# Role of Tree Seedling Nurseries in the White Oak Genetics and Tree Improvement Project

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## Abstract

White oak (*Quercus alba* L.) is a keystone species across the Eastern United States with high ecological and economic value. Various factors are converging to limit the species potential for long-term sustainability. To address the lack of successful white oak recruitment, a genetics and tree improvement program was developed and is being implemented by the University of Kentucky. The program has three phases (germplasm collection, genetic testing, and seed production). Tree seedling nurseries can contribute to each phase through production and deployment of improved white oak seedlings.

## Introduction

White oak (*Quercus alba* L.) functions as a keystone species in the ecosystems in which it occurs throughout forests in the Eastern United States (figure 1) where it provides unique habitat for a broad array of bird and invertebrate communities (Tallamy and Shropshire 2009), its acorns provide a critical food source for over 100 vertebrate species (Brose et al. 2014), and it influences energy flow and nutrient cycling (Hutchinson et al. 2012). In addition to its ecological value, white oak has significant economic value (Bumgardner 2019) with strong demand for high-quality white oak products (Tripp 2015).

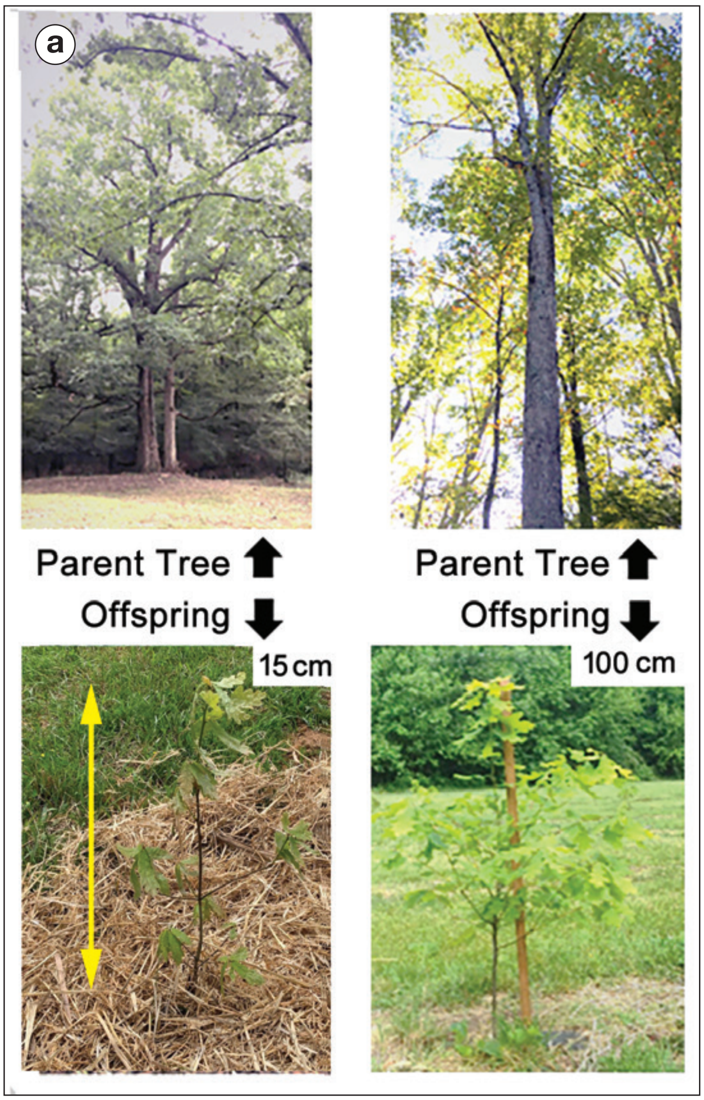
Despite its ecological and economic importance, long-term sustainability of white oak is uncertain. As overstory oaks are eliminated through natural mortality and harvesting, inadequate recruitment of competitive white oak seedlings results in stand compositional shifts toward more shade-tolerant species (Dey and Fan 2009). In these shaded conditions, the relatively poor competitiveness of white oak is due to slow aboveground juvenile growth, a preferential allocation of resources to the root system, and intermediate shade tolerance (Rebbeck et al. 2011).

In recognition of the lack of white oak recruitment and large-scale species compositional shifts, efforts to conserve and restore white oak ecosystems have been increasing (e.g., Hutchinson et al. 2012). In addition to a variety of management approaches that are being tested and implemented (e.g., Spetich 2020), organizations such as the White Oak Initiative (www.whiteoakinitiative.org) have been created to support the sustainable growth of the white oak resource (Fortuna 2021). Larger white oak seedlings

**Figure 1.** White oak's geographic range is the Eastern United States. (Source: USDA Forest Service, https://www.fs.fed.us/database/feis/pdfs/Little/quealb.pdf)

can improve the competitive status of oak regeneration relative to average-sized seedlings (Johnson et al. 2009) and can be planted to enrich advanced natural regeneration. This planting strategy might be especially critical when harvesting occurs in years of poor mast production, which is a regular occurrence with white oak (Greenberg and Parresol 2002). Development of high-quality white oak seedlings for artificial regeneration can be achieved through a combination of tree improvement and good nursery and planting practices (Sung et al. 2002). Not all trees that appear to be high quality in the forest will produce high-quality offspring. Seedlings from different parent trees can have very different growth rates when planted on the same site (figure 2a).

White oak genetic studies from the 1980s demonstrated significant genetic variation and high juvenile-mature correlations for height growth, indicating that genetic gains can be expected through selection at ages less than 15 years (Huang et al. 2016, O'Connor and Coggeshall 2011) (figure 2b). The collaborative White Oak Genetics and Tree Improvement Program (WOGTIP) was created at the University of Kentucky (UKY) to



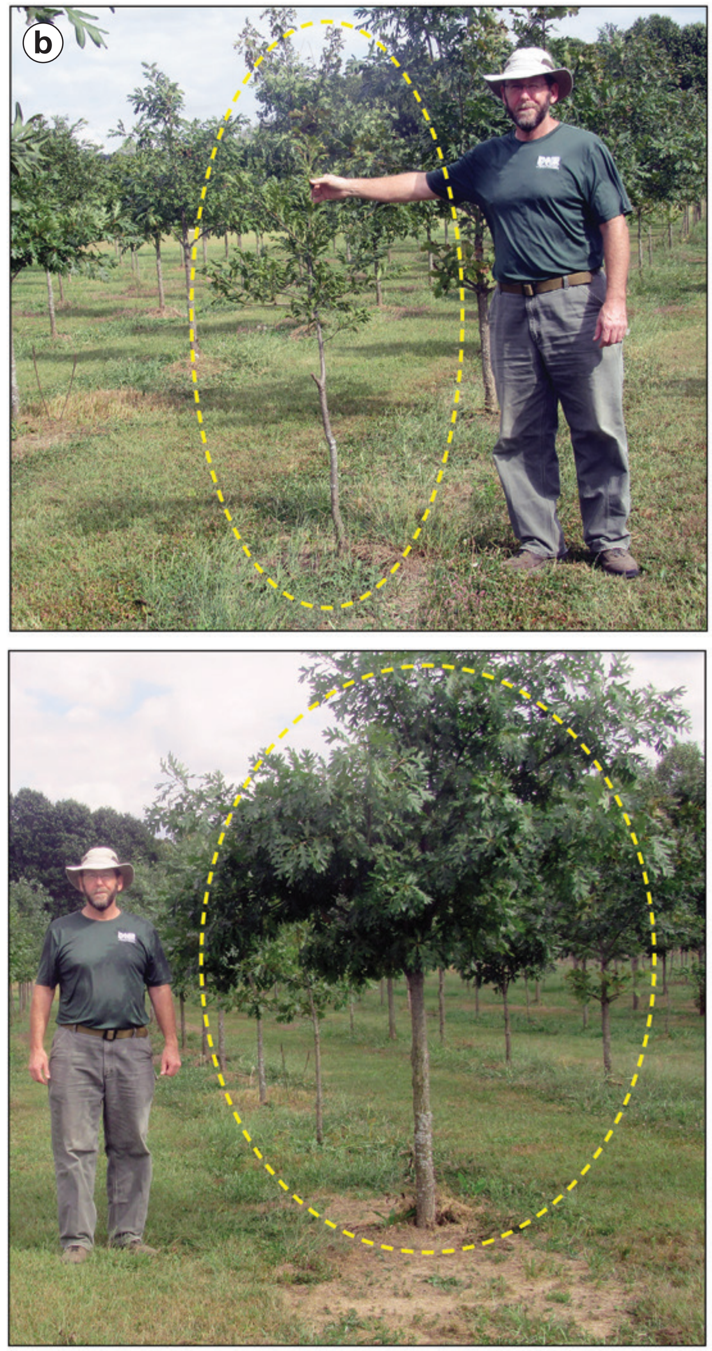




**Figure 2.** White oak has potential for growth improvement. (a) Seedling offspring from two high-quality white oak parents can vary significantly. The 1-0 seedlings were the same age and planted on the same site, yet they have very different growth rates. Similarly, (b) differences are evident in 10-year-old white oak trees grown from improved and unimproved acorns collected at the same time, grown in adjacent nursery beds, and outplanted as 1-0 seedlings on the same site (breeding and site set up by Phil O'Connor, Indiana Department of Natural Resources). The unimproved trees grew from a random sample of acorns sold to the nursery. The improved trees grew from acorns from controlled pollinations between two high-quality trees. (Photos by Laura E. DeWald, (a) 2021 and (b) 2019)

develop genetically improved seedlings to support multiple ongoing efforts focused on increasing success rates in white oak regeneration and recruitment over the long term. Specifically, the goals of WOGTIP are to develop a range-wide white oak tree improvement program that will collaborate widely and openly with industry (forest, wood, distilling); agencies and organizations (forestry, conservation, wildlife); and citizen scientists to quantify the levels and patterns of genetic variation as they may relate to important ecologic and economic traits, and to improve these traits for optimal seedling performance for recognized seed zones. A three-phase plan was developed to achieve the following goals of WOGTIP:

- Provide a sustainable supply of genetically improved white oak seedlings to meet current and future demands.
- Improve the ability to conserve and restore white oak in forests to achieve a variety of ecologic and economic goals across both regional and national scales.
- Provide genetic resources for future academic, operational, and industrial research and development.

## Phase 1 - Collecting and Archiving Germplasm

Acorns are being collected from across the entire geographic range of white oak. Volunteers collect approximately 1 gal (3.8 L) of acorns from one tree per county (figure 3) in as many counties as possible where white oak occurs naturally. To date, over 500 collections have been made with at least one collection from every State within white oak's geographic range (figure 4). The eastern seed zones database (Pike et al. 2020) is being used to help identify gaps in the collections. Due to the natural periodicity of white oak mast production; spatial and temporal pressures from wildlife for acorns; and effects of weather on acorn yields, Phase I will require 3 to 5 years to complete.

To quantify effects of collection year within the genetic tests in Phase 2, each year's acorn collections will come from at least 20 percent of the same trees that were collected from in the previous year(s). Acorn-collecting volunteers include nonprofit groups, Federal and State natural resources agencies, woodland owners, citizen scientists, participants in programs such as the Master Naturalists, youth organizations such as 4-H, and college students in


**Figure 3.** Volunteers collect enough acorns to fill a 1-gallon plastic bag and then ship the acorns to the University of Kentucky for the White Oak Genetics and Tree Improvement Program using prepaid priority mailing boxes. (Photo by Laura E. DeWald, 2019)

natural resources programs. Acorn collectors document their tree using the TreeSnap application for cell phones (treesnap.org), which automatically records the tree's GPS location. Because 50 to 90 percent of acorns can be infested with weevils (*Curculio glandium*) (Aldrich et al. 2003), volunteers conduct a float test to remove nonviable acorns from their collections. Volunteers document accession information specific to the seed tree. Shipping costs to send the acorns to UKY is funded through grants supporting WOGTIP. Seed collectors for tree seedling nurseries can also participate in the acorn collecting effort if they are willing to separate some of the white oak acorns they collect by parent tree. Nurseries can also help volunteer acorn collectors by recommending parent trees or areas where acorn production is consistent across years.

Annual acorn collecting began in 2019 and, as anticipated, acorn production has been highly variable. Late-spring frosts killed white oak flowers, heavy rains reduced pollen movement, late-summer droughts caused acorn development to abort, tropical storms denuded trees of acorn crops prior to maturity, and in 2020, Coronavirus COVID-19 policies negatively impacted participation by public employees. Despite these challenges, a total of 91 single-tree collections representing 9 States were sown in the nursery in 2019, and 112 collections representing 18 States were sown in 2020. The 2020 collections included the 20 percent recollections from 2019 trees needed for controls across years in the genetic tests.

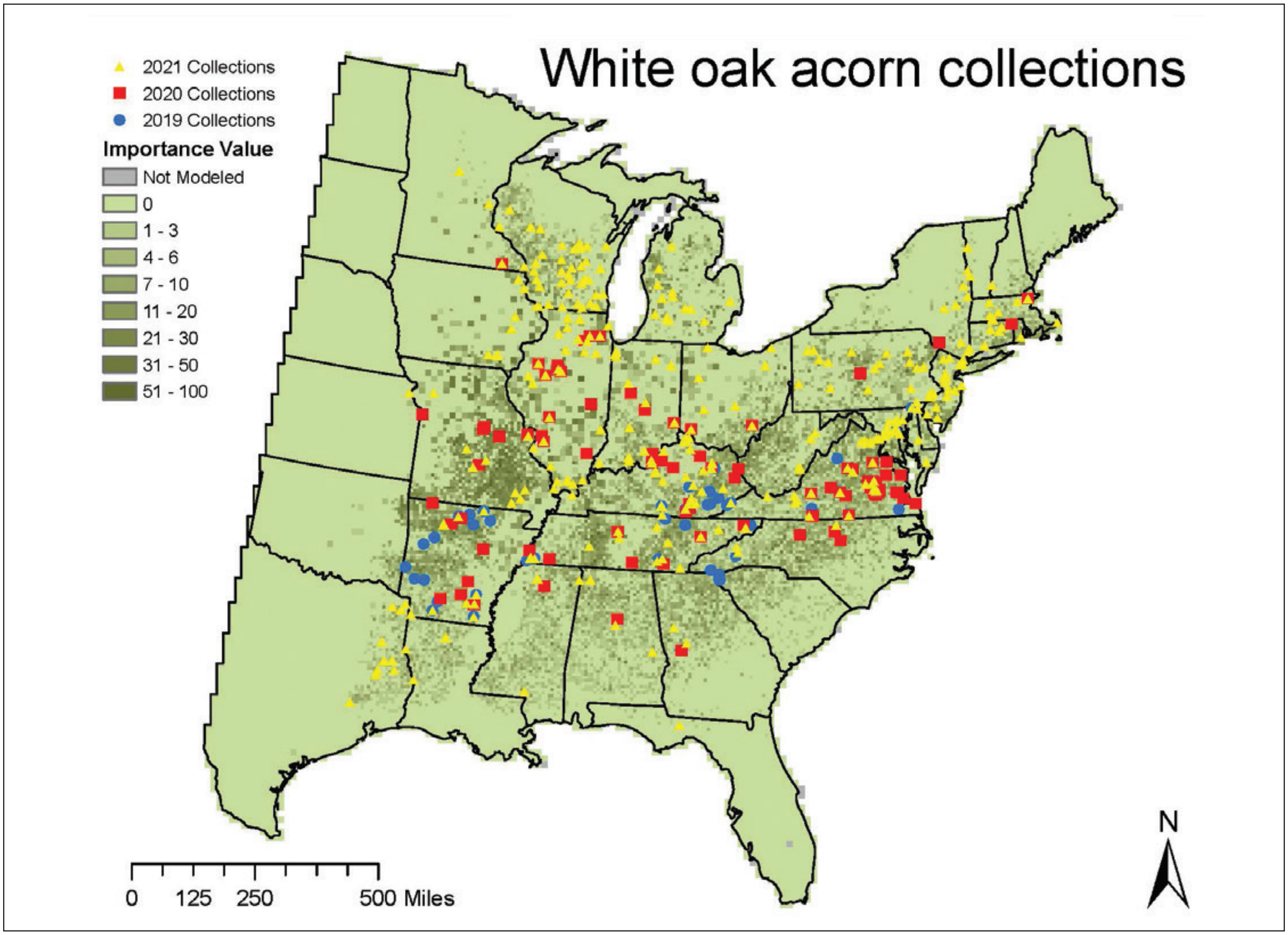



**Figure 4.** White oak acorns have been collected from multiple sites in the Eastern United States. The map also shows varying shades of green to indicate ecological importance of white oak in natural forests. Importance values are from the USDA Forest Service, Forest Inventory and Analysis database. (Source: USDA Forest Service, https://www.fs.fed.us/nrs/atlas/tree/v3/multi.php?spp=802)

At UKY, each acorn collection is weighed (figure 5). In addition, a random sample of 100 acorns per collection is weighed to estimate the number of acorns planted per collection. This weight data can also be used to quantify variations in acorn size. Since acorn size can affect initial seedling size, the data are also useful for comparing average seed weight with average 1-0 nursery seedling size for each seed lot. Mean seed source acorn weight in 2019 and 2020 ranged from 0.07 to 0.25 oz (2 to 7 g) and varied significantly ($p<0.001$) within and among seed sources.

The Kentucky Division of Forestry is partnering with WOGTIP to grow seedlings for the project at its Morgan County tree seedling nursery near West Liberty, KY. Acorns are hand sown in furrows created by the hardwood planting machine, sprayed with a rodenticide to reduce crow predation, and covered with soil (figure 6). After sowing, a layer of mulch is applied to reduce losses due to cold temperatures and wildlife predation. Except for hand-sowing, standard nursery operating procedures are used (i.e., irrigation, weed control, fertilization, lifting, and cold storage) until outplanting as 1-0 seedlings.

In the nursery, the half-sibling family groups (seed sources) of acorns sown in 2019 and 2020 did not affect emergence timing in the spring but did affect percent emergence. Average emergence was 37 percent (range 0 to 80 percent) and 50 percent (range 0 to 95 percent) from the 2019 and 2020 acorn collections, respectively. Mast production across the Eastern United States was much higher in 2020 than in 2019. Acorn quality also appeared to be higher (larger size, fewer weevils) in 2020, which may explain the difference in average emergence between the 2 years.


**Figure 5.** The weight of a random sample of 100 white oak acorns and the total weight of the acorn collection are used to determine the number of acorns planted from a parent tree. (Photo by Laura E. DeWald, 2019)


**Figure 6.** (a) White oak acorns are planted by hand to keep seedling groups from each parent tree separate from each other in the nursery. (b) After planting, mulch is applied to protect seedlings from predators and from cold winter temperatures. (c) The beginning and end of each seedling group is delineated by colored wooden markers. (d) Seedling groups can be clearly seen in May from acorns planted the previous autumn. (Photos by Laura E. DeWald, 2019–2021)

Similar to many tree seedlings nurseries, late-spring frosts at the Morgan County nursery can damage seedlings depending on their stage of development. Most white oak seedlings resprout from lateral buds at the root collar or along the base of stems, but this sprouting results in a high degree of forking and lateral branching. Thus, the nursery environment can affect assessment of genetic variation in seedling form at the time of outplanting. Continued assessment of the seedlings during Phase 2 could determine whether poor form at the time of planting can be outgrown if a seedling has strong apical dominance.

The same network of volunteers who collect acorns also collect scions from the same mother trees. Scions are being used to create two replicated, grafted clone banks to clonally replicate the parent trees (figure 7). Efforts are ongoing to propagate scions derived from all mother trees that contribute seeds to the WOGTIP. These clone banks will be used to create future clonal seed orchards for Phase 3.


**Figure 7.** (a) Using a shotgun, this private landowner collects white oak scion material for grafting. (b) Once collected, scions are grafted to clonally replicate the parent trees. (Photos by Laura E. DeWald, 2019–2021)

Grafting success is higher when scions are collected when the tree is fully dormant and when using scions collected from the upper crown where the branches get more sunlight, thus exhibiting greater annual growth. Meeting these criteria, however, can be problematic for most of the volunteers. As a result, most of the scion material collected to date has been of poor quality. Obtaining quality scion material suitable for grafting remains a significant challenge to WOGTIP. A network of volunteer arborists willing to climb or use a bucket truck and be paired with acorn collectors might be an effective way to get quality scion material. Personnel from tree seedling nurseries who have tree-climbing skills may be able to assist with scion collection. While not as desirable, material from the parent's best progeny could be an option when it is not possible to obtain scions directly from the parent tree.

## Phase 2 – Provenance and Progeny Testing

At the time of lifting from the nursery (approximately 18 months following seed collection), seedling roots are dipped in a hydrophilic polymer to reduce desiccation and drought stress. The 1-0 bare-root seedlings are then placed in cold storage until hand- or machine-planting into provenance/progeny tests plots in late March and early April. Results from these tests will describe patterns of geographic variation and adaptation among seed sources. This data will provide information about climate change responses (i.e., provenance comparisons) and will evaluate parent trees for traits of interest to stakeholders (i.e., progeny comparisons).

A master test site was established at the Maker's Mark Distillery Star Hill Farm near Loretto, KY, and will include seedlings from the entire rangewide acorn collection. In addition, a network of smaller regional tests will be established to elucidate patterns of genetic variation and to quantify genetic by environment interactions on adaptive and commercially important traits. The geographic target for seed-source inclusion in these regional tests is 80 percent from one to two seed zones surrounding the site, 15 percent from three to five seed zones away from the site, and 5 percent from seed zones representing a larger movement south to north. Efforts are ongoing to identify locations and partners for establishment of the regional progeny test sites that will ideally be distributed across the range of white oak. Several collaborative groups including the USDA Forest Service, State agencies, and academic institutions are in the planning stages to install progeny tests in 2023 (pending success of acorn collecting in 2021).

Some of these regional tests can be established in collaboration with State nurseries and potentially located at nursery sites.

Regional tests will vary in size depending on the number of seed sources included, with most occupying 2.5 to 4 ac (1 to 2 ha). Approximately 20 seedlings per seed source (i.e., single mother tree collection in a single year) will be planted in single tree plots at 8- by 8-ft (2.4- by 2.4-m) spacing. This relatively close spacing will encourage competition among the trees and allow the regional tests to be thinned to become seed production areas (SPAs) during Phase 3. Seedlings to be planted on previously nonforested sites will be root-dip treated with an ectomycorrhizae solution at the time of planting (MycoApply Injector Ecto®, Mycorrhizal Applications, Grants Pass, OR). This product has a diversity of species that occur in white oak forests.

Data collection from the progeny/provenance tests and subsequent analyses will be organized by UKY. In addition to survival, traits measured at the time of planting and thereafter at 3- to 5-year intervals include height and form. Additional traits, such as phenology, which are strong indicators of adaptation and thus important for making decisions about migration under future climates, will be measured within the first few years of test establishment. Other traits of interest to stakeholders and partners may also be measured. Selection based on survival, growth, and phenology can be done within 7 to 15 years for white oak (Huang et al. 2016). Continuation of the regional tests beyond 15 years will be up to the partner. Most tests will likely be rogued (based on estimated genetic values) to transition to SPAs during Phase 3 to provide a supply of genetically improved white oak acorns of known origins for seedling production in the appropriate seed zones. The Maker's Mark site is intended to be maintained as a long-term repository of the rangewide collection.

Of the acorns collected in 2019, 63 of the 91 seed sources produced enough seedlings to be outplanted at the Maker's Mark site and 2 other small sites in 2021. Even though the best seedlings (based on root and shoot characteristics) within a seed source at the nursery were selected for planting, there was still significant ($p<0.001$) variation in height and root collar diameter within and among seed sources after accounting for variation due to acorn weight (figure 8). The variation in seedling morphology did not, however, show any obvious geographic patterns. Forking and numbers of lateral branches were counted on each seedling to quantify form. A seedling with "acceptable form" was defined as having no forks and <1 lateral branch per 3.9 in (10 cm) of height. On average, 71 percent (range 20 to 100 percent) of seedlings met this definition of acceptable form. Form varied significantly among and within seed sources ($p<0.001$). On an individual basis, seedlings that met our definition of acceptable form were taller ($p<0.01$). Therefore, despite selecting higher quality seedlings at the nursery level, there is still significant variation within and among seed sources in the Phase 2 progeny tests.

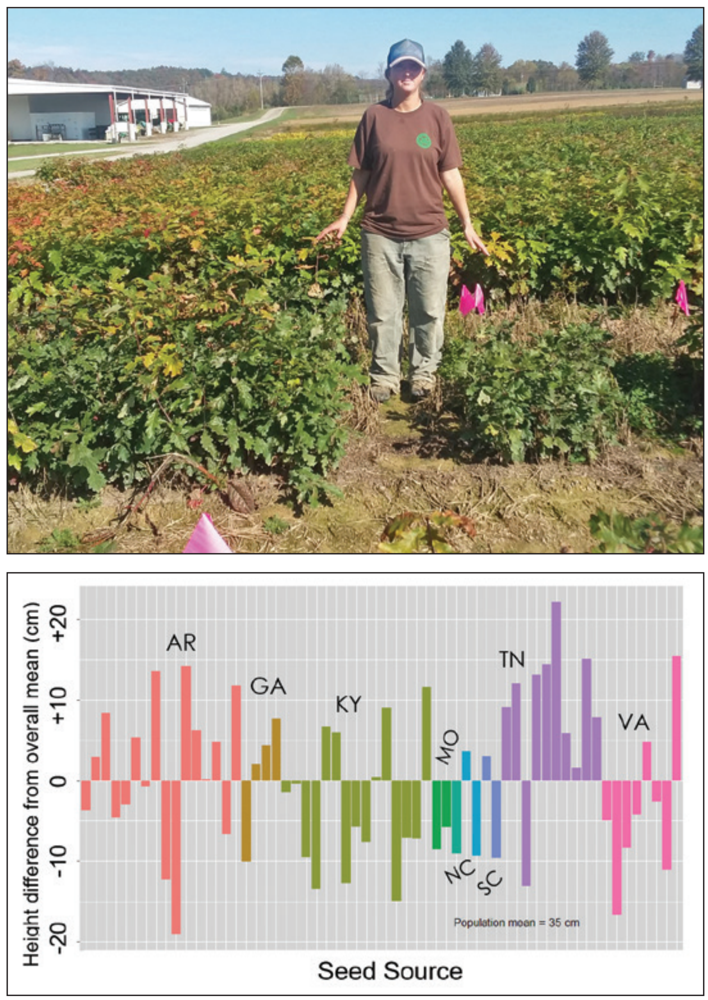



**Figure 8.** Genetic differences in white oak 1-0 seedling heights are evident in nursery trials. (a) The seed source on the left is from a tree in Kentucky and the seed source on the right is from North Carolina. (b) Nursery measurements in 2019 reveal height variation among seed sources. The two letter abbreviations are the State where the acorns originated for each color group. Bars with the same color represent seedlings from different trees within the State. (Photo by Laura E. DeWald, 2020)

## Phase 3 – Selecting Parents, Seed Production, and Seedling Deployment

Phase 3 of the WOGTIP plan will be implemented after progeny tests are evaluated and analyzed (approximately 7 to 10 years after establishment). Scion material from more than 20 parent trees have already been grafted onto root stock (see Phase 1), and the grafted clones are being planted on one of the farms at UKY as part of a clone bank that will ultimately provide material for the grafted seed orchards. Pollen mixing among top performing and locally adapted parents within these orchards will ensure high levels of genetic diversity and will produce broadly adapted seeds for reforestation. Future breeding programs could employ controlled pollinations to target specific traits and growing areas. Grafted seed orchards are best established on State lands near or at existing nurseries to ensure the sites are maintained for reliable, high-quality seed production.

Mast production is highly variable in white oak and demand for improved seedlings will be high. Therefore, multiple SPAs of genetically improved trees are needed to supplement the planned grafted seed orchards. Two types of SPAs can be established. Most of the regional progeny tests will likely be rogued at 15 years (based on genetic information from the test) and transitioned to become seed orchards. New production areas can also be established on private and public lands using collaborative partnerships with private woodland owners and public agencies such as the USDA Forest Service and State natural resources agencies. These additional acorn production areas can be planted using seed sources also included in nearby regional tests and then subsequently rogued prior to acorn production to remove poor performing seed sources using progeny test results. Both types of SPAs should be able to supplement the seed supply derived from the grafted seed orchards to ensure a consistent annual supply of acorns to nurseries for producing genetically improved white oak seedlings.

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