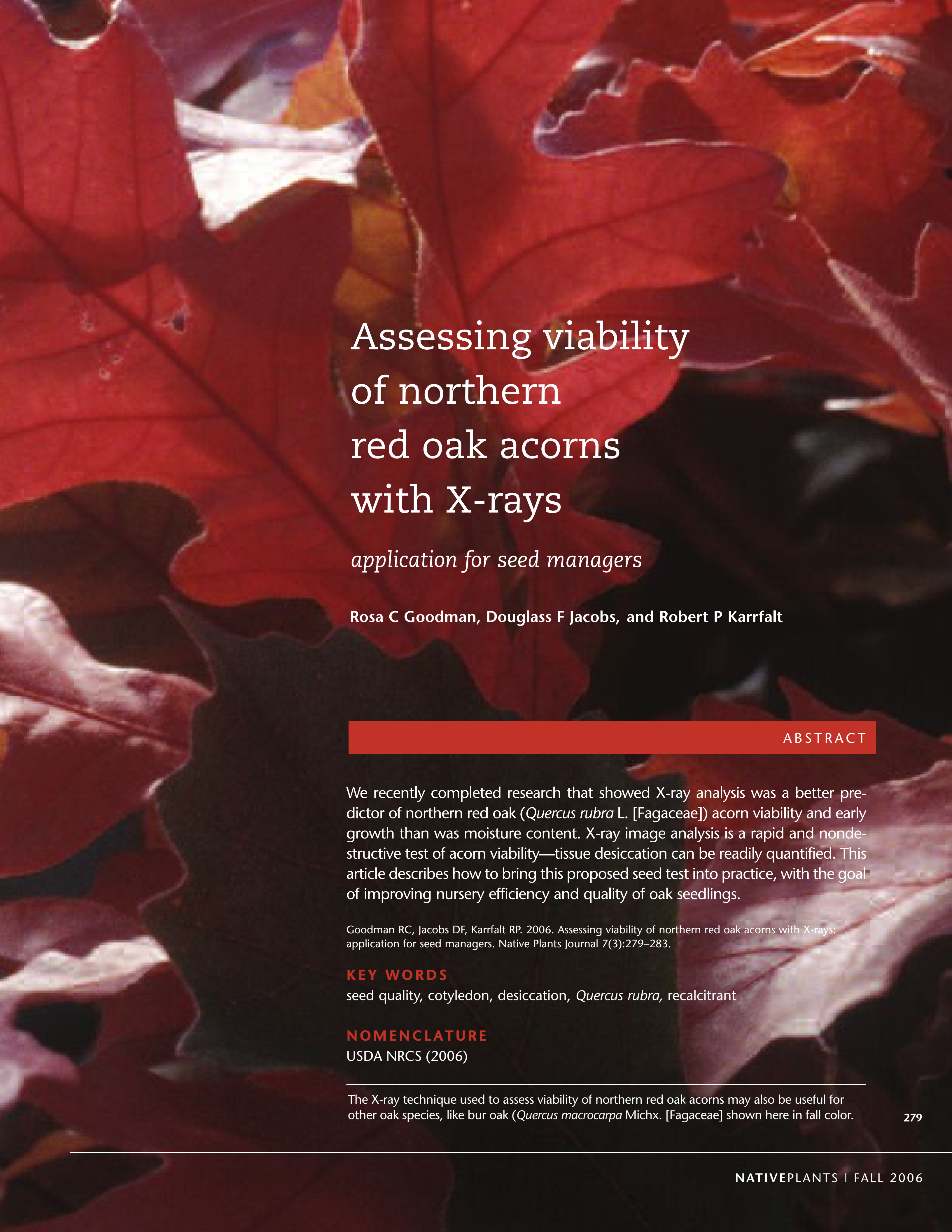

Assessing viability of northern red oak acorns with X-rays

application for seed managers

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

ABSTRACT

We recently completed research that showed X-ray analysis was a better predictor of northern red oak (*Quercus rubra* L. [Fagaceae]) acorn viability and early growth than was moisture content. X-ray image analysis is a rapid and nondestructive test of acorn viability—tissue desiccation can be readily quantified. This article describes how to bring this proposed seed test into practice, with the goal of improving nursery efficiency and quality of oak seedlings.

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seed quality, cotyledon, desiccation, *Quercus rubra,* recalcitrant

NOMENCLATURE
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The X-ray technique used to assess viability of northern red oak acorns may also be useful for other oak species, like bur oak (*Quercus macrocarpa* Michx. [Fagaceae] shown here in fall color.

Obtaining and maintaining seedlots of high viability is a critical first step to ensure nursery production. Unfortunately, oak (*Quercus* spp. L. [Fagaceae]) seeds are difficult to store and manage. As so, they have been termed "recalcitrant," meaning they cannot withstand moisture loss without loss of viability (Roberts 1973). Recalcitrant seeds must be collected, handled, and stored properly until sowing. Seed moisture content can influence and indicate seed maturity, longevity in storage, and necessity of treatments to stimulate germination (Bonner 1981).

Although seed moisture content is relatively easy to measure, tests for viability are often destructive to the seeds, not entirely accurate, and (or) time consuming to perform (Bonner 1998; Karrfalt 2004). One nondestructive test, however, is X-ray analysis. X-raying seeds to assess insect damage, maturity, and viability is not new to agriculture or forestry. In forestry, X-ray analysis has been used for decades to determine maturity and germination capacity of orthodox (not recalcitrant) conifer seeds (Belcher 1973; Duffield 1973; Belcher 1977; Sahlen and others 1995; Shen and Odén 1999). Belcher (1973) determined whether recalcitrant northern red oak (*Q. rubra* L.) acorns were developed or undeveloped by viewing X-ray images of acorns lying on their side, appearing either full or empty. The interpretation of images, however, must be more involved with recalcitrant seeds, where *degrees* of desiccation damage are important. Today, X-raying and processing equipment are more advanced, which increases application opportunities and ease of using X-ray machines.

Because acorns are large and susceptible to desiccation, we experimented to see if X-ray image analysis could be useful as a rapid and nondestructive test of whole acorns to predict seed viability and seedling performance, and how it compared with using moisture content alone as an index of viability (Goodman and others 2005).

We found that when results for X-ray image scores, moisture content, seed germination, and seedling growth measurements were analyzed, X-ray image scores showed promise as useful predictors of seed viability and early growth (Goodman and others 2005). The percentages of seedlings to reach each growth stage and final seedling size decreased as moisture content decreased and as X-ray scores increased (that is, desiccation damage increased). When seedling growth measurements were compared directly with our X-ray analysis, we found that the X-ray technique was a better predictor of seed viability and early seedling growth than moisture content alone (Goodman and others 2005). Differences were seen among families (half-sibs collected from the same mother tree) in relation to all variables tested, including growth stages, final sizes, and X-ray image scores.

Based on those results, we feel that X-ray analysis can provide a powerful and easy way to assess northern red oak acorn viability. With practice, we feel this technique could be used preliminarily by seed collectors to judge whether a group of acorns would be worth collecting and by seed buyers to decide whether a batch of acorns would be worth purchasing. Furthermore, because the entire procedure can be performed in a matter of minutes, it may be beneficial to X-ray seeds during several stages of the seed-handling process to examine the continuing conditions of seeds in storage. Because X-raying is nondestructive, it may be an especially useful test of viability on scarce or valuable acorns.

Below, we outline the technique for X-raying acorns and interpreting the results.

X-RAYING PROCEDURE AND IMAGE ANALYSIS

For our work with northern red oak acorns we used a Faxitron X-ray machine (MX-20, Faxitron X-ray Corporation, Wheeling, Illinois). Because X-ray images of acorns should show clear contrasts between fully hydrated cotyledons (white) and empty space (black), a balance must be found between exposure time and intensity. After some trial and error, we determined that 190 seconds and 28 kilovolt potential (kVp) yielded the best images. Optimal settings will depend on acorn size and the X-ray machine used.

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 non-dense material of uniform thickness should work. Acorns are large enough not to need magnification in the X-ray images, so the container of acorns can be placed directly on top of the photographic paper on the bottom of the X-ray machine (Figure 2). After exposure, the photographic paper should be processed, viewed, and labeled immediately to avoid confusion among samples (Figure 3).

In the X-ray images, cotyledons of healthy, non-desiccated acorns appear as a solid or nearly solid white image filling the interior of the pericarp. Lines of separation between the 2 cotyledons and (or) between the cotyledon and pericarp indicate that the cotyledons have desiccated and shriveled.

We found it useful to describe desiccation damage with a qualitative score based on shrinkage: separation of the 2 cotyledons from each other and separation of the cotyledons from the pericarp were classified as "no," "moderate," and "severe." We used this qualitative scoring system, rather than a strictly quantitative one, because of the irregularities in the acorns, such as the shape of the pericarp, varying thickness of the lines of separation, and indefinite areas of separation (grayish areas indicating incomplete separation through the entire aerial view of the acorn). We measured the

Figure 1. A sample of acorns, arranged vertically in indented carton, ready to be X-rayed. Photo by Rosa C Goodman

Figure 2. Indented carton of acorns on top of photographic paper on bottom of X-ray machine. Photo by Rosa C Goodman

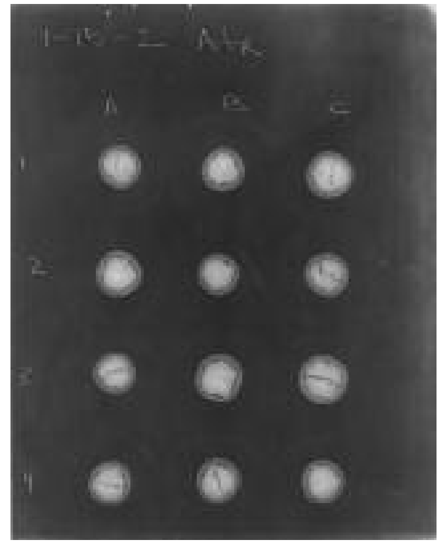

Figure 3. An example of a developed and labeled X-ray image. Photo by Rosa C Goodman

separation widths several times for each parameter (cotyledon to cotyledon separation, cotyledon to pericarp separation, and total distance inside the pericarp). The number of measurements taken depended on the variation observed. The measurements were then averaged (for each parameter on each acorn) and entered in the following formulas to calculate the X-ray score:

(width of cotyledon to cotyledon separation) / (total width inside pericarp) x 100 = X-ray score

and

(width of cotyledon to pericarp separation) / (total width inside pericarp) x 100 = X-ray score

Next, the X-ray score was used to sort 1) cotyledon to cotyledon; and 2) cotyledon to pericarp separation of each acorn into a class. Class 1 has no separation: X-ray score of $< 1\%$ to 1.5%; Class 2 has moderate separation: X-ray score between 1% and 7%; and Class 3 has severe separation: X-ray score $> 6\%$ to 7%. This way, there are 9 possible combinations of cotyledon to cotyledon and cotyledon to pericarp (1,1; 1,2; 1,3; 2,1; 2,2; 2,3; 3,1; 3,2; and 3,3).

See Figure 4 as an example. Though the quantitative guidelines may sound complicated, remember they are *just guidelines*. With some practice you can simply give each acorn a Class 1 (no), 2 (moderate), or 3 (severe) separation score for both the cotyledon to cotyledon and the cotyledon to pericarp separation.

Next, we found that averaging these 2 X-ray scores (1, 2, or 3 from both the cotyledon to cotyledon X-ray score and the cotyledon to pericarp X-ray score) provided the best correlation to acorn moisture content and seedling performance. These average scores can be considered for each seed analyzed or averaged over an entire batch of seeds. If you are X-raying samples to represent an entire batch of seeds, be sure to have a large enough sample size and to select seeds without bias.

In our work, we found that a higher percentage of Class 1 acorns (with little or no separation) germinated, grew faster, developed into viable seedlings, and produced seedlings of larger final size (height and diameter) than seedlings from the other classes of acorns. Many acorns in Class 3 (showing severe damage in the X-rays) did not even germinate; those that did germinate grew slower and (or) died before developing into viable seedlings; and those seedlings that did grow were smaller than those from the other acorn classes. The acorns from Class 2 (with moderate damage) performed in between the other classes of acorns.

OTHER FACTORS TO CONSIDER

Though scoring X-ray images has qualitative components, the procedure leaves very little room for error. For example, if an acorn falls to its side (not demonstrating the cotyledons from an aerial view), the mistake can be readily caught and remedied by simply placing the seed in the proper position and taking another X-ray image. Scoring may vary slightly from viewer to viewer, but variation can be minimized with training and communication. Remember that our scoring guidelines are somewhat subjective because you must consider the length, maximum and average width, and darkness (completeness) of separation when assessing damage. At least, relative changes among a single seedlot over time should be evident. Our proposed scoring method does not have to be fol-

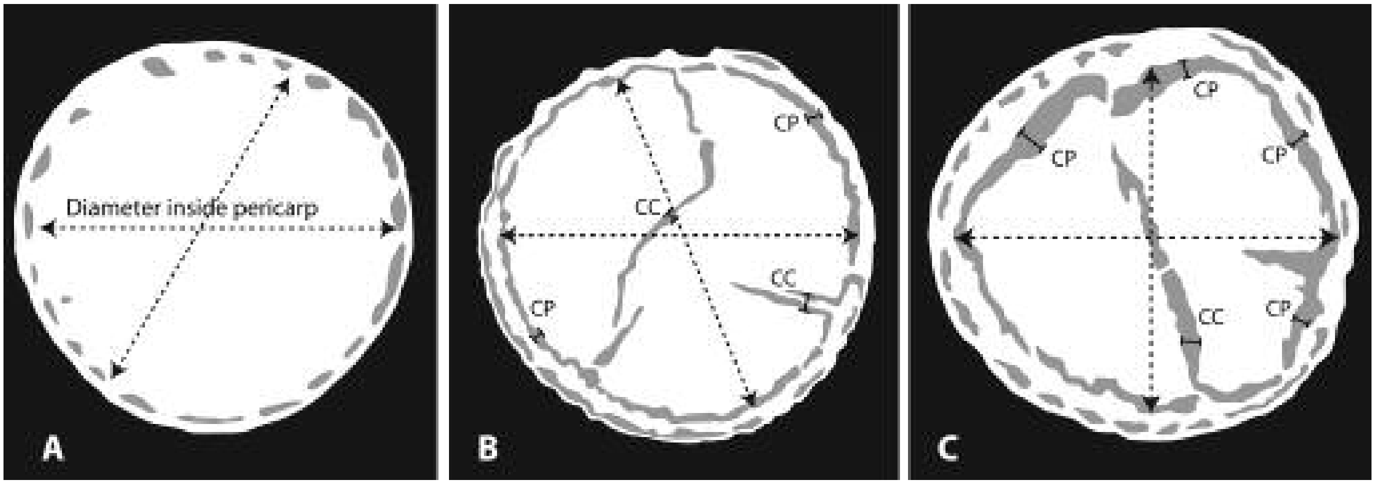

Figure 4. Examples of X-ray images of acorns with 3 of the 9 possible combinations of cotyledon to cotyledon separation (CC) and cotyledon to pericarp separation (CP). CC and CP are measured several times in each acorn; images show examples of where you could measure them. Dotted lines show the diameter inside the pericarp—irregular shaped acorns are measured 2 to 3 times and averaged. Acorns were classified qualitatively as: 1 (no separation); 2 (moderate separation); or 3 (severe separation). In the figure, scores corresponding to images, listed as "CC,CP," are as follows: A) 1,1; B) 2,2; and C) 3,3.

lowed rigidly; seed managers should feel free to adapt the analysis to suit their needs with the time, equipment, and labor resources available.

We observed differences among families (Goodman and others 2005), which means that genetics may play a significant role in seed viability and X-ray image analysis scores. As so, precision of the viability test could be enhanced by grouping seeds by family. Because the average of the X-ray scores was more strongly correlated with seedling growth stages than moisture content, we speculate that X-ray image analysis may reduce some of the variation observed among seed sources. Acorn viability is a function of other factors as well. Although any combination of these factors may affect recalcitrant seed viability, we think that X-ray image analysis was successful because it allowed us to view the internal seed morphological conditions—conditions that were a result of a probable combination of deleterious effects.

Although we examined only northern red oak, we believe this technique would work for other oak species and perhaps with other large recalcitrant seeds as well. Keeping detailed notes of scores and performance over time will improve the diagnostic ability of the technique. It is likely that further refinement of X-ray technologies and corresponding computer graphic imaging will make X-ray testing of seeds increasingly accurate and cost effective in the future.

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