Trees (2001) 15:131–136
DOI 10.1007/s004680100092

ORIGINAL ARTICLE

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The effects of desiccation on seeds of *Acer saccharinum* and *Aesculus pavia*: recalcitrance in temperate tree seeds

Received: 16 June 2000 / Accepted: 8 January 2001 / Published online: 8 March 2001

Abstract This study was undertaken to determine how the results from lipid, moisture, and differential scanning calorimetry analyses conducted on silver maple (Aceraceae: *Acer saccharinum* L.) and red buckeye (Hippocastanaceae: *Aesculus pavia* L.) compared with those obtained from previous studies on white and water oaks (Fagaceae: *Quercus alba* and *Q. nigra*), and the tropical zone species American muskwood (Meliaceae: *Guarea guidonia*) and carapa (Meliaceae: *Carapa guianensis*). Seeds were air-dried at room temperature for 9–11 days. At intervals, germination was tested, moisture determined, and lipids extracted. It was found that, like the other recalcitrant seeds, (1) viability was greatly reduced or lost after 11 days of drying, (2) percentage changes in individual fatty acids were not related to seed viability, and (3) results from the differential scanning calorimetry studies revealed a strong relationship between enthalpy/onset data from the embryo and cotyledon tissues and loss of viability. Also, silver maple seeds experienced a 50% reduction in viability by day 5 of drying and retained an axis moisture content over 25% throughout the experiment. However, unlike the other recalcitrant seeds surveyed, both silver maple and red buckeye had a significant reduction in the total amount (mg/g) of cotyledon lipids as the experiment progressed. However, no decrease in the unsaturated/saturated fatty acid ratio was found, so we conclude that in these species lipid peroxidation is not a marker of declining seed viability. Also, red buckeye seeds did not lose 50% viability until after day 8 of the experiment, and axis moisture content fell well below 20% as the seeds dried.

Keywords *Aesculus pavia* · *Acer saccharinum* · Seed storage · Recalcitrance · Moisture content

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Introduction

Temperate zone seeds can be divided into two storage classes: 'Orthodox' or desiccation-resistant seeds can be dried without damage to a moisture content of less than 12%, while 'recalcitrant' or desiccation-sensitive seeds cannot (Roberts 1973). Orthodox seeds commonly undergo a period of desiccation prior to being shed from the tree, but recalcitrant-seeded species do not have this final maturation drying stage in their evolutionary strategy and are thus vulnerable to moisture loss. This susceptibility makes any useful period of seed storage for many recalcitrant seeded species very short, while others, such as *Quercus nigra* L. (Bonner 1973), can survive up to 30 months under proper storage conditions. Forest tree genera with recalcitrant seeds are abundant in the tropics but less common in the temperate zone. They do exist, however, and some important temperate tree genera, *Castanea* (Jaynes 1969; Pritchard and Manger 1990), *Quercus* (Bonner and Vozzo 1987), *Aesculus*, and some *Acer* species (Bonner 1990), have seeds classified as recalcitrant.

Many hypotheses have been proposed to explain the physiological basis of recalcitrance. Theories concerning changes in membrane and storage lipids and of physical disruption of seed membranes have been proposed (Flood and Sinclair 1981; Seewaldt et al. 1981; Priestly and Leopold 1983), and changes in seed proteins and carbohydrates, and the complexities of water properties in seeds have been explored, with varying degrees of success (Roberts 1973; Farrant et al. 1985, 1988; Pammenter et al. 1991; Wesley-Smith et al. 1992). Recent work has modified both Robert's initial definition of recalcitrance and our perspective of the nature of recalcitrance. Pammenter et al. (1994) and Berjak and Pammenter (1997) recognized the damage caused by aberrant metabolic processes while seeds are in hydrated storage and as water is lost. Thus, while much progress has been made in understanding the nature of recalcitrance, the storage of some recalcitrant tree seed over a long period is an as yet insurmountable problem.

Pritchard (1991) determined that damage began in recalcitrant seed embryos at a much higher moisture content than that proposed by Roberts (1973); embryos of *Quercus rubra* exhibited damage when moisture content was still over 40%. Experiments by the authors with recalcitrant seeds from both temperate and tropical trees have yielded mixed results (Connor et al. 1996, 1998; Connor and Bonner 1998). While moisture and differential scanning calorimetry data exhibit a strong relationship with declining seed viability, results of lipid analyses have been conflicting. The objective of this study was to determine how results from lipid, moisture, and differential scanning calorimetry (DSC) analyses conducted on silver maple (Aceraceae: *Acer saccharinum* L.) and red buckeye (Hippocastanaceae: *Aesculus pavia* L.) compared with those obtained from studies on temperate zone white oak (Fagaceae: *Quercus alba* L.) and water oak (Fagaceae: *Quercus nigra* L.) (Connor et al. 1996), and the tropical zone species American muskwood [Meliaceae: *Guarea guidonia* (L.) Sleumer] (Connor and Bonner 1998) from Puerto Rico and carapa (Meliaceae: *Carapa guianensis* Aubl.) (Connor et al. 1998) from Brazil. Both silver maple and red buckeye are widely distributed in temperate North America and have recalcitrant seeds.

Materials and methods

Seeds were collected locally in Starkville, Miss. at or within 2 days of shedding. Seeds were spread on a laboratory bench lined with blotter paper and kept at ambient temperature (21±2°C) and ambient relative humidity (50±10%). Random samples of 180 silver maple and 85 red buckeye seeds were taken at regular intervals over a period of 9–11 days to span the condition of full viability to zero, or low, viability. The following tests were performed at each sampling period.

Germination

One hundred silver maple and 50 red buckeye seeds were randomly selected at each desiccation sampling time and germinated as two replications of 50 or 25 seeds, respectively, on moist Kimpak 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.

Moisture content

Moisture contents (MCs) were determined on six whole silver maple seeds and one whole red buckeye. Moisture was determined by procedures recommended for large seeds with high MCs (Bonner 1991; International Seed Testing Association 1993). Chopped seeds were dried in aluminum cans at 103±2°C for 17±1 h in a mechanical convection oven. MCs were expressed as percentages of fresh weight.

Other samples of eight red buckeye seeds and six silver maple seeds were dissected in order to determine distribution of moisture within the seeds during desiccation. Tissue samples were weighed to the nearest 0.1 mg on an electronic balance, immersed in 20 ml of anhydrous methanol (MEOH), and, 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. MCs were expressed as percentages of the tissue fresh weight. Due to deteriorated seed condition, moisture tests were run only through 9 days for silver maple.

Lipid extraction and gas chromatography analysis

At each sampling period, the cotyledonary tissue of 20 red buckeye and 50 silver maple seeds was chopped and immediately immersed in liquid nitrogen (LN_2). The tissue was then ground in a LN_2-cooled Wiley mill using a 20-mesh screen. Similarly, embryonic axes with a small amount of surrounding tissue were immersed in LN_2 and then ground in a LN_2-cooled mortar. Samples for the lipid analyses were extracted using a 2:1 chloroform/methanol solution and esterified as described in Connor et al. (1996). Analyses were performed using a 30 m × 0.53 mm × 0.5 µm J&W megabore DB-23 column. The initial column temperature, 110°C, was held for 5.50 min and then increased at 30°C/min to 150°C and held for 20 min. Injector temperature was set at 200°C and detector temperature at 220°C.

Differential scanning calorimetry

Samples of embryo and cotyledon tissue were sealed into 50 µl aluminum pans. A Perkin Elmer differential scanning calorimeter (DSC-7), calibrated using indium (melting point 156.6°C) and hexane (melting point –95.3°C) was used in the analyses. 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. Enthalpy values were calculated from two scans for each tissue at each sampling period.

Results

Both the silver maple and red buckeye seeds had high initial germination (89% and 94% respectively; Tables1, 2); after 9 days of desiccation, silver maple germination had dropped to 21%, while that of red buckeye was 24% after 11 days. Initial MC of intact silver maple seeds was 53.5%. Intact, axis, and cotyledon MCs of the silver maple seeds did not fall below 30% throughout the course of desiccation. Germination did decline with decreasing MC, but not to the extent expected. Initial MC of intact red buckeye seeds and embryos was 64.4% and 63.2% respectively, while cotyledon tissue averaged 58.4%. The buckeye seeds in this experiment experienced a severe drop in MC (Table 2). Intact MC after 11 days was 15.5%, axis MC had dropped to 12.5%, and cotyledon MC was 13.1–13.8%. The MC usually associated with recalcitrant seed mortality is 20% (Roberts 1973). Although buckeye seeds are classified as recalcitrant, MC fell well below this level and total mortality did not occur. However, the decline in seed viability with decreasing MC falls within the range found in other recalcitrant seeds (Tompsett 1984), and the seeds may best be classified as of intermediate recalcitrance.

Analyses revealed a significant decline in the mg/g amount of palmitic, oleic, linoleic, and linolenic fatty acids in the desiccating cotyledons of both red buckeye and silver maple (Table 3). This resulted in a significant reduction of the total fatty acid content in the cotyledons of both species.

Regression analyses of DSC and germination data for red buckeye revealed strong relationships between onset

Table 1 Dynamics of Karl Fisher seed moisture (g/g fresh weight) for silver maple (*Acer saccharinum*) samara, axes, and cotyledon tissue. Intact seed moisture content was determined by drying 17 h in a mechanical convection oven. Moisture contents are expressed as percentages of the tissue fresh weight. Numbers in parentheses are standard errors

Days of desiccation	Percent germination	Intact	Axis	Cotyledon	Samara
0	89	53.5 (0.4)	44.6 (5.3)	51.3 (1.4)	11.5 (2.3)
1	88	52.0 (1.0)	48.8 (4.3)	48.7 (2.3)	10.4 (0.6)
3	65	43.8 (0.6)	36.9 (5.5)	44.2 (1.4)	9.4 (0.5)
5	42	41.0 (1.6)	28.8 (4.5)	37.1 (2.8)	7.0 (0.8)
7	18	33.0 (1.4)	38.1 (3.8)	37.8 (2.6)	8.9 (0.6)
9	21	32.8 (4.9)	30.0 (5.3)	36.2 (3.8)	10.2 (0.5)

Table 2 Dynamics of Karl Fisher seed moisture (g/g fresh weight) for red buckeye (*Aesculus pavia*) pericarp, axes, proximal and distal cotyledon tissue. Intact seed moisture content was determined by drying 17 h in a mechanical convection oven. Moisture contents are expressed as percentages of the tissue fresh weight. Numbers in parentheses are standard errors. *PCOT* cotyledon tissue proximal to the axis; *DCOT* cotyledon tissue distal to the axis

Days of desiccation	Percent germination	Intact	Axis	PCOT	DCOT	Pericarp
0	94	64.4	63.2 (0.6)	58.5 (1.1)	58.3 (1.0)	45.2 (1.8)
2	88	54.4	53.0 (2.6)	57.3 (1.2)	55.0 (1.5)	26.2 (2.1)
4	88	43.9	38.8 (2.6)	56.8 (2.1)	51.2 (1.2)	23.1 (0.7)
6	82	31.8	29.2 (2.3)	43.0 (3.3)	39.5 (2.5)	20.1 (0.8)
8	58	23.3	19.0 (2.3)	23.9 (1.8)	26.4 (2.0)	17.2 (1.2)
11	24	15.5	12.5 (0.7)	13.8 (0.8)	13.1 (1.0)	13.8 (0.5)

Table 3 Amounts (mg/g dry weight) of individual fatty acids from *Acer saccharinum* and *Aesculus pavia* cotyledons (*=significantly different from fresh value at $P \leq 0.05$, **=significantly different from fresh value at $P \leq 0.01$)

Species	Days of drying	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic	Total
Acer	0	4.6	0.4	0.3	4.3	7.0	4.7	–	–	21.3
	1	2.1**	0.2**	0.1**	1.7**	3.0**	2.2**	–	–	9.3
	3	–	–	–	–	–	–	–	–	–
	5	2.4**	0.2**	0.2**	2.4*	3.7**	2.5**	–	–	11.4
	7	2.9**	0.2*	0.2**	3.0*	4.5**	3.0**	–	–	13.8
	9	1.9**	0.2**	0.2**	2.1*	3.0**	2.0**	–	–	9.4
	11	1.8**	0.1**	0.2**	1.9**	2.8**	1.9**	–	–	8.7
Aesculus	0	3.1	–	0.3	4.5	9.9	0.6	1.2	1.1	20.7
	2	2.0	–	0*	1.3**	5.4*	0.4	0.4*	0.5	10.0
	4	2.0	–	0*	1.4	5.5	0.3	0.3*	0.2*	9.7
	6	1.3*	–	0	1.1	3.6*	0.2*	0.3	0.2*	6.7
	8	2.3	–	0.2	2.0*	6.3	0.4	0.6	0.6*	12.4
	11	1.3**	–	0**	1.2	3.9	0.3**	0.4**	0.4*	7.5

Table 4 Peak onset and enthalpy values for desiccated *Acer saccharinum* and *Aesculus pavia* tissues. *PCOT* cotyledon tissue proximal to the axis; *DCOT* cotyledon tissue distal to the axis

Species	Days of drying	Axis		PCOT		DCOT	
		Onset (°C)	Enthalpy (J/g)	Onset (°C)	Enthalpy (J/g)	Onset	Enthalpy
Aesculus	0	0.6	175.1	0.4	183.3	0.6	181.7
	2	–5.5	131.0	–3.0	150.0	–1.0	151.4
	4	–10.5	93.4	–5.6	127.0	–8.6	71.3
	6	–23.1	16.8	–16.7	79.1	–10.4	76.6
	8	–20.7	13.0	–43.6	5.5	–13.1	54.9
	11	–33.9	1.6	–36.2	3.0	–38.4	3.2
Acer	0	0.7	134.5			–0.1	138.7
	1	–0.6	120.4			–0.8	104.2
	3	–0.9	143.9			–4.4	89.3
	5	–5.3	110.0			–0.7	95.8
	7	–7.9	95.5			–3.2	98.8
	9	–15.2	80.4			–9.3	66.7
	11	–15.6	43.7			–8.7	58.8

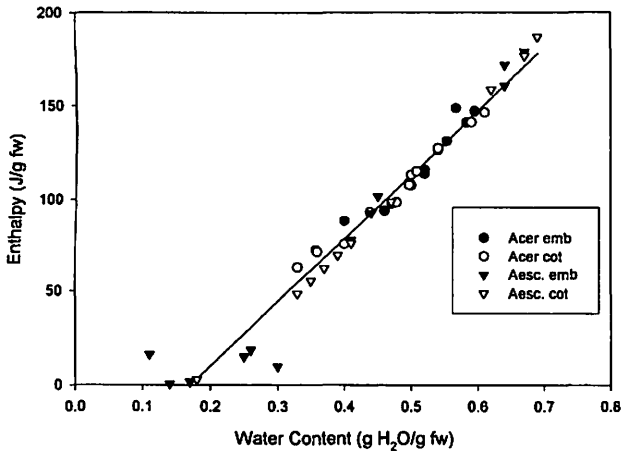

Fig. 1 Regression line relating the enthalpy values with the water content of the tissues examined by DSC. Slopes of regression lines for individual tissues did not vary significantly

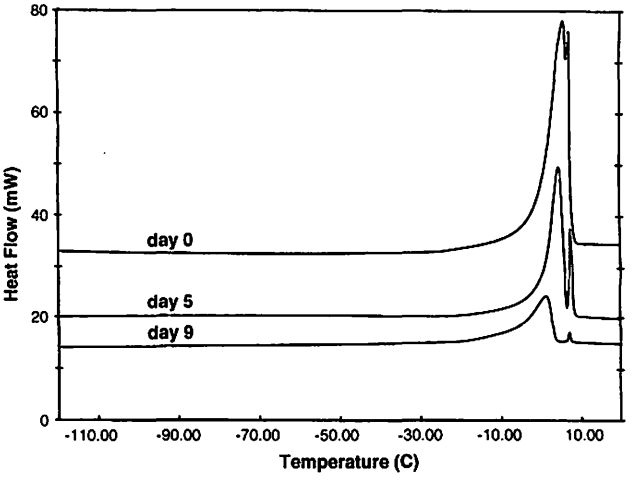

Fig. 2 DSC warming thermograms of silver maple cotyledon tissue at experiment days 0, 5 and 9. The sharp, pure water peak remained present throughout the entire drying experiment

and enthalpy values of drying seed tissues and both percent germination and length of desiccation period (Table 4). A decline in onset temperatures and enthalpy values was measured throughout the course of desiccation, corresponding to the moisture data reported in Tables 1 and 2. R^2 values relating onset/enthalpy values to percent germination ranged from 0.55 to 0.95. Like the drying red buckeye tissues, silver maple embryo and cotyledon onset and enthalpy values were strongly related to length of desiccation period and to percent germination. The relationship between the embryonic tissue and the length of desiccation was especially significant, as enthalpy and onset values declined throughout the course of the experiment (R^2=0.72–0.75). Further, there was a linear relationship between the enthalpy (J/g fw) of the melting endotherm and the water content (g H_2O/g fw) of the embryo and cotyledon tissue of both species. (Fig. 1).

Discussion

There were a number of similarities between the germination, moisture, lipid, and DSC data obtained from silver maple and red buckeye and those obtained from studies on other temperate and tropical zone species: (1) viability was greatly reduced or lost after 11 days of drying; (2) percentage changes in individual fatty acids were not related to seed viability; (3) DSC results revealed a strong relationship between enthalpy/onset data from the embryo and cotyledon tissues and loss of viability. While this is tempered by the fact that this is a general trend and cannot be used as a predictor of overall seedlot quality, (4) all species exhibited the three components of the melting endotherms described by Pammenter et al. (1991) and Berjak et al. (1992): the presence of a small, sharp peak centered at 0°C (pure water; Fig. 2), a broad endotherm (solution water), and a portion with no peak (non-freezable water). In addition, (5) silver maple seeds, like those of temperate white and water oak (Connor et al. 1996) and tropical American muskwood (Connor and Bonner 1998) and carapa (Connor et al. 1998) experienced a 50% reduction in viability by day 5 of the experiment and retained an axis MC over 25% even though viability had been almost lost.

The similarities, however, are complicated by indications that the pathways and mechanisms that determine recalcitrance may vary from genera to genera and indeed, in some cases, between species within a genera (Tompsett 1984; Hong and Ellis 1990). Unlike any of the other recalcitrant seeds surveyed, red buckeye seeds did not lose 50% viability until after day 8 of the experiment; and, more significantly, both intact seed MC and axis MC fell well below the suggested lethal level for recalcitrant seeds (20%; Roberts 1973) before all viability was lost. While these moisture data bring the recalcitrance of red buckeye into question, it must be noted that the species does not survive storage at 4°C for more than 1 year and that any drying period before seed storage results in complete loss of viability within 3 months (Connor, unpublished data).

Both silver maple and red buckeye had a significant reduction in the total amount of seed lipids as the experiment progressed. The cotyledon tissue of both seeds lost significant amounts of palmitic, oleic, linoleic, and linolenic fatty acids (Table 3). This may be due to translocation of fat-like substances from the cotyledons into the meristems for use as respiration metabolite or to lipid peroxidation, which may be responsible for seed deterioration during storage (Wilson and McDonald 1986; Ferguson et al. 1990; Finch-Savage et al. 1996; Li and Sun 1999). If the latter were true, one would expect a corresponding decrease in the ratio of unsaturated/saturated fatty acids in the lipid component. However, our studies found no such decline in the embryo or cotyledon tissue of either species or for any tissues of temperate

white oak and water oak (Connor et al. 1996) or tropical carapa (Connor et al. 1998) and guarea (Connor and Bonner 1998). Therefore, we conclude that for these species lipid peroxidation before desiccation-induced viability loss is not a marker of declining seed viability.

While the strong relationship between enthalpy/onset data from the embryo and cotyledon tissues and loss of viability is not unexpected, the presence of the sharp, pure water peak (Fig. 2) noted by Pammenter et al. (1991) and Berjak et al. (1992) in all the seed tissues is significant. Cumulative experiments by Vertucci (1989a, 1989b, 1990), Berjak et al. (1990, 1992), Pammenter et al. (1991), and Pammenter and Berjak (1999) have noted the five stages of water present in seeds and have indicated that removal of freezable water does not affect the viability of excised embryos if it is removed quickly. However, the seeds used in our experiments were large, and the period of time required for removal of even the pure water peak from these intact recalcitrant seeds appears too lengthy and stressful to preserve seed viability. The pure water peak remained present throughout the entire drying experiment in the embryo tissue of silver maple (30% MC), white oak (26% MC), and guarea (31% MC), while it disappeared only after 7 days from carapa (31% MC), after 4 days from buckeye (29% MC), and was absent from all but the fresh embryo tissue of water oak acorns (31% MC). Of the species listed, we have had the best success at storing the latter two, buckeye for up to 1 year at –2°C, water oak for 3 years at 4°C. The rapid disappearance of the pure water peak may be indicative of species more intermediate in their tolerance to desiccation. According to Vertucci et al. (1991), it may also indicate the presence of mature axes. They noted that there is more water associated with the sharp peak relative to the broad peak in immature axes; and, in physiologically mature axes, water disappears from the sharp peak at a greater rate than from the broad peak as the axes dry. Based on this definition, the embryos of both silver maple and red buckeye are physiologically mature. However, the tissues of red buckeye are able to dry at a faster rate than those of other recalcitrant species, and this may be indicative of species with seeds of intermediate recalcitrance.

The low MC reached by the embryonic tissues of red buckeye led to the inspection of seed morphology as a possible variant in the temperate recalcitrant seeds studied. It was hypothesized that the embryo positioning in the seed might have an effect on moisture loss from the embryo and on seed deterioration, and that seed morphology may strongly influence the uneven drying rates detected in recalcitrant seeds (Connor et al. 1996, 1998; Connor and Bonner 1998; Pammenter et al. 1998; Tompsett and Pritchard 1998). Using the system devised by Martin (1946), *Quercus* acorns are classified as investing (the embryo is erect and has thick cotyledons that overlap and encase the axis for at least half its length), maple seeds as folded (the embryo has thin, extensively expanded, folded cotyledons), and buckeye as bent (embryo is spatulate in shape but is bent in a jackknife form). This positioning might clarify patterns of embryonic moisture loss. In both oak and maple, the embryo is encased by the cotyledons and may be acting as a moisture sink (Connor et al 1998). Thus the embryo MC remains high as long as the seed coat remains intact. However, once expansion begins and the seed coat is broken, moisture loss is rapid and often fatal. The axis of buckeye seeds is relatively isolated from the cotyledon, and it is questionable if it is functioning as an efficient moisture sink since at times during the desiccation period its MC was up to 18% less than that of the cotyledon tissue (Table 2). It is, however, tightly enclosed in and surrounded by the seed coat which generally remains intact during drying.

In summary, the overall inconsistency of these results leads to the conclusion that we have as yet not clearly identified factors to explain the physiological basis of recalcitrance. The varying biochemical, physiological, and morphological features observed in recalcitrant seeds lacked commonality and resulted in an inability to identify all but the simplest unifying traits.

Acknowledgements We would like to recognize the assistance of Ms. Blossie Boyd; her technical skills and abilities were invaluable throughout the course of these experiments. The authors thank Dr. Eugenia Flores and Dr. John Vozzo for their constructive comments. Trade names are mentioned solely for information; no endorsement by the U.S.D.A. Forest Service is implied.

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