

Protocol for Oak MAST: Monitoring and Assessing Seed Traits

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Authors: Harmony J. Dalgleish¹ and Michael A. Steele²

¹Department of Biology, College of William and Mary, 540 Landrum Drive, Williamsburg, VA 23185; hjdalgleish@wm.edu; 757-221-2238

²Department of Biology, Wilkes University, 84 West South Street, Wilkes-Barre PA 18766; michael.steele@wilkes.edu; 570-408-4763

Introduction

Oak mast (i.e., acorns) is a key resource that profoundly influences a vast array of ecological interactions between consumers, predators, parasites and pathogens in today's forests. Masting refers to the episodic and synchronized production of large seed crops followed by years of low seed production (Kelly 1994). Masting can increase the probability of seedling establishment by increasing both pollination success and satiation of seed predators (Kelly 1994). In oak-dominated forests, oak mast creates periodic food pulses that drive ecosystem function in oak forests world-wide. Perhaps one of the best examples of this is the work of Ostfeld and associates who demonstrated that high acorn production years increase mice (*Peromyscus* spp.) populations, which in turn increase the probability of Lyme disease (Jones et al. 1998a). High mouse populations can decrease the incidence of gypsy moth outbreaks as the mice feed on the soil-dwelling pupal stage of the moth (Jones et al. 1998b). During mast failure, rodent populations plummet, Eastern chipmunks (*Tamias striatus*) switch to feeding on the eggs of ground nesting birds, the incidence of Lyme infection decreases, and gypsy moth outbreaks can increase.

Many of the ecological interactions that result from acorn production follow directly from the specific characteristics of the acorns themselves, including both chemical (e.g., tannin, lipid) and physical (germination schedules, and acorn size) traits. Most of the oaks in North America belong to the subgenus *Quercus*, and within this group either the Section *Quercus*, the white oaks (WO), or the Section *Lobatae*, the red oaks (RO). Acorns of white oak species tend to have lower levels of lipid and tannin, and exhibit no dormancy, sometimes even germinating while still attached to the tree. In contrast, acorns of red oak species, have higher lipid levels but also higher tannin concentrations, and exhibit variable periods of dormancy prior to germination. Numerous rodent species show a remarkable sensitivity to these characteristics (Smallwood and Peters 1986; Steele et al. 2001) but when such characteristics are carefully controlled in behavioral experiments, free-ranging Eastern gray squirrels (*Sciurus*

carolinensis) selectively cache dormant RO acorns over those of WO regardless of tannin and lipid levels, seed size, and handling costs (Hadj-chikh et al. 1996). Gray squirrels also show a similar response to RO acorns infested with weevil larvae and non-infested RO acorns, selectively caching sound acorns and eating infested acorns along with the weevil larvae (Steele et al. 1996). Steele et al. (2002) further demonstrated that artificial acorns in which the cotyledon is modified chemically (with lipids or tannins) does not alter this response as long as the shell (pericarp) is that of a RO acorn, suggesting that detection of dormancy is based on a cue in the pericarp.

Mammals, such as squirrels, chipmunks and deer, are not the only seed predators of acorns. During the summer before acorn maturation (the first summer for WOs, and the 2nd summer for ROs) a few species of insects deposit their eggs in the cotyledon of acorns where they develop into larvae and feed, eventually emerging from the acorn to complete their life cycle. The most common larvae to reside in the acorn are those of a beetle (order: Coleoptera) of the genera *Curculio* and *Conotrachelus*, the nut weevils. During late summer, adult weevils emerge from underground where they have spent the past 1 to 3 years in diapause. Upon emergence they climb a nearby oak tree and mate. Soon thereafter, the females then oviposit inside the acorn, depositing one or more eggs. These eggs then develop into larvae that feed on the mature acorn cotyledon, sometimes (but not always) causing considerable damage to the acorns. Weevil larvae are quite common in acorns, but the percentage of damaged acorns can vary greatly among years. Other less common larvae include those of moths (Order Lepidoptera), flies (Order Diptera), and wasps (Order Hymenoptera).

For both vertebrate and invertebrate acorn consumers, we have found that chemical gradients in acorns seem to promote partial consumption of acorns and subsequent survival of the apical half of the nut containing the embryo (Steele 1993, Steele et al. in prep.). Species that feed on acorns, including blue jays (*Cyanocitta cristata*) gray squirrels, white-footed mice (*Peromyscus leucopus*), and weevil larvae (e.g., *Curculio spp.*) each employ different techniques for handling and consuming the cotyledon but all selectively feed on the basal half of the acorn, especially when acorns are abundant. This results in partially damaged acorns that are potentially able to germinate and establish.

Overview of Objectives: With the above background in mind, we have been evaluating the latitudinal variation in acorn characteristics to assess the biogeographic variation in oak–disperser and oak–seed predator interactions, the evolution of such interactions, and the potential impact of climate change for strengthening or decoupling these interactions. Our goal is to document how acorn characteristics vary (1) across latitudinal gradients, (2) with acorn crop size in individual trees, and (3) in relation to insect infestation. Initial detection of

such patterns will lead to numerous predictions and hypotheses as we begin to understand the general patterns of biogeographic variation in acorn characteristics. We believe this EREN project will allow us to efficiently involve appropriate participants in a way that benefits our research goals and the instructional goals of participants, and at the same time provides opportunity for future collaborations with EREN participants.

Our specific objectives are to have participants (1) regularly monitor mast in a small number of individual oak trees (preferentially *Q. alba* and *Q. rubra*) with seed collectors, (2) analyze acorns for seed characteristics and patterns of weevil and other insect damage, and (3) ship acorns to Wilkes University for freeze drying and, ultimately, chemical analyses.

Protocol

Overview

By the first weekend in September, place two seed collectors under each focal tree. Focal trees should be marked to facilitate relocation in subsequent years. Measure the diameter at breast height (dbh), canopy area, and height (optional) of each tree (height and canopy area may be easier to measure after leaf-off). Optional additional measures include adding iButton temperature loggers to the north side of each tree and home-made dendrometer bands to each tree to monitor tree growth. Seed collectors should be monitored every two weeks with the collected seeds stored in a refrigerator. Store all seeds from a single tree and collection period in one bag labeled with the collection date, tree species identity, and tree number. In late November, all collected seeds can be counted and carefully measured in the lab, opened and inspected for damage and the presence of insect larvae (weevils, moths, flies, galls) with a subsample of sound acorns (5-10 seeds per tree) sent to Wilkes University for chemical analysis (the latter is optional, but strongly encouraged). Please send seeds by mid-December so they can be immediately freeze dried.

Part 1: Selecting trees, deploying mast traps, sensors and dendrometer bands and collecting seeds

Part 1 Overview

This part of the project is initiated in early September and completed by late November. Tree selection (Sections 1.1 and 1.2, below), mast traps construction (Section 1.3) and deployment (Section 1.4), and iButton and dendrometer placement occur in early September. Tree data can be taken in September or after leaf-off. Traps are visited every two weeks for seed collection and acorn quadrat counts until November (Section 1.6).

Equipment list for Part 1

mast traps (detailed construction instructions provided in section 1.3)
 mallet or sledge hammer
 dbh tape
 meter tape
 clinometer or hypsometer to measure tree height (optional)
 iButton temperature loggers (2 per tree) (optional, see section 1.5)
 dendrometer bands (1 per tree) (optional, see section 1.6)
 tree data sheets (see Appendix)
 bags to collect seeds (one bag per tree per week – paper is preferable, but
 plastic will also work. See notes in section 1.7)
 access to refrigerator or cold room for seed storage
 50 cm x 50 cm (0.25 m²) quadrat
 quadrat count datasheets (see Appendix)
 nitrile or latex gloves

Section 1.1 Species

The target species are

Quercus rubra, Northern Red Oak

Quercus alba, White Oak

Quercus macrocarpa, Bur Oak

At a minimum, we prefer that researchers use one of the species listed above. Ideally, researchers would use two or more species at their site(s). *Other species may also be used in addition to one or more of the species on the target list.* For example, researchers may have access to *Q. alba*, and *Q. velutina* and may choose to use these two species. Because one of the species is on the target list, this would qualify for full participation. We would also be interested in receiving material from other oak species if investigators live in areas that do not have any of our target species. However, acorns from other species will be freeze dried and saved for analysis at a later date because of financial constraints. Please contact either Harmony (hjdalgleish@wm.edu) or Mike (michael.steele@wilkes.edu) with species selection questions.

NOTE: Oaks can hybridize, so we encourage consulting with a local knowledgeable person when in doubt. Also, if a tree is questionable, simply choose another!

Appendix 1 contains photos and descriptions to aid tree identification.

Section 1.2. Tree selection

Select at least 5 trees per species to monitor. All trees should have the following characteristics:

1. canopy trees
2. forested setting (not campus or park trees)
3. canopies of monitored trees should not overlap
4. minimize overlap of canopies with other oaks of the same species to ensure that acorns are collected from the target tree.

Mark all trees with a unique number (use flagging, tags, or anything you have on hand) so that you can return to the same tree to collect acorns each year.

Section 1.3. Seed collectors

If you already have access to seed collectors (mast traps), you MAY use them! There is no need to build new ones. We will standardize our calculations based upon the area of the collector opening. Aim to have *minimum* total surface area of collection space per tree of at least 0.3 m². If you are using the instructions below and purchase bins the size indicated, that will equate to using **2 collectors per tree**, as each collector has an opening of approximately 0.18 m².

If you do not have seed collectors, below are instructions for building them.



Figure 1. Seed collectors. 10 gallon plastic totes are mounted on poles and covered inside with chicken wire to prevent animal pilfering.

Materials list to create 1 seed collector

- 1 – 10 gallon plastic storage bin (20 ¾” x14 ½” x 13 ⅛”) (e.g., Sterilite Co. available at Walmart or Amazon) Brand and color are unimportant. Each brand has slightly different dimensions and that is ok. However, do purchase the deep bins (about 12 – 13 inches) not the shallow versions.
- 1 – 2 in x 2 in x 8 ft pressure-treated lumber cut in half. Each collector

uses only a 4 ft section so the other half can be used to construct another collector.

- 1 – 6 in x 6 in x ½ inch thick exterior grade plywood – suggest purchasing a 1 x 6 inch plank and cutting it into 6 inch sections.
- 1 – piece of chicken wire cut to dimensions slightly larger than the seed collector
- 2 – 5/16 inch x 1 ¼ inch long bolts, washers, and wingnuts
- 2 – 1 ½ – 2 inch long galvanized nails

Method

In the shop or lab:

Drill 2 holes large enough to accommodate your bolts in the square plywood plate, one inch from the corners on two opposite corners (Figure 2). It is best to create a single template and then use this to mark where holes belong on all plywood plates and the bottom of the plastic totes.

Use the plywood template to drill two matching holes in the bottom of all the totes.

Drill 4 additional small (1/8 inch) holes near the outside edge of each tote to allow rainwater to drain.

Put a bolt, washer, and nut in the bolt holes on the tote.

Cut all 2 x 2 x 8 in poles half at a 45° angle to create a point on the end of each that facilitates driving the post into the ground.

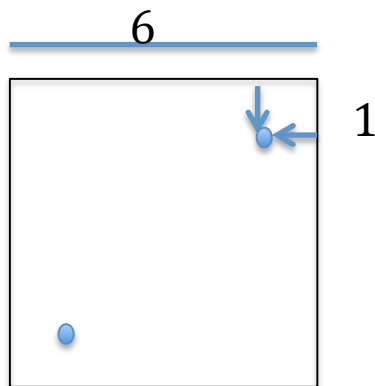


Figure 2. Diagram of plywood plate template. Drill two holes with a 5/8 inch drill bit in each plate AND on the bottom of each tote. Smaller drain holes should also be drilled on the outside edge of the bottom of the tote.

In the field:

Using a small sledge hammer, pound the flat end of the 2 x 2 x 4 posts with the pointed end directed into the ground. The pointed end should be driven 6–

12" into the ground so that the stake is vertical and as straight as possible. If your ground is rocky or particularly tough, it may help to pound a metal bar or pole of similar diameter to the wooden pole into the ground first to create a hole for the wooden pole.

Nail the plywood plate to the flat end of the stake with two nails. (Figure 3).

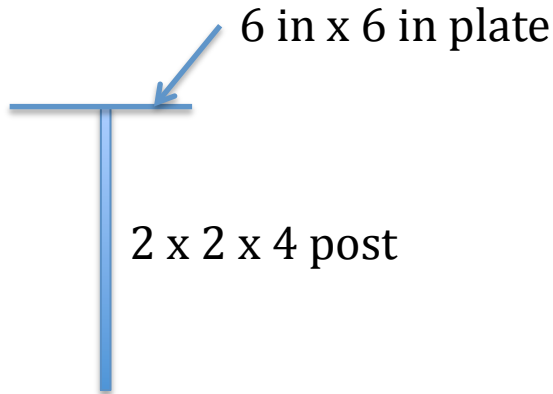


Figure 3. When nailed to the post, the plate makes a stand to hold the collector.

Line up the holes on the plywood plate with the holes in the collector and attach it to the plate using the bolts. Tighten the wingnuts.

Place the chicken wire snugly inside the seed collector several inches from the bottom and at least 6 inches from the top of the collector (Figure 1) . This allows acorns to fall through the chicken wire and to the bottom of the collector while preventing access to the acorns by birds, larger rodents and deer.

Check that all is secure and install the next trap.

Section 1.4. Deploying Mast Traps

When: By the first weekend in September (goal! If you're later, it is ok, but aim for as early as possible). Collectors should remain in the field until seeds have stopped falling (usually this is by late November in PA). It may be much later in other parts of the range.

How: Position two seed traps under the tree canopy in a way that maximizes the opportunity for sampling acorns from each tree. The direction and distance from the trunk is not important, but do try to position the traps under different parts of the tree's canopy.

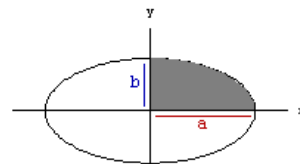


Figure 4. Measure canopy area as an ellipse with the trunk in the center.

For some trees, for example, this may be on either side of the trunk.

Tree measures: Either when traps are placed, or after leaf-off, measure the diameter at breast height (DBH), canopy area, and height (optional) of each tree. Measure DBH at 1.37 m (4.5 ft) using a DBH tape. Estimate canopy as the area of an ellipse, $\text{Area} = \pi ab$, where a and b are the shortest and longest distances from the trunk to a canopy edge (Figure 4). It is also critical to measure the openings of your collector and record this data on the data sheet!!

Section 1.5 iButtons

If you are able to purchase iButtons to record temperatures at your trees from Sept – June, it will add a great deal to the study. If you are willing to collect temperature data, but do not have funds to purchase the iButtons, let Harmony and Mike know.

Ideally, place 2 iButtons per tree (in case one fails, which does happen occasionally). If you do not already have iButtons, you can purchase the simplest temperature logging model (DS1921G-F# ~ \$22.00 each if you order 10) that will log temperatures between -40°C and 85°C . You will also need to purchase an interface cable to program your iButtons and download the data. There are several interface options to choose from including the basic DS1402-RP3+ Cable (~\$6.50), which will also require the LinkUSB – 1-Wire USB Interface (~\$30.00). Harmony has had good luck using the freeware Maxim 1-Wire Viewer software, which can be downloaded from several sites that sell iButtons and accessories including Maxim Integrated: <http://www.maximintegrated.com>. There are several vendors online for the iButtons and interface cables, including Maxim Integrated and iButtonlink.com.

Program the iButtons to log temperature every 240 minutes (every 4 hours) and do not permit writing over data. This will allow for 341 days of reading before the iButton memory is full. If you would like step-by-step instructions for programming and downloading data from iButtons using the 1-Wire Viewer software, email Harmony.

iButtons are not waterproof and should be coated in PlastiDip before being placed in the field, but after being programmed. PlastiDip is available at Home Depot and other hardware stores. Purchase the actual dip, not a spray. The spray doesn't work as well for these small items.

To coat the iButtons, first wrap a thread or thin string around the circumference of the button

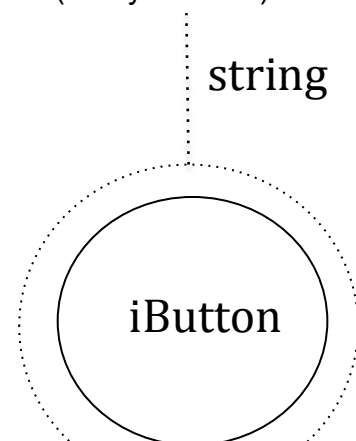


Figure 5. Tie the string around the iButton as illustrated by the dotted line. Pull the string tight so that you can dip the iButton into the PlastiDip and hang it to dry. Apply two coats of PlastiDip.

(Figure 5). Dip the entire iButton into the PlastiDip by holding on to the string. Tie the string to a rack or bar where it can dry (we fashioned one from a spare bit of hardware cloth). Dip the iButton in a second coat after the first is dry. Then take a paint pen or silver sharpie and write a number on the outside of the button so that it can be matched to the tree when you take it back to the lab for downloading.

iButton placement and removal: iButtons should be placed on the north side of the tree at breast height (1.35 m). You can glue them to a piece of wood and nail them to the tree or you can attach them to a post and pound the post in the ground next to the north side of the tree. All iButtons should be deployed before leaf off and removed and downloaded by June 1 the following year.

Section 1.6. Dendrometer bands

Dendrometer bands accurately measure tree growth and will enable us to examine the relationship between acorn crop size and tree growth. Although dendrometer bands can be purchased, they are quite expensive (>\$50 each). Two papers detail how to make your own for a fraction of the cost. Here are the citations:

EVELYN R. ANEMAET AND BETH A. MIDDLETON. 2013. Dendrometer Bands Made Easy: Using Modified Cable Ties to Measure Incremental Growth of Trees. *Applications in Plant Sciences*, 1(9) 2013. Botanical Society of America. DOI: <http://dx.doi.org/10.3732/apps.1300044>

Or

CATTELINO, P. J. , C. A. BECKER, AND L. J. FULLER. 1986. Construction and installation of homemade dendrometer bands. *Northern Journal of Applied Forestry* 3: 73 – 75.

The Anemaet and Middleton (2013) paper compares the two methods. They provide similar results, so if you would like to add dendrometer bands to your trees, you may use either method. See the papers for detailed construction instructions.

Section 1.6. Monitoring Seed Collectors

Collectors should be checked at least every two weeks. With each visit, students should carry the necessary tools to reposition baskets as required (small sledge hammer, tools to tighten bolts, etc), as well as a 1 m² quadrat, nitrile or latex gloves, and acorn count datasheets.

Ground quadrat counts

Ground quadrat counts can give us an index of acorn removal compared to seeds in the collector that have been protected from animals. During your first visit to each seed trap, choose a random distance between the trunk and the edge of the canopy in each of two randomly chosen cardinal

directions from the trunk. Mark two opposite sides of the quadrat location with a pin flag as you will return to the same area each subsequent visit. Place a 50 cm x 50 cm quadrat (0.25 m² – quadrat can also be circular as long as the area is the same) on the ground and count the number of whole acorns that appear visually sound. Count the number of partial acorns. DO NOT count visually unsound, rotten, or last year's acorns. Also count the number of immature acorns (small, and/or green). These indicate aborted acorns and are often more common early in the season. It is acceptable to remove the acorns from the quadrat to facilitate counting, but **if acorns are touched, latex or nitrile gloves should be worn** to minimize olfactory contamination that could interfere with rodent removal of seeds. Replace the acorns into the quadrat after counting.

After quadrat counts are done, check the collectors. All seeds in the collectors (not the quadrats) should be removed and stored in a refrigerator at ~4°C.

Seeds from all collectors under a single tree can be placed in the same paper bag, but seeds from each collection time and tree should be kept separate. Plastic bags can also be used, but store them upright and unsealed to prevent mold and germination. Condensation will accelerate the germination and/ or rotting process.

Label each bag with the date, location, tree species, tree identifier, and collector initials.

Part 2: Processing collected acorns

Overview

This part of the project occurs in late November or early December and can be conducted in a class or lab if desired. If seeds are still falling, but you'd like to process the majority of the seed crop with your students in a lab, feel free to have the students process what has been collected up to that point. Then, have another student process the acorns collected after seed fall ends.

For each seed we will measure seed mass and size, and determine if it is sound, weevil-infested, or damaged by other insects or fungus. We also assess whether insects or exit holes are in the top half or bottom half of the acorn.

Materials list for Part 2

calipers

balance

razor blades and/or a PVC pipe cutter to cut acorns

dissecting scopes (useful, but not necessary)

acorn data sheets (see Appendix)

tally counters (useful if it is a mast year and there are A LOT of acorns)

sharpies

Section 2.1. The insects

Weevils

The most common larvae to reside in the acorn are those of beetles (order: Coleoptera) of the genera *Curculio* and *Conotrachelus*, the nut weevils. Weevil larvae are usually yellow or whitish, fat and round with a brownish head (Figure 5). They do not have prolegs like the moth larvae.

Figure 5. Left: close up of weevil larvae. Middle: a recently emerged weevil larvae that has exited the whole it excavated in the acorn above. Note that exit holes indicate that a weevil has exited the acorn. Right: an adult beetle on an immature acorn. <http://www.ipm.iastate.edu/ipm/info/insects/beetles/acorn-and-nut-weevils> and <http://bugguide.net/node/view/342502/bgimage>



Moths

A less common insect found in acorns is a moth of the genus *Valentina* (order: Lepidoptera). This larva is thinner than that of the weevil, but often longer and grayish in color (Figure 6). Moth larvae have three pairs of small prolegs that further distinguish it from weevils, which have no visible legs.

Figure 6. Moth larvae. Left: Note the prolegs. Photo by Dana @ natureworks.org
Right: <http://bluejaybarrens.blogspot.com/2010/09/acorn-moth-larva.html>.



Flies

Fly larvae (order: Diptera) may occur in a few acorns and are translucent, teardrop shaped and very small in length (~5mm). When fly larvae occur they are usually found in high numbers within a single acorn but their prevalence among acorns is quite low.

Wasps

Also low in prevalence are the larvae of unknown wasp species, which appear as small, hard, round spheres.

Section 2.2. Processing acorns

Step 1. Verify that the collected seeds are the correct species for the tree. In other words, if the tree was listed as a red oak, are all collected acorns red oak acorns? Discard any acorns that are not of the correct species. Count all the remaining acorns from each collection time and add the data to the data sheet (see Appendix). Only count whole or partially eaten acorns; do not count caps. Include undeveloped (small and/or green) acorns, as this may indicate aborted acorns, sometimes common early in the season.

Step 2. Set aside acorns for chemical analysis. Acorns from trees that have more than > 5 acorns can have some sent to Mike for analysis. Randomly sample 5 acorns per tree and place them in their own paper bag. Label each bag with the date, location, tree number, tree species, and the EREN member name and contact number. Bags with acorns should be shipped to:

MA Steele

Department of Biology, Wilkes University

84 West South Street

Wilkes-Barre PA, 18766

All seeds shipped to Mike must be shipped by December 15 of the collection year in order to be usable for chemical analysis.

Step 3. After setting aside the acorns for chemical analysis, number all acorns with a sharpie.

Step 4. Examine the acorn for germination. If the acorn has germinated, measure the radical length (mm). Note also whether there are multiple radicals indicating multiple embryos in the acorn (Figure 7). Record the data on the data sheet.

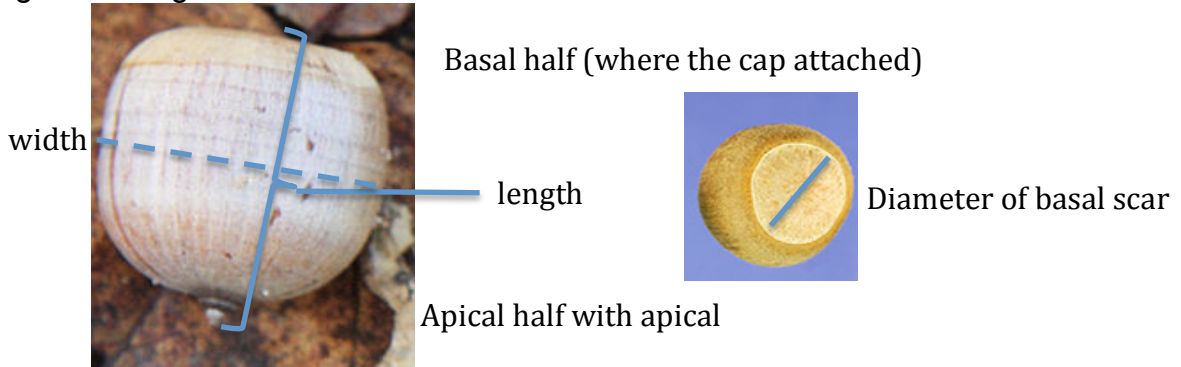


Figure 7. Germinating white oak acorns with one or more radicals emerging. Photo by MA Steele.

Step 5. Weigh the acorns individually and record the mass of each acorn to the nearest 0.001 g on the data sheet.

Step 6. Using a caliper, measure the longest length and width of the acorn (Figure 8).

Figure 8. Diagram of acorn measurements



Step 7. Inspect the outside of the acorn for any weevil exit holes. Record the number of exit holes in the basal half (where cap attached) or apical half (with apical tip) of the acorn on the data sheet (Figure 8).

Step 8. Cut the acorn along the 'equator' of the acorn, producing a basal half with the basal scar, and an apical half with the apical tip, containing the embryo. Razor blades or knives can be used. A PVC pipe cutting tool works well too (Figure 9). Next peel the shell from one half (save the shell for Step 9), dice the cotyledon into small pieces, and record the number of insect larvae found in that half. If they are weevil larvae make sure you have an exact count of the number in that half of the acorn. Now complete the dissection for the second half. Also note if you encounter more than one embryo. Record all data on the data sheet.



Figure 9. Example pipe cutting tool.

Step 9. Using a caliper, measure the diameter of the basal scar, the thickness of the basal scar, and the shell thickness at the equator where the acorn was cut in half. Record the information on the data sheet.

Step 10. When you have completed all the acorns for a single tree, fill out the tree-level information on the Acorn Processing Tree Datasheet (see Appendix). Enter the total number of acorns collected per tree, the total sound (mature only), total immature, and the total of each of the different types of infestation.

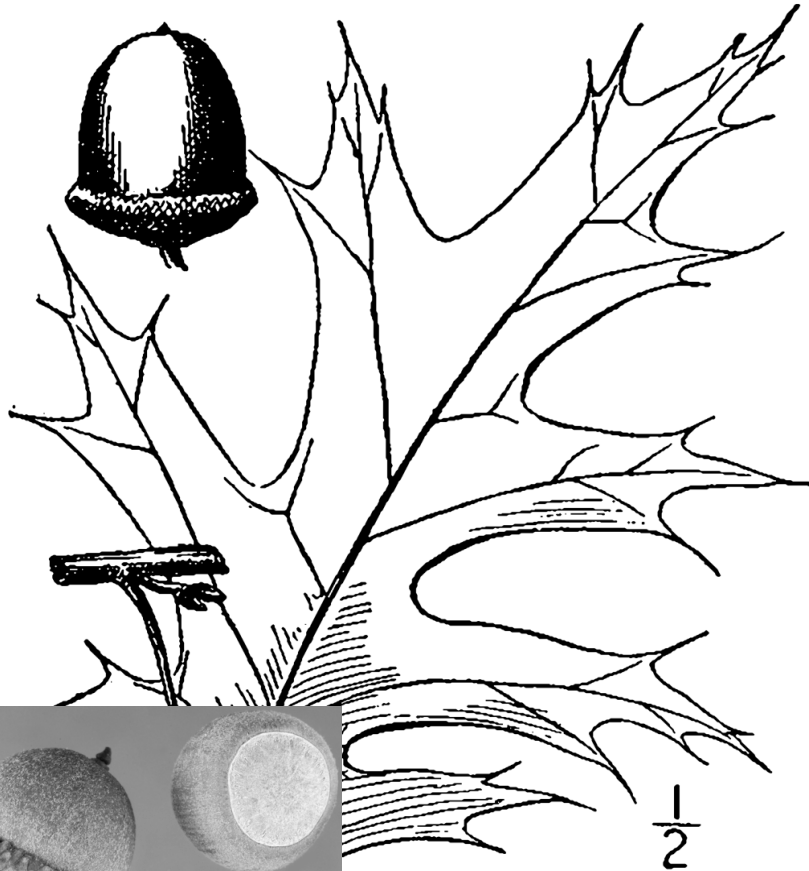
Part 3: Data entry

All data should be entered into the Oak Mast data spreadsheet and emailed to Harmony (hjdalgleish@wm.edu) by December 31 of the year of collection. See Appendix 3 for metadata. When data entry is complete add the year and the

EREN Member last name to the data file, for example,
"OakMAST_Data_YEAR_Dalgleish.xlsx".

Temperature data from iButtons should be sent by June 1 the year following collection. Only data taken while iButtons were in the field should be sent. Again, add collection year and EREN member name to the file names.

Quercus rubra, Northern Red Oak

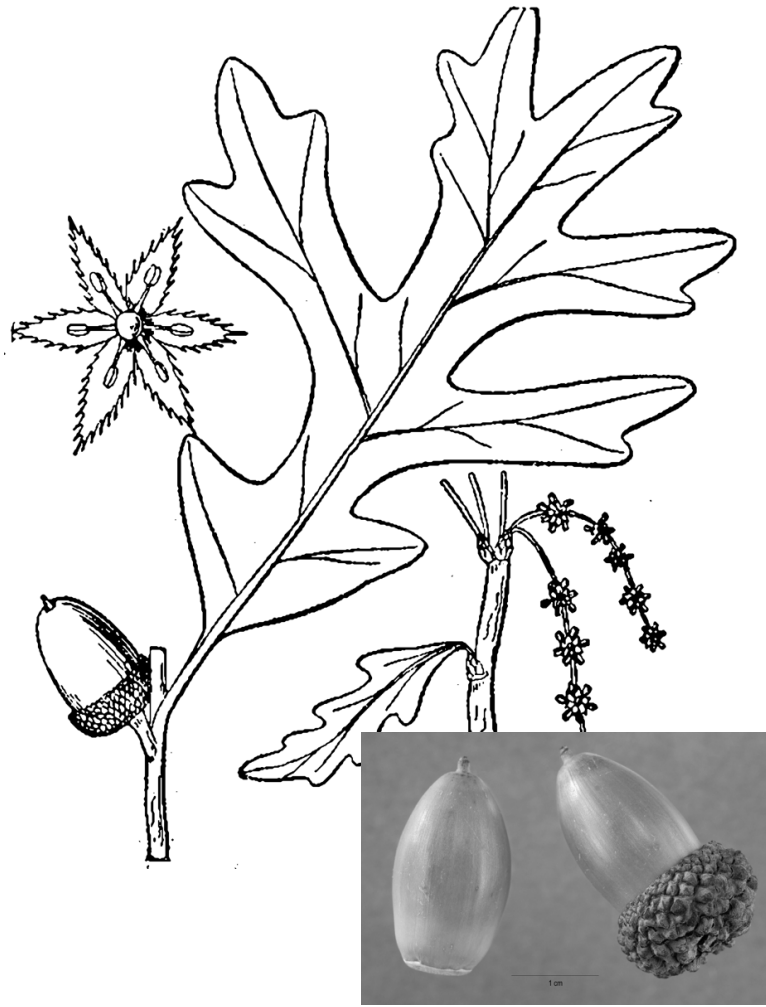


- Leaves
 - Lobes bristle-tipped.
 - 3-5 pairs of lobes with sinuses extending $\frac{1}{4}$ to $\frac{2}{3}$ of the way to the midrib.
- Bark
 - Bark often reddish between shallow furrows. Very large trees may have deeply furrowed bark while young trees have smooth bark.
- Buds
 - Reddish brown and smooth – without hairs. Black oak leaves can look similar, but their buds are hairy.
- Acorns
 - 2-3 cm, ovoid to ellipsoid; cups shallow, saucer-shaped, covering only $\frac{1}{4}$ of the nut. These acorns look like they're wearing Scottis tams. Check for last year's acorns on the ground near the tree.



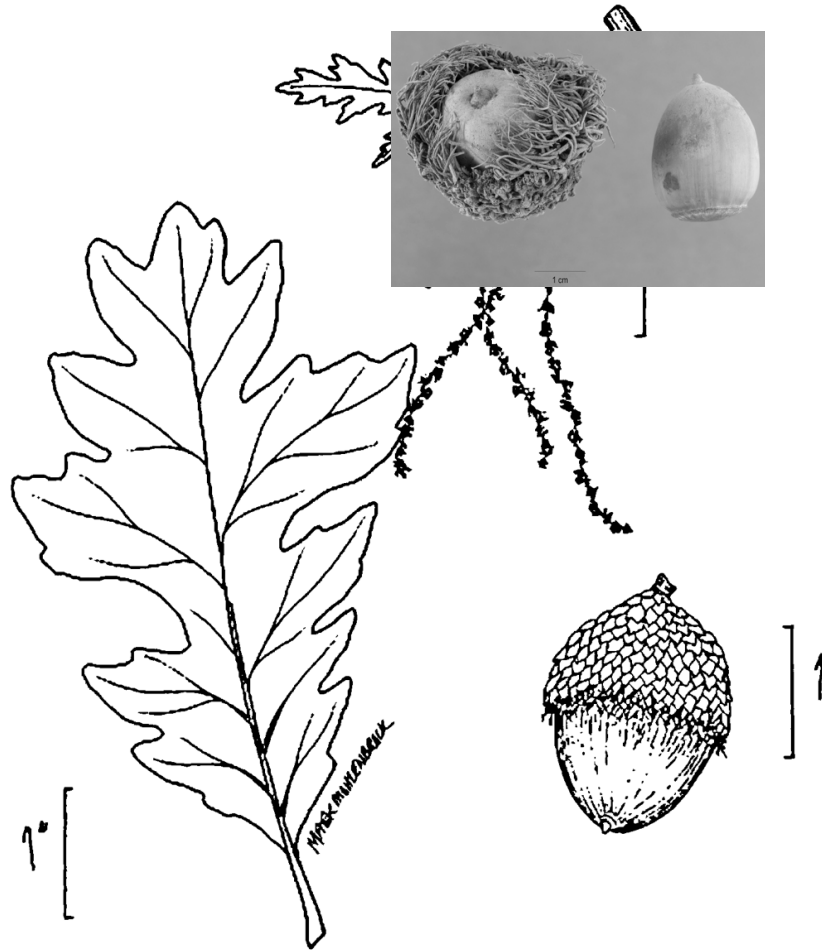
USDA-NRCS PLANTS Database / Britton, N.L., and A. Brown. 1913. *An illustrated flora of the northern United States, Canada and the British Possessions*. 3 vols. Charles Scribner's Sons, New York. Vol. 1: 617. Acorn inset: Steve Hurst @ USDA-NRCS PLANTS Database

Quercus alba, white oak



- Leaves
 - Lobes NOT bristle-tipped.
 - 3-5 pairs of usually deeply-cut ascending lobes.
- Bark
 - Light gray and flaky, particularly above. Looks like slate.
- Acorns
 - 1.5-3 cm, ellipsoid; cups bowl-shaped, cover 1/8 to 1/2 of the nut, with warty or knobby scales. Check for last year's acorns on the ground near the tree.

Quercus macrocarpa, Bur Oak



- Leaves
 - Lobes NOT bristle-tipped.
 - 4-7 pairs irregular lobes, the pair of sinuses near the middle deeper and wider.
- Bark
 - Gray, flaky, deeply furrowed or ridged and checked.
- Acorns
 - 1.5 – 3.5 cm, depressed-ovoid; cups covering $\frac{1}{2}$ to most of the nut with upper scales tapering to fringelike tips. These acorns look like they're wearing fringed stocking caps. Check for last year's acorns on the ground near the tree.

Appendix 2. Additional Resources

- L. Z. Hadj-Chikh, M. A. Steele, and P. D. Smallwood. 1996. Caching decisions by grey squirrels: a test of the handling time and perishability hypotheses. *Animal Behaviour* 52: 941–948.
- P. A., Jansen, F. Bongers, and L. Hemerik. 2004. Seed mass and mast seeding enhance dispersal by a neotropical scatter–hoarding rodent. *Ecological Monographs* 74: 569–589.
- C. G. Jones, R. S. Ostfeld, M. P. Richard, E. M. Schaubert, and J. O. Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk, *Science* 279:1023–1026.
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Appendix 3. Oak MAST Metadata

All data for Oak MAST should be entered into the Oak MAST Excel workbook, "OakMAST_Data.xlsx". This workbook contains three sheets: Tree Data, Acorn Data, Quadrat Count Data. Below is the metadata for these worksheets. When data is entered, add the year and the EREN Member last name to the data file, for example, "OakMAST_Data_YEAR_Dalgleish.xlsx".

Tree Data Metadata

EREN_member_name – the first and last name of the EREN member supervising the local data collection.

Institution – affiliated college or university

student_name – the first and last name of the student collecting the data.

date_deployed – date that the seed collectors were placed in the field starting with the four digit year, two digit month, two digit day: YEARMDD. Thus September 7, 2013, would be 20130907.

tree_num – a unique number for each tree.

tree_location – name of the site where the tree is located, for example, William and Mary College Woods (include GPS coordinates if possible).

tree_city – name of the nearest town or city.

tree_state – Two letter postal abbreviation for the state in which the tree is found.

species – full Latin name of the tree species

dbh – diameter at breast height in cm measured at 1.37 m

height_optional – the height of the tree in meters. This is optional

canopy_area – the area of the canopy in square meters calculated as the area of an ellipse. Note that on the field data sheet, there is a place to record the short and long radii of the canopy.

collector_area – calculate the total collector surface area *per tree* in m². THIS INFORMATION IS CRITICAL. Even if you followed the protocol specifications exactly, measure the area of the collector opening for your actual seed collector and multiply by the total number of collectors under

each tree. Report this value in m^2 and recall that there are 10,000 cm^2 per m^2 .

deployment_notes – any field notes on the trees. In particular, whether mature acorns have already started falling and are present on the ground or whether green immature acorns are on the ground. Any other observations welcome here too.

N_acorns – the total number of mature acorns collected in all collectors combined for this tree

N_immature – the total number of immature acorns collected in all collectors combined for this tree. Immature acorns are smaller and may also be green.

chemistry – where a subsample of acorns sent to Mike Steele for chemical analysis? 1 = yes, 0 = no.

dendrometer – was a dendrometer band installed? 1 = yes, 0 = no.

iButton – Were iButtons installed? 1 = yes, 0 = no.

Acorn Data Metadata

Some of these fields are repeated from the tree data sheet, but please add the data here too.

ERENMember – the first and last name of the EREN member supervising the local data collection.

student_name – the first and last name of the student collecting the data.

location – name of the site where the tree is located, for example, William and Mary College Woods.

date – date of acorn dissection starting with the four digit year, two digit month, two digit day: YEARMMD. Thus December 7, 2013, would be 20131207.

acorn_num – a unique number for each acorn within a tree.

tree_num – a unique number for each tree. These numbers should match those on the tree data sheet.

species – full Latin name of the tree species

germ – 1 = seed has germinated; 0 = seed has not germinated.

rad_length – length of radical. If there is no radical put NA in this field.

mass – mass of seed to the nearest 0.001g

length – length of seed including apical tip in mm

width – width of seed at widest point in mm

basal_thick – the thickness of the basal scar from the outside to the inside in mm

basal_diam – diameter of the basal scar in mm

hole_basal – number of weevil exit holes in the basal half of the acorn (the half with the basal scar)

hole_apical – number of weevil exit holes in the apical half of the acorn (the half with the apical tip).

weevil_basal – the number of weevil larvae in the top half of the acorn

weevil_apical – the number of weevil larvae in the bottom half of the acorn

moth – the number of acorn moth larvae in the entire acorn

fly – the number of fly larvae in the entire acorn

galls – the number of little 'round balls' in the acorn

N_radical_embryo – the total number of embryos in the acorn as evidenced by the embryos in the nut or emerging radicals. Do not count an embryo and its radical as two, but only as one.

comments – any comments about individual acorns. Comments about whole trees should be placed in the tree data sheet.

Quadrat Count Metadata

date – date of quadrat count starting with the four digit year, two digit month, two digit day: YEARMMD. Thus October 7, 2013, would be 20131007.

tree_num – a unique number for each tree. These numbers should match those on the tree data sheet.

species – full Latin name of the tree species

quad_area – area of the quadrat in m^2 . Recall that there are 10,000 cm^2 per m^2 .

quad_direction – the cardinal direction for the quadrat count: N, S, E, W

N_sound – the number of sound acorns in the quadrat

N_eaten – the number of partially eaten acorns in the quadrat

N_immature – the number of immature acorns in the quadrat. These are small and may be green.

Appendix 4. Datasheets

- A. tree datasheet
- B. acorn datasheet
- C. acorn processing tree datasheet
- D. quadrat count datasheet

