Test of the Float Method of Assessing Northern Red Oak Acorn Condition

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The float method of assessing the condition of acorns and other tree seeds is widely used, yet its efficacy has not been rigorously studied. In this study, 18,334 northern red oak (Quercus rubra *L.) acorns collected from 2 sites in West Virginia were tested using the float method and then dissected to assess true condition. Immersion in water was found to be a reliable means of identifying insect-infested, diseased, and otherwise damaged northern red oak acorns. It was most effective in identifying undeveloped or aborted acorns and those infested by the stony cell gall wasp* (Callirhytis fructosa *Weld.), fly larvae (Drosophilidae, Psychodidae, and/or Anthomyiidae), and lepidopterous larvae-the filbertworm* (Melissopus latiferreanus *Walsingham) and acorn moth* (Valentinia glandulella *Riley). Use of the float method was also found to result in the unnecessary rejection of variable numbers of apparently sound acorns. On average, half of the 4,257 sound acorns used in this study failed the test. Based on these results, it is recommended that large collections of acorns from many sources be made to compensate for genetic differences and desiccation due to microsite conditions. Alternately, acorns may be soaked in water to raise moisture content before testing.* Tree Planters' Notes 46(4):143-147; 1995.

The float method of assessing acorn condition has been widely recommended as a fast, inexpensive, and nondestructive means of differentiating between sound and insect-infested or otherwise damaged acorns (Korstian 1927, Schopmeyer 1974, Stockton and Morgan 1979, Bonner and Vozzo 1987). Batches of acorns are placed in water; those that sink to the bottom are deemed viable whereas those that float are assumed to be insect-infested, aborted, malformed, or diseased. Although true viability of the embryo cannot be assessed using the float method, it may be used to differentiate between sound acorns and those that have a reduced probability of germination.

This method of sorting acorns has been used in most studies of oak *(Quercus)* seedling establishment and is widely used by planters and nursery managers. However, the reliability of the method has not been rigorously studied. Therefore, it is unknown how many damaged acorns remain undetected and are futilely planted out nor how many sound acorns are inadvertently rejected after failing to sink. In addition, damage due to certain agents, such as infestation by specific insects, may tend to be detected whereas damage caused by other agents may remain concealed. Therefore, it is possible that the reliability of the method may vary as the species composition of acorn-infesting insects and the incidence of disease fluctuate.

This study was conducted in October and November 1993 to determine the reliability of the float method in assessing the condition of northern red oak *(Quercus rubra* L.) acorns. The primary objective of the study was to determine what percentage of northern red oak acorns damaged by several different agents could be expected to pass the test (sink) and, therefore, be falsely assumed sound. Also of interest was the percentage of sound acorns that could be expected to fail the test (float) and be unnecessarily discarded.

Methods

Study site. This study was conducted on the West Virginia University (WVU) Forest located in north-central West Virginia along the westernmost range of the Allegheny Mountains. This 7,600-acre experimental forest is part of the Coopers Rock State Forest, which straddles Interstate 68 in Monongalia and Preston Counties (figure 1).

Two study sites were chosen. The first site was located in the Lick Run watershed of the WVU Forest, where cove hardwood stands were selected on a northeast-facing slope. This study area was characterized by very high site indices (81 to 97) for northern red oak *(Quercus rubra* L.*)*, an abundance of mature yellow-poplar *(Liriodendron tulipifera* L.) and northern red oak, and a moderate ground cover of herbaceous and woody vegetation. The SAF cover type was yellow-poplar-white

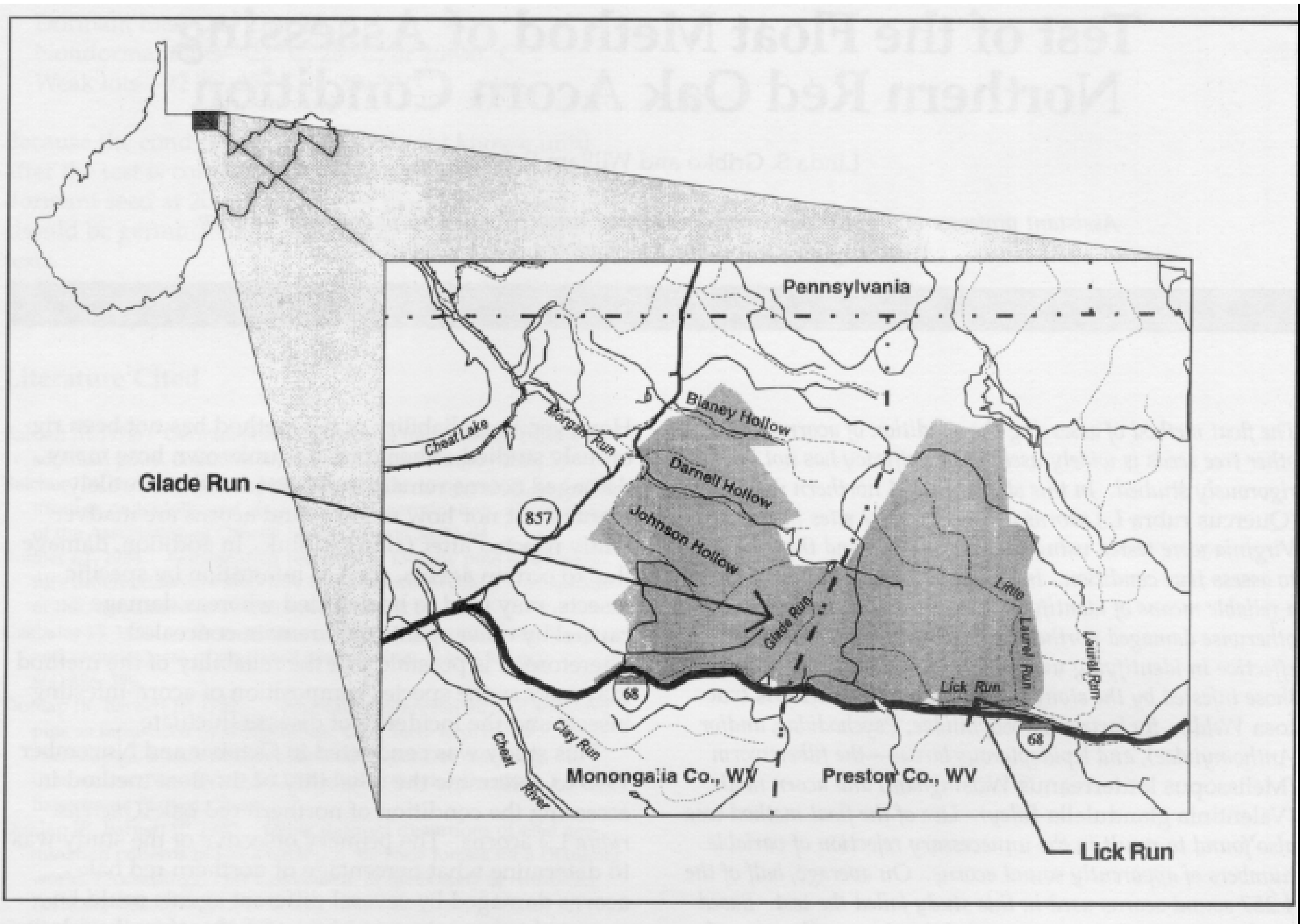

Figure 1—*Location of the West Virginia University Forest (shaded area).*

oak-northern red oak (Eyre 1980). This site was located on a mid-slope position with an average slope of 12% (McNeel 1993). The soils were Dekalb stony sandy loams (Baur 1959). This study area will henceforth be referred to as the Lick Run site.

The second site was located in the Glade Run watershed of the WVU Forest. The stands selected in this area were located on a drier northwest-facing slope and were characterized by lower site indices (68 to 73) for northern red oak, a relative absence of yellow-poplar, a significant component of mature black cherry *(Prunus serotina* Ehrh.), and sparse ground cover. The SAF cover type on this site was also yellow-poplar-white oak-northern red oak (Eyre 1980). This site was located on a higher mid-slope position with an average slope of 17% (McNeel 1993). Soils were Dekalb stony sandy loams; however, the A horizons were thinner and contained more stone than those on the Lick Run site. This study area will henceforth be referred to as the Glade Run site.

Acorn collection. Ten 0.5-acre (0.2 ha) square plots were established in the study area; 6 on the Lick Run site and 4 on the Glade Run site. Each plot was divided into quarters and a mature (> 12 inches dbh), mast-producing, dominant or codominant northern red oak was located within each quarter. In mid-October 1993, all acorns, regardless of apparent condition, were manually collected from the ground under the crown of each of the 40 study trees. Acorns collected under the 4 study trees on each plot were then combined to provide a representative sample of acorns produced on each plot.

The acorns were tested 3 times over a 2-week period using the float method (Korstian 1927, Schopmeyer 1974, Stockton and Morgan 1979). All acorns were submersed in water immediately after collection. Those that either floated on the surface or remained suspended in the water column were classified floaters; those that sank to the bottom were considered sinkers. The floaters were removed for dissection and the sinkers were covered and refrigerated for 1 week to allow further development of any larvae contained within. These acorns were then immersed in water a second time. As was done previously, all acorns that floated were separated for dissection and all that sank were held for 1 additional week, after which the final floatation test was conducted.

Dissection of acorns. Of the 19,774 northern red oak acorns collected, 14,775 floated in water (or failed the test) and 4,999 sunk (passed the test). One thousand, four hundred and forty (1,440) randomly selected sinkers were used in an unrelated germination study and so were not available for dissection. This left 3,559 sinkers and the 14,775 floaters to be dissected with anvil shears and classified based on condition. Each acorn was cut in quarters and given one of the following classifications (Dorsey and others 1962):

1. undamaged
2. damaged by acorn weevil larvae (*Curculio* spp.)
3. containing galls of the stony cell gall wasp (*Callirhytis fructosa* Weld.)
4. infested with fly maggots (presumably Drosophilidae, Psychodidae, and/or Anthomyiidae)
5. damaged by caterpillars— the filbertworm (*Melissopus latiferreanus* Walshingham) or the acorn moth (*Valentinia glandulella* Riley)
6. undeveloped with shriveled cotyledons
7. diseased
8. infested with gnat maggots (presumably Cecidomyiidae or Mycetophilidae)
9. aborted

In cases where infestation by more than one insect species or type was apparent, an individual acorn was tallied in more than one category. For example, an acorn infested with fly maggots but containing weevil exit holes was noted as having been infested by both weevils and fly maggots. Damage was classified based on photographs published by Dorsey and others (1962).

Statistical analysis. Eight of the classes included sufficient observations to allow statistical analysis. The acorns were kept segregated by plot so that analysis of variance comparisons could be made between the mean percentages of sinkers and floaters within the 8 condition classes. Because of the large differences in sample sizes among the plots, all means and associated statistics were weighted by sample size. Analyses of variance were conducted using these weighted statistics.

Results

Overall, the float method appears to be a reliable method of culling damaged or insect-infested northern red oak acorns from collections. An average 91.6% (SE_M = 7.7%) of the acorns damaged by any agent floated in water and correctly failed the test (table 1). The technique was particularly good at identifying undeveloped (μ_f = 99.7%, SE_M= 0.2%) or aborted acorns (μ_f = 99.8%, SE_M = 0.2%) and those infested by gall wasps (μ_f = 99.0%, SE_M = 0.7%) or dipterous larvae (μ_f = 99.5%, SE_M= 0.3%) (table 1). Nearly 100% of the acorns damaged by these agents failed the test, regardless of plot. On average, the test was also effective in distinguishing acorns infested by lepidopterous larvae, although there was more variability in efficacy among plots with small sample sizes (μ_f = 99.4%, SE_M = 1.0%). The test was least effective and least consistent in the identification of diseased (μ_f = 89.8%, SE_M= 3.9%) or weevil-infested (μ_f = 88.4%, SE_M = 3.3%) acorns; however, these success rates appear high enough for most applications. Analyses of variance were all highly significant within these condition classes (P = 0.0001). Individual Fvalues will not be reported here.

The float method was neither reliable nor consistent in the discrimination of sound northern red oak acorns. On average, only 56.0% (SEM = 9.1%) of the apparently sound acorns sank and, therefore, passed the test (table 2). Success rates on individual plots ranged from 4.9%0 (n = 451) to 92.8% (n = 741), and there was no significant difference between percentages of sound acorns that floated or sank (F = 0.78, P = 0.3896).

Discussion

The float method is based on the presupposition that damaged acorns contain more air space than do sound acorns; therefore, damaged acorns should be more apt to float in water. This generally proves to be the case when acorns are infested by gall wasps, fly larvae, or lepidopterous larvae. The presence of wasp galls in particular changes the entire structure of the cotyledons; the resulting ligneous mass of galls is lightweight and often surrounded by air space rather than healthy hydrated plant tissue. Infestation by the filbertworm can cause rapid desiccation due to the caterpillar's habit of chewing an entrance hole through the acorn shell. In addition, this species feeds rapidly and replaces the cotyledon with light, fibrous frass that is often held together with strands of silk. Fly larvae and larvae of the acorn moth often infest acorns vacated by weevil larvae. The combined feeding of the weevils and the secondary invaders converts much of the acorn contents from moist cotyledon to light, dry frass. In addition, the large exit hole(s) excavated in the acorn shell by the departing weevil larvae provide a significant route of desiccation.

Undeveloped and aborted acorns also tend to consistently contain more air space than do sound acorns.

Table 1- *Percentages* of *acorns that correctly failed the float test by condition; mean percentages and standard errors are weighted by plot-level sample size*

Condition	Total no.	Mean sample size	Standard error (SE_M)	Mean percentage failed	Standard error (SE_M)
Weeviled	8395	839.5	18.1	88.4	3.3
Diseased	1832	183.2	30.0	89.8	3.9
Dipterous larvae	1295	129.5	13.7	99.5	0.3
Undeveloped	1182	118.2	12.8	99.7	0.2
Aborted	627	62.7	10.4	99.8	0.2
Wasp galls	406	40.6	11.1	99.0	0,7
Lepidopterous larvae	332	33.2	7.8	99.4	1.0
Total	14077			91.6	7.7

Table 2-*Percentages of sound acorns that passed the float test by plot; mean percentages and standard errors are weighted by sample size*

Site & plot	No. of sound acorns	Percentage passed
Lick Run		
1	806	39.8
2	451	4.9
3	208	42.8
4	499	46.5
5	741	92.8
6	843	70.3
Glade Run		
7	72	11.1
8	139	16.5
9	106	47.2
10	392	90.8
Total	4257	
Mean	426	56.0
SE_M	93	9.1
Confidence interval		38.2 - 73.8

Generally, acorns in this condition have very small or shriveled cotyledons and appear to be "dried up" (Dorsey and others 1962). These acorns are often severely desiccated and can be picked from the sample visually based on their dull appearance or the presence of a cap.

The fact that the float method failed to perform consistently in the identification of weeviled or diseased acorns is not particularly surprising. Initially, weeviled acorns exhibit very little evidence of desiccation and the narrow tunnels created in the cotyledons during feeding are densely packed with heavy moist frass. Acorns in this condition could still be heavy enough to sink in water and would, therefore, falsely pass the test. As the larvae develop and continue to feed, the tunnels become much more substantial and the frass produced by the weevils becomes larger and looser. Heavily weeviled acorns, therefore, tend to float even in the absence of exit holes. Once weevils begin to emerge, acorn contents dry rapidly; acorns with exit holes seldom sink and falsely pass the test.

Substantial numbers of diseased acorns may also sink in water, especially if the cotyledons are fully infected. In this study, many of the apparently diseased acorns were very wet internally and, consequently, dense and heavy. Conversely, acorns that apparently succumbed to other bacteria or fungi developed large air spaces as the cotyledons pulled away from the shell. These were more apt to float and, therefore, correctly failed the test.

Overall, however, the float method appears to be a reasonably reliable means of removing damaged acorns from a collection. This is particularly true when used in combination with visual checks of acorn condition. For example, many diseased acorns that pass the test are dark and discolored, many undeveloped acorns retain their caps, and oviposition scars are often visible on the shells of weevil-infested acorns. In fact, the float method should be used as a supplement to these more obvious indicators of condition.

The efficacy of the test in identifying sound acorns varied greatly among the plots (table 2). This probably reflects genetic variability and differential site conditions. Structurally, many sound acorns that floated contained more open space either between the two cotyledons or between the cotyledons and the shell. This condition did not appear to affect viability, but it did cause the acorns to float. In addition, the size of the acorns varied widely from tree to tree and from plot to plot. Although no record of acorn size was made in this study, this variable may have had some affect on the outcome of the test.

In the absence of structural differences, drier microsites on some of the plots may have resulted in the slight desiccation of certain samples of acorns, allowing them to float in water. This may be evidenced by the fact that 92.8% (n=741) of the sound acorns collected from a very moist site in the Lick Run watershed sank whereas only 4.9% (n=451) from a drier site in the same watershed sank.

Regardless of the cause, use of the float method as a means of ascertaining acorn condition can be expected to result in the rejection of, on average, approximately half of the sound acorns in a collection. In the absence of a more reliable test, it may be prudent to collect many more acorns than might otherwise be needed for germination and to collect acorns from many sites and sources to avoid local anomalies in acorn structure and hydration. In addition, Bonner and Vozzo (1987) suggest that acorns collected from the ground should be soaked in water for 16 to 24 hours before testing. They note that this extra step is particularly critical when conditions are extremely dry at collection. Although not tested in the present study; use of this technique could be expected to significantly reduce the number of sound acorns that fail the test.

Summary

The float method of assessing acorn condition was found to be a reliable means of identifying insect-infested, diseased, and otherwise damaged northern red oak acorns. It was most effective in identifying undeveloped or aborted acorns and those infested by gall wasps, fly larvae, and lepidopterous larvae. Satisfactory, although more variable, results were obtained from acorns that were diseased or infested with *Curculio* spp. weevil larvae.

Use of the float method was also found to result in the unnecessary rejection of variable numbers of apparently sound acorns. On average, half of the apparently sound acorns used in this study failed the test. Based on these results, it is recommended that large collections of acorns from many sources be made to compensate for genetic differences and desiccation due to microsite conditions and that acorns be soaked for 16 to 24 hours before testing to increase moisture content.

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