PART FOUR: FRONTIERS OF CONSERVATION

Changes in Desiccating Seeds of Temperate and Tropical Forest Tree Species

K. F. Connor
F. T. Bonner
J. A. Vozzo
I. D. Kossmann-Ferraz

INTRODUCTION

Seeds have traditionally been divided into two storage classes (Roberts, 1973). Orthodox seeds, which include those of most temperate

K. F. Connor, F. T. Bonner (retired), and J. A. Vozzo are Plant Physiologists, U.S. Forest Service, Forestry Sciences Laboratory, P.O. Box 928, Starkville, MS 39760-0928 USA.

Isolde Kossman-Ferraz is Scientist, Instituto Nacional de Pesquisas da Amazônia, Coordenacão de Pesquisas em Silvicultura Tropical, Manaus-AM, Brasil.

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tree species, undergo a period of desiccation before being shed from the tree; they can be dried easily to moisture contents of less than 12% and stored for long periods of time. Recalcitrant seeds, however, do not undergo a significant maturation drying phase, are sensitive to moisture loss and low temperatures and have a short storage lifespan. Most tropical and a few important genera of temperate tree species, notably *Quercus*, *Aesculus*, some *Acer* (Bonner, 1990) and *Castanea* (Pritchard and Manger, 1990) have recalcitrant seeds.

The physiological basis of seed recalcitrance is as yet unknown. Hypotheses suggesting possible causes have been proposed (Roberts, 1973; Flood and Sinclair, 1981; Finch-Savage et al., 1994; Berjak and Pammenter, 1997), but the end result is that intact recalcitrant seeds cannot be stored for long periods of time. Thus, if the seed crop of a recalcitrant species fails, nurseries will be unable to draw upon a storage reserve of seeds in order to meet the demands of growers. A series of experiments on both temperate and tropical recalcitrant-seeded tree genera were initiated with the goal of relating changes in the physiology, biochemistry, and ultrastructure of desiccating seeds to loss of viability. Two tropical species, *Carapa guianensis* Aubl. and *Guarea guidonia* (L.) Sleumer (American muskwood) and two temperate species of oak, *Quercus nigra* L. (water oak) and *Quercus alba* L. (white oak) were selected for our experiments. This paper summarizes results (Connor et al., 1996, Connor et al., 1998a, Connor et al., 1998b) obtained over a 7-year period.

MATERIALS AND METHODS

Seeds were either collected locally or express-shipped to the Starkville laboratory shortly after shedding. They were imbibed overnight in tap water at room temperature before the experiment to ensure full hydration. Desiccation of the seeds was carried out on a laboratory benchtop at a room temperature of 27 ± 2°C and an ambient RH of 40 ± 10%. Random subsamples of seeds were periodically removed to provide specimens between full imbibition/high germination and reduced viability from desiccation. Each subsample was further divided for the following tests:

1. *Germination*: At each sampling time, seeds were germinated as two replications of 17-50 seeds each, depending on the species

and seed abundance. Seeds were placed on trays lined with Kimpak®/blotter paper and germinated in a Stults® wet box set on a 20-30°C temperature cycle with 8 h of light at 30°C and 16 h of dark at 20°C. Germination was considered complete when the cotyledons emerged.

2. *Differential scanning calorimetry (DSC) and moisture content (MC) analyses*: Tissue samples were sealed into aluminum pans and placed in a Perkin Elmer® DSC-7. 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 (heat content) value of the melt were determined. Moisture content (MC) of whole seeds was determined on three to six subsamples by cutting seeds into halves or quarters and drying in an oven set at 107°C. MC was expressed as a percentage of fresh weight. To determine distribution of moisture within the seeds, tissues were dissected, rapidly weighed to the nearest 0.1 mg on an electronic balance, and immersed in 20 ml of anhydrous methanol (MEOH). After dehydration in the MEOH for 48 h, MCs were measured on an aliquot of the MEOH by Karl Fisher analysis with an Aquastar® V1B automatic titrator (Association of Official Agricultural Chemists, 1965).
3. *Gas chromatography (GC)*: Seeds were chopped, the pieces immediately immersed in liquid nitrogen (LN_2), and the entire sample ground either by a LN_2-cooled Wiley mill equipped with a 20-mesh screen or with a LN_2-cooled mortar and pestle. A portion of the sample was placed in 2:1 chloroform ($CHCl_3$)/MEOH and the lipids extracted and purified as described in Connor et al. (1996).
4. *Ultrastructure* (*Carapa* and *Quercus* only): Three-mm^3 plugs of tissue were immediately placed in a 2.5% glutaraldehyde solution of pH 7.2 phosphate buffer, fixed in osmium tetroxide, dehydrated in acetone, embedded in Epon®, sliced at 0.5 to 0.75 m, and stained with uranyl acetate (Vozzo and Song, 1989). Sections were observed at 1.2 MeV using high-voltage transmission electron microscopy (HVEM).

Experiments were replicated in time; results summarized below represent two years of data for *Carapa*, *Guarea*, and *Q. alba* and three years for *Q. nigra*.

RESULTS AND DISCUSSION

Germination

Initial seed germination was high in all species (Table 1); fungal contamination in year 1 *Guarea* seeds resulted in a drop in viability to 3% by day 3 of the experiment despite a 30 second surface sterilization wash in a 10% solution of commercial bleach. In year 2, seed coats were removed prior to germination testing, and viability remained higher than that of year 1 seeds throughout the experiment. Generally, viability of these recalcitrant seeds did not fall below 50% of the original fresh value until at least 5 days of exposure to ambient laboratory conditions.

DSC and Moisture Content (MC)

Karl Fisher analyses demonstrated that the axis MC of all species was high throughout the experiment (Figure 1). Despite lower mois-

TABLE 1. Germination (%) results of temperate and tropical recalcitrant seeds as they were desiccated.

Drying Time (days)	*Q. nigra*			*Q. alba*		*Carapa*		*Cuarea*	
	1991	1992	1993	1993	1994	1992	1994	1992	1993
Fresh	90	97	94	79	90	80	69	57	74
1	96	--	--	86	--	--	--	23	54
2	97	--	--	90	92	--	--	--	34
3	93	--	--	84	--	70	42	3	38
4	89	--	--	--	68	--	--	--	--
5	83	68	71	74	68	60	15	--	--
6	46	--	--	--	32	--	--	0	20
7	52	49	48	36	--	40	5	--	--
8	48	--	--	--	14	--	--	0	24
9	21	15	19	38	--	--	--	--	--
10	23	--	--	--	0	10	--	--	--
11	13	--	--	--	--	--	0	--	--
12	--	4	5	--	0	--	--	--	--

Each number represents the average germination of two replications.
Number of seeds per replication varied from 17 to 50 depending on species and availability.

ture in the surrounding cotyledonary tissue, axis MC never fell below 20%. In fact, when water oak cotyledon tissue reached the lethal MC of 15% (Agmata 1982), the axis MC was still 27% (Connor et al., 1996). In *Carapa*, axis MC was still at least 45% when seed viability had been reduced to 5%. It appears that the axes in these recalcitrant species may act as moisture sinks; and despite differences in seed sizes and chemical composition, the MC of all tissue in these recalcitrant-seeded species remained relatively high even when viability dropped below 50% of the original value (Table 2). Predictably, statistics re-

FIGURE 1. Axis moisture dynamics for desiccating temperate and tropical recalcitrant seeds.

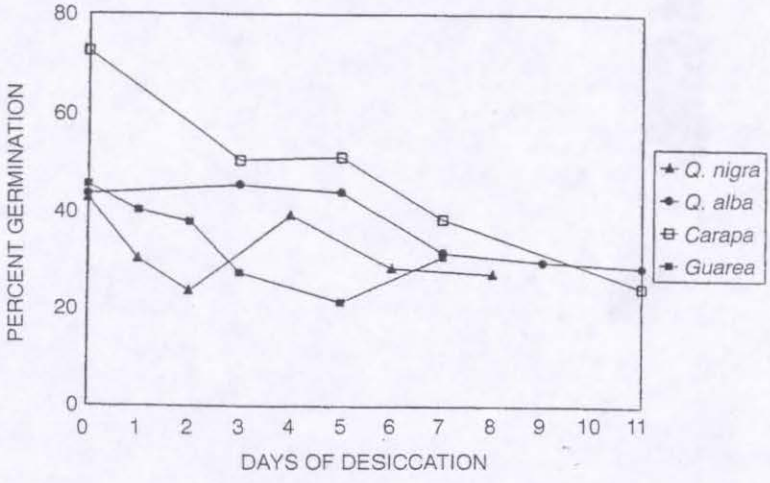

TABLE 2. Moisture contents of seeds after a 50% reduction in viability.

			% Moisture (fresh weight)			
Species	Year	% Germination	Intact	Axis	PCOT[1]	DCOT[1]
Carapa	1994	15	28.6	51.4	59.4	35.4
Guarea	1993	20	20.5	21.6		21.3
Q. alba	1993	36	28.0	34.1	37.7	33.7
Q. nigra	1992	49	16.0	29.3	21.7	13.0

[1] PCOT = cotyledon tissue proximal to the axis; DCOT = cotyledon tissue distal to the axis.

vealed that the relationship between declining MCs of the various seed tissues and declining viability was high (r^2 = 0.46-0.99).

DSC thermographs also showed a strong but variable relationship between moisture enthalpy (heat content) values of the seed tissues and seed viability (r^2 = 0.25-0.99). Generally, the axes and intact seeds appeared most sensitive to moisture loss. However, while useful for showing general declines in seed viability, DSC analyses could not pinpoint drops in viability when germination remained above 50%. Enthalpy values only gradually declined in the axes, while viability dropped more dramatically. High enthalpy values in the axes might seem good indicators of high seed viability, but they are mostly a reflection of high MC. Since the axes may act as moisture sinks, high enthalpy values are maintained despite sharply declining seed viability. Therefore, such analyses cannot be used as predictors of seed deterioration.

Lipid Analyses

Petroleum ether soxhlet extractions on fresh seeds yielded 0.1901 g lipid/g dry wt of seed tissue in *Guarea*, 0.284 g/g in *Q. nigra*, 0.057 g/g in *Q. alba*, and 0.486 g/g in *Carapa*. Analyses determined that there were no significant patterns of gain or loss of individual fatty acids as the seeds desiccated and viability was lost (Connor et al., 1996; Connor et al., 1998a; Connor et al., 1998b). Also, results could vary from year to year. While 1994 *Carapa* seeds revealed a strong relationship between viability and a decline in palmitic and stearic acids and an increase in oleic acid (r^2 = 0.60, 0.84. and 0.98, respectively), the pattern was not repeated in 1992, where r^2 values dropped as low as 0.05. At this time, the lipid analyses have provided little insight into the cause or nature of tree seed recalcitrance.

Electron Microscopy

The cotyledonary tissues of both *Carapa* and *Q. nigra* exhibited cell wall trauma after only 3 days of drying. Axis tissue in these 2 species, however, showed greater resistance to desiccation-imposed damage. This strengthens the significance of the moisture analysis results, indicating that the high MC of the axes and the possibility that they might be moisture sinks may protect them from desiccation damage.

Like the cells of *Q. nigra*, there were enlarged spherosomes and highly vacuolated cytoplasm present in the *Q. alba* samples. However, there was no evidence of cell wall trauma in either the cotyledon or axis tissue even after 7 days of drying. Thus, whereas in *Carapa* and *Q. nigra*, high in lipid, cell wall damage was evident, no damage was visible in low-lipid *Q. alba*. Perhaps in these seeds, changes in lipid composition may not be an important indicator of seed deterioration; but the overall hydrophobic nature of lipids in general may be an important factor in cell wall trauma.

CONCLUSIONS

- Viability for all species tested did not drop below 50% of the original level until seeds had dried at least 5 days.
- Relationships between axis and intact seed MCs and seed viability were generally strong.
- Axis MC rarely dropped below 20%; this suggests axes may act as moisture sinks.
- DSC analyses cannot be used as predictors of seed deterioration.
- Lipid analyses provided little insight into the nature of seed recalcitrance but the hydrophobic nature of lipids may be a factor in cell trauma, since seeds high in lipid showed damage after only three days of drying.
- None of the tests provided clear clues to explain the physiological basis of recalcitrance.

REFERENCES

Association of Official Agricultural Chemists. 1965. Official methods of analysis of the association of official agricultural chemists. Association of Official Agricultural Chemists, Washington, DC.

Agmata, A. 1982. Physiological Effects of Drying *Quercus Nigra* L. Acorns. MS Thesis, Mississippi State Univ. 59 pp.

Berjak P., and N.W. Pammenter. 1997. Progress in the understanding and manipulation of desiccation-sensitive (recalcitrant) seeds. 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: Kluwer Academic Publishers: 689-703.

Bonner, F.T. 1990. Storage of seeds: potential and limitations for germplasm conservation. For. Ecol. Manag. 35: 35-43.

Connor, K.F., F.T. Bonner and J.A. Vozzo. 1996. Effects of desiccation on temperate recalcitrant seeds: differential scanning calorimetry, gas chromatography, electron microscopy, and moisture studies on *Quercus nigra* and *Quercus alba*. Can. J. For. Res. 26: 1813-20.

Connor, K.F., F.T. Bonner and J.A. Vozzo. 1998a. Effects of desiccation on the recalcitrant seeds of *Carapa guianensis* Aubl. and *Carapa procera* DC. Seed Tech. 20(1): 71-82.

Connor, K.F., F.T. Bonner and J.A. Vozzo. 1998b. Effects of desiccation on recalcitrant seeds: results from lipid and moisture studies on *Guarea guidonia* (L.) Sleumer. Seed Tech. 20(1): 32-42.

Finch-Savage, W.E., G.A.F. Hendry and N.M. Atherton. 1994. Free radical activity and loss of viability during drying of desiccation-sensitive seeds. Proc. Royal Soc. Edinburgh 102B: 257-60.

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

Pritchard, H.W., and K.R. Manger. 1990. Quantal response of fruit and seed germination rate in *Quercus robur* L. and *Castanea sativa* Mill. to constant temperatures and photon dose. J. Exp. Bot. 41: 1549-57.

Roberts, E.H. 1973. Predicting the storage life of seeds. Seed Sci. Tech. 1: 599-514.

Vozzo, J.A., and M.J. Song. 1989. High-voltage electron microscopy of cell walls in *Pinus taeda* seeds. In: Turner, J.W., ed. Tropical Seed Research. ACIAR Proc. No. 28: 78-80.