# Effects of desiccation on temperate recalcitrant seeds: differential scanning calorimetry, gas chromatography, electron microscopy, and moisture studies on *Quercus nigra* and *Quercus alba*

**K.F. Connor, F.T. Bonner, and J.A. Vozzo**

**Abstract**: Investigations into the nature of desiccation-sensitive, or recalcitant, seed behavior have as yet failed to identify exact causes of this phenomenon. Experiments with *Quercus nigra* L. and *Quercus alba* L. were conducted to examine physiological and biochemical changes brought about by seed desiccation and to determine if there were predictable changes in seed moisture content, in enthalpy (heat content) of seed moisture, in the lipid fraction, or in seed ultrastructure as viability declined. *Quercus nigra* intact acorn moisture contents at 50% and 5% viability were 15% and less than 14%, respectively; those of intact *Q. alba* at 50% and 0% viability were much higher, 32% and 22%, respectively. Generally, it was found that as the seeds of both species dried, the moisture content of the axes remained high (26–27%), even after 9 days of drying. In *Q. nigra* acorns, there was little difference in average percent moisture lost per day among axes, proximal cotyledon tissue, and distal cotyledon tissue. *Quercus alba* acorns, however, lost moisture more rapidly from the axes than from the cotyledons. This was probably caused by the longitudinal splitting of the pericarp during the drying process. Lipids composed 28.4% of the dry weight of *Q. nigra* and 5.7% of *Q. alba* dry weight. Neither individual fatty acids nor total fatty acid content exhibited definite patterns of change over the course of the experiment. The most prevalent saturated fatty acid in both species was palmitic acid, and the most common unsaturated fatty acid was generally oleic acid. Electron microscopy studies of *Q. nigra* showed cell wall trauma after 3 days of drying (moisture content 23%); by day 7, when moisture content had dropped to 15.6%, there was a definite dissolution of cytoplasmic density and a reduction of spherosome concentration. *Quercus alba* exhibited similar responses to drying, but cell wall integrity was maintained. Differential scanning calorimetry studies revealed strong relationships between onset and enthalpy values of all acorn tissues and percent germination, as did regressions involving moisture content and seed germination.

**Résumé** : Les recherches sur la nature des graines sensibles à la dessiccation, ou sur le comportement des graines récalcitrantes, n'ont pas réussi jusqu'à maintenant à identifier la cause exacte de ce phénomène. Des expériences ont été réalisées avec *Quercus nigra* L. et *Quercus alba* L. afin d'étudier les changements physiologiques et biochimiques provoqués par la dessiccation de la graine et de déterminer s'il y avait des changements prévisibles dans le contenu en humidité de la graine, dans l'enthalpie (le contenu en énergie calorifique) de l'eau dans la graine, dans la fraction des lipides ou dans les caractéristiques ultrastructrales de la graine à mesure que la viabilité diminue. Le contenu en eau de glands intacts de *Q. nigra* avec un taux de viabilité de 50% et 5% était respectivement de 15% et de moins de 14%. Dans le cas de *Q. alba*, les glands avec un taux de viabilité de 50% et 0% avaient un contenu en eau beaucoup plus élevé, soit respectivement 32% et 22%. Généralement, à mesure que les graines des deux espèces séchaient, le contenu en eau des axes demeurait élevé (26–27%), même après 9 jours de séchage. Avec les glands de *Q. nigra*, il y avait peu de différence dans le pourcentage moyen de perte en eau entre les axes, le tissu proximal des cotylédons et le tissu distal des cotylédons. Les glands de *Q. alba*, cependant, ont perdu de l'humidité plus rapidement au niveau des axes que des cotylédons. Cela était probablement dû à la fissuration longitudinale du péricarpe durant le séchage. Les lipides constituaient respectivement 28,4% et 5,7% du poids sec chez *Q. nigra* et *Q. alba*. Ni le contenu en acides gras individuels ni le contenu total en acides gras n'ont exhibé de patrons définis de changement pendant la durée de l'expérience. L'acide gras saturé le plus important chez les deux espèces était l'acide palmitique et

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l'acide gras non saturé le plus fréquent était généralement l'acide oléique. Dans le cas de *Q. nigra*, les observations en microscopie électronique révélaient un traumatisme dans la paroi cellulaire après 3 jours de séchage (contenu en humidité de 23%); après 7 jours, alors que le contenu en eau était descendu à 15,6%, on pouvait définitivement observer une dissolution de la densité cytoplasmique et une réduction de la concentration des sphérosomes. La réponse au séchage était semblable pour *Q. alba* mais l'intégrité de la paroi cellulaire était maintenue. Le balayage par calorimétrie différentielle montrait une forte relation entre les valeurs d'enthalpie au début et le pourcentage de germination, de la même façon que les régressions qui impliquaient le contenu en eau et la germination des graines.

[Traduit par la Rédaction]

## Introduction

The majority of temperate tree seeds can be easily dried to a low moisture content (<10%) and stored at low temperatures; they are commonly called orthodox, or desiccation-resistant seeds. A few, however, are sensitive to moisture loss and low temperatures and are thus recalcitrant, or desiccation-sensitive seeds (Roberts 1973). These few, yet notable, exceptions include *Castanea* (Jaynes 1969; Pritchard and Manger 1990), *Aesculus*, some *Acer* species (Bonner 1990), and *Quercus* (Olson 1974).

The seeds of the genus *Quercus* have a wide range of storability. Acorns of the red oak subgenus (*Erythrobalanus*) can be stored for greater than one season, although viability loss can be high (Bonner and Vozzo 1987), while acorns of the white oak subgenus (*Leucobalanus*) germinate soon after falling from the tree and usually cannot be stored from season to season (Rink and Williams 1984).

Exact causes of recalcitrance remain unknown; speculation on changes in lipids, proteins, sugars, or bound water properties as seeds dry have all been explored, with varying degrees of success (Berjak et al. 1992; Crowe and Crowe 1986*a*, 1986*b*; Crowe et al. 1988; Farrant et al. 1985, 1988; Flood and Sinclair 1981; Priestly and Leopold 1979, 1983; Seewaldt et al. 1981; Vertucci 1992). However, the fact remains that, as yet, storage of temperate recalcitrant seeds from one growing season to the next is difficult. The objective of this study was to examine the temperate recalcitrant seeds of *Quercus* for physiological and biochemical changes brought about by desiccation, and relate them to loss of seed viability. In particular, we wanted to determine if changes in moisture content, enthalpy (heat content) of seed moisture, lipid composition, or ultrastructure of various acorn tissues that occurred when viability was lost could be related to the basis of recalcitrant behavior. We selected a red oak with a high lipid content, *Q. nigra* L. (water oak), and a white oak with a high carbohydrate content, *Q. alba* L. (white oak), for our experiments. Water oak can be stored at 4°C for up to 3 years before losing viability (Bonner and Vozzo 1987), while white oak will begin to germinate within a few months of harvesting while still under refrigeration.

## Materials and methods

Experiments were performed on 1991, 1992, and 1993 *Q. nigra* acorns and on 1993 and 1994 *Q. alba* acorns, which were collected locally after they had been shed. Before storage at 4°C, acorns were imbibed in tap water overnight; visibly damaged acorns and floaters were discarded. Earlier experiments determined that *Q. nigra* acorns varied greatly in size, and that very large and very small acorns dried and lost viability at different rates. Therefore, before the experiment began, acorns were screened and only those 13–15 mm in diameter were used. The smaller number of *Q. alba* acorns available made such discrimination impossible; however, acorn size was fairly uniform in the *Q. alba* collections, and both very small and very large acorns were discarded. Desiccation was carried out by spreading the acorns in a single layer on a laboratory bench at a room temperature of 25 ± 2°C and at a relative humidity of 45 ± 10%. Random samples of 210 *Q. nigra* and 125 *Q. alba* acorns were taken at selected intervals over a period ranging up to 12 days to span the condition of full viability to no viability. The following tests were performed.

### Germination

One hundred *Q. nigra* acorns were randomly selected at each sampling time for the germination test. They were germinated as two 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® and incubated under a diurnal cycle of 20°C for 16 h in the dark and 30°C for 8 h with light. 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. Germination tests of *Q. alba* acorns were similar in all respects but two: acorns were not cut in half, and only 50 acorns were tested at each sample period for germination (two replications of 25 seeds each). *Quercus alba* acorns were left intact because of pericarp splitting, cotyledon separation, and radicle emergence during the course of this experiment.

### Moisture content

Moisture contents of whole *Q. nigra* acorns were determined on five (1991) or six (1992) subsamples of five each. Moisture measurements were not taken in 1993 samples. For whole *Q. alba*, determinations were made on two (1993) and four (1994) subsamples of five acorns each. The randomly selected acorns were cut into halves and moisture was determined by procedures recommended for large seeds with high moisture contents (Bonner 1981; International Seed Testing Association 1993). Acorn pieces were dried in aluminum cans at 103 ± 2°C for 17 ± 1 h in a mechanical convection oven. Moisture contents were expressed as percentages of fresh weight.

Other samples of whole acorns were dissected to determine distribution of moisture within the acorns during desiccation. Tissue samples were weighed to the nearest 0.1 mg on an electronic balance and immediately immersed in 20 mL of anhydrous methanol (MEOH). Tissues analyzed were the embryonic axis, cotyledon proximal to the axis (PCOT, within 10 mm), cotyledon distal to the axis (DCOT, underneath the cup scar), and pericarp. From five to eight acorns were used for these measurements each sample day. Axis weights ranged from 5 to 20 mg in

*Q. nigra* and 15 to 50 mg in *Q. alba*, but most were in the 10- to 30-mg range. Cotyledon tissue samples ranged from 10 to 100 mg with most in the 30- to 40-mg range. Pericarp samples were proportionally larger (100–300 mg) because their moisture contents were lower than those of the other acorn tissues.

After an extraction period of 48 h, moisture was measured on aliquots of the MEOH by Karl Fischer analysis with an Aquastar V1B® automatic titrator. Moisture contents were expressed as percentages of the tissue fresh weight.

### Lipid extraction and gas chromatography analysis

Embryonic axes were removed from 75 *Q. nigra* and 50 *Q. alba* acorns at each sampling time. All but a small portion of cotyledon tissue was dissected from the embryo. The remaining cotyledon tissue was chopped and then, like the embryonic axes after dissection, immediately immersed in liquid nitrogen ($LN_2$). The axes were finely ground in a $LN_2$-cooled mortar and the cotyledons in a $LN_2$-cooled Wiley mill using a 20-mesh screen. All of the ground embryonic axis tissue and a sample of the ground cotyledons were stirred in hot isopropyl alcohol for 10 min (Fishwick and Wright 1977), filtered, and the alcohol was vacuum evaporated. The evaporation flask was rinsed with 2:1 chloroform ($CHCl_3$) – MEOH and the contents were added to the seed sample along with 3 mg of an internal standard, heptadecanoic acid. The samples were homogenized for 3 min in a blender and then filtered. The filtrate was measured volumetrically, washed, and purified in the manner of Folch et al. (1957) using one wash of 0.9% NaCl and two washes of a 1:1 solution of MEOH – 0.9% NaCl. Butylated hydroxytoluene (5 mg/L) was added as an antioxidant to all solvents used in the extractions (Pearce and Abdel Samad 1980). Lipids were esterified using 14% boron trifluoride in MEOH (premixed; Metcalfe and Schmitz 1961), dried, and redissolved in 0.5 mL $CHCl_3$. Samples from 1991 and 1992 were analyzed on a Hewlett Packard® 5880A gas chromatograph (GC) using a 4 mm × 2.44 m glass column packed with GP 3% SP2310 – 2% SP2300 on 100/120 Chromosorb WAW (Supelco®, Inc., Bellefonte, Pa.). Samples from 1993 and 1994 were analyzed on a Hewlett Packard 5890 GC equipped with a 30-m HP-5 capillary column, crosslinked with 5% phenylmethyl silicone. The initial temperature for the glass column was 170°C; this was held for 2 min, increased at 3°C/min to 191°C, at 4°C/min to 216°C (held for 4 min), and then increased to 240°C at 5°C/min and held for 20 min. Injector and detector temperatures were 250°C. The program for the capillary column was the same, but the final temperature increase to 240°C was omitted, and injector and detector temperatures were 230°C. Response factors were calculated from injections of low erucic rapeseed oil and AOCS oil reference mix No. 3 (Sigma Chemical Co., St. Louis, Mo.).

### Electron microscopy

Five seeds of 1991 *Q. nigra* and five of 1993 *Q. alba* were taken at random on days 0, 1, 3, 5, and 7 from the bulk collection of drying acorns. A 3-$mm^3$ plug was extracted from the embryonic axis proximal to the bract scars, and a 3 × 3 × 5 mm section of the cotyledon was dissected from the same acorn but distal to the bract scars. All sections were immediately placed in a 2.5% glutaraldehyde solution of pH 7.2 phosphate buffer.

Tissues were fixed in osmium tetroxide, dehydrated in acetone, embedded in Epon®, sliced at 0.5 μm, and stained with uranyl acetate (Vozzo and Song 1989; King 1981). Thick sections were then observed at 1.2 MeV using high-voltage transmission electron microscopy (HVEM) to determine the effects of desiccation on cell ultrastructure and membrane integrity.

**Table 1.** Germination (%) of *Q. nigra* and *Q. alba* acorns.

| Drying time (days) | *Q. nigra*[a] | | | *Q. alba*[b] | |
|---|---|---|---|---|---|
| | 1991 | 1992 | 1993 | 1993 | 1994 |
| Fresh | 90 | 97 | 94 | 79 | 90 |
| 1 | 96 | — | — | 86 | — |
| 2 | 97 | — | — | 90 | 92 |
| 3 | 93 | — | — | 84 | — |
| 4 | 89 | — | — | — | 68 |
| 5 | 83 | 68 | 71 | 74 | 68 |
| 6 | 46 | — | — | — | 32 |
| 7 | 52 | 49 | 48 | 36 | — |
| 8 | 48 | — | — | — | 14 |
| 9 | 21 | 15 | 19 | 38 | — |
| 10 | 23 | — | — | — | 0 |
| 11 | 13 | — | — | — | — |
| 12 | — | 4 | 5 | — | 0 |

**Note**: Each number represents the average germination of two replications.

[a]Fifty seeds per replication.

[b]Twenty-five seeds per replication.

### Differential scanning calorimetry (DSC)

Tissue samples from 1992 *Q. nigra* and 1993–1994 *Q. alba* acorns were collected at each sampling time and sealed into 50-μL aluminum pans. A Perkin-Elmer® DSC-7, calibrated using indium (melting point 156.6°C) and hexane (melting point −95.3°C) was used to analyze axes, PCOT, and DCOT tissue. Samples were cooled from 30°C to −150°C at 10°C/min, held at −150°C for 5 min, then warmed back to 30°C at the same rate. The onset temperature for melting water in the seed and the enthalpy value of the melt were determined for these dried acorns and also for acorns dried the requisite number of days and then rehydrated in water overnight. This latter procedure tested the efficacy of rehydrating the acorns prior to germination.

## Results and discussion

### Germination

We dried acorns of *Q. nigra* and *Q. alba* at room temperature over a period of days, and then examined them for changes in germination, moisture content, enthalpy of seed moisture, lipid composition, and ultrastructure. Samples were taken more frequently in the 1st year of the experiments since the effect of the drying conditions on percent germination was unknown. Thus, in 1991, *Q. nigra* samples were taken for 11 straight days, while in 1992 and 1993, samples were taken only from fresh acorns and from those dried for 5, 7, 9, and 12 days (Table 1). Initial germination was high in all years for both species. The rate of decline in viability was fairly uniform from year to year. While the majority of *Q. nigra* acorns remained intact, *Q. alba* acorn pericarps cracked and the radical emerged throughout the drying process. Still, viability was retained for at least 9 days in *Q. alba* and for at least 12 days in *Q. nigra*. A 50% reduction in viability was reached between day 5 and day 7 in *Q. alba* and at day 7 in *Q. nigra*.

**Fig. 1.** Dynamics of seed moisture for 1992 *Quercus nigra* intact acorns (○), pericarp (□), axes (●), and proximal (▽) and distal (▼) cotyledon tissue (on *x* axis, 1 = fresh acorn values).

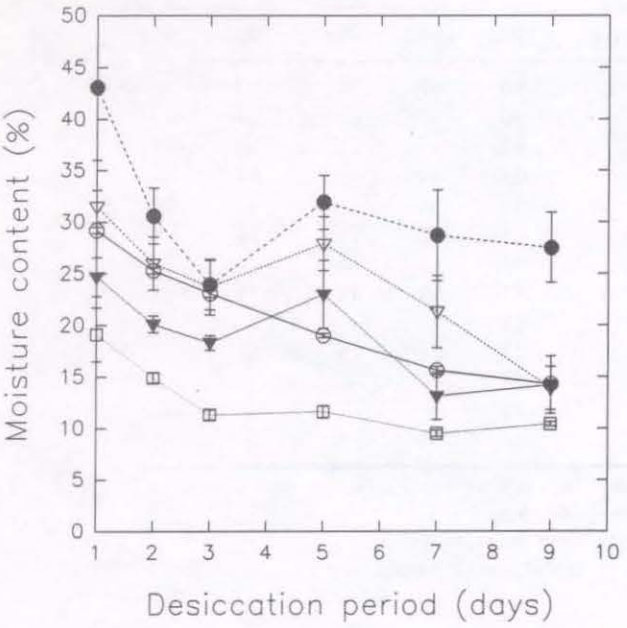
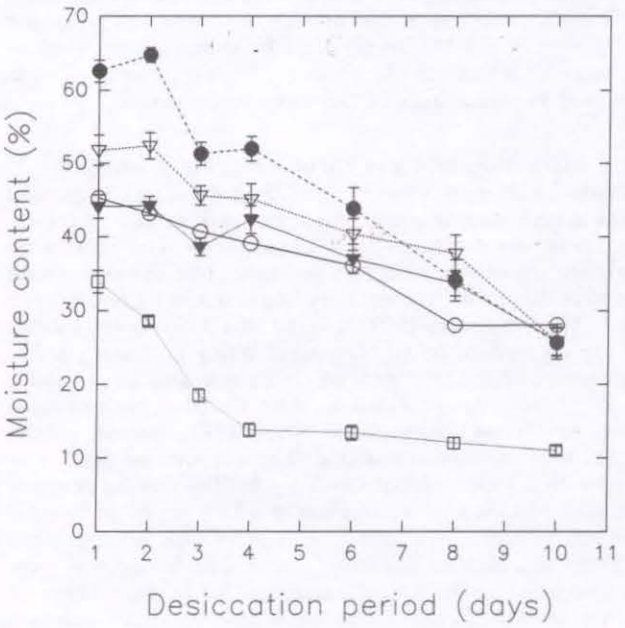



**Fig. 2.** Dynamics of seed moisture for 1993 *Quercus alba* intact acorns (○), pericarp (□), axes (●), and proximal (▽) and distal (▼) cotyledon tissue (on *x* axis, 1 = fresh acorn values).




**Moisture content**

Internal moisture dynamics of the desiccating acorns were similar in all years, so data are shown only for 1992 *Q. nigra* and 1993 *Q. alba* (Figs. 1 and 2). The pericarps rapidly lost moisture in both species, and minimum water contents were reached in 3–4 days. Intact acorns of *Q. nigra* began desiccation at an average moisture content just below 30% and fell to 14% at day 9 (Fig. 1). Loss of moisture was most rapid in all tissues between days 1 and 3, with the greatest loss from the axis (43–27%). Rates of loss then declined, and in the last 6 days, cotyledon tissues lost moisture at a slightly faster rate than the axis. Over the entire 9 days of drying, there was little difference in average percent moisture lost per day among axis, PCOT, and DCOT tissues. The high values on day 5 are the most notable variations and probably are the result of a random draw of larger acorns that day. Other tests have shown that large acorns dry much more slowly than small ones as long as the pericarps do not split. The most interesting data are those that show that the axes moisture contents are highest of all the tissues from the beginning to the end of the experiment. The lethal moisture content of intact *Q. nigra* acorns has been estimated to be around 15% (Agmata 1982). When these acorns reached that level at day 9, axis moisture content was still 27% (Fig. 1). During the final days of desiccation (days 7 and 9), rate of moisture loss from the axes slowed relative to that in cotyledons, as if the axes were sinks for internal moisture.

Moisture levels in acorns of *Q. alba* were much higher than those of *Q. nigra*, but the relationships of the tissues were similar (Fig. 2). The biggest difference between *Q. alba* and *Q. nigra* was that moisture loss from axes of *Q. alba* was faster than that from the cotyledons, and all three tissues were equal at the end of desiccation (26%). Rapid drying of axes probably occurred because practically all *Q. alba* pericarps were split by radicle emergence during desiccation, thus exposing the axes directly to the dry atmosphere. This condition rarely occurred in the more dormant acorns of *Q. nigra*. Thus, in *Q. alba*, axis moisture would be higher than intact acorn moisture at full imbibition or early in desiccation; but, when the pericarp split, axis moisture content would be similar to that of intact acorns as the lethal moisture level was approached. Since there was still 38% germination at day 9 for *Q. alba*, a good estimate of the lethal moisture content was not possible in 1993. In the 1994 test, viability was lost when axes moisture fell to 28–30%; intact acorn moisture was 21–24% at this point. These results suggest that the lethal moisture contents of axes were about the same for both species, but that the corresponding intact acorn figures were higher for *Q. alba*.

**Lipid analyses**

Respiration data for *Q. nigra* acorns indicate that the primary metabolic substrate is lipid (Bonner and Vozzo 1987). Results from our petroleum ether soxlet extractions found that lipids comprise 0.284 g/g dry weight of *Q. nigra* acorns and 0.057 g/g dry weight of *Q. alba* acorns. Conversion of 3 years of *Q. nigra* and 2 years of *Q. alba* lipids to fatty acid methyl esters (FAMEs) for GC analysis yielded similar results each year for each species, so data are shown for 1993 cotyldeon tissue only. In *Q. nigra* we found, on the average, 53.0 mg FAMEs/g cotyledon tissue (Table 2) and 52.8 mg FAMEs/g embryonic axis tissue. This almost equal distribution between cotyledon and axis was not found in the *Q. alba* acorns; the 2-year average

**Table 2.** Mean saturated and unsaturated fatty acid contents (mg/g dry weight) in 1993 *Q. nigra* and *Q. alba* cotyledons during acorn desiccation.

| Time (days) | Palmitic | Stearic | Oleic | Linoleic | Linolenic | Total |
|---|---|---|---|---|---|---|
| | | | ***Q. nigra*** | | | |
| 0 | 7.31 | 0.87 | 28.86 | 13.22 | 0.00 | 50.26 |
| 7 | 5.43 | 0.67 | 21.44 | 9.61 | 0.00 | 37.15 |
| 9 | 7.15 | 0.90 | 34.45** | 10.41 | 0.13 | 53.04 |
| 12 | 7.84 | 0.87 | 35.32** | 11.05 | 0.22* | 55.30 |
| | | | ***Q. alba*** | | | |
| 0 | 3.27 | 0.11 | 7.17 | 8.07 | 1.12 | 19.74 |
| 1 | 2.42 | 0.07 | 5.41 | 5.90 | 0.88 | 14.68 |
| 2 | 2.94 | 0.10 | 5.65 | 7.00 | 1.03* | 16.72 |
| 3 | 1.81** | 0.04 | 4.39** | 3.65** | 0.43** | 10.32 |
| 5 | 3.42 | 0.11 | 10.21** | 7.77 | 0.90** | 22.41 |
| 7 | 4.11 | 0.17 | 11.94** | 8.96 | 1.06* | 26.24 |
| 9 | 2.35* | 0.00* | 4.93 | 5.29 | 0.64 | 13.21 |

**Note**: * and **, significance at $p < 0.05$ and $p < 0.01$, respectively. Probabilities were calculated using individual fatty acid content as a percent of the total.

of 17.8 mg/g in the cotyledon tissue (Table 2) was more than double that of the axis FAMEs (7.6 mg/g).

Since decline in *Q. nigra* acorn viability was slower than expected and fatty acid analyses of the 1st week's samples from 1991 showed little or no changes, efforts and analyses in 1992 and 1993 were concentrated on acorns exposed to a more prolonged drying period. No definite patterns of change in degree of unsaturation were discernable in either total fatty acid content or in individual fatty acid contents of *Q. nigra* cotyledons (Table 2). The most prevalent saturated and unsaturated fatty acids in both the axes and the cotyledons were palmitic acid and oleic acid, respectively. An increase in palmitic acid content in 1991 cotyledons was offset by a similar decline in 1992 when day 9 and day 12 samples were analyzed. This reduction, coupled with declining acorn germination, gave a fairly high coefficient of determination ($r^2 = 0.69$), but the 1993 samples showed little difference between fresh and day-12 acorns ($r^2 = 0.05$). The 20% increase of oleic acid from 1993 fresh to day-12 samples and the decrease of linoleic acid were not related to acorn germination. A similar randomness was found in the total and individual fatty acid contents of the *Q. nigra* axis samples. This resulted in low $r^2$ values for the fatty acids of the axes as well. These results suggest that changes in *Q. nigra* fatty acids during desiccation were not significant factors in the expression of recalcitrance.

Just as in *Q. nigra*, the lipid data offered no insights into the recalcitrant nature of *Q. alba* seeds. *Quercus alba* acorns had a low lipid content, and a slight change in a fatty acid could dramatically affect overall lipid makeup of the cotyledons (Table 2) and axes. Palmitic acid was the most common saturated fatty acid in both the embryonic axes and cotyledons. The most prevalent unsaturated fatty acid shifted between oleic acid and linoleic acid in 1993 cotyledons; in 1994 cotyledons, oleic acid was always highest.

**Table 3.** Peak onset and enthalpy values for dry and rehydrated (rhyd.) *Q. nigra* acorn tissues.

| Tissue[a] | Time (days) | Onset (°C) | | Enthalpy (J/g) | |
|---|---|---|---|---|---|
| | | Dry | Rhyd.[b] | Dry | Rhyd.[b] |
| Axis | 0 | −6.49 | — | 90.14 | — |
| | 5 | −10.48 | −10.11 | 61.09 | 65.55 |
| | 7 | −13.98 | −11.55 | 44.50 | 51.92 |
| | 9 | −23.58 | −19.36 | 12.54 | 24.60 |
| | 12 | −22.82 | −22.56 | 10.42 | 18.07 |
| PCOT | 0 | −6.84 | — | 76.47 | — |
| | 5 | −12.50 | −10.75 | 49.50 | 45.85 |
| | 7 | −19.17 | −18.12 | 23.36 | 27.67 |
| | 9 | −18.60 | −20.13 | 23.57 | 18.58 |
| | 12 | −20.26 | −19.70 | 9.50 | 17.14 |
| DCOT | 0 | −6.92 | — | 63.49 | — |
| | 5 | −15.11 | −10.06 | 41.97 | 50.56 |
| | 7 | −17.36 | −13.97 | 32.09 | 39.52 |
| | 9 | −17.07 | −16.68 | 22.46 | 24.35 |
| | 12 | −16.53 | −16.46 | 20.96 | 23.56 |

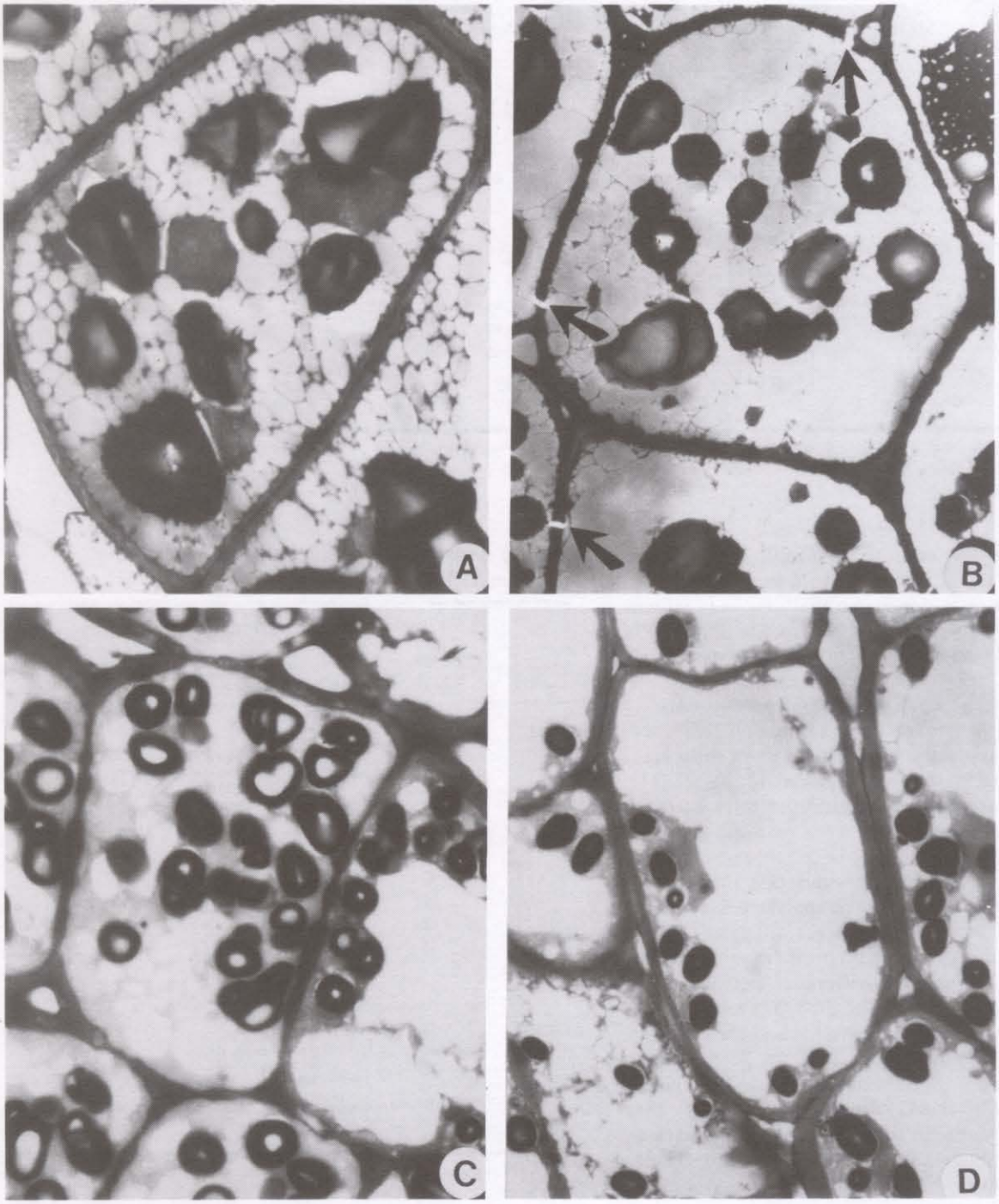
[a]PCOT, cotyledon tissue proximal to the embryonic axis; DCOT, cotyledon tissue distal to the embryonic axis.

[b]Rehydrated acorns were soaked overnight in water.

## Electron microscopy

Undifferentiated parenchyma cells of both the cotyledon and the embryonic axis were observed for cellular anatomy; specifically to evaluate densities, distribution patterns, and cell wall integrity. Temperate recalcitrant *Quercus* acorns broadly followed a pattern similar to tropical recalcitrant

**Fig. 3.** Parenchyma cells of *Quercus* seeds before and after ambient drying treatments. (A) *Quercus nigra* 0-day; (B) *Q. nigra* 3-day. Breaks in cell walls are evident (arrows); (C) *Q. alba* 0-day; (D) *Q. alba* 3-day. Vacuolated cytoplasm and a reduction in spherosome density are evident but cell walls are intact. All ×7500.




*Carapa* seeds (J.A. Vozzo, to be published[2]). For *Q. nigra*, Figs. 3A and 3B show less cytoplasmic density and reduced spherosome concentration as samples dry. Although embryonic axis cells show more drying resistance than cotyledon cells, all sections have large spherosomes and empty vacuoles, which are highlighted as precipitation sites resulting from osmium-uranylic cell preparations. A distinct and common cell wall trauma was evident after day 3 (Fig. 3B). The cotyledons appeared much more challenged than the embryonic axis cells for the same drying period.

[2] J.A. Vozzo. Comparative morphological changes during ambient drying of tropical and temperate recalcitrant seeds. *In* Recent advances in tropical tree seed technology. To be published.

**Table 4.** Peak onset and enthalpy values for dry and rehydrated 1993 and 1994 *Q. alba* acorn tissues.

| | 1993 | | | | | 1994 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Onset (°C) | | Enthalpy (J/g) | | | Onset (°C) | | Enthalpy (J/g) | |
| Tissue[a] | Time (days) | Dry | Rhyd.[b] | Dry | Rhyd. | Time (days) | Dry | Rhyd. | Dry | Rhyd. |
| Axis | 0 | 3.3 | — | 160.3 | — | 0 | 3.0 | — | 167.1 | — |
| | 1 | 4.7 | 2.7 | 182.9 | 218.7 | 2 | −3.5 | −2.8 | 149.0 | 149.8 |
| | 2 | 3.8 | 3.2 | 160.4 | 213.4 | 4 | −3.9 | −1.6 | 126.0 | 141.9 |
| | 3 | 4.0 | 1.7 | 170.2 | 183.8 | 6 | −5.1 | −4.6 | 108.4 | 127.3 |
| | 5 | −5.4 | 0.6 | 105.8 | 169.6 | 8 | −6.1 | −6.8 | 92.1 | 82.4 |
| | 7 | −5.4 | 0.1 | 91.8 | 148.0 | 10 | −6.6 | −9.4 | 73.3 | 67.0 |
| | 9 | −7.8 | 2.9 | 71.3 | 189.3 | 12 | −9.0 | −10.7 | 61.9 | 54.8 |
| PCOT | 0 | 5.3 | — | 133.6 | — | 0 | 4.4 | — | 110.8 | — |
| | 1 | 2.9 | 3.7 | 150.8 | 154.1 | 2 | 4.7 | 4.8 | 90.2 | 116.9 |
| | 2 | 6.1 | 4.7 | 137.4 | 165.6 | 4 | 5.2 | 4.8 | 79.1 | 80.1 |
| | 3 | 1.9 | 5.9 | 128.2 | 104.9 | 6 | 5.2 | 5.1 | 67.0 | 83.0 |
| | 5 | 6.0 | 6.0 | 92.8 | 98.6 | 8 | −9.7 | −5.5 | 34.1 | 38.1 |
| | 7 | −5.9 | −1.4 | 66.1 | 65.4 | 10 | −6.3 | −8.2 | 51.3 | 38.5 |
| | 9 | −4.7 | −6.7 | 69.9 | 52.5 | 12 | −21.2 | −10.1 | 10.0 | 31.1 |
| DCOT | 0 | 5.9 | — | 89.2 | — | 0 | 3.8 | — | 84.0 | — |
| | 1 | 6.3 | 6.8 | 104.8 | 74.9 | 2 | 4.9 | 4.9 | 86.7 | 86.5 |
| | 2 | 5.1 | 6.4 | 104.2 | 107.0 | 4 | 5.3 | −1.6 | 73.2 | 47.4 |
| | 3 | −0.4 | 4.3 | 67.5 | 66.1 | 6 | 5.4 | −3.7 | 60.3 | 29.9 |
| | 5 | 5.5 | 6.0 | 73.3 | 78.0 | 8 | −6.9 | −9.5 | 30.8 | 28.3 |
| | 7 | 2.6 | −0.1 | 56.4 | 29.2 | 10 | −8.6 | −10.1 | 32.8 | 39.8 |
| | 9 | −12.6 | −7.4 | 23.8 | 52.1 | 12 | −13.4 | −10.1 | 26.2 | 34.7 |

[a]PCOT, cotyledon tissue proximal to the embryonic axis; DCOT, cotyledon tissue distal to the embryonic axis.
[b]Rehydrated acorns were soaked overnight in water.

*Quercus alba* cells representing the embryonic axis and cotyledons also show decreasing spherosomes and highly vacuolated cytoplasm (Figs. 3C, 3D). However, unlike *Q. nigra*, the tissues of *Q. alba* do not show cell wall trauma after even 7 days of drying, as the entire membrane remains intact.

*Quercus nigra* dried normally with an intact pericarp, while *Q. alba* split its pericarp longitudinally during the drying process. *Quercus nigra* intact acorns and cotyledons dried to a lower moisture content than those of *Q. alba*, but the axes of both species consistently had more moisture than the cotyledons from beginning to end of drying. This moisture was sufficient in the embryonic axes to sustain a more structurally uniform cell wall than in cotyledons, where moisture content was too low to maintain cell wall integrity (lethal moisture level = 15%).

As moisture moved in and out between the two tissues in *Q. nigra*, the net movement resulted in cell wall challenges and eventual trauma. Alternating wetting and drying exerted sufficient moisture gradient to affect cell walls at or near the lethal moisture level. *Quercus alba* axis and cotyledon tissue moisture content, however, never fell below 33% at day 7, and cell wall damage was not evident.

A comparison could be made with total lipids present in *Q. nigra*, *Q. alba*, and *Carapa*. HVEM images showed a similar pattern of spherosome decline and cell wall trauma after 3 days of drying for both *Q. nigra* and *Carapa*. Both species are relatively lipid enriched (28.4% and 48.6% on a dry-weight basis, respectively; J.A. Vozzo, see footnote 2). Cell wall integrity was not challenged for *Q. alba* with 5.6% total fat.

### Differential scanning calorimetry

Rehydration prior to germination of *Q. nigra* acorns resulted in elevated enthalpy values for the embryonic axis tissue and also in less negative endotherm onset values for both axis and cotyledon tissue (Table 3). Enthalpy values, however, never recovered to their fresh levels once acorns started to dry; and rehydration was much less effective in affecting onset temperatures once viability dropped below 50% (day 7). Similar results were seen in 1993 *Q. alba* axis, PCOT, and DCOT tissue (Table 4), but effects were less clear cut in the 1994 data.

Regression analyses of DSC and germination data revealed strong relationships between onset and enthalpy values of tissues and percent germination at the time of sampling (Tables 3, 4). In both species, a decline in onset temperatures and enthalpy values was measured throughout the course of desiccation. Seed germination was especially linked to both onset temperature and enthalpy of the embryonic

axis tissue. However, regardless of the acorn tissue being tested, DSC enthalpy data always produced higher $r^2$ values than onset temperatures when regressed on seed germination ($r^2$ = 0.72–0.99). Although the relationship was not quite so strong as those of the DSC data, analyses confirmed that intact acorn and embryonic axis moisture content were also strongly related to seed germination ($r^2$ = 0.67–0.90 and $r^2$ = 0.38–0.96, respectively).

In conclusion, systematic changes in lipid content composition during desiccation were lacking in both *Q. nigra* and *Q. alba*. However, other physiological changes could be related to the recalcitrant nature of the seeds. Viability was retained for only 9–12 days in both species, and a 50% reduction in germination occurred between days 5 and 7; this reduction in viability coincided with the failure of rehydration to bring onset temperatures back to their fresh levels (Tables 3, 4). The steady decline in enthalpy values throughout the experiment suggests loss of cell osmotic integrity. The high moisture content of the axes even after 9 days of drying was common to both species, as was the strong relationship of moisture content and DSC onset and enthalpy values to germination. Interestingly, when germination was reduced by 50%, *Q. nigra* intact acorn moisture content had dropped below 20%, but that of *Q. alba* was still over 30%. Although the intact *Q. nigra* acorns reached the lethal moisture content (15%) by day 9, axis moisture content never dropped below 25%. The lethal moisture content of intact *Q. alba* acorns was much higher (22%), but the axis moisture content at 0% viability was, like that of *Q. nigra*, over 25%. The electron microscopy studies confirmed what the steady decline in DSC enthalpy values suggested: that the osmotic integrity of the cells had been lost. Cell wall trauma was evident in the cotyledons, which lost moisture rapidly and in which enthalpy values had dropped dramatically by day 5; trauma was less evident in the axes, and enthalpy values did remain higher than those of cotyledon tissue throughout the experiment, especially in *Q. alba*. We can speculate that the membrane integrity in *Q. alba* cells may be due to the osmotic protection provided by the high carbohydrate content of the seeds. Carbohydrates have been shown to provide protection against the stress of dehydration (Crowe et al. 1984; Koster and Leopold 1988; Blackman et al. 1992), and they account for 80–90% of *Q. alba* seed dry weight (Clatterbuck and Bonner 1985).

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