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*Tools and Technology*

# Should We Use the Float Test to Quantify Acorn Viability?

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**ABSTRACT** The float test is the most widely used method to discriminate between viable and nonviable acorns. It provides an objective, simple, rapid, and inexpensive test to inform experiments and management strategies dependent on quantification of viable acorns. However, the accuracy of the float-test method is understudied. To test the accuracy of the float-test method, during autumn of 2013 we collected 300 acorns from white oak (*Quercus alba*), native to our study area within the city limits of Raleigh, North Carolina, USA, and sawtooth oak (*Q. acutissima*), not native to the study area. An untrained observer visually inspected acorns (visual test) to assign viability subjectively and then float-tested the respective acorn. After conducting visual and float tests, we planted the acorns in a test plot protected from predation. In the test plots, 56% of white oak acorns and 60% of sawtooth acorns germinated. Both the float test and visual methods accurately predicted viability in both oak species. However, the visual test (white $R^2 = 0.83$, sawtooth $R^2 = 0.85$) explained more variation in observed germination than the float test explained (white $R^2 = 0.65$, sawtooth $R^2 = 0.70$). Our data indicate the float test, though objective, is less accurate than an untrained observer at predicting the viability of acorns. We tested the potential for the methods to be paired to further improve prediction accuracy and determined the float test provided no additional information to visual inspections. When dissecting or germinating acorns is not possible, our data indicate that visually inspecting acorns is better than float-testing to determine viability. © 2017 The Wildlife Society.

**KEY WORDS** germination, mast, oaks, *Quercus*, regeneration, viability.

Oaks (*Quercus* spp.) are a foundation genus that modulate ecosystem processes in structure and function in many deciduous forest types (Ellison et al. 2005, McShea et al. 2007). Perhaps the most important function of oaks comes through the production of a valuable energy-rich food source (i.e., acorns) for wildlife during autumn and winter seasons, when other energy-rich foods are commonly scarce. Many wildlife species are partially or completely dependent on acorns because of the paucity of other foods available during this time of the year (Martin et al. 1961), with many wildlife populations increasing and decreasing commensurate with acorn production (McShea 2000). Acorns are of broad importance in deciduous forest types; therefore, researchers have raised concern about decreases in oak populations, particularly in the face of rapid climate change (McShea et al. 2007). Oak declines make research evaluating the production of viable acorns for the long-term regeneration and perpetuation of oak species and maintenance of wildlife foods important. Thus, ensuring methods to estimate acorn viability are reliable is necessary with the growing need for related research.

The float test is the most widely used method to rapidly determine acorn viability (Gribko and Jones 1996). It provides simple, inexpensive discrimination between viable and nonviable acorns and does not require observers to follow acorns to germination or destroy acorns by dissection (Schopmeyer 1974, Stockton and Morgan 1979, Bonner and Vozzo 1987). To conduct the float test, acorns are submerged in water and all acorns that float are rejected as nonviable. Although this method has proven effective in rejecting nonviable acorns, Gribko and Jones (1996) reported that as many as 50% of apparently sound acorns are also rejected by the use of the float test. The float test may incorrectly reject viable acorns for 2 reasons: 1) insect damage increases the likelihood of an acorn floating but does not affect its probability of germination in most cases (Hou et al. 2010);

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and 2) arid microclimate decreases the percent of the acorn that is water, making it more likely to float (Bonner and Vozzo 1987). Either scenario could introduce substantial bias when measuring the overall viability rate of acorns. However, Gribko and Jones (1996) determined acorn viability by dissection, which may also be biased in determining acorn viability because it is inherently untestable (i.e., once dissected, the acorn cannot be tested via germination). Thus, further evaluation of the float test is needed.

We sought to test the accuracy of the float-test method to predict acorn viability in 2 oak species, one native to North America and the other native to eastern Asia (i.e., white oak [*Q. alba*] and sawtooth oak [*Q. acutissima*], respectively). We compared the predictive power of the float test with a subjective assignment of acorn viability based on visual inspection. Also, we coupled the 2 assessments of viability to determine whether their accuracy was improved in combination.

## METHODS

In autumn of 2013, we collected 10 acorns from each of 30 white oaks and 30 sawtooth oaks ($n = 600$) in an urban environment. All trees were free from competition (i.e., full sun) and within the city limits of Raleigh, North Carolina, USA. An untrained undergraduate researcher performed a visual assessment to determine viability of each acorn (hereafter, visual test) using acorn characteristics such as casing discoloration and damage (inspected visually), mass (inspected by touch), relative size (inspected visually and by touch), and internal seed development (inspected by touch and chemical senses). When assessing for discoloration and damage, the researcher determined an acorn to be nonviable if its casing was much lighter in color than an average acorn of the target species (Fig. 1) or if it had visual damage such as cracks, chips, or gouges. Additionally, the researcher determined to be nonviable any acorns with dark or discolored cap connection points (Fig. 1). The researcher determined an acorn to be nonviable based on mass assessment if it was much lighter than an average acorn of the target species and based on size if it was much smaller. The researcher determined an acorn to be nonviable based on assessment of the internal seed development if it rattled when shaken (indicating improper seed development). The researcher then placed each acorn in water and determined it to be viable if it sank and nonviable if it floated (hereafter, float test).

When viability predictions from each method were complete, we uniquely marked and planted each acorn during November 2013. We planted acorns in 1 of 2 adjacent 1.2-m × 2.4-m × 20.3-cm raised trays filled with soil (1 tray for each species). Trays were divided into 10.2-cm × 9.8-cm grid cells using nylon twine tied to either side of the tray to track the fate of each individual acorn. We pressed each acorn

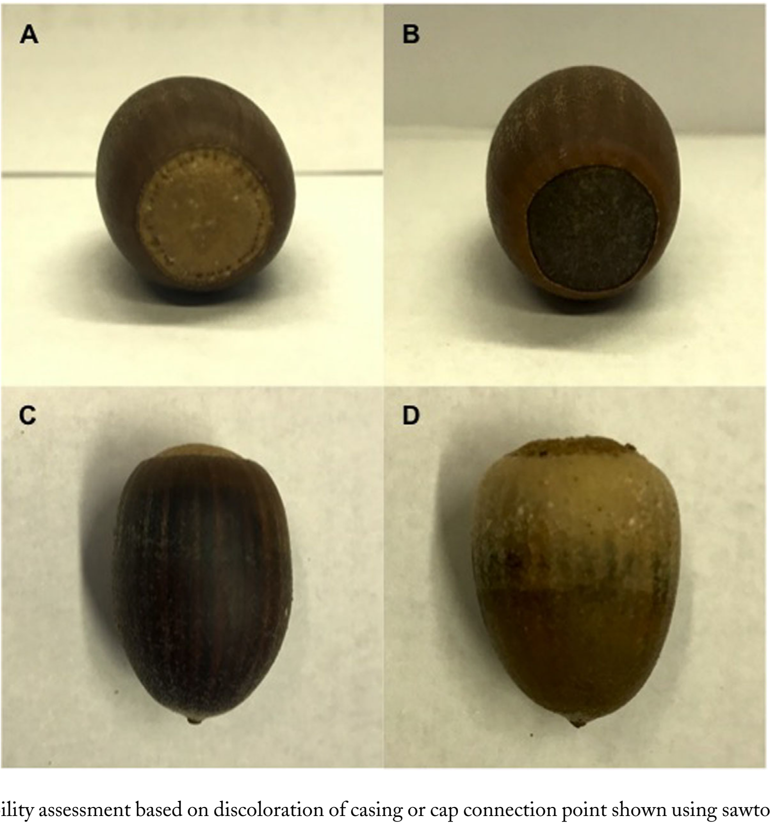

**Figure 1.** Examples of acorn viability assessment based on discoloration of casing or cap connection point shown using sawtooth oak acorns. Viable acorn with brightly colored cap connection point (A). Nonviable acorn with discolored or dark cap connection point (B). Viable acorn with average casing coloration (C). Nonviable acorn with discolored casing (D). Acorns were collected during autumn of 2013, from each of 30 white oaks and 30 sawtooth oaks within the city limits of Raleigh, North Carolina, USA.

into the soil even with the surface within a grid cell to ensure seed to soil contact (Li and Ma 2003). We marked each grid cell uniquely to the respective acorn so the predictions from the visual and float-test methods could be linked to the germination of the specific acorn. We covered the white oak tray with white oak leaves and sawtooth oak tray with sawtooth leaves. We placed both trays under shade trees to simulate their respective natural microenvironment conditions (Li and Ma 2003). We covered the trays with poultry wire cages that were fastened to the outside edge of the tray walls to minimize acorn predation (Shaw 1968). All acorns received water naturally from rainfall events, which were sufficient during this time to provide enough water for germination to occur. We monitored each acorn weekly until it germinated (i.e., the emergence of a visible radicle). Acorns stopped germinating by the end of April (Gómez 2004), so we stopped monitoring at the beginning of May 2014 and assumed acorns that did not germinate were nonviable.

We used binary logistic regressions in JMP (SAS Corporation, Cary, NC, USA) to compare the predictive power of the float-test method and the visual method in predicting acorn viability. We tested 3 models; 1 that used the actual germination of each white oak acorn as the binary response variables (1 = successful germination), 1 that used the actual germination of each sawtooth oak acorn, and 1 with both species pooled. Each model assessed the predictive power of the float test and visual test on actual acorn germination. In each of the single-species models, we controlled for effects of the individual trees by including a categorical variable for individual tree. In the model pooling the species, we included visual-test and float-test predictions as predictor variables to quantify the unique variance that each method explained while controlling for the other.

## RESULTS

For white oak acorns, the float-test method predicted that 61% would germinate, whereas the visual method predicted 57.2% would germinate. The actual percentage of white oak acorns that germinated was 57.2% (162 of 283), excluding 17 acorns that were lost to depredation. The float-test and visual methods misclassified 25 (9%) and 14 (5%) white oak acorns, respectively. For sawtooth oak acorns, the float-test method predicted that 63.7% would germinate, whereas the visual method predicted 60.7% would germinate. The actual percentage of sawtooth oak acorns that germinated was 60.5% (179 of 296), excluding 4 acorns that were lost to depredation. The float-test and visual methods misclassified 21 (7%) and 12 (4%) sawtooth oak acorns, respectively. Overall, the float test misclassified 10 of 237 (4%) acorns that were actually viable and 30 of 341 (9%) acorns that were actually nonviable. The visual test misclassified 10 of 237 (4%) acorns that were actually viable and 11 of 341 (3%) acorns that were actually nonviable. Thus, the float test and the visual test were similar in Type II error, but the visual test improved on the Type I error.

The visual test ($R^2 = 0.83$, $P < 0.001$, $R^2 = 0.85$, $P < 0.001$) was more accurate than the float test ($R^2 = 0.65$, $P < 0.001$, $R^2 = 0.70$, $P < 0.001$) for white oaks and sawtooth oaks, respectively. When testing the unique variation explained by each method across species, the float test ($\chi^2_{575} = 1.7$, $P = 0.19$) did not explain any unique variation compared with that of the visual test ($\chi^2_{575} = 108.1$, $P < 0.001$).

## DISCUSSION

Although the float test predicted acorn viability fairly accurately, greater predictive power may be required to make biologically meaningful inferences. For example, Bonner and Vozzo (1987) demonstrated that site characteristics determined acorn moisture, which significantly affected the accuracy of the float-test method. In this case, improper inferences could be made based on moisture levels in the soil rather than acorn viability itself. They recommended soaking acorns in water for approximately 24 hr before testing, which is not feasible for most studies that do not remove acorns from the site. Further, Gribko and Jones (1996) showed that half of the viable acorns they tested failed the float test and most of these had insect damage that did not affect the apical cotyledon, which must be damaged by the insect to negatively affect germination (Hou et al. 2010). Therefore, because the float test is sensitive to environmental conditions and insect-damaged acorns, the float test is less reliable than the visual test in field-based research.

In contrast, the visual test was based on external acorn characteristics (i.e., insect damage, enzymatic deterioration, seed malformation) rather than moisture-related qualities associated with the float test. Also, the visual test (but not the float test) allows the observer to distinguish between acorns with insect damage near the apical cotyledon and damage to other sectors of the acorn. This could be a key advantage of the visual test over the float test because the sector of the acorn damaged determines the likelihood of viability following insect damage (Hou et al. 2010).

Management of oak-dominated deciduous forests is necessary to maintain wildlife populations dependent on acorns and increase oak regeneration. For managers to make proper management decisions, monitoring acorn viability as accurately as possible is required. The visual test of viability allows managers to conveniently and accurately assess the germination potential of oak acorns. Though it is subjective in nature, the visual test requires little training and potentially removes biases inherent to the float-test method (e.g., environmental conditions such as ground moisture content and discriminating between the sector of insect damage). We would suggest that managers adopting our visual test provide some simple training on average viable acorn appearance, mass, and size for the target species to individuals collecting these data.

Using the visual test to avoid the inconsistencies in the float test may be particularly important when monitoring viable acorn production responses to forest management activities. For example, canopy-reducing treatments have been shown to increase acorn production of individual oaks (Goodrum et al. 1971, Healy 1997, Bellocq et al. 2005, Lombardo and McCarthy 2008), but also may influence environmental characteristics such as moisture in the litter layer of the treated stands (Bréda et al. 1995). Therefore, when comparing acorn production among canopy-reducing treatments, the float test may positively bias the estimate of viable acorns in treatments that increase soil moisture.

Another practical use of visually assessing acorn viability is to preemptively track the production of individual trees before timber harvests so that good and excellent producers can be retained. Healy (1997) and Bellocq et al. (2005) demonstrated that individual trees within a species can vary in their genetic potential to produce acorns, and tree characteristics, canopy position, and microsite conditions are poor predictors of the sometimes orders-of-magnitude disparity in production among individuals (Lashley et al. 2009). Thus, the only way to reliably select trees to be retained is to estimate production of each individual (Lashley et al. 2009). In these cases, the float-test method may lead to retaining the incorrect trees if a tree's viable acorn production is being biased by the tree's microclimate. This is a plausible concern with the float test because microclimate affects acorn moisture and acorn moisture affects the reliability of the float test (Bonner and Vozzo 1987).

When monitoring viable acorn production where removing acorns from the site is not feasible, we suggest using the visual test for discriminating viable and nonviable acorns. Though subjective by nature, the visual method outperformed the float-test method, even with an untrained observer. Moreover, visual inspections do not require a water source, making it more convenient in remote field studies.

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