Evaluating desiccation sensitivity of *Quercus rubra* acorns using X-ray image analysis

Rosa C. Goodman, Douglass F. Jacobs, and Robert P. Karrfalt

Abstract: Desiccation of northern red oak (*Quercus rubra* L.) acorns can impact seed viability. We examined use of X-ray image analysis of cotyledon damage in dried acorns to predict germination capacity and seedling vigor. Acorns collected from five half-sib sources were X-rayed before and after drying to one of four moisture content (MC) levels (30%, 25%, 20%, or 15%) or maintained as nondesiccated controls (35%–38% MC). X-ray images were scored qualitatively according to degree of cotyledon–cotyledon and cotyledon–pericarp separation. Following sowing, acorns were evaluated for number of days to reach each of three developmental stages (emergence of radicle, epicotyl, and first leaf flush) and growth for 80 d. Both MC and family significantly affected all variables. The percentage of acorns to reach each developmental stage, as well as final height and root-collar diameter declined with decreasing MC and dropped most notably between 20% and 15% MC. X-ray separation scores more effectively predicted the percentage of acorns to reach each of the first three developmental stages than MC level (R^2 = 0.49–0.63 vs. 0.40–0.59). Our results confirm the recalcitrant nature of northern red oak acorns and demonstrate the potential of X-ray image analysis to provide a rapid and nondestructive means to successful predict acorn viability.

Résumé : La dessiccation des glands du chêne rouge (*Quercus rubra* L.) peut affecter la viabilité des graines. Les auteurs ont étudié l'utilisation de la radiographie des glands desséchés pour observer les dommages aux cotylédons et pour prédire la capacité germinative et la vigueur des semis. Les glands, collectés à partir de cinq provenances uniparentales, ont été radiographiés avant et après avoir été séchés jusqu'à l'un des quatre degrés d'humidité suivants : 30, 25, 20 ou 15 %, ou ils ont été gardés comme témoins non séchés (35–38 % d'humidité). Les radiographies ont été cotées qualitativement selon le degré de séparation entre les cotylédons ou entre le cotylédon et le péricarpe. Après l'ensemencement, les glands ont été évalués sur la base du nombre de jours nécessaires pour atteindre chacun des trois stades de développement (émergence de la radicule, de l'épicotyle et apparition de la première feuille) et de la croissance durant 80 jours. Le degré d'humidité et la provenance ont affecté de façon significative toutes les variables. Le pourcentage de glands à atteindre chaque stade de développement, aussi bien que la hauteur finale et le diamètre final du collet, ont diminué avec la baisse du degré d'humidité et ce pourcentage a chuté de façon la plus marquée entre 20 et 15% d'humidité. Le classement à l'aide de la radiographie a prédit plus efficacement le pourcentage de glands à atteindre chacun des trois premiers stades de développement que le degré d'humidité (R^2 = 0,49–0,63 vs. 0,40–0,59). Leurs résultats confirment la nature récalcitrante des glands du chêne rouge et démontrent le potentiel de la radiographie pour fournir un moyen rapide et non destructif de prédire avec succès la viabilité des glands.

[Traduit par la Rédaction]

Introduction

Current scientific knowledge of propagation requirements for temperate deciduous tree species is much less advanced compared with that of many coniferous species (Wilson and Jacobs 2006). In the United States, nursery-grown hardwood seedlings are becoming increasingly important for high quality timber, conservation, and wildlife habitat and forage (Gardiner et al. 2002; Jacobs et al. 2004; Ross-Davis et al. 2005). Seedling demand often exceeds supply (Gardiner et al. 2002; Michler and Woeste 1999), resulting in pressures on nursery growers to increase supply and efficiency of hardwood seedling production. Maintaining high viability of collected seed represents one potential limitation to ensuring adequate production of high-quality hardwood nursery stock for outplanting.

Seeds that cannot withstand moisture loss without loss of viability are termed recalcitrant (Roberts 1973). Recalcitrant seeds do not undergo maturation drying and are, therefore, metabolically active and sensitive to desiccation (Farrant et al. 1988). This seed type is less studied or understood compared with "orthodox" seed, largely because species exhibiting this trait are less common, particularly in seasonal climates (Tweddle et al. 2003). However, several important temperate genera produce recalcitrant seed, including *Castanea* (Prit-

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chard and Manger 1990), *Aesculus*, some *Acer* spp., and *Quercus* (Bonner 1990). *Quercus*, the oak genus, is further broken down into two subgenera: *Leucobalanus*, the white oaks (germinating soon after dissemination in fall), and *Erythrobalanus*, the red oaks (germinating in spring).

Bonner (1981) stated that seed moisture content (MC) can influence and indicate seed maturity, longevity in storage, and necessary pretreatments. This is especially important for recalcitrant seeds; thus, much of the research on recalcitrant seeds has focused primarily on handling, storage, and resulting viability. For example, Bonner and Vozzo (1987) recommended storing acorns of the red oak group at MC above 30% to ensure high viability. Pritchard (1991) found that northern red oak (*Quercus rubra* L.) germination began to decrease when seed MC dropped below 30% and viability was minimal at MC of 10%–15%. A study on cherrybark oak (*Q. pagoda* Raf.) ascertained that germination declined severely when short-term MC fell below 17% (Sowa and Connor 2003).

Loss of viability is not solely a function of total seed MC. Several processes concurrently influence viability, including embryonic axis MC (Farrant et al. 1988; Pritchard 1991), presence of soluble sugars (Sun et al. 1994), drying conditions (Farrant et al. 1985; Bonner 1990; Pritchard 1991; Liang and Sun 2002), MC before storage (Farmer 1975), and length of storage (Farmer 1975; Bonner and Vozzo 1987; Connor and Bonner 1999; Connor and Sowa 2002; Sowa and Connor 2003). With proper storage, red oak acorns can usually maintain viability for up to 1 year (Connor 2004). Desiccation tolerance can be maintained under proper storage conditions, including both storage container (Bonner and Vozzo 1987) and storage temperature (Bonner 1973; Bonner and Vozzo 1987; Finch-Savage 1998; Connor and Sowa 2002). Imbibing seeds with water before storage (Gosling 1989) and priming acorns with polyethylene glycol (Pritchard 1991) have also been found to increase desiccation tolerance of northern red oak.

A more recent trend in research on recalcitrant seeds has been to study physiological and biochemical responses to desiccation (Berjak et al. 1990; Connor 2004). Fourier transform infrared spectrometry (FT-IR) has proven useful in examining changes in membrane lipid and protein secondary structure during desiccation (Connor and Sowa 2002, 2003; Sowa and Connor 2003). Some findings have determined that desiccation acts to damage recalcitrant seeds by disturbing their metabolism and cellular structure, which occurs at different moisture levels (Farrant et al. 1988; Pammenter and Berjak 2000; Liang and Sun 2002). In a study comparing orthodox soybean (*Glycine max* (L.) Merrill 'Chippewa 64') seeds to recalcitrant northern red oak seeds, Sun et al. (1994) concluded that desiccation tolerance is largely a function of cell membrane stability and may be associated with the accumulation of soluble sugars, namely oligosaccharides, and cytoplasmic vitrification or glass formation in the cell membranes.

Finch-Savage (1998) recognized that recalcitrant seeds can have variable germination and poor seedling emergence in the nursery, which limits the efficiency of tree seedling production. Variable genetics, environmental conditions, and handling can potentially affect the quality of these seeds. Seed lots with high viability can still germinate and emerge poorly because of improper seed treatment (Finch-Savage 1998), and seed handling would benefit from a rapid and nondestructive assessment of seed quality. Myriad tests are available to gauge seed quality including germination rate, seedling growth, accelerated aging, leachate conductivity, tetrazolium staining, and excised embryo (Bonner 1998; Karrfalt 2004). Bonner (1998) asserted that precise evaluation and application of tests is not yet possible because of the wide genetic variability in tree seeds. In addition, many of these tests are destructive and (or) time consuming. Thus, it is important to continue to evaluate new testing alternatives that may improve both precision and efficiency of seed viability testing.

Since seeds of *Quercus* spp. are large and susceptible to dehydration, X-ray images could possibly reveal accurate information about MC and viability. X-raying seeds to evaluate potential viability was investigated in the 1970s (Belcher 1973, 1977; Duffield 1973). For example, Belcher (1973) viewed radiographs of northern red oak acorns lying on their side to determine whether seeds were full or empty, indicating that the seeds were developed or undeveloped, respectively. Since that time, equipment and techniques have advanced. X-ray analysis has been used to determine anatomical maturity and germination capacity of conifer seeds (Sahlen et al. 1995; Shen and Odén 1999). The IDX (incubation (I), drying (D), X-raying (X)) method was used to separate viable and nonviable Scots pine (*Pinus sylvestris* L.) seeds; seeds with low optical density (a light image) were considered viable (Sahlen et al. 1995). More recently, X-ray research has examined seed tissue to assess desiccation tolerance (Sun et al. 1994; Panza et al. 2002). Sun et al. (1994) used X-ray diffraction to study membrane stability and its relationship to desiccation tolerance.

We used X-ray image analysis to examine desiccation damage of whole northern red oak acorns. Since northern red oak acorns are large and prone to water loss, we suspected that cotyledons would shrink with moisture loss and separate at the "critical MC" (i.e., MC below which viability was lost). Thus, our specific study objectives were to determine: (*i*) the effects of MC on northern red oak acorn germination and early seedling development and (*ii*) whether an X-ray image analysis could serve as an accurate test to predict northern red oak acorn viability and early seedling vigor.

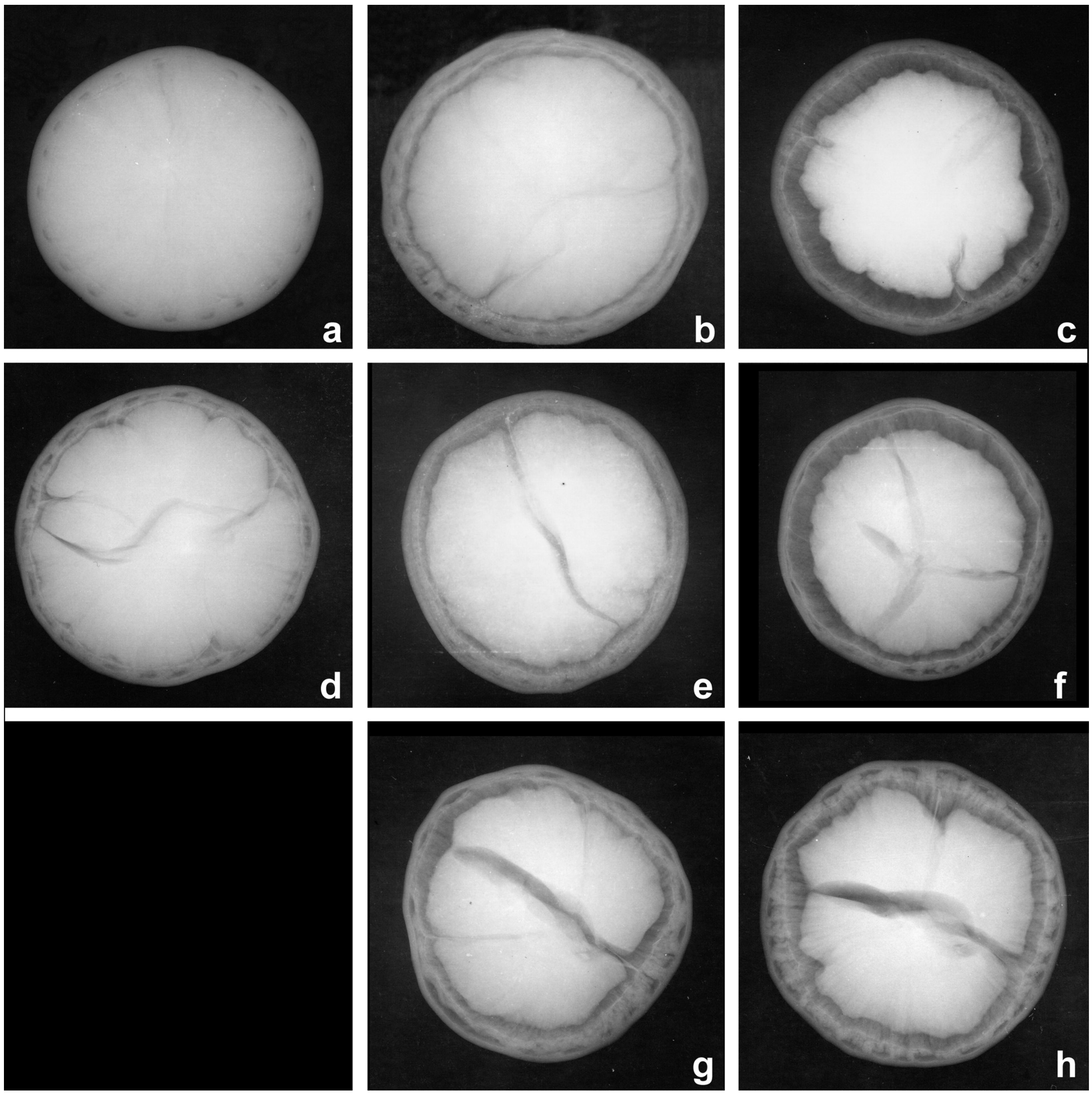
Materials and methods

Seed material

In the Central Hardwood Region of the United States, oaks are a dominant forest component and are planted extensively by landowners (Jacobs et al. 2004). Northern red oak, one of the most widespread oaks and probably the most important ornamental and timber species in the genus (Hardin et al. 2001), was chosen for the study.

Acorns from five trees on or near Purdue University in West Lafayette, Indiana, USA (40°25′N, 86°55′W) were collected in late October and early November 2003, when MC had fallen to or below 40%, a point at which acorns from species in the red oak subgenus exhibit optimal germination (Bonner 1974; Bonner and Vozzo 1987). Seeds were stored in sealed, permeable polyethylene bags at 3 °C (as per

Fig. 1. Examples of X-ray images of acorns with each possible combination of cotyledon-cotyledon (CC) and cotyledon–pericarp (CP) separation score. Scores were qualitatively assigned to designate no, moderate, and severe separation as 1, 2, and 3, respectively. Scores corresponding with the respective images, listed as “CC, CP”, are as follows: (*a*) 1,1; (*b*) 1,2; (*c*) 1,3; (*d*) 2,1; (*e*) 2,2; (*f*) 2,3; (*g*) 3,2; and (*h*) 3,3. No acorn scored 3,1.



Bonner and Vozzo 1987). Debris and insect-infested seeds were removed by water flotation and visual examination.

From the sound seeds, samples were drawn by the recommended procedures of the International Seed Testing Association (ISTA 1993). Briefly, 15 samples of 12 acorns each were randomly selected from each of the five families. One sample of eight acorns was drawn from each family to determine initial MC of whole acorns. Acorns were cut into quarters, roughly 15 g of tissue was weighed to determine fresh mass (FM), dried for 18 h at 103 ± 2 °C, cooled in a desiccant cabinet, and weighed for dry mass (DM). All mass lost (initial FM – DM) was assumed to be lost moisture. MC was expressed on a FM basis. Initial MC (i.e., control MC) varied by family between 35% and 38% MC.

Acorn drying

The entire sample was weighed, and a target fresh mass (FM) was calculated to determine when the sample had reached the target MC using the following formula:

$$\text{Target FM} = \frac{(\text{Initial FM}) \times (100 - \text{Initial MC})}{100 - \text{Target MC}}$$

Table 1. Analysis of variance (ANOVA) results from examination of moisture content (MC) and half-sib family effects on percentage (%) of samples to reach radicle emergence (RE), epicotyl shoot emergence (ES), first leaf flush (1LF), and second leaf flush (2LF); number of days to reach RE, ES, and 1LF; seedling height and root-collar diameter (RCD) at 80 d; and cotyledon–cotyledon (CC) and cotyledon–pericarp (CP) X-ray image separation scores.

	Source of variation ($p > F$)		
Response variable	MC	Family	MC × family
% to RE	0.0001	0.0001	0.1172
% to ES	0.0001	0.0001	0.4948
% to 1LF	0.0001	0.0001	0.3294
% to 2LF	0.0001	0.0001	0.1932
Days to RE	0.0026	0.0001	0.0449
Days to ES	0.0001	0.0001	0.0384
Days to 1LF	0.0001	0.0001	0.0318
Height	0.0001	0.0002	0.0120
RCD	0.0001	0.0487	0.0880
CC	0.0001	0.0001	0.3636
CP	0.0001	0.0001	0.0087

Target MC levels were 30%, 25%, 20%, and 15% MC. All 20 samples (excepting the control) in one replication were prepared and set out to dry in a single day; control samples were X-rayed and subsequently returned to cold storage without intentional desiccation.

Samples were placed on a laboratory bench and dried under ambient conditions (21 °C, 30%–35% relative humidity), until reaching target FM. Drying times ranged from 2 to 19 d depending on treatment. Dried samples were X-rayed, packaged, and replaced in cold storage until all samples in the replication were ready to be transplanted.

X-ray images

X-rays were taken both before and after desiccation using the same procedure; control MC samples were X-rayed only once. Each acorn was labeled and placed cup scar down in a 3 cell × 4 cell indented carton and X-rayed (MX-20, Faxitron X-ray Corp., Wheeling, Illinois, USA) before imposed desiccation. The sample to be X-rayed was placed on a 20.3 cm × 25.4 cm sheet of photographic paper at the bottom (no enlargement) of an X-ray machine (set to 190 s and 28 kV potential for best contrast and clarity). Images were developed in an instant processor and labeled.

Each acorn in the postdesiccation X-ray image was scored qualitatively according to the width of separation relative to the overall size of the seed as follows: cotyledon–cotyledon (CC) separation and cotyledon–pericarp (CP) separation (scored independently) as 1, 2, or 3 (no, moderate, or severe separation, respectively) (Fig. 1). Quantitative guidelines of ((width of separation)/(total width of acorn inside pericarp) × 100%) for 1, 2, and 3 were as follows: <1%–1.5%, 1%–1.5% to 6%–7%, and >6%–7% separation. These quantitative guidelines could not be used absolutely, because length of separation, maximum and average width, and darkness (completeness) of separation were all considered in scoring.

Planting and growth measurements

In total, 900 containers (656 mL each, 6 cm diameter × 25 cm length; D40, Steuwe and Sons, Inc., Corvallis, Oregon, USA) were filled with media (Scotts Metro-Mix® 366, The Scotts Co., Marysville, Ohio, USA) and watered to saturation. On 9–11 January 2004, pericarps were clipped to induce germination (Jones and Brown 1966); acorns were sown in individual pots and placed horizontally with the cut face fully submerged in moist soil. Acorns from each replication were sown in a single day, and all three replications were sown within 3 consecutive d. Greenhouse conditions were set to 16 h photoperiods with 26 °C, 60% relative humidity (light) : 20 °C, 50% relative humidity (dark) and photosynthetic photon flux density of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All pots were watered to saturation every 2 d.

Over the course of 80 d, seedlings were assessed approximately every 2 d for cumulative number of days since sowing (by replication) required to reach three stages of development: radical emergence (RE), epicotyl shoot emergence (ES) (Hanson et al. 1986), and full emergence of the first flush (1LF). After 80 d, height and root-collar diameter (RCD) were measured, and the number of leaf flushes was recorded (second and third leaf flushes as 2LF and 3LF, respectively).

Data analysis

The experiment was established as a randomized complete block design with three replications and a 5 × 5 factorial structure (MC × family). Analysis of variance (ANOVA) was conducted among sample means of each treatment for MC and family variables, and where significant effects were detected in the ANOVA ($p = 0.05$), treatment means were ranked according to Waller–Duncan's multiple range tests at $\alpha = 0.05$. The relationship between CC and CP X-ray scores and growth data was quantified with a goodness-of-fit statistic, the R^2 value. Tests for normality, linearity, and constant variance were performed, and transformations were unnecessary. SAS® software version 8.1 (SAS Institute Inc. 2001) was used for analysis of all data.

Results

Moisture content

Viability and growth of northern red oak seeds proved to be highly dependent on MC (Table 1). Percentages to reach each stage of growth (RE, ES, 1LF, and 2LF) were reduced as MC decreased, declining steadily throughout the decreasing MC level continuum, with the sharpest drop between 20% and 15% MC (Fig. 2). At each MC level, percentages to reach each stage of growth decreased through the four growth stages (RE to 2LF); the greatest decrease between percentages to reach subsequent growth phases occurred between 1LF and 2LF. MC significantly influenced the number of days needed to reach each of the growth stages (Table 1); a greater number of days were required for samples to reach each growth stage as MC level decreased (Fig. 3). MC significantly influenced the final size of surviving seedlings at 80 d (Table 1); both height and RCD were reduced at lower MC levels (Fig. 4).

X-ray image analysis

Separation scores determined from X-ray images demonstrated a clear association with germination rates and initial seedling vigor. Analyzed separately, both CC and CP separation scores exhibited good R^2 values for predicting growth

Fig. 2. Mean percentage (±SE) of northern red oak acorns to reach each growth stage within 80 d as influenced by moisture content (MC). MC of the control treatment (C) ranged from 35% to 38%. Growth stages were radicle emergence (RE), epicotyl shoot emergence (ES), and full emergence of the first (1LF) or second (2LF) leaf flush.

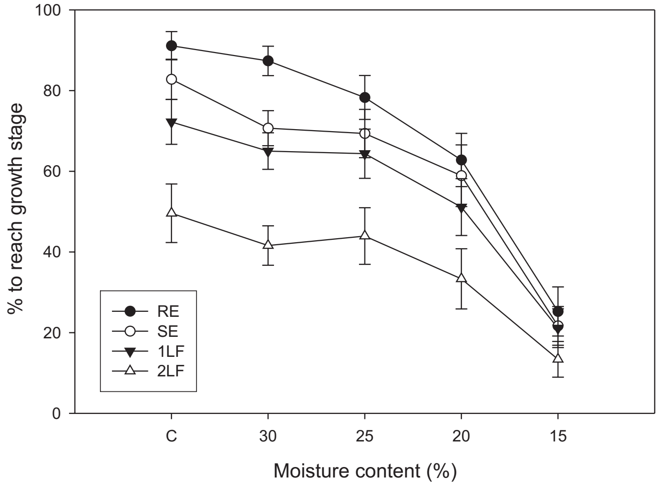


Fig. 3. Mean number of days (±SE) for northern red oak acorns to reach each growth stage within 80 d as influenced by moisture content. MC of the control treatment (C) ranged from 35% to 38%. Growth stages were radicle emergence (RE), epicotyl shoot emergence (ES), and full emergence of the first (1LF) or second (2LF) leaf flush.

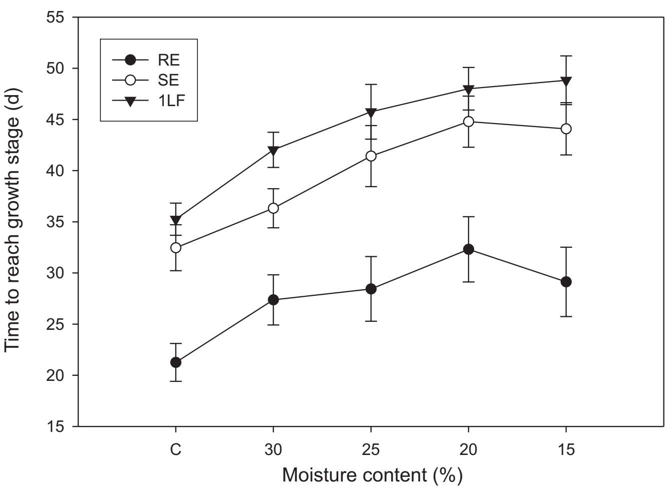
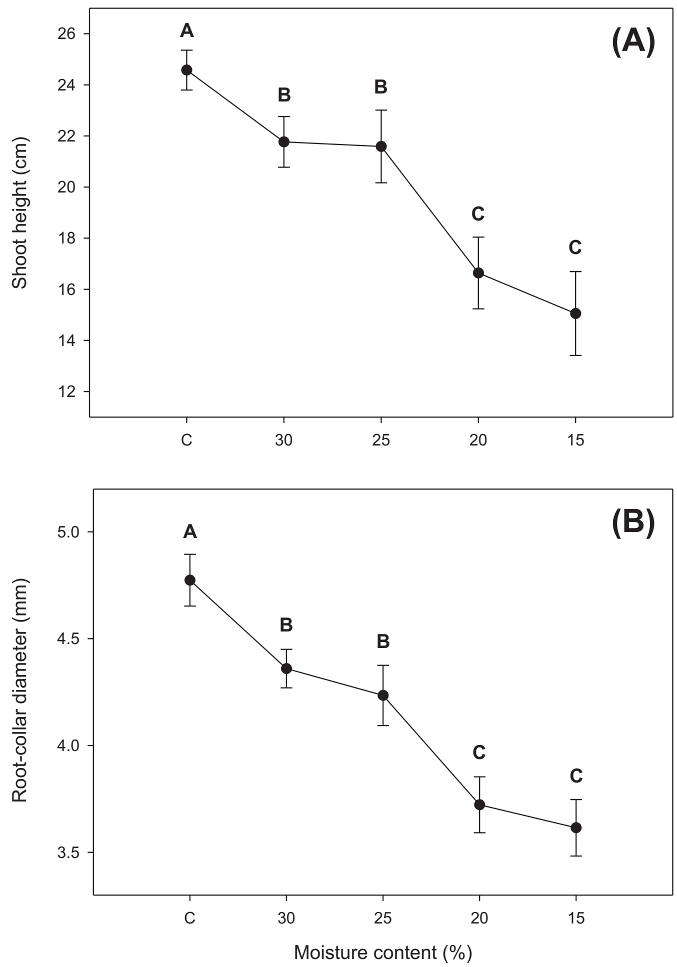


Fig. 4. Mean shoot height and root-collar diameter (RCD) (±SE) of northern red oak seedlings after 80 d as influenced by moisture content. Moisture content (MC) of the control treatment (C) ranged from 35% to 38%. For either shoot height or RCD, treatments with similar letter groupings did not differ at α = 0.05.



measurements, but averaging the two separation scores (average X-ray score, AXS) yielded the highest R^2 values (Table 2). The relationships between AXS and all percentages to reach each growth stage were stronger when fitted to binomial than to linear regression trend lines. Percentages to reach each growth stage declined with increasing AXS score, exhibiting moderately high R^2 values for RE, decreasing slightly for SE and 1LF, and falling noticeably for 2LF (Table 2, Fig. 5). A greater number of days were needed to reach each growth stage as AXS increased, but R^2 values were lower than that for percentage to reach each growth stage (data not shown). Similar trends were observed when examining the relationship between growth measurements and MC. However, R^2 values for growth and AXS were higher than those between growth and MC for all variables analyzed (Table 2).

Family variation

Half-sib families exhibited significant differences in growth within the same MC treatments (Table 1). All variables were significant for the family main effect; number of days to the first three stages of development (RE, ES, and 1LF), height, and CP separation score also exhibited significant MC × family interactions.

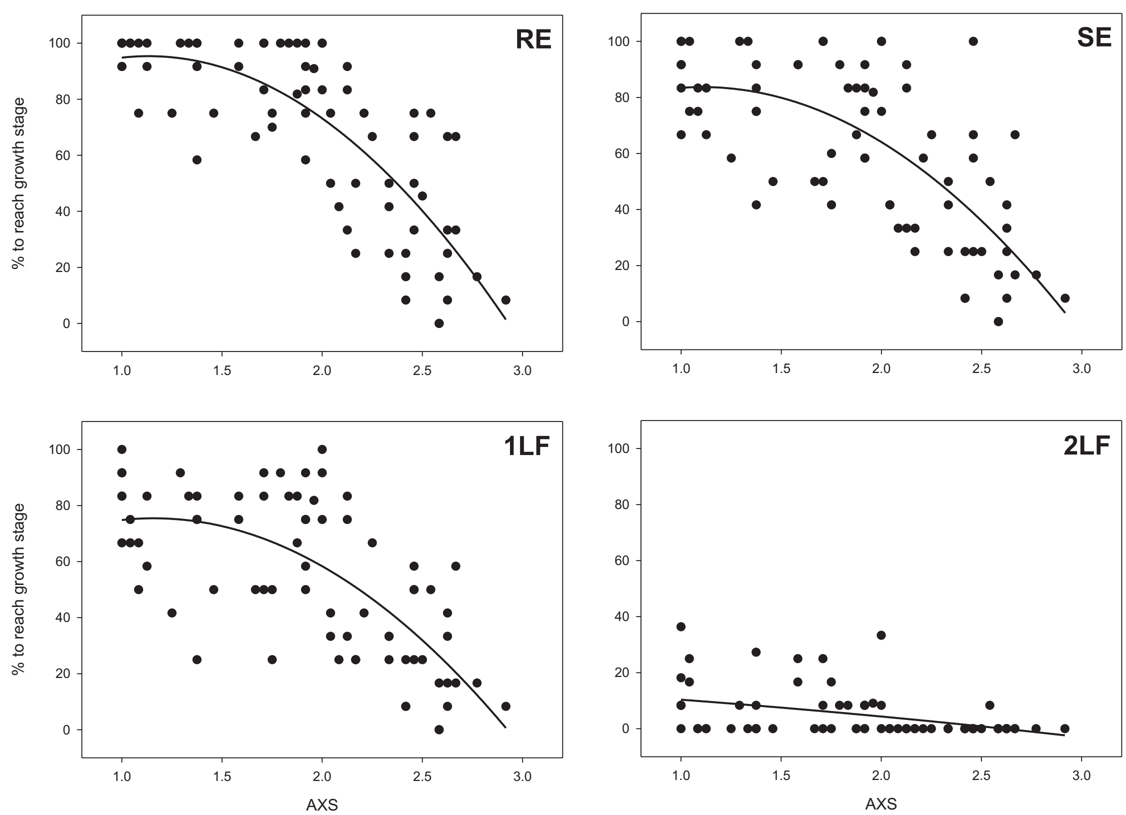
Families 2, 4, and 5 displayed higher percentages to reach RE for control and 30% MC compared with families 1 and 3 (Fig. 6). Family 1 percentage to reach RE began to decline most rapidly after 25%, but family 5 exhibited very high RE until after 20% MC (Fig. 6). Similar results were seen in all other stages measured. Families that exhibited lower percentage RE also required a greater number of days to reach RE; analogous relationships were seen with all other growth stages (data not shown).

Table 2. Polynomial regression equations and R^2 values for mean percentage of acorns to reach each growth stage versus average X-ray score (AXS), and R^2 values for cotyledon–cotyledon (CC) or cotyledon–pericarp (CP) X-ray image separation scores and moisture content (MC).

		Goodness-of-fit statistic (R^2)			
Growth stage	AXS regression equation	AXS	CC	CP	MC
RE	$y = -29.56x^2 + 66.99x + 57.41$	0.6335	0.5385	0.5959	0.5883
SE	$y = -24.59x^2 + 54.38x + 53.62$	0.5021	0.4365	0.4598	0.4555
1LF	$y = -24.24x^2 + 56.23x + 42.84$	0.4863	0.4234	0.4404	0.4001
2LF	$y = -0.69x^2 - 3.95x + 14.98$	0.1724	0.1528	0.1737	0.1063

Note: Growth stages were radicle emergence (RE), epicotyl shoot emergence (ES), and full emergence of the first (1LF) or second (2LF) leaf flush. R^2 values for percentages of samples to reach each growth stage were consistently higher for the dependent variable AXS compared with MC.

Fig. 5. Mean percentage of acorns to reach each growth stage versus average X-ray score (AXS). Growth stages were radicle emergence (RE), epicotyl shoot emergence (ES), and full emergence of the first (1LF) or second (2LF) leaf flush. AXS represents mean score from cotyledon–cotyledon (CC) and cotyledon–pericarp (CP) separation where no, moderate, and severe separation were designated as 1, 2, and 3, respectively. Regression equations are given in Table 2.



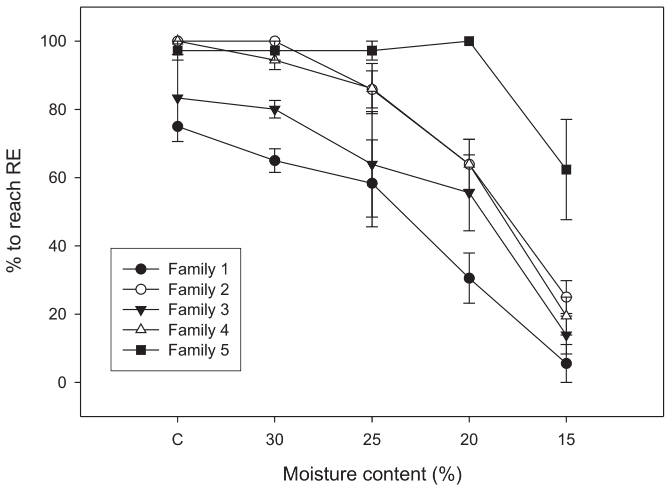
AXS of each family increased with decreasing MC level. The same families with higher percentage RE and fewer number of days to RE also resulted in lower than average AXS. Percentage to reach RE graphed against AXS showed a similar pattern of differentiation among families as RE graphed against MC, but the shapes of the AXS curves displayed fewer irregularities (data not shown).

Discussion

Moisture content

Our study results confirmed the recalcitrant nature of northern red oak seeds, exemplified by decreasing viability as desiccation increased (Figs. 2–4). While a critical MC (i.e., a precise point at which seeds lost viability) could not be determined, all variables declined steadily with decreasing MC, declined most drastically between 20% and 15% MC, and exhibited very poor viability at 15% MC. Though little previous research has examined the effects of MC on acorn viability of northern red oak, these results concur with the findings of published studies on other oak species. For example, Sowa and Connor (2003) found that cherrybark oak seeds exhibited close to 100% germination until MC reached 20%; germination dropped rapidly when MC decreased below 20%, and viability was almost completely lost near 10% MC (year 1). Bonner (1996) found that viability of water oak (*Q. nigra* L.) acorns also declined steadily as MC decreased and almost all viability was lost between 10% and 15% MC.

Fig. 6. Mean percentage (±SE) of northern red oak acorns from each family (1–5) to reach radicle emergence (RE) within 80 d as influenced by moisture content (MC). MC of the control treatment (C) ranged from 35% to 38%.



In dealing with recalcitrant seed, Pammenter and Berjak (2000) also concluded that a "critical water content" did not exist. However, refining MC determination specifically to the embryo (rather than to the whole acorn) may help to isolate a more accurate MC at which acorn viability is lost. For example, Finch-Savage (1998) found that oaks can tolerate 40%–45% embryo MC without loss of viability. In our study, if critical MC of the embryonic tissue explained viability and growth performances, then it is possible that anatomical differences in the acorns, or other unobserved differences, may have contributed to some distinction between performances among families of the same (whole acorn) MC level. Variations in shape, size, and pericarp thickness, which were evident among the families studied, may have caused the embryos of some acorns to dry to lower MC levels than that of other samples, while whole seeds were drying to a target MC.

In addition to differences between MC of the whole seed and MC of the embryo, recent research offers an alternative explanation for loss of viability during desiccation, which emphasizes the importance of drying rate. According to Liang and Sun (2002) two types of damage occur during desiccation: (*i*) mechanical stress attributed to the loss of water cellular volume, and (*ii*) physiochemical damage of tissues resulting from an unregulated metabolism. Under normal slow-drying conditions, it is the second type that kills recalcitrant seeds (Pammenter and Berjak 2000). Slow drying can cause an accumulation of detrimental effects that occur at intermediate MC, while rapid drying results in less accumulation of damage, and lower MC can be tolerated (Pammenter and Berjak 2000). In this case, variation in seed size between samples may have also required some samples to undergo longer drying times, spent at intermediate MC where these detrimental effects occur. Bonner (1996) also found that water oak acorns exhibited lower germination as a result of longer drying times; however, the differences were minimal at the lower drying temperature of 27 °C. Comparatively, the effect of drying rate in our study may have been even less influential given our drying temperature (21 °C).

Unlike previous studies, the majority of which only considered germination, we observed seedling growth to the second leaf flush. This additional information is important when interpreting results of desiccation damage, because we found that many seeds that germinated did not develop into vigorous seedlings (Fig. 4). Some seeds produced radicles, epicotyl shoots, and even the first leaf flush without developing viable root systems to support independent growth (i.e., hardened radicles with no fine roots or growing root apices); cotyledon reserves alone were likely responsible for growth. Predicting growth to the first three growth stages (i.e., RE, ES, and 1LF) was moderately reliable using AXS, CC, CP, or MC, though less so for 2LF (Table 2; Fig. 5).

Family variation

Viability and early growth was influenced by MC and likely by drying rate, days spent drying, and initial MC; however, no single factor explained loss of viability and subsequent seedling performance. Given the significance of family (Table 1; Fig. 6) and, in several cases, family × MC interactive effects (Table 1), genetic variability may be a very important factor in desiccation tolerance of oak seed. Other studies have also found considerable variability in desiccation tolerance within a species (Bonner and Vozzo 1987; Farrant et al. 1988; Bonner 1998; Pammenter and Berjak 2000). Bonner (1998) asserted that genetic variation was expressed through variable maturity and dormancy. Seeds demonstrated increasing sensitivity to desiccation as the seed developed (Farrant et al. 1988); those in a more advanced stage of germination lost viability at higher MC levels (i.e., less desiccation tolerant) (Farrant et al. 1985). Along with environmental influences, some individual trees may be genetically "superior" in terms of storability, desiccation tolerance, and viability. This suggests the importance of selecting or establishing superior seed stock to help improve seed viability and seedling performance.

X-ray image analysis

Regardless of how and why recalcitrant seeds lose viability, for the purpose of successful and efficient seedling propagation, it is critical to detect when seeds have lost viability and (or) will exhibit reduced vigor following germination. The test we proposed and studied, X-ray image analysis, has shown potential for seed quality assessment of many species. For example, Scots pine seeds have been sorted into well- and poorly developed seeds by examining embryo size in an X-ray image (Shen and Odén 1999). X-raying seeds to detect damage, embryo condition, and (or) whether the seeds were full or empty, has been standardized and employed for many coniferous tree species (Gao 1998; Shen and Odén 1999), agricultural crops (Verma et at. 1975), and wild plant species (Linington et al. 1995). As mentioned previously, seed tests used with agricultural and coniferous species are often less effective when applied to seeds of broadleaf tree species, especially for recalcitrant seeds. One study on oriental beechnuts (*Fagus orientalis* Lipisky), a recalcitrant seed, used X-ray images to separate nonviable seeds from viable seeds, where seeds with clean and intact cotyledons were considered viable (Soltani et al. 2003). Initial X-ray studies on whole acorns (Duffield 1973; Belcher 1973, 1977) have not adequately addressed the problem of recalcitrance.

A study on English oak (*Quercus robur* L.) acorns attempted to evaluate the use of the X-raying method as a test for seed viability and to standardize techniques in Hrvatska and Yugoslavia (Borzan et al. 1990). X-raying methods were similar to our study, except acorns were laid on their sides, rather than placed vertically. From this angle, it was possible to detect damage from insects and desiccation, which appeared as a black gap between the cotyledon and pericarp. By classifying these seeds as nonviable, the X-ray method predicted germination fairly accurately but tended to project slightly higher than actual germination percentages. In comparison, we also found that CP separation effectively predicted germination, but averaging both CC and CP yielded more accurate results (Table 2). Furthermore, to account for acorn sensitivity to MC, our X-ray image analysis scored desiccation damage as moderate (2) or severe (3) (Fig. 1). Nonetheless, the methods of Borzan et al. (1990) were at least as accurate as several other seed tests and showed promise as a reliable method of testing seeds of *Quercus* spp.

While recalcitrance is a multifaceted behavior that cannot be captured entirely in an X-ray image, this study demonstrated a prominent relationship between damage assessed in the X-ray image analysis and both seed viability and seedling performance (Table 2; Fig. 5). This relationship was stronger than that with MC (Table 2). This reflects the premise that many factors (in addition to MC) influence loss of viability. Seed morphological condition likely reflected a cumulative result of all factors influencing loss of viability; hence, conditions seen in the X-ray images may have provided a more comprehensive representation of damages accrued during desiccation. Another possible explanation for the success of X-ray image analysis is that it may have reduced family variation. Families that remained viable and performed well even after being dried to low MC levels also exhibited less separation (i.e., less damage) in the X-ray images. Our two interpretations of why X-ray image analysis worked well are likely compatible. Responses following desiccation are likely under a great deal of genetic control and those responses are, in turn, expressed morphologically.

Even with the strong predictive ability of X-ray image analysis scores for seed viability and seedling performance, if a great degree of accuracy is required, X-ray image analysis should be used only as a supplemental method to provide insight into seed viability and morphological conditions. To more efficiently relate seed X-ray images to performance, a digital image analysis system to quantify damage and (or) an appropriate quantitative formula relating CC and CP separation to performance could be developed.

Conclusions

While further research is needed to fully understand and properly manage recalcitrant seeds, it is clear that, given the direct relationship between MC and seed viability and seedling performance, proper handling and storage of northern red oak acorns is essential. X-ray image analysis of acorns demonstrated a strong ability to predict seed viability; stronger, in fact, than MC. This suggests that an X-ray image analysis has potential to be used as a rapid and nondestructive test to predict seed viability and seedling performance for seeds of northern red oak and, likely, other species in the red oak grouping. With continued technological improvements, X-ray image analysis may eventually be applied to large-scale seed management operations and, perhaps immediately, in other small-scale research projects where assessment of seed viability is required.

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