Using X-Ray Image Analysis to Assess the Viability of Northern Red Oak Acorns: Implications for Seed Handlers

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Abstract: This paper discusses the potential to use X-ray image analysis as a rapid and nondestructive test of viability of northern red oak (*Quercus rubra L.*) acorns and the methodology to do so. Acorns are sensitive to desiccation and lose viability as moisture content (MC) decreases, so we examined X-ray images for cotyledon damage in dried acorns to predict seed viability and early seedling growth. When compared to greenhouse growth measurements, the X-ray image analysis proved to be related to seed germination and early seedling growth, even more so than MC. These results suggest that X-ray image analysis may provide a more accurate test for acorn viability than other popular tests. This paper describes how to bring this proposed seed test into practice with the hope of improving nursery efficiency and quality of oak seedlings.

Keywords: acorn, cotyledon, desiccation, *Quercus rubra,* recalcitrant, red oak, X-ray

Introduction

Hardwood plantings are becoming increasingly popular for timber production, conservation, and wildlife benefits (Gardiner and others 2002; Jacobs and others 2004; Ross-Davis and others 2005). In fact, demand for hardwood seedlings often exceeds supply (Michler and Woeste 1999; Gardiner and others 2002). This trend puts pressure on nursery suppliers to increase efficiency in production of hardwood seedlings. Obtaining and maintaining seedlots of high viability is a critical first step to ensure and improve nursery production. Unfortunately, scientific knowledge of hardwood seeds is far behind that of coniferous and agricultural seeds.

The seeds of oaks (*Quercus* spp.) are particularly difficult to store and manage. Hence, they have been termed "recalcitrant" seeds, meaning that they cannot withstand moisture loss without loss of viability (Roberts 1973). Recalcitrant seeds are metabolically active and sensitive to desiccation because, unlike orthodox seeds, they do not undergo maturation drying (Farrant and others 1988). Species with seeds of this classification are in the vast minority, but include the following temperate genera: chestnuts (*Castanea* spp., Pritchard and Manger 1990), buckeyes (*Aesculus* spp.), some maples (*Ater* spp.), and oaks (Bonner 1990). The oak genus is further broken down into two subgenera: the white oaks (*Leucobalanus),* which do not experience any dormancy and germinate in fall, and the red or black oaks *(Erythrobalanus),* which do undergo fall dormancy and do not germinate until the spring.

Methods of seed collection and storage are very important in maintaining high viability of recalcitrant seeds. Seed moisture content (MC) can influence and indicate seed maturity, longevity in storage, and necessity of pretreatments (Bonner 1981). MC is especially critical for recalcitrant seeds, though MC is not the only factor affecting the viability of recalcitrant seeds. Furthermore, recalcitrant seeds must be handled and stored properly between collection and sowing.

Testing seeds for MC and viability is important for efficient nursery production. Currently, several tests are available to evaluate seed quality, including germination rate, seedling growth, accelerated aging, leachate conductivity, tetrazolium staining, and excised embryo (Bonner 1998; Karrfalt 2004). Unfortunately, many ofthese tests are destructive to the seeds, not entirely accurate, and time consuming to perform. Investigating new techniques to evaluate seed viability may improve the accuracy and efficiency of such testing for oak acorns.

This paper explores the use of X-ray image analysis as a potential test for acorn MC and viability. X-raying seeds to assess insect damage, maturity, and viability is not new to agriculture or forestry. In forestry practices, X-ray analysis has been used to determine maturity and germination capacity of orthodox conifer seeds (Sahlen and others 1995; Shen and Oden 1999). However, the situation is more complicated with recalcitrant seeds, where degrees of desiccation damage must also be assessed. In the 1970s, investigations were undertaken to use X-ray images to determine viability of agricultural and tree seeds (Belcher 1973, 1977; Duffield 1973). Pertaining to recalcitrant seeds, Belcher (1973) determined whether the northern red oak acorns were developed or undeveloped by viewing X-ray images of acorns lying on their side that were either full or empty. Today, X-raying and processing equipment are more advanced, which increases opportunities and ease of using X-ray machines.

Because acorns are large and susceptible to desiccation, an X-ray image analysis may be useful as a rapid and nondestructive test of whole acorns to predict seed viability and seedling performance. Specifically, the degree of separation seen between the cotyledon and pericarp (CP) and between the two cotyledons (CC) may be used to indicate MC and desiccation damage of acorns. This paper describes the background, methodology, and possible application of X-ray image analysis of northern red oak acorns as per Goodman and others (2005).

X-Raying Procedure

To examine desiccation damage and potential viability of acorns, the acorns should be analyzed individually by the following procedure. To obtain a clear view of the cotyledons in the X-ray image, acorns should be arranged vertically (cup scar down) in the X-ray machine. An indented Styrofoam carton works well to keep the acorns in an upright position (figure 1), although any nondense material of uniform thickness should work. Acorns are large enough that magnification is not necessary in the X-ray images; the container of acorns can be placed directly on top of the photographic paper on the bottom of the X-ray machine (figure 2). X-ray images of acorns should show clear contrasts between fully hydrated cotyledons (white) and empty space (black), so a balance must be found between exposure time and intensity. For northern red oak acorns in a Faxitron X-ray machine (MX-20, Faxitron X-ray Corporation, Wheeling, IL, USA), 190 seconds and 28 kilovolt potential (kVp) were found to yield the best images (Goodman and others 2005). Optimal settings are dependent upon the size of the acorn and X-ray machine being used. The photographic paper can be processed, viewed, and labeled immediately (figure 3).

X-Ray Image Analysis

In the X-ray images, the cotyledons of healthy, nondesiccated acorns appear as a solid or nearly solid white

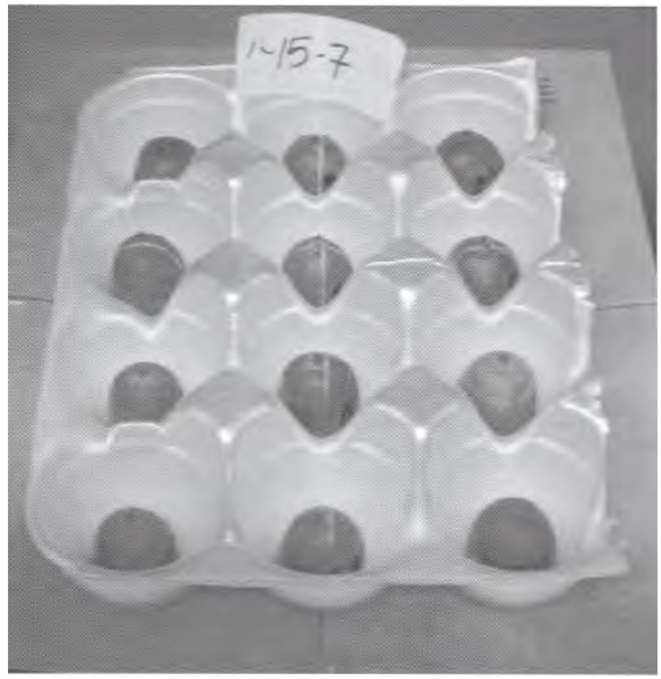

Figure 1—A sample of acorns, arranged vertically in an indented carton, ready to be X-rayed.

Figure 2—Indented carton on top of photographic paper on bottom of X-ray machine.

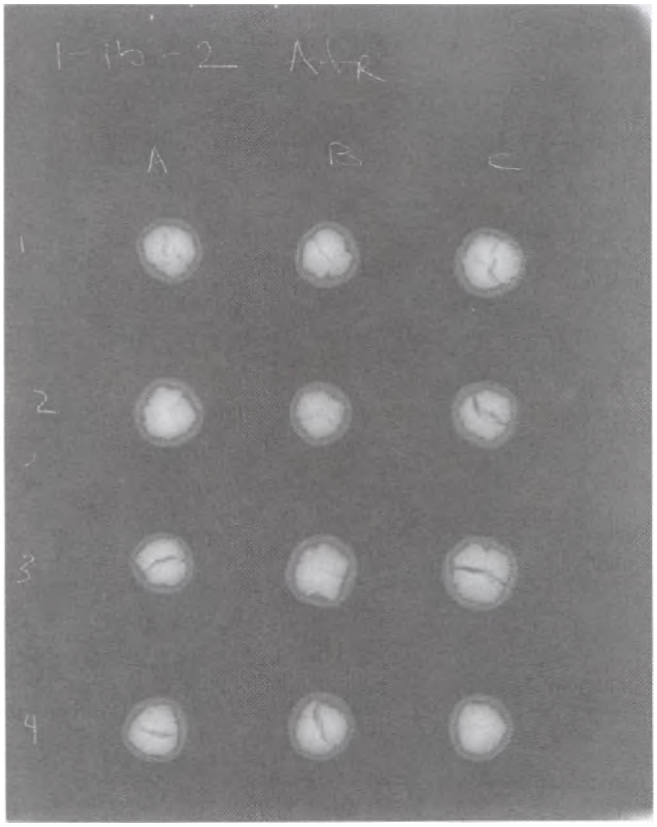

Figure 3—An example of a developed and labeled X-ray image.

image filling the interior of the pericarp. However, lines of separation between the two cotyledons or between the cotyledon and pericarp indicate that the cotyledons have desiccated and shriveled.

X-ray images can be qualitatively scored to assess the desiccation damage of whole acorns by the following technique proposed by Goodman and others (2005). The CC separation and CP separation should be scored individually, that is, 1, 2, or 3 as no, moderate, or severe separation, respectively (figure 4). Quantitative guidelines, (width of separation)/(total width of acorn inside pericarp) $\times$ 100 percent, were as follows: < 1 to 1.5 percent, 1 to 1.5 percent to 6 to 7 percent, and > 6 to 7 percent for 1, 2, and 3, respectively. However, these guidelines could not be used absolutely because the length, maximum and average width, and darkness (completeness) of separation should all be considered when assessing damage. Averaging the two X-ray image scores CC and CP (Average X-ray Score = AXS) was found to show the strongest correlation to acorn moisture content and seedling performance (Goodman and others 2005).

Findings and Discussion

When results for X-ray image scores, MC, seed germination, and seedling growth measurements were analyzed, X-ray images scores showed promise to be useful to predict seed viability and early growth (Goodman and others 2005). Percentages to reach each growth stage and final seedling size decreased as MC decreased and as X-ray scores increased (that is, desiccation damage increased). The opposite trend was seen for the number of days needed to reach each growth stage and X-ray image separation scores (that is, more damage), which both increased as MC decreased. When growth measurements were compared directly to AXS, we found that the AXS was a better predictor of seed viability and early seedling growth than MC.

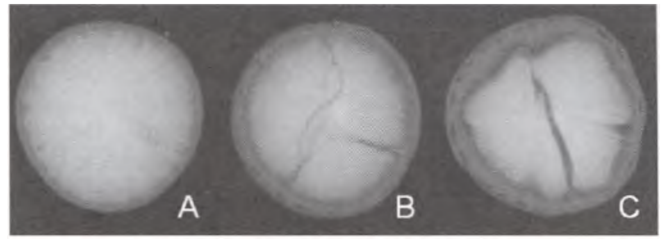

Figure 4—Examples of X-ray images of acorns with three of the nine possible combinations of cotyledon-cotyledon (CC) and cotyledon-pericarp (CP) separation scores. Acorns were qualitatively scored 1, 2, or 3 to designate no, moderate, or severe separation, respectively. Scores corresponding to images, listed as "CC, CP," are as follows: A) 1,1; B) 2,2: and C) 3,3.

This study confirmed that northern red oak acorn viability is very negatively affected by decreasing MC. Hence, acorns should be handled and stored with care in order to avoid desiccation damage. Because viability is highly variable, acorns should be evaluated for MC and viability during the seed handling process to help increase production efficiency. We found that X-ray image analysis of whole acorns predicted seed viability and early seedling performance moderately well, and the relationship was stronger than with MC.

Acorn viability is not solely a function of MC. Many factors have been found to affect recalcitrant seed viability including embryonic MC (Farrant and others 1988; Pritchard 1991), presence of soluble sugars (Sun and others 1994), drying conditions (Farrant and others 1985; Bonner 1990; Pritchard 1991; Liang and Sun 2002), MC before storage, and length of storage (Bonner and Vozzo 1987; Connor and Bonner 1999; Connor and Sowa 2002; Sowa and Connor 2003). While any combination of these factors may affect recalcitrant seed viability, we think X-ray image analysis was successful because it allowed us to view the internal seed morphological conditions that resulted from a probable combination of deleterious effects.

Application

We believe X-ray image analysis may be used as a rapid and nondestructive test of acorn viability and seedling performance. It may be a more accurate test than other tests, for example, MC, cut, or float tests. Because the test yields immediate results, it may be beneficial to X-ray seeds during several stages of the seed-handling process. For example, it could be useful to X-ray a sample of acorns before painstakingly collecting many more acorns from a natural stand or before purchasing a batch of acorns brought to a nursery. Since X-raying is nondestructive, it may be an especially useful test of viability of scarce or valuable lots of acorns.

We hope that the proposed technique will be used in small-scale research projects and nursery operations in the near future. If X-ray image analysis proves to be useful in these situations, it may be beneficial to create a digital image analysis system to quantify the X-ray images and develop an appropriate quantitative formula relating CC and CP separation to performance to improve accuracy and efficiency of this technique. With continued technological advancements, a cost efficient system for X-ray image analysis may eventually be applied to large-scale seed management operations to provide a rapid and nondestructive prediction of acorn viability and seedling performance.

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