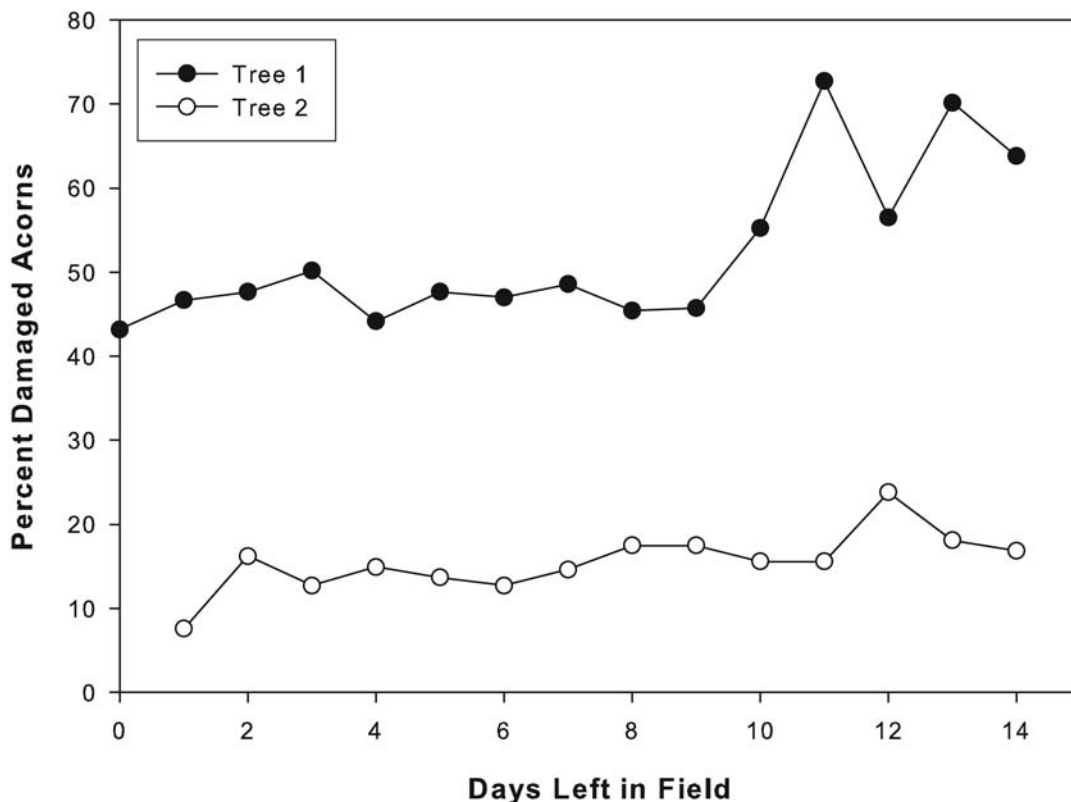


Figure 2. Percentage of insect-damaged acorns collected from trees 1 and 2. Data points to the left of the graph represent acorns that remained on the ground the least amount of time (shed last) before being brought into the laboratory for analyses.

Experiment 3

Initial acorn germination for the freshly collected white oak acorns was 80% (fig. 3). Drying acorns were dead after 9 days on the laboratory bench, while wet acorns had over 90% germination throughout the experiment. MC in the wet acorns remained high and fairly constant; moisture in the drying acorns fell from 44.6% to 22.2%.

Sucrose concentrations of the dry acorn embryonic axes and cotyledons were higher than those of the wet acorns throughout the experiment. Differences became significantly higher on day 5 of the experiment and remained so thereafter. Sucrose concentrations of the embryonic axes and cotyledon tissue were similar at the start of the experiment, but sucrose of the embryonic axes became significantly higher than that of the cotyledon tissue in both wet and dry acorns as the experiment progressed.

Discussion

Collecting and handling protocols for acorns are critical. If acorns are not collected for immediate use and MC falls before storing, seed quality can be negatively affected. Cherrybark and water oak acorns retained high viability for 2 years if stored fully hydrated (fig. 1). However, while the initial 48-hour drying period before storage reduced germination of water oak and cherrybark oak acorns only by 9% and 2%, respectively, dried water oak acorns stored

at 4 °C (39 °F) and dried cherrybark acorns stored at either temperature had significant losses in viability after 1 year. After 2 years, germination of water oak acorns dried before storing was 25% or less, and dried cherrybark oak acorns were dead. Although losses of acorns stored fully hydrated to insects and fungi may be significant, drying acorns before storing results in critical quality reduction.

Interesting differences were observed between the two white oak trees used in experiment 2. Acorn shed began a full week earlier on Tree 1 than on Tree 2. Because the two trees are separated by less than 100 m (330 ft), and thus exposed to the same meteorological conditions, we attribute this to either significant morphological differences between the two trees in flowering times and acorn development, or to the high incidence of bug damage on Tree 1 (fig. 2).

MC was high in acorns from both trees throughout the experiment, primarily due to the amount of rainfall that occurred over the entire collection period, keeping the ground moist and relative humidity high. We believe because of this, and mild temperatures throughout the collection period, acorn germination also remained high. We still suggest that time and frequency of acorn collections should reflect weather conditions throughout the collection period. If temperatures are hot and drought conditions prevail, frequent collections should be the order of the day. While the results from this experiment have provided some interesting information, the tests obviously need to be repeated before definite conclusions can be drawn.

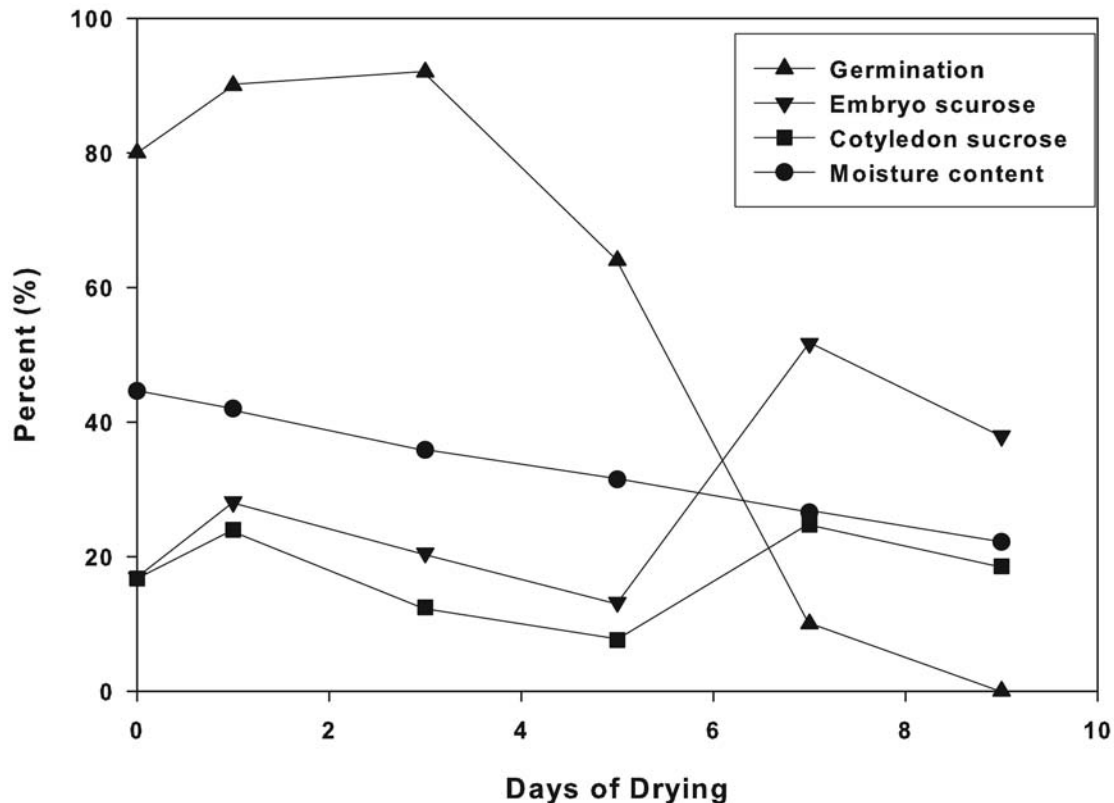


Figure 3. Germination, moisture content, and sucrose content of white oak embryonic axis and cotyledon tissues. Half of the acorns were allowed to dry out, while the remaining half were kept hydrated (wet) throughout the 9-day experiment.

We found a clear effect of desiccation on the sucrose contents of the embryonic axes and cotyledons in white oak acorns. The dry acorns overall had higher sucrose contents than the wet acorns throughout the experiment (fig. 3). Additionally, sucrose content in both the wet and dry embryonic axes was always higher than that of the cotyledon tissue. We suggest that, while this increase did not prevent loss of acorn viability, it did serve to initially protect against cellular collapse in the acorn tissues. Li and Sun (1999) reported that desiccation sensitivity of *Theobroma cacao* embryonic axes might be due to a decrease of enzymatic protection against oxidative stresses rather than a lack of sugar-related protective mechanisms during desiccation. They did not, however, find significant shifts in carbohydrate content during desiccation of axes.

Electron micrographs taken of desiccating embryonic axis and cotyledon tissue of white oak acorns (Connor and others 1996) show that cell membranes of the embryo and cotyledon tissues remain intact despite the stress imposed by lowering moisture contents. However, while the combination of high sucrose content and high axis moisture content may protect membranes in white oak acorn tissues, the mechanism is obviously not successful in preserving viability, which dropped rapidly after day 5 of the experiment.

References

- Berjak P, Farrant JM, Mycock DJ, Pammenter NW. 1989. Homiohydrous (recalcitrant) seeds: the enigma of their desiccation sensitivity and the state of water in axes of *Landolphia kirkii* Dyer. *Planta* 186:249-261.
- Bonner FT, Vozzo JA. 1987. Seed biology and technology of *Quercus*. New Orleans (LA): USDA Forest Service, Southern Forest Experiment Station. General Technical Report SO-66. 21 p.
- Connor KF, Bonner FT, Vozzo JA. 1996. Effects of desiccation on temperate recalcitrant seeds: differential scanning calorimetry, gas chromatography, electron microscopy, and moisture studies on *Quercus nigra* and *Quercus alba*. *Canadian Journal of Forest Research* 26:1813-1820.
- Dereuddre J, Hassen M, Blandin S, Kaminski M. 1991. Resistance of alginate-coated somatic embryos of carrot (*Daucus carota* L.) to desiccation and freezing in liquid nitrogen: 2. thermal analysis. *CryoLetters* 12:135-148.
- Ellis RH, Hong TD, Roberts EH. 1990. An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany* 41:1167-1174.
- Fang J-Y, Wetten A, Hadley P. 2004. Cryopreservation of cocoa (*Theobroma cacao* L.) somatic embryos for long-term germplasm storage. *Plant Science* 166:669-675.
- [ISTA] International Seed Testing Association. 1993. International rules for seed testing. *Seed Science and Technology* 21 (supplement, rules). 258 p.
- Li C, Sun WQ. 1999. Desiccation sensitivity and activities of free-radical scavenging enzymes in recalcitrant *Theobroma cacao* seeds. *Seed Science Research* 9:209-217.
- Mycock D, Wesley-Smith J, Berjak P. 1995. Cryopreservation of somatic embryos of 4 species with and without cryoprotectant pretreatment. *Annals of Botany* 75:331-336.
- Roberts EH. 1973. Predicting the storage life of seeds. *Seed Science and Technology* 1:499-514.
- Shibli RA, Al-Juboory KH. 2000. Cryopreservation of 'nabali' olive (*Olea europea* L.) somatic embryos by encapsulation-dehydration and encapsulation-vitrification. *CryoLetters* 21(6):357-366.
- Touchell DH, Chiang VL, Tsai C-J. 2002. Cryopreservation of embryogenic cultures of *Picea mariana* (black spruce) using vitrification. *Plant Cell Reports* 21:118-124.
- Towill LE, Bajaj YPS, editors. 2002. Cryopreservation of plant germplasm II. *Biotechnology in agriculture and forestry series, volume 50*. London (United Kingdom): Springer. 390 p.
- Towill LE, Forsline P, Walters C, Waddell J, Laufmann J. 2004. Cryopreservation of *Malus* germplasm using a winter vegetative bud method: results from 1915 accessions. *CryoLetters* 25:323-334.
- Wesley-Smith J, Vertucci CW, Berjak P, Pammenter NW, Crane J. 1992. Cryopreservation of desiccation-sensitive axes of *Camellia sinensis* in relation to dehydration, freezing rate and the thermal properties of tissue water. *Journal of Plant Physiology* 140:596-604.
- Withers LA, King PJ. 1980. A simple freezing unit and routine cryopreservation method for plant cell cultures. *CryoLetters* 1:213-220.