



Scaling Chinese Chestnut Breeding

New Selections, Seedling Orchard Populations, and Partnerships

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The [University of Missouri Center for Agroforestry](#) (UMCA) has actively curated and evaluated Chinese chestnut and other complex hybrid cultivars since the late 1990s. From the beginning, these efforts set out to identify cultivars well-suited to the Missouri River Hills, an ideal environment for growing chestnut with the purpose of encouraging grower adoption of those that consistently bear high-quality nuts. However, the benefits of these activities went far beyond initial grower recommendations to the establishment of a foundation for continued genetic improvement.

Since 2008, UMCA has distributed open-pollinated seed from its collection of 57 cultivars to growers across the eastern half of the United States. Growers outside of ideal environments often plant seedlings rather than grafted cultivars due to delayed graft failure, an incompatible interaction at the graft union that arises over time to cause tree crown decline unexpectedly. Seedling cultivation sidesteps issues of graft failure; however, it introduces heterogeneity into the orchard. While seedling families inherit their parents' characteristics, inheritance occurs variably within and between families causing some offspring to perform better than their cultivar parents and others worse. This reality occurs on a trait-by-trait basis. To counterbalance the orchard-level heterogeneity introduced by seedling families, growers often establish their seedling orchards at two to four times the final planned density. The poor performing trees can be culled, and the better performing trees retained. This application of selection offers an extraordinary opportunity for on-farm genetic improvement, and UMCA and dozens of growers are working on folding these selection events into a coordinated participatory breeding network.



UMCA cultivar repository in May of 2021. The collection cultivar houses ramets of 57 cultivars. An additional 17 named cultivars or accessions have been provided by grower partners and are in propagation in our nursery.

On-farm selection and breeding of chestnut offers highly valuable benefits in coordinated settings. First, it increases the breeding program's capacity to grow large generations of offspring (1000s of seedlings annually) by overcoming space limitations at universities and institutes. These large seedling generations enhance opportunities for genetic gain, testing of good parental combinations, and finding unique, improved offspring. Second, on-farm selection allows breeders to evaluate seedling performance within

growers' actual environments, as opposed to solely in the environment of the university's research farm. Growers benefit from cultivating these seedling orchards of breeding materials through direct access to the advanced generation populations and, of course, by selling the resulting nuts as a food crop.

Last year, [Dr. Ron Revord](#), the chestnut breeder at UMCA, and colleagues were awarded [a nearly 1 million dollar grant](#) from the USDA Agriculture Marketing Service to initiate evaluations of on-farm seedlings descending from UMCA cultivar repository. From 2021 to 2023, the team will evaluate around 1000 trees at more than 40 farms and in 12 states. The project seeks to use genotypic and phenotypic data to assemble core collections (i.e., select sets representing the observed diversity) of around 10% of the study size for conservation and next-generation breeding. The project also aims to formalize on-farm breeding within an official [Chestnut Improvement Network](#). While on-farm breeding has advanced chestnut improvement to date, making it possible to cultivate Chinese chestnut seedlings commercially, the activities have been ad hoc and spontaneous. A coordinated network that operates under a common breeding scheme and consistent selection criteria would have greater efficiency and rate of genetic gain. A goal of Revord and UMCA is to bring this leadership to the grower network, along with scientific methods, resources, and institutional support.

In its simplest form, the Chestnut Improvement Network includes participating growers where (1) last generation's seedlings (selections previously identified by growers) are under evaluation and (2) next generation's seedling orchards are being established. The next-generation material we seek to establish moves beyond assorted open-pollinated seeds from UMCA cultivar repository to one of three structured population types: **(1) grafted seed orchard populations, (2) diversity panels of seedlings families, or (3) full-sibling families as part of a pedigree scheme that are generated from controlled crosses.** Each population type offers its advantages for genetic improvement and production.

Seed orchards (1) comprise a limited set of grafted cultivars (i.e., three to five), where the goal is to control the pollen cloud and derive large volumes of offspring between the respective combinations of included cultivars. These orchards allow us to deploy large seedling populations from improved breeding selections and better cultivars. Critically important, seed orchards allow us to conduct progeny tests and assess parental combining ability, determine which combinations of cultivars produce superior performing offspring (referring to both average progeny performance and the degree of variation).



UMCA 'Peach' × 'Qing' × 'Kohr' grafted cultivar seed orchard located in New Franklin, MO. Where the controlled pollen cloud produces seedling populations that can enable progeny tests, if a systematic approach is taken to organizing and field planting seedlings in a balanced design.

UMCA maintains a ~2-acre seed orchard of the Chinese chestnut cultivars 'Peach' × 'Qing' × 'Kohr', representing the first opportunity to implement such progeny tests across multiple environments. These cultivars are among the upper tier of UMCA collection, each with good or excellent nut quality and productivity. While 'Peach' and 'Kohr' carry some defects, like fall-flowering and average blossom end rot (BER) incidence, respectively, 'Qing' has many exceptional characters, including low BER, and late bud-break (frost avoidance). Together, the seedling population descending from these cultivars produces a great commercial crop and simultaneously serves as a major step forward in contributing to genetic studies to find the several top parents base on progeny performance and environments.

The second population type, the **diversity panel** (2), comprises seedling families from a range of elite cultivars. Like seed orchards, diversity panels in this context descend from plots with grafted cultivars; however, these orchards typically contain a greater amount of genetic diversity (12-36 cultivars), so the pollen cloud isn't as tightly controlled. The major emphasis of diversity panels is to initiate genetic improvement schemes in environments that have only tested a narrow germplasm set, and where historical performance data is sparse. The diverse genetic base of this population type offers a broad lens to see location-specific constraints and opportunities. The diversity panel is excellent for the applied selection of regionally adapted individuals/families. Much can be learned from genetic analysis of these populations to inform breeding, such as trait inheritance, traits genetic architecture, and even DNA markers associated with traits of high economic importance. The orchard's commercial production and quality will continually improve as selection is applied to these populations and seedlings are culled.

The final population type includes **full-sibling families** (3) derived from controlled crosses made by Dr. Revord's lab in UMCA cultivar repository. This population type establishes a new pedigree breeding scheme. Elite cultivars with complementary characteristics are paired, and pollen from "parent A" is collected and placed on receptive female flowers of "parent B." Specialized pollination bags then enclose the pollination event to prevent contamination from airborne pollen. Generally speaking, complementary individuals are those that



A look at controlled pollination activities in UMCA repository in 2021. Aerial boom lifts are used to reach high in the canopy. Over 3000 pollination bags were used, each with several female flowers.

express opposite traits of interest (or defects). By crossing them, we intend to find unique recombinant offspring within their full-sibling families that inherit the desired traits of both parents (but not the defective trait(s)). Traits of interest extend to late bud break time, narrow ripening window, canopy architecture, nuts that drop free from the burs, bearing habit, pest/disease resistance, high nut quality and low defect rate, and beyond. Dr. Revord's lab is scaling this pedigree scheme, with 3,000 pollination bags deployed in the HARC repository this year and 65 crosses made. The resulting breeding generation is estimated at around 6,000 full-sibling offspring and will be a milestone population for Chinese chestnut genetic improvement in the eastern U.S. We expect the annual number of full-siblings generated to increase 2-3 fold in the coming years gradually.



A view of one of Eusner Farm's several fields to be planted with UMCA breeding populations in 2022.

In the meantime, this first breeding generation of ~6,000 full-siblings will be established in 2022 at a single farm in Olive Hill, KY, kicking off a unique and exciting new collaboration. Matt Eusner of [Eusner Farms](#) has agreed to make his farm home to the population and available to UMCA researchers to conduct long-term genetic research and evaluate the seedling families. This collaboration is the first of its kind for Chinese chestnut and quickly establishes a flagship research farm for the Chestnut Improvement Network. Eusner has begun retrofitting his farm in anticipation of these seedlings, erecting a deer-proof fence around the property's perimeter, prepping the land for the establishment, and making plans to construct on-site housing for visiting researchers. Eusner Farms will soon be a centerpiece of Chinese chestnut breeding and genetics research in the eastern half of the U.S. The population to be planted at Eusner Farms is a partial systematic diallel, where the 65 crosses made this year used 22 cultivars in 2-6 crosses each. This classic genetic research design is deeply informative for genetic research and highly valuable for applied breeding. The seeds/seedlings will be tracked with barcode labels and a state-of-the-art germplasm management database, E-Brida, from seed harvest this fall through nursery cultivation and field establishment in fall 2022. The barcode labels are invaluable for



maintaining accurate seedling identification until a field map can be established. The labels and database provide similar quality control once data collection begins. QR codes on the labels ensure accurate data entry using the E-Brida field application, which automatically archives data to the cloud following entry. As the Chestnut Improvement Network scales beyond Eusner Farms, the E-Brida database will help minimize the growing pains involved with quality control of breeding stock identification and accurate data entry and archiving.

UMCA and Matt Eusner are also partnering to scale the Chestnut Improvement Network's annual breeding stock supply beyond what UMCA can achieve in-house. While seedlings for/from the population types described above will be available through UMCA, Eusner's nursery in Georgia will provide the program services to grow an annual supply of breeding material that numbers in the tens of thousands. As a network grower himself, Matt understands the importance of quality control requirements of breeding material management. He is working with the Revord lab to take the extra steps of QR code labeling and tracking using E-Brida. This partnership helps overcome a major potential bottleneck in implementing the grower improvement network by generating adequate supply volume. We are thrilled about the potential to expand the footprint of the Chestnut Improvement Network.



UMCA seedling populations a part of the Chestnut Improvement Network growing at Eusner's Georgia nursery in July of 2021.

If you are interested in becoming a chestnut grower a part of the Chestnut Improvement network, please email us at UMCA for more information.

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