HOW DOES PROLONGED EXPOSURE TO NATURAL CONDITIONS AFFECT ACORN MOISTURE AND VIABILITY?

Kristina Connor, Jillian Donahoo, and Gretchen Schafer[1]

Abstract—After placing acorns on a lab bench to dry, we saw changes in moisture content, germination, and seed biochemistry in as few as 3 days. As drying progressed, we found that irrevocable damage to membrane lipids and protein secondary structure occurred in just 5 days. However, we did not know if these experiments mimic what happens to acorns in the field. We cleared an area beneath two open-grown white oak (*Quercus alba* L.) trees and collected acorns after they were shed. Acorns found each morning under the trees were marked with spots of paint, different colors for each day. Five hundred acorns were marked each day, and the remaining acorns were raked from the site. The experiment continued until acorns shed from the trees dropped below 500. We found that, unlike the laboratory experiment, acorn germination showed no distinct pattern of decreasing viability. Additionally, moisture content of the acorns from both trees remained relatively high, never dropping below 35 percent. The high moisture content was no doubt in some part due to the 8 days of precipitation that occurred during each tree's collection period. We also confirmed that insect damage was more prevalent on acorns first shed from the trees.

INTRODUCTION

Desiccation-resistant seeds can be dried without damage to a moisture content (MC) of ≤ 12 percent and stored for long periods of time, while the viability of recalcitrant, or desiccation-sensitive, seeds is affected by moisture loss and/or cold temperatures (Roberts 1973). This susceptibility to drying makes any period of storage for some temperate 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). 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, Golovina and others 1997, 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).

In earlier laboratory experiments on temperate recalcitrant seeds (Connor and Sowa 2003, 2004; Sowa and Connor 2003), we found that marked changes in seed biochemistry can occur in just 3 days, and irrevocable damage to membrane lipids and protein secondary structure can occur in just 5 days. In these laboratory experiments, acorns were spread on lab benches and left to dry at room temperature and ambient relative humidity. Moisture was completely excluded until acorns were rehydrated prior to germination testing. While these experiments have provided much information on biochemical changes that occur during desiccation, we do not know if they reflect what happens to acorns in the field. The objectives of this experiment were to determine what changes in MC and germination occurred in acorns left exposed to natural conditions. We also evaluated the percentage of collected acorns that had insect damage.

MATERIALS AND METHODS

Acorn collections began when the daily number of acorns shed reached at least 500 under each of the two open-grown white oak (*Q. alba* L.) study trees near Starkville, MS. The collected acorns were marked with Uni-Paint® PX-21 Opaque oil-base paint-marking pens (Mitsubichi Pencil Company), a different color or color combination for each day. Remaining acorns were raked aside so that only freshly shed acorns were collected each day. Marked acorns were transported to an area with few predators and placed under the canopy of a (non-oak) tree. The experiment on each tree ended when acorn fall dropped below 500 acorns per day. On the last day of collection (October 26, 2004, for tree one and October 31, 2004, for tree two), all marked acorns were brought to the lab. The following experiments were performed on each day's collection of acorns:

1. Germination tests: White oak acorns were soaked overnight in tap water prior to viability testing. The end of the acorn with the cup scar was cut off and discarded. The acorns were then placed cut-side-down on moist Kimpac® under a diurnal cycle of 20 °C for 16 hours in the dark and 30 °C for 8 hours with light. 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.
2. Moisture content: Whole acorns chopped into pieces were used to determine MC. Samples were oven dried at 103 °C for 17 ± 1 hours (Bonner 1981, International Seed Testing Association 1993). Measurements were made on five replications of three acorns each.
3. Damage: Bug 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.
4. Weather information: Minimum and maximum temperatures, relative humidity, and rainfall data were obtained from the Mississippi State University weather site.

[1] Project Leader, USDA Forest Service, Southern Research Station, Auburn, AL 36842; and Technicians, USDA Forest Service, Southern Research Station, Mississippi State, MS 39762, respectively.

Citation for proceedings: Connor, Kristina F., ed. 2006. Proceedings of the 13th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS–92. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 640 p.

RESULTS AND DISCUSSION

Acorn Germination and MC: Laboratory Experiments

White oak acorns, among the most recalcitrant of the Fagaceae, are high in carbohydrates and, when freshly collected, have a higher MC than acorns that have a high lipid content such as water oak (*Q. nigra* L.) or cherrybark oak (*Q. pagoda* Raf.) (Connor and Sowa 2004, Connor and others 1996). In laboratory experiments, where moisture was excluded, viability dropped below 50 percent when MC reached 27 percent (fig. 1). This happened, on average, in just 5 to 6 days. Additionally, biochemical and physiological changes were observed in as few as 3 to 5 days in these artificially dried acorns (Connor 2004, Connor and Sowa 2003, Sowa and Connor 2003).

Acorn Field Experiments

Acorn shed—The two trees used in this experiment were large, open-grown trees no more than 100 m apart. Both had a bumper crop of acorns. The experiment spanned 21 days and began when acorn drop reached 500 acorns per day. The experiment on tree 1 ran from October 12 through October 26; the experiment on tree 2 ran from October 19 through November 1.

Germination and MC—Unlike in the laboratory experiments, germination tests on acorns left in the field gave variable results and no distinct pattern of decreasing viability (fig. 2). Additionally, MC of the acorns from both trees remained relatively high, never dropping below 37 percent on tree 1 and 35 percent on tree 2 (fig. 3). This is well above the MC obtained in laboratory drying experiments and, for the most part, the higher percent germination results reflect this. In the laboratory experiments, germination did not drop below 50 percent until moisture fell to 27 percent. The results from the field experiment are no doubt attributable to the moderating effects of the rainfall that occurred throughout the experiment. Although three times as much rainfall occurred over the collection period for tree 1 (October 12 through October 26, 2004) than for tree 2 (October 19 through October 31, 2004) (figs. 4 and 5), MC remained high in acorns from both trees. This was not surprising, because during the collection period some precipitation occurred on 8 days at both trees, and although relative humidity did drop as low as 34 percent during the day, the maximum daily relative humidity never fell below 90 percent (fig. 6).

We believe that the random declines in acorn viability we observed are more a reflection of problems in the germination cabinets we used than of physiological changes. The number of acorns we tested filled every tray in the four germination cabinets. This restricted light penetration to some areas of the trays. Also, moisture accumulated on trays, resulting in mold growth. We think germination would have been uniformly high if moisture-wicking problems were controlled and mold growth reduced.

Insect Damage—Although the two trees were within 100 m of one another, insect damage on tree 1 was significantly higher than on tree 2 (fig. 7). On average, 66 percent of the

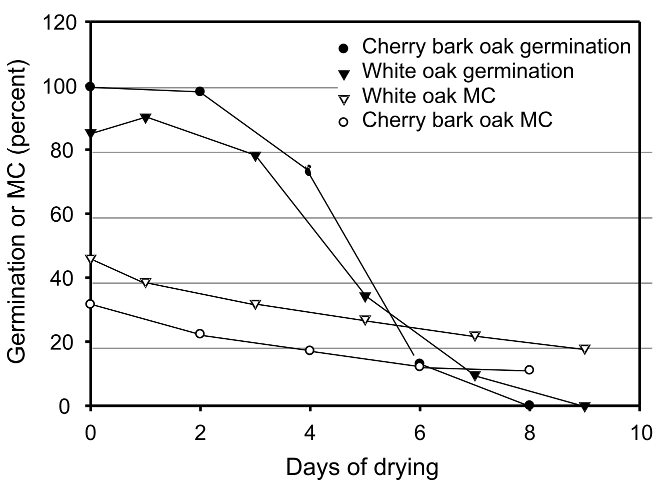

Figure 1—Germination and moisture content (MC) of white oak and cherrybark oak acorns desiccated in the laboratory over 8 to 10 days. From Connor and Sowa (2004).

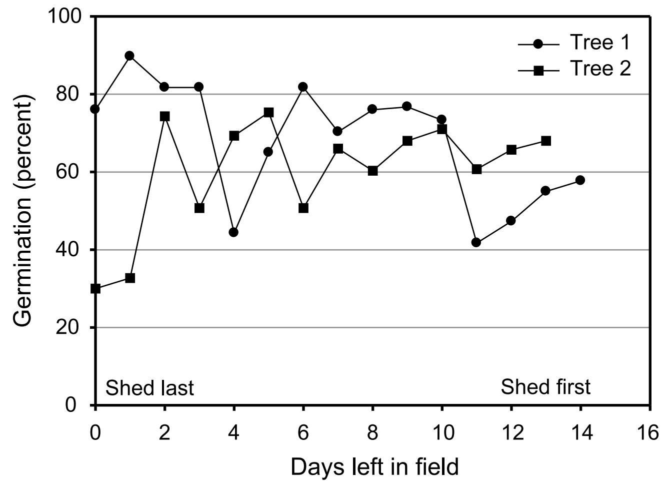

Figure 2—Germination of acorns from field experiment trees 1 and 2. Data points to the left of the graph represent acorns that remained on the ground the least amount of time (shed last) before being brought into the laboratory for analyses.

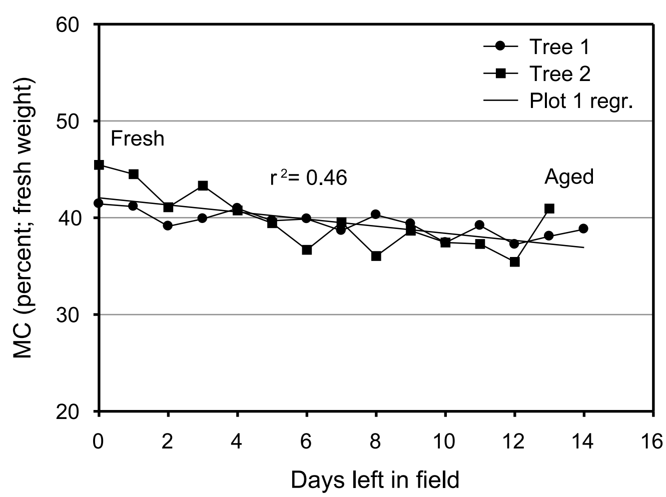

Figure 3—Moisture content (MC) (fresh-weight basis) of acorns collected from trees 1 and 2. Data points to the left of the graph represent acorns that remained on the ground the least amount of time (shed last) before being brought into the laboratory for analyses.

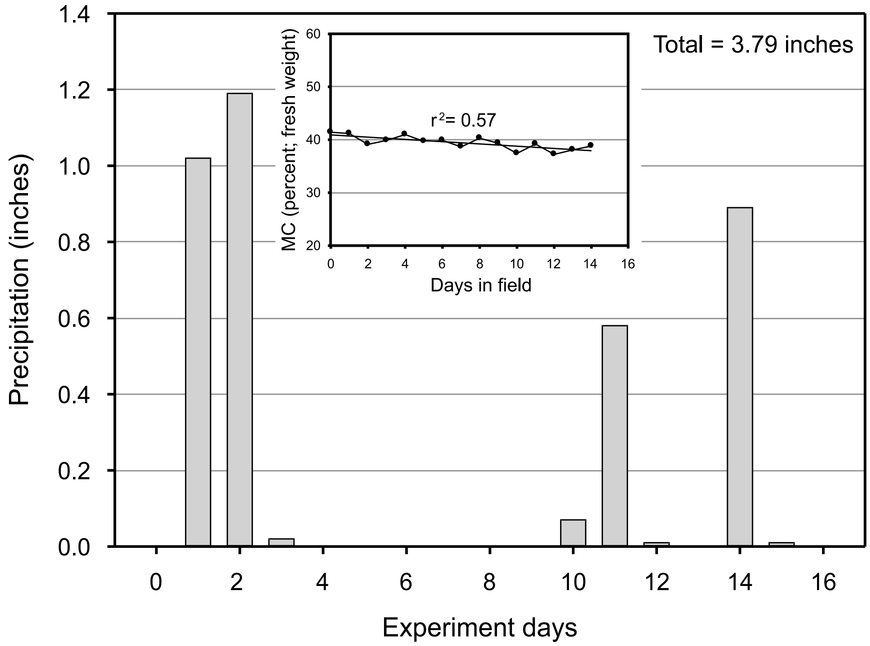

Figure 4—Precipitation during the acorn collection period for tree 1. MC = moisture content.

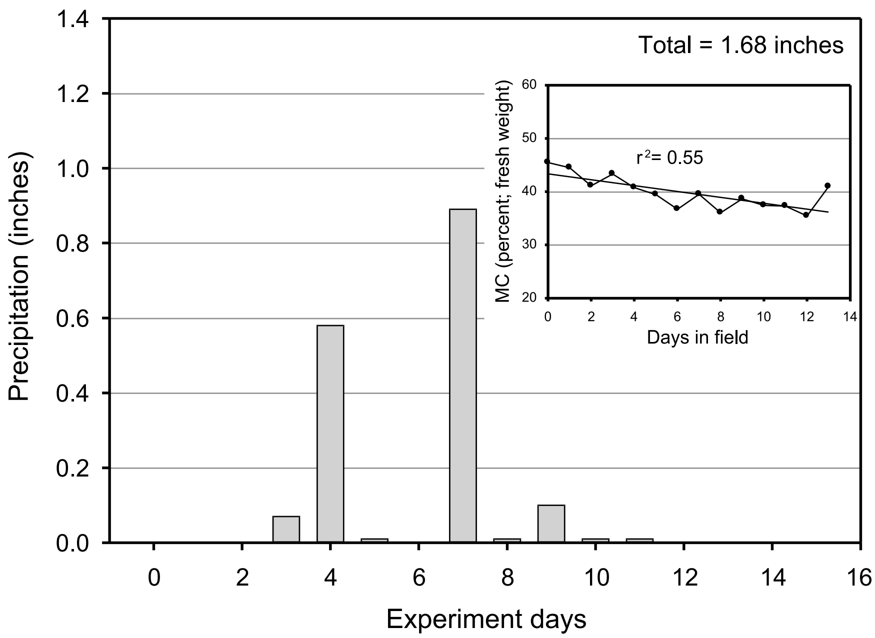

Figure 5—Precipitation during the acorn collection period for tree 2. MC = moisture content.

acorns collected from tree 1 in the first 4 days of the experiment were damaged, and this percentage did not drop below 40 percent for the duration of the experiment. Damage was much lower on tree 2, averaging 19 percent 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.

SUMMARY

We observed some interesting differences between the two *Q. alba* trees used in this experiment. Acorn shed began a full week earlier on tree 1 than on tree 2, despite the fact that they were < 100 m apart and exposed to the same meteorological conditions. This must signify significant morphological differences between the two trees in flowering times and acorn development. The high incidence of insect damage on tree 1 compared to tree 2 was surprising—again because of their proximity, but also because we have often collected acorns from tree 1 for experiments in good acorn production years and have never noted such a high percentage of damage.

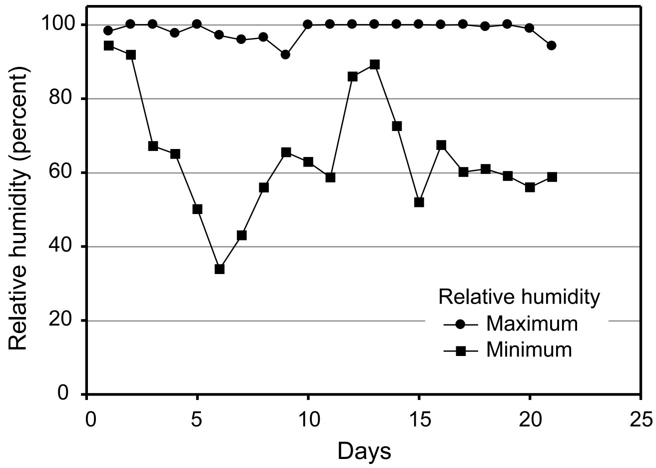

Figure 6—Maximum and minimum relative humidity data for the acorn field experiment.

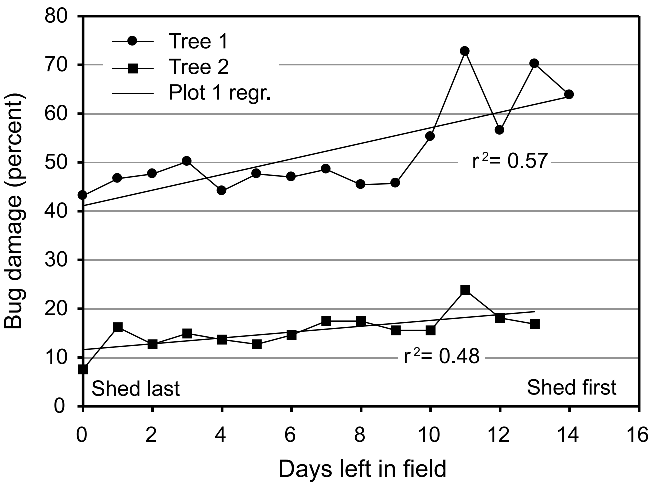

Figure 7—Percent of insect-damaged acorns collected from trees 1 and 2. Data points to the left of the graph represent acorns that remained on the ground the least amount of time (shed last) before being brought into the laboratory for analyses.

Moisture content remained fairly high in acorns from both trees, primarily, we believe, due to the amount of rainfall occurring during the experiment. These rainfall events also occurred over the entire collection period, thus keeping the ground moist and relative humidity high.

We believe that the high acorn germination in this experiment reflects the mild temperatures and significant rainfall that occurred during acorn drop, and also that care should be taken to make frequent collections during years when high temperature and low rainfall occur during acorn drop. While the results from this experiment have provided some interesting information, such tests obviously should be repeated before drawing any definite conclusions.

ACKNOWLEDGMENT

We thank Michael Brown, Department of Geosciences, Mississippi State University, for all weather information used in this experiment.

LITERATURE CITED

Bochicchio, A.; Vernieri, P.; Puliga, S. [and others]. 1997. 2. Desiccation tolerance in immature embryos of maize: sucrose, raffinose, and the ABA-sucrose relation. In: Ellis, R.H.; Black, M.; Murdoch, A.J.; Hong, T.D., eds. Basic and applied aspects of seed biology. Proceedings of the fifth international workshop on seeds; Reading, 1995. London: Kluwer Academic Publishers: 13-22.

Bonner, F.T. 1981. Measurement and management of tree seed moisture. Res. Pap. S0-177. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Experiment Station. 11 p.

Connor, K.F. 2004. An update on oak seed quality research: hardwood recalcitrant seeds. In: National proceedings of the forestry and conservation nursery associations 2003. RMRS-P-33. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 111-116.

Connor, K.F.; Bonner, F.T. 1999. Effects of temperature and moisture content on the storability of hardwood seeds. In: Haywood, J.D., ed. Proceedings of the 10th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-30. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 123-126.

Connor, K.F.; Bonner, F.T.; Vozzo, J.A. 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: 1813-1821.

Connor, K.F.; Sowa, S. 2003. Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. Tree Physiology. 23: 1147-1152.

Connor, K.F.; Sowa, S. 2004. The physiology and biochemistry of desiccating white oak and cherrybark oak acorns. In: Connor, K.F., ed. Proceedings of the 12th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-71. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 473-477.

Farrant, J.M.; Berjak, P.; Pammenter, N.W. 1985. The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*. South African Journal of Botany. 51: 432-438.

Farrant, J.M.; Pammenter, N.W.; Berjak, P. 1988. Recalcitrance – a current assessment. Seed Science and Technology. 16: 155-166.

Finch-Savage, W.E.; Pramanik, S.K.; Bewley, J.D. 1994. The expression of dehydrin proteins in desiccation-sensitive (recalcitrant) seeds of temperate trees. Planta. 193: 478-485.

Flood, R.G.; Sinclair, A. 1981. Fatty acid analysis of aged permeable and impermeable seeds of *Trifolium subterraneum* (subterranean clover). Seed Science and Technology. 9: 475-477.

Greggains, V.; Finch-Savage, W.E.; Quick, W.P.; Atherton, N.M. 2000. Putative desiccation tolerance mechanisms in orthodox and recalcitrant seeds of the genus *Acer.* Seed Science Research. 10: 317-327.

Golovina, E.A.; Wolkers, W.F.; Hoekstra, F.A. 1997. Behavior of membranes and proteins during natural seed aging. In: Ellis, R.H.; Black, M.; Murdoch, A.J.; Hong, T.D., eds. Basic and applied aspects of seed biology. Dordrecht: Kluwer Academic Publishers: 787-796.

International Seed Testing Association. 1993. International rules for seed testing. Seed Science and Technology. 21 [supplement, rules]. 258 p.

Pammenter, N.W.; Berjak, P.; Ross, G.; Smith, M.T. 1994. Why do hydrated, recalcitrant seeds die? Seed Science Research. 4: 187-191.

Pierce, R.S.; Abdel Samad, M. 1980. Changes in fatty acid content of polar lipids during ageing of peanut (*Arachis hypogea* L.). Journal of Experimental Botany. 41: 1549-1557.

Roberts, E.H. 1973. Predicting the storage life of seeds. Seed Science and Technology. 1: 499-514.

Seewaldt, V.; Priestly, D.A.; Leopold, A.C. [and others]. 1981. Membrane organization of soybean seeds during hydration. Planta. 152: 19-23.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. New Phytologist. 73: 377-420.

Sowa, S.; Connor, K.F. 2003. Recalcitrant behavior of cherrybark oak seed: an FT-IR study of desiccation sensitivity in *Quercus pagoda* Raf. acorns. Seed Technology. 25(2): 110-123.