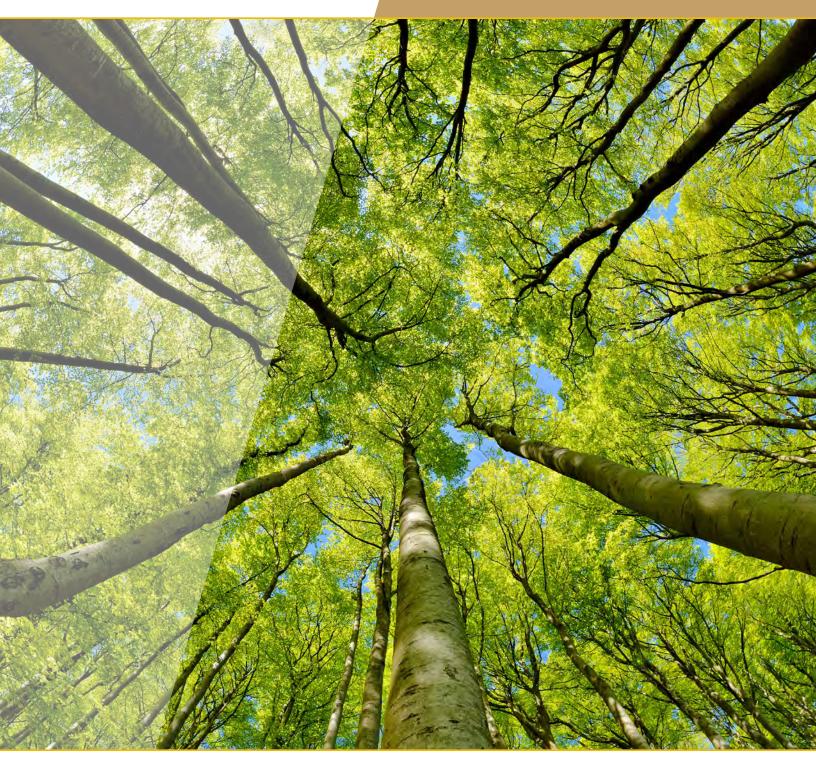


# **2024 ANNUAL REPORT**



In partnership with:



Forestry and Natural Resources



Forest Service U.S. DEPARTMENT OF AGRICULTURE

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### **ABOUT HTIRC**

The mission of the HTIRC is to advance the science and application of tree improvement, management, and protection of hardwood forests, with emphasis in the Central Hardwood Forest Region (CHFR).

HTIRC's strategic objectives are to:

- Produce hardwood trees with desirable traits, using both classical tree breeding and novel tree improvement techniques.
- Improve management strategies and techniques to enhance the ecological sustainability and economic benefits of hardwood forests.
- Develop and demonstrate strategies to address existing and emerging threats to hardwood forests.
- Engage stakeholders and address their needs through communicating research findings and management recommendations.
- Educate future leaders in tree improvement, management, and protection of hardwoods.

We are also committed to connecting our partners, collaborators, and stakeholders with the people, information, and products of the HTIRC through our technology-transfer efforts.

The Hardwood Tree Improvement and Regeneration Center (HTIRC) was conceived in 1998 to address a perceived void in hardwood tree improvement research in the Central Hardwood Forest Region (CHFR) and is committed to enhancing the productivity and quality of CHFR trees and forests for the economic and environmental benefits they provide. Research in tree breeding, tree nursery practices, tree plantation establishment and management, and Central Hardwoods silvicultural systems is aimed at increasing the regeneration success rate for high-quality hardwood trees and forests.

Hardwood Tree Improvement and Regeneration Center Purdue University htirc.org

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715 Mitch Daniels Blvd. West Lafayette, IN 47907-2061



### **DIRECTOR'S REPORT**

I am pleased to share the 2024 HTIRC Annual Report, which details the work of the center over the past year. You may notice a change in the format of this year's report. Instead of the data-heavy project reports of the past, we have opted for a broader overview of each, aiming to better connect you with the relevance and impact of our work.

This year has been one of meaningful progress for the HTIRC as we expanded our research, Extension and educational efforts to support our mission. With guidance from our executive committee, we funded three new projects in 2024 and continued to support research through our stakeholder-driven, project-based funding model. Since 2019, we have supported more than 20 projects. In this report, we highlight progress on nine ongoing projects that align with our strategic objectives and reflect our commitment to addressing stakeholder needs.

Training the next generation of researchers and practitioners remains a core mission of the HTIRC. This year, we provided support through our funding model to four MS and eight PhD candidates, eight postdoctoral researchers and six undergraduate research technicians.

HTIRC also remained actively engaged with the Center for Advanced Forestry Systems (CAFS), the only forestry-based National Science Foundation (NSF) Industry/University Cooperative Research Center (I/ UCRC). CAFS brings together industry stakeholders and NSF funding to address broad forestry challenges and offers valuable opportunities for supplemental grants and undergraduate research experiences. In 2024, CAFS held its annual industry advisory board meeting in Madison, Wisconsin, providing a vital forum for knowledge-sharing, collaboration and shaping the future of forestry research.

A significant milestone this year was our new partnership with Tree Pro, which will distribute select hardwood seedlings from our breeding program starting in March 2025. This marks our first commercial seedling release in 25 years and will make select black walnut, black cherry, northern red oak, white oak, butternut and American chestnut seedlings available to landowners throughout the Central Hardwood Forest Region. This collaboration represents a major step forward in expanding our impact, ensuring that high-quality seedlings are available to enhance the resilience and long-term value of hardwood forests.

Our commitment to stakeholder engagement and technology transfer remains strong. In 2024, we gathered for our annual meeting in March, updated key online resources and engaged with stakeholders through both online and in-person programs. These efforts continue to provide valuable research-based information on hardwood tree management, improvement, protection and more.

None of the work highlighted in this report would have been possible without the dedicated efforts of our executive committee, partners, collaborators, scientists, staff and students whose work drives our mission forward. Thank you for your continued support of the HTIRC. We look forward to working together to advance our mission in the coming year.

Best,

Matthew Ginzel HTIRC Director



### **EXECUTIVE COMMITTEE**

To help us deliver on our strategic objectives, an HTIRC Executive Committee was formed from members of our existing Advisory Board. Duties of the Executive Committee include the timely oversight of all HTIRC activities, as well as providing input to the Forestry and Natural Resources department head and HTIRC leadership in the form of recommendations as they relate to annual research budget allocations. The members are:

John Brown (Pike Lumber) Dan Dey (USDA Forest Service) James Jacobs (USDA Forest Service) Dana Nelson (USDA Forest Service) Guillermo Pardillo (ArborAmerica) John Siefert (Indiana DNR Division of Forestry)

## PARTNERS AND COLLABORATORS

AMERICAN CHESTNUT FOUNDATION: The goal of the ACF is to restore the American chestnut tree to our eastern woodlands to benefit our environment, our wildlife and our society.

ARBORAMERICA, INC .: Is devoted to the development of genetically superior, intensively cultivated, fine hardwood plantings that are offered as a long-term investment opportunity.

FRED M. VAN ECK FOREST FOUNDATION: Supports our research program in hardwood tree improvement and regeneration efforts.

INDIANA CHAPTER OF THE NATURE CONSERVANCY: The mission of TNC is to conserve the lands and waters on which all life depends.

INDIANA DEPARTMENT OF NATURAL RESOURCES, DIVISION OF FORESTRY: The division's mission is to manage, protect and conserve the timber, water, wildlife, soil and related forest resources for the use and enjoyment of present and future generations, and to demonstrate proper forest management to Indiana landowners.

INDIANA FORESTRY AND WOODLAND OWNERS ASSOCIATION: IFWOA's mission is to promote good stewardship of Indiana woodlands.

INDIANA HARDWOOD LUMBERMEN'S ASSOCIATION: A trade association whose members share a passion for creating the world's finest hardwood products and a determination to maintain the sustainable productivity of our nation's forest resources.

NATIONAL HARDWOOD LUMBER ASSOCIATION: NHLA's mission is to serve members engaged in the commerce of North American hardwood lumber through education, promotion, advocacy and networking.

NELSON IRRIGATION: Recognized as a world leader in state-of-the-art water application products for agriculture and industrial applications.

**OHIO DIVISION OF FORESTRY:** The Ohio Division of Forestry promotes and applies management for the sustainable use and protection of Ohio's private and public forest lands.

STEELCASE, INC.: The global leader in office furniture, interior architecture and space solutions for offices, hospitals and classrooms.

**TREE PRO:** The leading U.S. manufacturer of tree protection products.

SAM SHINE FOUNDATION: Upon the direction of the founder, Sam Shine, this foundation's mission is of long-term conservation, preservation and restoration of natural ecosystems while focusing on maintaining and enhancing native wildlife habitats associated with unique lands and waters.

USDA FOREST SERVICE EASTERN REGION STATE, PRIVATE AND TRIBAL FORESTRY: Collaborates with states, other federal agencies, tribes, landowners and other partners to protect, conserve and manage forests and community trees across 20 Northeast and Midwest states and the District of Columbia.

WALNUT COUNCIL: A science-based organization that encourages research, discussion and application of knowledge about growing hardwood trees.

### **RESEARCH TEAM** LEADERSHIP AND STAFF

Matthew Ginzel | Director Heather Dawson | Administrative assistant Lenny Farlee | Sustaining hardwood Extension specialist Elizabeth Jackson | Engagement specialist Wendy Mayer | Communications coordinator Rhonda Taylor | Laboratory manager genomics and FESSL

### **PROJECT SCIENTISTS**

Vikram Chhatre | USDA Forest Service, research geneticist Anna Conrad | USDA Forest Service, plant pathologist John Couture | Plant-Insect chemical ecology Ellen Crocker | University of Kentucky | Forest health Extension Songlin Fei | Measurements & quantitative analysis Morgan Furze | Plant physiology Rado Gazo | Wood processing Ayman Habib | College of Engineering Brady Hardiman| Urban ecology Eva Haviarova | Wood products engineering Joseph Hupy | School of Aviation and Transportation Technology Douglass Jacobs | Forest biology Michael Jenkins | Forest ecology Shaneka Lawson | USDA Forest Service, research plant physiologist Jingjing Liang | Quantitative forest ecology Carolyn Pike | USDA Forest Service, Eastern Region State, Private and Tribal Forestry Michael Saunders | Forest Biology/ Ecology of natural systems Guofan Shao | Forest measurement and assessment/GIS Song Zhang | College of Engineering Mo Zhou | Forest economics and management

#### **POSTDOCTORAL RESEARCH ASSOCIATES**

Dennis Heejoon Choi Aziz Ebrahimi Mojtaba Zamani Faradonbeh Behrokh Nazeri Sylvia (Minjee) Park Bina Thapa **Austin Thomas** Andrei Toca

#### **GRADUATE STUDENTS**

Olivia Bigham | PhD Aishwarya Chandrasekaran | PhD Elias Bowers Gaffnev | MS Scott Gula | PhD Yunmei Huang | PhD Ellie Joll | PhD Mikaela Scherzinger | MS Thad Swart | MS Wang Xiang | PhD Zhihen Yin | PhD

#### **TECHNICAL STAFF**

Brian Beheler | Senior farm operations administrator Don Carlson | Forester Bryce Chupp | Technician Clayton Emerson | Assistant property manager Katie Grong | Research associate Brianne Innusa | Research associate Caleb Kell | Operational tree breeder Ron Rathfon | Extension forester Caleb Redick | Research associate James Warren | USDA Forest Service Cameron Wingren | Unmanned aerial systems data collection specialist

### NSF Center for Advanced Forestry Systems,

#### **2024 UPDATE**

The HTIRC at Purdue University, along with Oregon State University, co-founded the only forestry-based National Science Foundation (NSF) Industry/University Cooperative Research Center (I/UCRC). The NSF I/ UCRC Center for Advanced Forestry Systems (CAFS) was established in 2006 to address challenges facing the wood products industry, landowners and managers of the nation's forestland. CAFS originally included North Carolina State University, Oregon State University, Purdue University and Virginia Tech. Since then, CAFS expanded to nine distinct university sites that include the above in addition to Auburn University, University of Georgia, University of Idaho, University of Maine and University of Washington.

HTIRC Purdue was part of CAFS during Phase I (2006-2011) and Phase II (2012-2017). At the end of 2019, NSF awarded our Phase III CAFS proposal, which continues our involvement with CAFS through 2025. CAFS couples support of HTIRC partners with investments from NSF to support research projects that aim to solve complex, industrywide problems. Funding from NSF CAFS supports projects that address CAFS research themes as part of our HTIRC project-based funding model. In addition to the core funding from NSF for CAFS, there is opportunity to apply to NSF for supplemental grants that support fundamental research and research experience for undergraduate students.

A CAFS Industrial Advisory Board (IAB) reviews ongoing and completed activities and selects new projects. In addition, the IAB provides input to NSF on the functioning of the center. The IAB strongly influences the priority given to various research projects. Each university site appoints a representative to the IAB, which provides direction to CAFS's operation and research activities. Guillermo Pardillo, member of the HTIRC Executive Committee, serves as our representative to the IAB.

In CAFS Phase III, HTIRC Purdue participates in three collaborative research projects with partners across other CAFS university sites. The HTIRC Purdue site is leading a project related to using hyperspectral imaging to evaluate forest health risk, which aligns with two HTIRC funded projects (PI John Couture) and has been presented at CAFS meetings by Sylvia Park, who completed her PhD with Purdue HTIRC in 2024. Another project involves assessing and mapping regional variation in site productivity, a project led by North Carolina State University, for which we contributed from a funded HTIRC project on soil suitability indices for black walnut (PI Shaneka Lawson) and another project supported by Indiana DNR to investigate hardwood plantation performance on mine reclamation sites across Indiana (PI Douglass Jacobs). The last project deals with intraspecific hydraulic responses of commercial tree seedlings to nursery drought conditioning, which is led by the University of Idaho site. The HTIRC Purdue site is participating with results for black walnut funded by a USDA NIFA grant (PI Douglass Jacobs) with a Purdue HTIRC postdoctoral scientist, Andrei Toca, presenting the results at CAFS meetings.

In 2024, we held our CAFS IAB annual meeting in Madison, Wisconsin, June 11-12, which included a full day and a half of presentations from CAFS scientists and a field tour on structural round timber (SRT) and remote sensing. Our next IAB meeting will be June 10-12, 2025, in Kona, Hawaii, and will feature a field day hosted by the Tropical Hardwood Tree Improvement and Regeneration Center (TropHTIRC).

NSF CAFS website: https://iucrc.nsf.gov/centers/center-for-advanced-forestry-systems



### **Institute for Digital Forestry**

The Institute for Digital Forestry (iDiF) works collaboratively across the Purdue colleges of Agriculture, Engineering, Liberal Arts, Libraries, Polytechnic and Science. IDiF has active efforts across eight Midwest, Northeast and Southeast states with Indiana project partners including the Indiana Department of Natural Resources, ArborAmerica, Naval Support Activity Crane, Indianapolis Zoo, the Hardwood Ecosystem Experiment, the Southeast Purdue Agricultural Center and Purdue Forestry and Natural Resources' Martell Forest.

One promising venture is advancing with Purdue's new Center on AI for Digital, Autonomous and Augmented Aviation (AIDA3) and United Kingdom partner Windracers, a global cargo drone operator. iDiF is developing a forest measurement sensor package to fit the Windracers ULTRA fixed wing drone donated to Purdue. The long-duration flight capability of the uncrewed drone will lead to efficient scanning of forest tracts at the landscape level with the data specificity of iDiF's stand-level quadcopter drones piloted onsite from the ground.

A tool immediately available for forest stakeholders and professionals alike is the iForester iPhone app available for free download. iDiF launched the app with an initial tree diameter module but will be rapidly deploying modules for commercial features, such as merchantable height, grading and tree scaling volume for Doyle, Scribner, and international scales. A little further down the development pipeline is species identification solely through bark imagery. iForester syncs seamlessly with the Purdue Data to Science (D2S) platform for access, visualization and sharing.



### **2021 HTIRC-FUNDED RESEARCH GRANTS – FINAL REPORTS**

### BACKPACK SYSTEM FOR HIGH RESOLUTION FOREST INVENTORY

**Principal investigator(s):** Ayman Habib, professor, civil engineering, Purdue University, ahabib@purdue.edu; Songlin Fei, professor, Forestry and Natural Resources, Purdue University, sfei@purdue.edu

Manual forest inventory is labor intensive and costly. Automation, which takes advantage of recent advances in light detection and ranging (LiDAR) systems and data analytics, should make forest inventory less expensive, more precise and faster, while allowing for fine-resolution inventory at a scale.

The backpack system for high resolution forest inventory research project is focused on developing a BackPack system equipped with LiDAR, RGB camera(s) and a positioning and orientation system (i.e., integrated global navigation satellite system and inertial navigation system - GNSS/INS). To date, several systems have been developed, with the latest prototype going into a commercialization phase, where two systems are being requested by an academic research institute.

In addition to the hardware development, researchers focused on two data processing aspects. First, a trajectory enhancement and mapping (TEAM) data processing system has been developed to mitigate the intermittent access to GNSS signal under forest canopy Second, following the TEAM-based point cloud generation, a data processing pipeline has been developed to derive fine-resolution biometrics of detected individual trees. The results from the data processing chain include fine-resolution point clouds with good positional accuracy (in both the relative and absolute sense). The point clouds are then ingested into a data processing pipeline to derive inventory metrics such as individual tree detection, localization and segmentation. The point clouds for the segmented trees are finally used to derive tree height, diameter at breast height (DBH) and stem curve.

The developed system, together with the data processing workflow, enables natural resources professionals, managers and landowners to derive cost-effective inventory metrics for large areas while ensuring the precision of derived information. These innovations will reduce the cost and time for field crew data acquisition, yet provide more complete information about forest stands than was feasible before and ensure the availability of actional information for better decision making.

Another component of the research focused on expanding the application scope of the developed BackPack unit to allow for its utilization on an e-bike (BikePack) as well as an autonomous robotic system. The BikePack system has been utilized for a Purdue University campuswide data acquisition. The collected data have been augmented with airborne data for a more complete representation of the scanned environment (e.g., building rooftops



#### Goals:

The project objectives are to: optimize system integration, data logging and deployment of a BackPack LiDAR system; develop data processing and biometrics extraction algorithms; and to share tools and methods with HTIRC researchers and stakeholders in trainings and workshops.

#### Methods:

The research focused on developing a backpack system equipped with LiDAR, RGB camera(s), position and orientation system (i.e., integrated global navigation satellite system and inertial navigation system - GNSS/INS).

In addition to the hardware development, a trajectory enhancement and mapping (TEAM) data processing system has been developed to mitigate the intermittent access to GNSS signal under the forest canopy. Following the TEAMbased point cloud generation, a data processing pipeline has been developed to derive fineresolution biometrics of detected individual trees.

and other infrastructure). The developed system was tested in a wide range of environments, including plantations and natural forests in Indiana, Great Smoky Mountains National Park, the Brazilian Amazon and chimpanzee habitats in Senegal. Preliminary results from the hardware and software developments are guite promising. The HTIRC research enabled a chain of other state, federal and international funding opportunities (e.g., forest managers, anthropologists, carbon credit accounting and small forest owners).

So far, the system and data analytics developed have focused on geometric characteristics of trees. The last phase of the research deals with integrating LiDAR point clouds with imagery captured by RGB digital cameras. The integration process will allow for groupspecies identification (e.g., using bark images and leaf structure). The augmentation with imaging systems will be expanded to include multi/hyperspectral cameras. This augmentation will allow for deriving more information, such as specific species and disease condition.

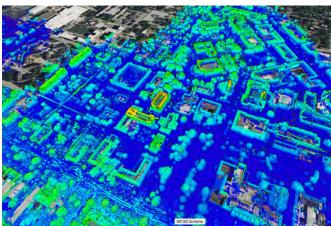
"This project has been rewarding for the investigators and research team from two aspects," Ayman Habib said. "It allowed us to expand our prior research, which focused on using mobile mapping systems for infrastructure assessment and monitoring to mapping, monitoring and management of forest ecosystems. It also enabled us to provide a wall-to-wall development of data acquisition and processing framework for forest inventory that is ready for commercial adoption."

#### **Key Findings in 2024**

- To date, the team has developed seven BackPack LiDAR systems. Data acquisition protocols and user manuals have been established for each. The latest prototype has moved into a commercialization phase.
- In addition to the deployment of the BackPack LiDAR, UAV LiDAR missions have been conducted.
- Strategies have been developed for trajectory enhancement and mapping (TEAM) to mitigate GNSS signal outages while collecting data under the forest canopy. The results from the data processing chain using the developed BackPack systems include fineresolution point clouds with good positional accuracy (in both relative and absolute sense).
- Data analytics strategies have been developed for forest biometrics extraction, including ground/ nonground filtering, individual tree detection, localization and segmentation. The point clouds can then be used to derive tree height estimation, DBH evaluation and stem curve.
- A web portal has been developed for the visualization of point clouds and derived products.
- The BackPack system has been incorporated into a BikePack unit. The entire Purdue campus was covered by the BikePack system during the fall semester.
- A pipeline has been developed to ensure the alignment of the BackPack/BikePack LiDAR point cloud with legacy geospatial data (e.g., 3DEP LiDAR data).
- Individual tree segmentation results have been augmented with imagery data, which will be used to identify tree species.
- Research findings have been disseminated in several presentations, conference proceedings and peer-reviewed journal papers.







#### **Publications**

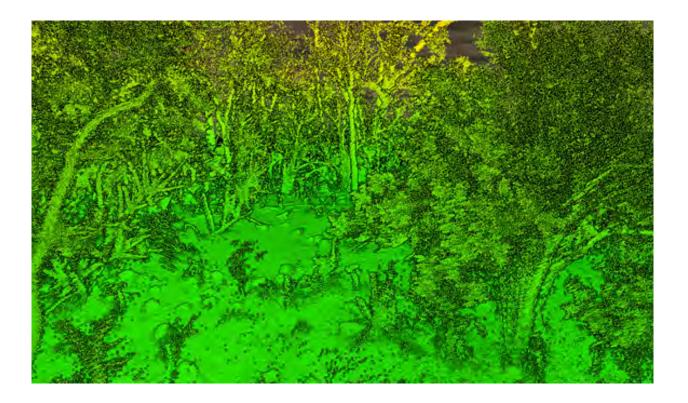
- Integration of Near Proximal and Proximal LiDAR Sensing for Fine-resolution Forest Inventory. Accepted for publication, Photogrammetric Engineering and Remote Sensing Journal (PERS). Habib, A.; Zhao, C.; Hanafy, H.; Eissa, A.; Aboelmaaty, Y.; Shao, J.; and Fei, S.; 2024.
- Large-scale inventory in natural forests with mobile LiDAR point clouds, Science of Remote Sensing, Volume 10, 2024, 100168, ISSN 2666-0172, https://doi.org/10.1016/j.srs.2024.100168. Shao, J.; Lin, Y.C.; Wingren, C.; Shin, S.Y.; Fei, W.; Carpenter, J.; Habib, A.; Fei, S.; 2024.
- Forest feature LiDAR SLAM (F2 -LSLAM) for backpack systems. ISPRS Journal of Photogrammetry and Remote Sensing, Volume 212, 2024, Pages 96-121, ISSN 0924-2716, https://doi.org/10.1016/j.isprsjprs.2024.04.025. Zhou, T.; Zhao, C.; Wingren, C.P.; Fei, S.; and Habib, A., 2024.

#### **Future Research**

The last phase of the research deals with integrating LiDAR point clouds with imagery captured by RGB digital camaras. The integration process will allow for group-species identification (e.g. using bark images and leaf structure). The augmentation will allow for deriving more information such as specific species and disease condition.

#### **Key Partners/Collaborators**

Guofan Shao, professor emeritus, Forestry and Natural Resources, Purdue University Joey Gallion, forest inventory program manager, Indiana Department of Natural Resources Collaborators at Great Smoky Mountains National Park, in the Brazilian Amazon and at the chimpanzee habitats in Senegal



### TESTING EFFICACY OF ENRICHMENT PLANTINGS FOR STAND REGENERATION IN HARDWOOD FORESTS

**Principal investigator:** Douglass Jacobs, Fred M. van Eck Professor, Forestry and Natural Resources, Purdue University (djacobs@purdue. edu)

**Co-authors:** Don Carlson, forester, Forestry and Natural Resources, Purdue University; Ron Rathfon, Extension forester, Forestry and Natural Resources, Purdue University; Caleb Redick, research associate, Forestry and Natural Resources, Purdue University; Brianne Innusa, research associate, Forestry and Natural Resources, Purdue University; Michael Saunders, professor, Forestry and Natural Resources, Purdue University

A mature central hardwood forest typically consists of hickory, maple, tulip-poplar and oak species in the canopy. Oaks have a high economic value and they provide food and habitat for numerous wildlife species. However, many oak species fail to regenerate naturally due to factors such as deer browse and competition with surrounding vegetation. Enrichment plantings, or plantings of nursery-grown oak seedlings to supplement natural regeneration in forests, coupled with deer exclusion fences and crown thinning, may provide the optimal conditions for oak regeneration success.

Researchers have used an existing network of HTIRC/Purdue Department of Forestry and Natural Resources enrichment planting trials to establish a long-term study to examine the performance of enrichment oak plantings, including their responses to deer exclusion and a crown release from competing trees. These plantings also have been used in HTIRC/Purdue Extension field days to communicate research findings to forest managers.

Since 2021, researchers have visited eight sites across the Central Hardwood Forest Region that received enrichment plantings in the past 9-21 years. They measured the growth, competition status, survival and vigor of these planted trees and analyzed the effects of various silvicultural treatments. A crown release also was performed at three sites.

In 2022, after eight years, planted northern red oak (*Quercus rubra*), white oak (*Quercus alba*), chinkapin oak (Quercus muehlenbergii) and American chestnut (*Castanea dentata*) all had taller heights, greater stem diameters and a higher percentage of trees in competitive positions in the canopy (codominant or dominant) when grown within fenced enclosures. Researchers also found that tree height decreased with increasing competition, but some competition may be needed to force the trees to allocate resources to height to maintain sun exposure.

Another site that underwent midstory and crown thinning in fenced and unfenced plots showed that 12 years after thinning, northern red oaks exhibited the most growth in unfenced crown thinned treatments. White oaks had the largest diameters in this treatment as well. The tallest white oaks were found in the fenced crown thinned treatments. On a site that underwent gap harvests of varying size, researchers found that larger gaps resulted in a higher probability of survival than small or medium gaps. Lastly, at another site, fencing increased the survival probability of northern red oak and white oak.

In 2024, researchers revisited a few of the sites to measure height, diameter, crown width, survival and crown status. Planted American chestnut and chinkapin oak had a higher percentage of mortality than white oak and northern red oak. American chestnut and northern red oak had more



Purdue technicians Scott Engwall and Chelsea Zaldivar walk between enrichment planting experimental plots at the Southeast Purdue Agricultural Center (SEPAC). Photo credit: Brianne Innusa.

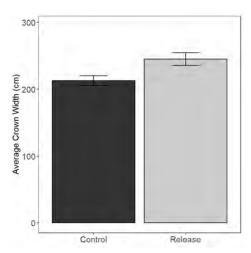


Figure 1: The average crown width of planted trees at the Southeast Purdue Agricultural Center (SEPAC) in 2024 from the control and release treatments.

growth in terms of height and DBH compared to white oak and chinkapin oak across all levels of fencing and vegetation management. The planted trees receiving a crown release had greater groundline diameters and crown widths than those that received no crown release.

"This research provides a framework to supplement natural oak regeneration," principal investigator Doug Jacobs said. "From our current results, we can recommend that forest managers crown thin stands that are 12-plus years of age to open more light in the canopy for oak growth — and to fence seedlings when possible. This research also shows that crown release can be successfully performed on both planted and naturally regenerating trees as young as 8 years."

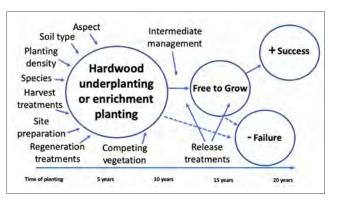


Figure 2. Factors that help to explain relative success of underplanting and enrichment plantings.

Researchers say next steps should include following up on the responses of released trees for two more years, as the effects of crown release often require a few years to manifest.

#### **Key Findings in 2024**

Release of planted trees resulted in greater growth than the control (not released) treatments, which shows that crop tree release can be done successfully on enrichment planting as soon as eight years after planting.

At the Southeastern Indiana Purdue Agricultural Center (SEPAC), the release treatments showed an increase in the average crown width relative to the control treatments (Figure 1).

The Southern Indiana Purdue Agricultural Center (SIPAC) seedlings planted in 2002 showed that harvest gap size had a significant effect on planted tree survival. Probability of survival was higher in large gaps than in medium or small gaps. The effect of stock type was smaller but also significant. Probability of survival was higher for container northern red oaks seedlings than bareroot northern red oak seedlings.

At the Harrold property, deer exclusion fencing increased survival of red oaks and white oaks over 10 years. Without fencing, white oaks had lower survival than tuliptree and black walnut.

Unfenced seedlings had larger crown widths and larger crown ratios than unfenced seedlings on Nelson Stokes property seedlings planting in 2011. This is a surprise given that deer browse usually restricts initial crown growth.

#### **Goals:**

Oak is a keystone species that provides valuable habitat and food for wildlife and has a high economic value. This project examines eight enrichment plantings in the Central Hardwood Region of Indiana. This research is important for maintaining oaks in the overstory and understory of our oak-hickory forests and it is one of the few existing experiments to document these changes for more than 10 years.

The primary goal is to increase the survival, growth and vigor of oak species while analyzing the effect of fencing and vegetation control. Analyzing these methods over a longer term allows landowners and foresters to make better informed land management decisions.

Researchers anticipated that the crown release would increase the growth of natural and artificial trees in the enrichment planting, allowing the enrichment planting to succeed, due to higher nutrients and sunlight available to the saplings.

#### Methods:

Some enrichment plantings were done in conjunction with a previous shelterwood harvest, a preparatory cut where the smaller trees in the understory and midstory are removed to increase the amount of sunlight reaching the forest floor. Each site was revisited one time, 10-20 years after the original planting. The original experimental design and seedlings were identified and measured for DBH, ground diameter (where appropriate), competitive status, crown width, crown ratio and height. Using this data, researchers assessed the long-term performance of these sites and are currently making management recommendations.

At three of the eight enrichment plantings, researchers performed a crown release in 2022 on half of the planted trees with competitive crown positions to assess their ability to respond to the release and to maintain their status as part of the maturing stand.

Release treatments consisted of partial (two- or three-sided) crown release. Trees that were not competitive were excluded. In addition to the planted trees, natural regeneration (stump-sprouts and seeded trees) of a similar age and competitive canopy were selected for release, allowing researchers to compare the responses between planted trees and natural regeneration.

These experimental trees were all measured at the time of release and again after two growing seasons.

### BETTER BLACK WALNUT BY BREEDING WITHOUT BREEDING

**Principal investigator:** Vikram Chhatre, research geneticist, USDA Forest Service (vikram.chhatre@usda.gov)

**Co-authors:** Keith Woeste, former PI, national program lead for genetics, research and development deputy area, USDA Forest Service; Richard Cronn, research geneticist, Pacific Northwest Research Station, Corvallis, Oregon; Denita Hadziabdic, associate professor, entomology and plant pathology, University of Tennessee; Mo Zhou, associate professor, Forestry and Natural Resources, Purdue University; James McKenna, operational tree breeder, USDA Forest Service (retired); James Warren, biological scientist, USDA Forest Service.

There is a long history of tree breeding and improvement in the United States. Pioneering tree improvement programs focused primarily on southern pines, which are the bread and butter of the construction timber industry. These programs implemented classical tree breeding and selection for economically important traits, a long and arduous process often requiring decades of work before any gains are realized, primarily due to long generation times of many forest tree species. Genomics-based methods have the potential to accelerate this process by incorporating genetic marker data from the family structure when estimating breeding values using statistical methods such as best linear unbiased prediction, or BLUP. The resulting method, G-BLUP, may help accelerate identifying superior parent trees in a breeding program.

The initial goal of this project was to apply this method termed "breeding without breeding" to the numerous black walnut provenance trials established by the HTIRC using phenotypic (growth and form) and genotypic (genetic variation) data to predict superior parents. Researchers anticipate, at least in theory, that well-performing trees can be identified and potentially used by stakeholders in commercial plantations.

Using prior knowledge of the familial relationships within HTIRC provenance trials, researchers collected leaf or bud tissues from 83 mother trees and more than 1,000 progeny. They recorded growth (height) and form (straightness and low branching patterns) from the same individuals.

The tissue is used for isolating genomic DNA, which is subjected to targeted genotyping on a MassArray panel to identify genetic variants in the coding regions of the genome. Resulting data is then used to verify the known mother-progeny relationship, potentially identify the pollen parent and to feed into statistical models to estimate G-BLUPs, which provide a measure of genetic superiority of the parents (breeding value).

Researchers are currently gathering data to estimate the G-BLUP breeding values. However, based on the genetic data available, they were able to verify parent x progeny relationship for at least some of the samples.

"Being able to corroborate field data using genomic data is an important step forward," principal investigator Vikram Chhatre



said. "While we were able to verify some of the parent x progeny relationships, it remains a work in progress. One unexpected result was the low degree of statistical confidence we saw in parental assignment analysis. We are currently reassessing this analysis and hope to provide more details in the summer of 2025."

Future research inquiries would investigate whether this method is suitable for species that do not have a well-established breeding program. Focusing on an array of measurable traits also would broaden the genetic variability to be studied using this statistical model.

#### 2024 Findings

Researchers do not yet have all the data necessary to estimate the G-BLUP breeding values. However, based on the genetic data they had available, they were able to verify parent/progeny relationships for at least some of the samples. Being able to corroborate field data using genomic data is an important step forward.

One unexpected result was the low degree of statistical confidence that researchers saw in parental assignment analysis. They are currently reassessing this analysis.

#### Key Partners/Collaborators

Richard Cronn of the USDA Forest Service from the Pacific Northwest Research Station was instrumental in developing the MassArray genotyping panel that is being used in this study.

James McKenna painstakingly planted many of the HTIRC trials that are part of this study.

Other collaborators include: Denita Hadziabdic and Sarah Boggess of the University of Tennessee at Knoxville, Mo Zhou of Purdue University and James Warren of the USDA Forest Service.



#### Goals:

The overarching goal of tree breeders is to develop planting stock with highly desirable traits (disease resistance, environmental adaptation, superior quality timber, to name a few) as rapidly as possible. If increasing the efficiency and speed of the selection and breeding process is the goal, then "breeding without breeding" is one of the potential tools available to us. This project aims to serve as a "proof of concept" that this technique could work in eastern hardwoods and will therefore be impactful for stakeholders. Depending upon the results of the project, it may be possible to develop the G-BLUP metric further with implications for commercial sector.

This project is aimed at using statistical models that have been tested in a small number of cases. The "breeding without breeding" method (conceptualized by El-Kassaby et al., 2009) has so far been tested only to a limited extent (e.g., Douglas fir, Norway spruce and Scotch pine). From a theoretical standpoint, the method presents a conceptual advancement in the field.

A novel contribution of this project would be to demonstrate that the method could work in eastern hardwood species. It should be noted that among genomics-based methods in the area of forest tree breeding, genomic selection (GS) and genome wide association studies (GWAS) have been successful and broadly implemented. Those technologies, however, require considerably more financial investment for deep sequencing of individuals. The "breeding without breeding" does not require deep sequencing since it uses pre-developed MassArray genotyping method.

#### Methods:

Researchers collected leaf or bud tissues from 83 mother trees and over 1,000 progeny. They recorded growth (height) and form (straightness and low branching patterns) from the same individuals.

The tissue is used for isolating genomic DNA, which is subjected to targeted genotyping on a MassArray panel to identify genetic variants in the coding regions of the genome. Resulting data is then used to verify the known motherprogeny relationship, potentially identify the pollen parent and to feed into statistical models to estimate G-BLUPs, which provide a measure of genetic superiority of the parents (breeding value).

# INDIANA'S FUTURE FORESTS: EMPTIED NICHE OCCUPATION IN AN ASH-LESS WORLD

**Principal investigators:** Michael Saunders, professor, Forestry and Natural Resources, Purdue University (msaunder@purdue.edu); Michael Jenkins, professor, Forestry and Natural Resources, Purdue University

**Co-authors:** Christopher Webster, professor, College of Forest Resources and Environmental Science, Michigan Technological University; Robert Morrissey, owner, Branch Scientific Editing; Thaddeus Swart, research assistant, Forestry and Natural Resources, Purdue University

Emerald ash borer (EAB; *Agrilus planipennis*), a jewel beetle native to northeastern Asia, has led to the decline of ash species (*Fraxinus* spp.) in forests throughout the Central Hardwood Forest Region. Using long-term datasets predating EAB introduction, researchers aim to identify forest responses to overstory species loss resulting from emerald ash borer. This study explores the impacts of ash mortality on forest regeneration and how these shifts in regeneration may contribute to future structural and compositional changes.

In 1996-97, permanent monitoring plots were established across Indiana state parks and nearby reference areas on north-facing slopes of mesic, closed canopy, hardwood forests. These locations were remeasured in 2010-11 and in 2023. Species level data were collected for the seedling, sapling and overstory layers on plots in parks that contained ash trees in 1996-97.

Species responses were found to be highly variable across parks. Blue ash (*Fraxinus quadrangulata*) has largely survived attack by EAB and was widely present as living trees throughout this study. One positive outcome is that invasive species were not as common as expected in the seedling layer.

While considerable ash regeneration was observed within the seedling layer in the 2010-11 resample, in 2023, the recruitment of those seedlings to the sapling layer was found to have largely failed. Instead, shade-tolerant and invasive species have become established

#### Goals:

Using long-term datasets predating EAB introduction, researchers aimed to identify the response of forests to overstory species loss resulting from emerald ash borer. The study explored the impacts of ash mortality on forest regeneration and how these shifts in regeneration may contribute to future structural and compositional changes.

#### Methods:

Permanent monitoring plots were established across Indiana state parks and reference areas in 1996-97 on north-facing slopes of mesic, closed canopy, hardwood forests. These were remeasured in 2010-11 and in 2023. Species level data were collected for the seedling, sapling and overstory layers on plots in parks that contained ash trees in 1996-97.

In 2024, researchers investigated EAB-induced canopy gaps in the Davis-Purdue Research Forest (DPRF) using data from prior censuses and field measurements. All ash trees over 60 centimeters diameter at breast height were mapped and canopy gaps were measured using the Runkle ellipse method. Quadrats were established at 5-meter intervals to assess seedling and sapling density. Gaps were classified by size, single- or multi-tree nature and decay class of dead ash trees. Spatial data were collected via drone imagery and analyzed with ArcGIS. Linear models assessed the relationships between gap size, regeneration and edge tree basal area. Statistical analyses included ordination and similarity testing.



in the sapling layer, with few ash seedlings successfully recruited into the larger size class.

The seedling layer had variable composition and density across parks but generally contained high densities of ash and sugar maple. Most parks were dominated by the same groups of species in 2010-11 and 2023 and show only slight deviation between sampling periods, except for Potato Creek State Park.

Analysis suggests that ash may become functionally extinct in Indiana, even in late successional forests. Early findings prompted further investigation into the implications of ash species loss on the ecological health of closed canopy hardwood forests.

In 2024, researchers concentrated on documenting the ecological impacts of emerald ash borer (EAB) on the Davis-Purdue Research Forest (DPRF), a 21-hectare relict forest remnant located in eastern Indiana, where EAB has caused widespread mortality in canopy ash trees since 2012, drastically altering stand dynamics and threatening biodiversity. Work focused on determining the fate of EAB-induced canopy gaps and how that



disturbance may be influencing tree regeneration and overall species composition.

Findings indicate that shade-tolerant species such as *Acer saccharum* (sugar maple) dominate the understory, while historically dominant canopy species, including oaks and ash, are rarely regenerating. Gap dynamics, influenced by factors like gap size and decay class, revealed trends in lateral infill and seedling density, though some results were statistically insignificant.

This study underscores a shift toward shade-tolerant species, particularly those that grow in environments with moderate moisture levels, in response to EAB-induced disturbance. This shift has implications for long-term forest composition and management.

Despite similarities to other invasive pests, such as Dutch elm disease and chestnut blight, EAB impacts are unique in their near suppression of all ash, contrasting with elm's ability to persist in smaller size classes.

"This research demonstrates the long-lasting ecological changes invasive species can induce," primary investigator Mike Saunders said. "This emphasizes the need for further study to guide forest management and conservation strategies in the face of such disturbances."

#### **Key Findings in 2024**

Findings indicate that shade-tolerant species such as *Acer saccharum* dominate the understory at Davis-Purdue Research Forest, while historically dominant canopy species, including oaks and ash, are rarely regenerating. Gap dynamics, influenced by factors like gap size and decay class, revealed trends in lateral infill and seedling density, though some results were statistically insignificant.

#### **Partners/Collaborators**

Indiana Department of Natural Resources

Davis Purdue Agricultural Center for accommodating researchers during field work activities

Christopher Webster, professor, College of Forest Resources & Environmental Science, Michigan Technological University; and Robert Morrissey, owner, Branch Scientific Editing, who provided advice and historical data

Jinha Jung and the Institute for Digital Forestry for access to the UAS-based imagery for the site

Thaddeus Swart, research assistant, FNR, Purdue University

## 2022 HTIRC-FUNDED RESEARCH GRANTS - FINAL REPORTS

### UNLOCKING BUTTERNUT CONSERVATION THROUGH CUTTING-EDGE SCIENCE

**Principal investigators:** Aziz Ebrahimi, postdoctoral research associate, Forestry and Natural Resources, Purdue University (aebrahi@purdue.edu). Douglass Jacobs, Fred M. van Eck Professor of Forest Biology, Forestry and Natural Resources, Purdue University (djacobs@purdue.edu); Anna Conrad, research plant pathologist, USDA Forest Service; Carolyn Pike, regeneration specialist, USDA Forest Service, Eastern Region — State, Private and Tribal Forestry; John Couture, associate professor, Entomology and Forestry and Natural Resources, Purdue University.

**Co-authors:** Mojtaba Zamani Faradonbeh, postdoctoral research associate, Forestry and Natural Resources, Purdue University; Elisabeth G. Joll, PhD student, Forestry and Natural Resources, Purdue University; James Warren, biological scientist, USDA Forest Service.

Researchers at the HTIRC are harnessing innovative technology to protect one of North America's most threatened trees. Butternut (*Juglans cinerea*) faces an existential threat due to butternut canker disease (BCD) and are threatened across much of their range. With dwindling natural populations and limited regeneration, the time to act is now.

Butternut trees are critical to forest ecosystems, offering habitat and food for wildlife while contributing to the biodiversity and sustainability of forests. They are essential species for indigenous tribes that use them for food and medicine resources. Their loss poses significant risks to the ecological balance of North American hardwood forests. Furthermore, butternut's genetic diversity holds the potential for understanding disease resistance, which could benefit other threatened species. Protecting this iconic tree is not just about conserving a species but preserving a vital piece of our natural and cultural heritage.

Genotyping has become a crucial and efficient tool in efforts to identify genetic diversity and the genetic background within HTIRC's extensive collection, laying the groundwork for future genome-wide association studies. Researchers analyzed genetic material from two main butternut plots planted at Martell Forest using advanced DNA markers. These studies have allowed the team to identify hybrids and pure butternuts and the overall genetic diversity in the plots. They also have also discovered hybrids and a few pure butternuts less susceptible to BCD.

In addition to genotyping, researchers used chemotyping (chemical phenotyping) to analyze disease severity and environmental interactions. Spectral data obtained from near-infrared (NIR) spectroscopy have proven to be a noninvasive and effective method for identifying pure butternut from its hybrid. This method also highlighted spectral shifts linked to disease severity, validating the potential for NIR as a rapid diagnostic tool. However, more data should be collected to prove this method in the future.

"This work integrates traditional morphology with state-of-the-art genomics and digital forestry techniques, supporting chemotyping for forest conservation," co-principal investigator Aziz Ebrahimi said. "It provides a scalable framework for identifying resistant genes for butternut canker disease and could be a model for conserving other endangered hardwood species."

In the future, HTIRC's innovative strategies will guide reforestation efforts and conservation programs. By identifying disease-resistant

#### **Goals**:

The primary goal of this research is to leverage existing butternut research plantings established over the past two decades to support conservation and resistance breeding efforts. The team aimed to integrate morphological, genotypic and chemotypic methods to distinguish pure butternut trees from hybrids, assess their genetic pedigrees and identify disease resistant hybrids.

Anticipated outcomes included the identification of hybrids with Japanese walnut genetics that exhibit resistance to butternut cancer disease. Researchers also sought to develop scalable tools for detection and disease screening using spectroscopy and advanced genotyping techniques.

#### Methods:

*Morphology:* Conducted detailed monitoring of bud break progression in butternut trees using manual observations and biweekly drone imagery from mid-April to mid-May. Monthly drone-based RGB imaging extended data collection into the summer, capturing foliage development and leaf senescence in the fall.

*Genotyping:* Used genomic single nucleotide polymorphism (SNP) panels and genotypingby-sequencing (GBS) to analyze the genetic diversity and hybridity of ~1,500 trees.

*Chemotyping:* Leveraged near-infrared spectroscopy (NIR) to analyze foliar spectral data collected seasonally from northern and southern Indiana sites to determine if hybrid and pure butternut could be distinguished and to assess disease incidence and severity. Complementary chlorophyll content and leaf weight measurements provided additional insights into tree health.

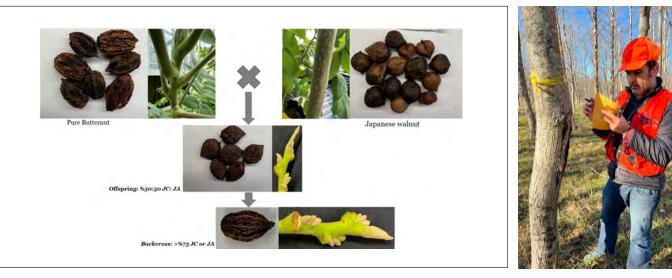


Figure 1: Hybridization process between pure butternut (Juglans cinerea) and Japanese walnut (Juglans ailantifolia). The initial cross produces a 50:50 hybrid offspring (JC: JA), displaying mixed traits of both species. Further backcrossing increases the proportion of one parent species (>75% JC or JA), advancing toward disease resistance and maintaining desired genetic characteristics.

trees and preserving the genetic integrity of butternut, researchers are setting the stage for long-term restoration. Future plans include using drone images (RGB, multispectral and hyperspectral images), genotyping additional trees and conducting genotype-phenotype association studies to deepen the understanding of resistance mechanisms. HTIRC's work not only aims to restore butternut but also sets a precedent for the conservation of other threatened species, demonstrating how science and technology can work together to preserve natural heritage for future generations.

#### **Key Findings in 2024**

Genotyping revealed clear distinctions among pure butternut, hybrids and Japanese walnut accessions, enabling the identification of promising disease resistant hybrids or pure butternut that are less susceptible.

Chemotyping highlighted spectral shifts linked to species, validating the potential for NIR as a rapid fieldbased tool.

Disease incidence and severity were correlated with genetic backgrounds, with some hybrids showing significant resistance to BCD.

Drone imagery demonstrated a high potential for identifying phenological patterns, further supporting hybrid differentiation.

#### **Future Research**

Genotype-phenotype association analysis for identifying genes related to BCD resistance and adaptive traits

Refining breeding strategies to enhance disease resistance while preserving genetic diversity

Conducting long-term monitoring of families (both hybrid and pure butternut) that are less susceptible to BCD and planting them in provenance trials to evaluate disease resistance under climate change scenarios.

Scale up drone-based imaging and spectroscopy tools for broader applications in forest health

Improve finding use multi-omics approaches and transcriptome-wide association analysis

#### **Key Collaborators/Partners**

Keith Woeste (Washington office of the USDA Forest Service); Nicholas LaBonte (USFS National Forest System); Songlin Fei and Joseph Hupy (Purdue Institute for Digital Forestry); Martin Williams and Nathalie Isabel (Canadian Forest Service)

### **iFORESTER: AI-ASSISTED SMARTPHONE APP FOR AUTOMATED TREE INVENTORY**

Principal investigators: Song Zhang, professor, mechanical engineering, Purdue University (zhan2053@purdue.edu); Songlin Fei, professor, Forestry and Natural Resources, Purdue University.

Co-authors: Victor Chen, Cheryl Qian, Rado Gazo, Wang Xiang, Zhiheng Yin, Zizun Zhou

Forest health and sustainability rely on accurate and efficient monitoring, with tree inventories playing a critical role. The initial goal of this research is to develop a practical and efficient tool for accurate tree inventory and valuation, addressing the critical need for sustainable forest management. Leveraging AI and smartphone technology, the iForester app was anticipated to deliver precise DBH and height measurements through contactless methods. These measurements are fundamental, paving the way for future functionalities such as species identification, tree grading and biomass estimation, ultimately supporting informed decision-making and promoting ecological and economic sustainability.

In 2023, iForester released version 1.0 of its AI-assisted smartphone app, capable of accurately measuring tree DBH using LiDAR and RGB data. In 2024, the focus shifted to enhancing mobile AI-based segmentation algorithms and developing a height measurement method. Researchers from Purdue University tested these advancements using diverse tree data from Indiana, combining field observations with iPhone video scans for analysis. The team assessed segmentation and height estimation accuracy, identifying influencing factors. Within expectation, the AI significantly improved the segmentation accuracy (Figure 1) and tree height can be accurately measured using a photogrammetrybased method to overcome the limited measurement range of the iPhone LiDAR sensor (Figure 2). Using mobile Segment Anything Modeling (SAM), 547 out of 562 trees were successfully segmented, over 97% accuracy. For height measurement, experimental results demonstrated an error margin within 1 foot for the first 16 feet of the log.

The findings are crucial, as DBH and height measurement form the foundation of the project. Improved segmentation leads to more accurate DBH and height measurements, while the completion of the height measurement algorithm establishes the data processing workflow, guiding the design of the corresponding user interface and data-saving formats for practical use. The next phase of research focuses on integrating height measurement and AI-based segmentation algorithms into the iForester app. Once the height measurement module is complete, the focus will shift to tree grading algorithms for evaluating log values using DBH and height information. Concurrently, efforts are underway to develop a species classification algorithm based on tree surface vein patterns and size data.



Figure 1: Enhanced tree trunk segmentation achieved through different prompt types (point and box) using mobile SAM.

#### Goals:

The goal of this research is to develop an artificial intelligence assisted smartphone app, capable of delivering precise, contactless measurements of tree diameter at breast height (DBH) and height, By leveraging AI and integrated LiDAR and RGB data, the team anticipated significant improvements in tree trunk segmentation accuracy and predicted that photogrammetry-based methods would overcome the range limitations of iPhone LiDAR sensors for height measurement.

#### Methods:

The team gathered tree data from Indiana using a combination of field observations and iPhone video scans with LiDAR and RGB data. They employed mobile Al-based segmentation algorithms and utilized a photogrammetry-based method to capture and analyze tree measurements. The collected data was then used to study segmentation processes, height estimation and the factors influencing these methodologies.

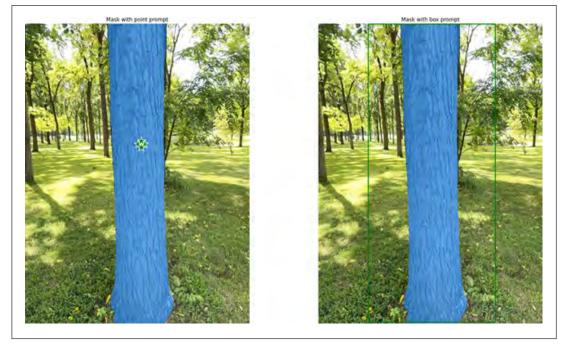


Figure 2: A key frame of the field tree captured from the phone scan, with a nearby rod used for measuring tree height during field observations.

"The iForester app provides landowners and forestry professionals with an efficient tool to streamline tree inventory and valuation, enabling precise DBH and height measurements through contactless methods," principal investigator Song Zhang said. "This innovation replaces labor-intensive traditional practices, improving accuracy and decision-making. By later integrating features such as species classification and tree grading, the app supports sustainable forest management and resource optimization. Its adoption can enhance community forestry practices, contributing to ecological and economic sustainability by helping stakeholders better estimate tree value and manage resources effectively, ultimately delivering financial benefits and fostering ecological stewardship."

"iForester" App is available at the App store; detailed information is at the website: https://digitalforestry.org/iforester/

#### **Key Findings in 2024**

Segmentation: The mobile AI-based segmentation achieved 97% accuracy, with 547 out of 562 trees successfully segmented using the Mobile-SAM algorithm.

Height measurement: A photogrammetry-based method demonstrated an error margin of within one foot for the first 16 feet of the log, overcoming the iPhone LiDAR sensor's limited range.

#### **Future Research**

Integrating the height measurement and AI-based segmentation algorithms into the iForester app to enhance usability and functionality.

Developing tree grading algorithms to evaluate log values using DBH and height measurements, providing valuable insights for forest resource valuation.

Creating a species classification algorithm by analyzing tree surface vein patterns and size data to further support forest management decisions.

#### **Key Collaborators/Partners**

This project unites a multidisciplinary team of researchers from Purdue University, leveraging expertise in mechanical engineering, computer graphics technology, forestry and design.

## **2024 HTIRC-FUNDED RESEARCH GRANTS – FINAL REPORTS** RESISTANCE TO BUTTERNUT CANCER DISEASE: RESTORING BUTTERNUT

### **RESISTANCE TO BUTTERNUT CANCEP TO THE EASTERN U.S.**

**Principal investigator:** Anna Conrad, research plant pathologist, USDA Forest Service (anna.conrad@usda.gov)

**Co-authors:** Douglass Jacobs, Fred M. van Eck Professor of Forest Biology, Forestry and Natural Resources, Purdue University; Aziz Ebrahimi, postdoctoral research scholar, Forestry and Natural Resources, Purdue University; Mojtaba Zamani Faradonbeh, postdoctoral research associate, Forestry and Natural Resources, Purdue University.

Butternut (*Juglans cinerea*) is native to eastern North America and is well-known for its hard-shell nuts and timber value. A member of the walnut family, butternut is found in mixed hardwood forests and in riparian areas. Historically, the species reached as far south as northern Mississippi and northward into southeastern Canada.

Over the last half a century, butternut has been decimated by butternut canker disease. Because of this, butternut is now listed as endangered on the International Union for the Conservation of Nature Red List and in Canada under the Species at Risk Act. The disease is caused by a fungus that causes cankers (lesions) on the branches and stems of trees. These cankers, particularly when abundant and located on the main tree trunk, can lead to tree death.

Despite the disease being present across the range of butternut, not all butternut trees have died. Site conditions may influence the disease. For instance, dry, upland sites are less favorable for the disease. In addition, butternut readily hybridizes with Japanese walnut (*J. ailantifolia*), a non-native tree introduced into the eastern United States in the 19th century. Japanese walnut is known to have higher levels of resistance to butternut canker disease compared to butternut. Research has shown that some naturally occurring butternut/ Japanese walnut hybrids have higher levels of disease resistance, as well.

As a result, restoration of butternut has focused on identifying disease resistant trees, including both butternut and hybrids. This research project is focused on identifying the most resistant butternut and hybrid butternut families, and evaluating how resistance holds up when trees are planted in sites that are suitable for butternut restoration.

To date, researchers have screened more than 300 families for disease resistance. The most resistant families are often hybrid, but some butternut may also have acceptable levels of disease resistance. To evaluate this, the team is identifying the genetic background of the most resistant families and are using that information, as well as the geographic origin of the trees, to select the most suitable trees for study. Sites in Connecticut, Vermont and Ohio are planned, with trees expected to go into the ground starting spring 2026.



#### **Goals**:

The goal of this research is to evaluate how stable resistance is in the most resistant butternut and hybrid butternut families in HTIRC's collection. Researchers anticipate that resistance will vary depending on site locations.

#### Methods:

Disease incidence and severity was collected from butternut trees as part of the HTIRC butternut program. This information was used to identify the most resistant butternut families. Ongoing work to determine the genetic background of trees in HTIRC's collection was leveraged so that knowledge of both genetic background and geographic location can be considered when selecting families for the study. "This research will guide butternut restoration activities in the future," principal investigator Anna Conrad said. "To date, much of our screening for butternut resistance has occurred in plantings that are exposed to heavy levels of disease pressure. These plantings likely do not reflect disease across the broad range of butternut. Therefore, this study will be essential to determine how resistance holds up in sites where butternut may be planted in the future. In addition, these plantings can be used as a future source of butternut seed."

#### **Key Findings in 2024**

Researchers identified partners to host three plantings of resistant butternut in Ohio, Vermont and Connecticut. In addition, a partner was identified to help propagate grafted trees for the study.

Currently researchers are finalizing quantitative genetic analysis to determine the most resistant families to select for grafting, which will begin in winter/spring 2025.



#### **Future research**

This research is still in early stages, but it is anticipated that the plantings established as part of this project will serve as a germplasm resource for future studies focused on restoring butternut within the eastern United States.

#### **Collaborators/Partners**

Jim Warren, biological scientist, USDA Forest Service, Northern Research Station John Butnor, research plant physiologist, USDA Forest Service, Northern Research Station Todd Hutchinson, research ecologist, USDA Forest Service, Northern Research Station Elena Karlsen-Ayala, research plant pathologist, USDA Forest Service, Northern Research Station Carolyn Pike, regeneration specialist, USDA Forest Service, Eastern Region — State, Private and Tribal Forestry

Nick LaBonte, regional geneticist, USDA Forest Service, National Forest System Region 9 Marcus Warwell, regional geneticist, USDA Forest Service, National Forest System Region 8 Caleb Kell, operational tree breeder, Forestry and Natural Resources, Purdue University Carrie Fearer, assistant professor, Forest Resources and Environmental Conservation, Virginia Tech Karl Fetter, postdoctoral researcher, Plant Computational Genomics, University of Connecticut Martin Williams, forest genomics research scientist, Natural Resources Canada Susanna Kerio, assistant agricultural scientist, Connecticut Agricultural Experiment Station Jim McKenna

### IMPACT OF SHADE ON WHITE OAK (QUERCUS ALBA) SEEDLING DEVELOPMENT IN VARIABLE LIGHT CONDITIONS

**Principal investigators:** C. Dana Nelson, project leader/research geneticist, USDA Forest Service, Southern Research Station (charles.d.nelson@usda.gov); Ellen V. Crocker, assistant professor of Forest Health Extension, University of Kentucky (e.crocker@uky.edu)

**Co-author:** Austin Thomas, ORISE postdoctoral scholar, USDA Forest Service Southern Research Station

A preferred strategy for white oak reforestation is enhancement planting in forest gaps. These gaps vary in size and therefore light availability on the forest floor. The Hardwood Tree Improvement and Regeneration Center (HTIRC) and White Oak Genetics and Tree Improvement Program (WOGTIP) are working to improve the performance of white oak seedlings through selection and breeding.

To provide the best testing environment for selecting genotypes that will perform well in variably shaded conditions, researchers need to know the extent to which families respond differently to light conditions to determine the best level of light for testing families' performance in gap plantings.

To answer these questions, researchers with the U.S. Forest Service Southern Research Station Forest Health Center (FHC) at the University of Kentucky and the HTIRC at Purdue University are evaluating the performance of white oak families (seedlings from individual mother trees) grown under varying light conditions (experimental shade treatments).

Traditionally, progeny tests are grown under open-field or full sunlight conditions, but white oak (*Quercus alba*) typically regenerates under at least partially shaded conditions, necessitating the need for determining optimal light conditions for testing white oak families.

"This research will provide needed information to tree improvement programs," principal investigator Dana Nelson said. "Specifically, should they test white oak families in traditional full-sun (open field conditions) for performance in gap plantings, or do they need to test families under lower or variable light conditions? The outcome of this research will begin to answer this question and lead to refinements in the progeny testing strategies developed by tree breeders."

White oak acorns collected from several mother trees in three areas of the Central Hardwood Region (central Missouri, Indiana, and eastern Kentucky) are being grown into seedlings for testing the families' responses to light conditions ranging from full sun to 80% shade. Survival, growth, biomass, and photosynthesis rates will be measured to determine the performance of each family and test for family/ light level interactions.

Significant interactions will suggest the need for testing families under conditions most like the intended regeneration light environment. Alternatively, lack of interactions will simplify future testing protocols, as a single light level will provide appropriate performance data for any anticipated light condition.

The acorns for this research were collected in fall 2024, with the shade treatments being planned for the 2025 and possibly the 2026 growing seasons. Additional families may be collected and entered into testing in fall 2026, depending on funding and acorn mast availability.



#### Goals:

This project aims to examine the growth responses of white oak seedlings under varying shade conditions using 0%, 20%, 50% and 80% shade netting, simulating varying shade levels in forest gaps.

White oaks will represent about 20 open-pollinated families collected from across a west-to-east (wet-to-dry) gradient of the Central Hardwood Region. Survival, height growth, tree biomass and photosynthetic rates will be quantified across seed sources, families and shade treatments.

The findings of this study will result in a better understanding of the genetic contribution to shade tolerance and how variation in the physiological response to shade translates to gap silviculture.

#### Methods:

*Objective 1* of this research is to develop a light response curve for white oak. Researchers will evaluate how seedlings from 20 half-sib families representing three seed sources (central Missouri, Indiana and eastern Kentucky) respond to different levels of light. Approximately 40 acorns per family were sown in 2024 with a goal of achieving 15 healthy seedlings per family in each shade treatment. Measurements of photosynthesis rates and above- and below-ground biomass will provide insight into genetic variation in shade tolerance and help refine recommendations for forest gap silviculture.

*Objective 2* will evaluate half-sib family performance under high and low light conditions. Researchers will test the performance of the same 20 families from Objective 1 under high (full sun) and low-moderate (50% shade) light. They will evaluate genetic parameters such as heritability, genetic correlations and genotype-by-environment interactions for important seedling traits.

Germination (emergence), growth (height, diameter) and biomass will be measured over two growing seasons to assess family performance and refine breeding strategies.

#### **Key Findings in 2024**

Researchers collected acorns from 20 families representing central Missouri, Indiana non-selected and elite (seed orchard source) and eastern Kentucky seed sources.

The acorns were sown into two studies; a third study is pending sowing once conditions permit at the Morgan County Tree Nursery in eastern Kentucky. The first two experiments were sown in containers at UK's South Farm greenhouse facility due to excessive rain and snow. In late January, field conditions permitting, researchers plan to sow a second experiment in the nursery for Objective 2.

#### **Future Research**

Assuming researchers show that light conditions matter for selecting families, larger-scale validation trials will need to be established, further refining the approach. Second, validation of family performance in actual gap plantings will need to be tested. And finally, an efficient protocol will need to be developed and implemented as part of operational tree improvement programs.

#### **Key Partners/Collaborators**

**Primary partners:** HTIRC, Kentucky Division of Forestry, University of Kentucky Departments of Forestry and Natural Resources and Horticulture, U.S. Forest Service Southern Research Station.

**Secondary partners:** White Oak Genetics and Tree Improvement Program (WOGTIP), the Daniel Boone National Forest and the University of Missouri School of Forest Resources.



# COMBINING ENRICHMENT PLANTING WITH OVERSTORY GAPS TO RESTORE DIVERSE CENTRAL HARDWOOD FORESTS

**Principal investigator:** Mike Saunders, professor, Forestry and Natural Resources, Purdue University (msaunder@purdue.edu)

**Co-authors:** Stacy Clark, research forester, USDA Forest Service, Southern Research Station; Don Carlson, forester, Forestry and Natural Resources, Purdue University; Mikaela Scherzinger, assistant forester, Forestry and Natural Resources, Purdue University; Brian Beheler, forest property manager, Purdue University; Clayton Emerson, assistant forest property manager, Forestry and Natural Resources, Purdue University; Robert McGriff, installation forester, Naval Support Activity (NSA) Crane.

Oak regeneration is problematic throughout eastern North America, and researchers fear that the probability of a large-scale replacement of oak by more shade-tolerant tree species, such as sugar maple (*Acer saccharum*) and American beech (*Fagus grandifolia*), may be imminent.

Decades of research in the Ozarks and Appalachia show that successful regeneration of oak species on mesic habitats, or environments with moderate moisture, is possible but often requires the use of shelterwood regeneration systems in conjunction with repeated application of prescribed fire.

This approach has not been as reliable in xeric, or very dry, sites within eastern North America, however. In addition, many landowners do not have the capacity or desire to use fire, or to apply various management techniques in their woodlands enough to achieve success. The use of gap-based silvicultural systems, which involve creating gaps in the canopy to mimic natural disturbances, in combination with enrichment planting, or planting trees to increase the number of a desired species in a forest, may serve as a viable alternative for the regeneration of oak and other hardwood species.

By utilizing the existing enrichment planting study of white oak (*Quercus alba*) at Naval Support Activity Crane in southern Indiana and also establishing a network of plantings in recently harvested gaps on four Purdue Forestry and Natural Resources woodland properties across the state, researchers aim to better define proper management techniques (cultural regimes) for white oak and black walnut in underplanting and enrichment planting contexts. They also hope to provide a network of enrichment planting sites that can be used for physiological and genetic studies, which, in time, may be used for studies of precommercial crop tree release and other intermediate treatments.

In addition, researchers look to corroborate past research on white oak completed at Naval Support Activity Crane, where white oak seedlings were marginally competitive in a partial shade environment after three years. In that case, white oak seedlings could not compete in full sun environments even with two years of competition control. With that project now in its fourth growth season after planting, researchers will compare the midterm effects (i.e., years 5-6) of light levels and competition control on planted white oak in the context of both enrichment planting and underplanting.



#### Goal:

Researchers aim to better define proper management techniques (cultural regimes) for white oak and black walnut in underplanting and enrichment planting contexts. They also hope to provide a network of enrichment planting sites that can be used for physiological and genetic studies.

#### Methods:

The study is planned for six stands across four FNR properties through the state, which vary in site quality and overstory composition. In each stand, three 0.4 – 0.7 hectare openings, or gaps, have been or will be harvested. All overstory is removed within each gap. Edges around the gaps are being feathered to reduce overstory canopy cover to 50%. The midstory is being removed in these feathered areas as well. Initial hypotheses are that growth and survival of the planted white oak will be inversely related to overstory light availability, increase with a longer period of competition control and vary by seed source. with local sources having the best growth and survival rates.

The second objective of the project will build on the Crane study by utilizing a wider variety of seed sources as well as multiple species, which will be planted across a much wider array of site conditions. This work will begin on three Purdue properties: Miller Woodlands, Southeast Purdue Agricultural Center (SEPAC) and Okocha.

It is anticipated that delayed planting of black walnut and/or butternut will allow the persistence of white oak; competition control will result in an increase in growth and survival on productive sites as opposed to less productive sites; and growth and survival of white oak will be greatest on the feathered edge, with black walnut (Juglans nigra) and butternut (Juglans cinerea) doing best in the gap interior. Survival and growth responses will be assessed by species and site conditions over the next four years, covering each of three growing seasons.

This part of the project began with the removal of the entire overstory and midstory in small areas (2-3 acres) and thinning the overstory and midstory to reach a 50% overstory canopy cover in a zone around each area. In May 2024, researchers underplanted 2-0 white oak throughout the harvested areas and into the adjoining unharvested areas. Trees were either tubed or placed inside fenced research plots.

Researchers anticipate that white oak will need release from competitors for a longer period than walnut, likely 2-3 years or more. It is also expected that white oak will survive and be more successful in areas with partial shade from overstory. Walnut, on the other hand, is predicted to be more successful in areas without overstory shade.

In 2025, the team will gather year 1 growth measurements on the white oak, plant black walnut seedlings into the fenced research plots on the existing sites and add three more planting sites to the study (i.e., SEPAC 2, Miller 2, and Martell).

"We expect these studies to provide guidance to professionals and landowners on cultural practices for enrichment planting in forested gaps and under existing canopy," principal investigator Mike Saunders said. "Furthermore, the Crane study, in particular, can provide strong guidance on future crop tree release treatments with the goals of achieving white oak dominance."

Black walnut and/or butternut hybrids will be added to the plantings one growing season after the 2-0 white oak. In sum, each site will have 650-700 white oak and 100-200 black walnut or butternut planted across gap edges. In all cases, ground vegetation and woody competitors will be controlled for the first two growing seasons after white oak planting.

Analysis of two-year growth and survival trends will primarily test for early differences among species and gap positions (North, East and West).

#### **Collaborators:**

Stacy Clark of the SRS of the USDA Forest Service

Matt Salima, master's degree student, Purdue.

Mikaela Scherzinger, assistant forester, Purdue Forestry and Natural Resources, who was key to installing the study on the first three sites.

Brian Beheler, senior farm operations administrator; Don Carlson, forester; and Clayton Emerson, assistant property manager, Purdue FNR, also helped immensely with the work.





### ENGAGEMENT AND EXTENSION

The role of HTIRC outreach is to connect our partners, collaborators and stakeholders with the people, information and products of the HTIRC.

#### **HTIRC NEWS**

For the latest HTIRC news, check out these resources on the HTIRC website:

- E-newsletters htirc.org/resources/newsletters/
- News archive httrc.org/news-archive/
- Annual reports htirc.org/annual-report/

### **EXTENSION PRODUCTS**

In 2024 we produced or updated a variety of online resources, including videos on hardwood management, invasive species and tree identification. These are posted at https://htirc.org/resources/landowner-information/ and https://www.purdue.edu/fnr/extension/. They include:

- ID That Tree series: Over 130 videos highlighting native and invasive trees and shrubs.
- videos intended for landowners.
- issues relevant to woodland owners.
- These publications are utilized by landowners and resource managers.

#### New Extension products released in 2024

 Deer Impact Toolbox, part of the Integrated Deer Management Project at Purdue University. The toolbox includes:

Four publications: Introduction to White-Tailed Deer Impacts, Understanding White-Tailed Deer and Their Impact on Indiana Woodlands, Monitoring White-Tailed Deer Impacts, Managing White-Tailed Deer Impacts on Indiana Woodlands

Two videos on deer impact monitoring methods Deer browse monitoring web tool

EAB University Webinars on: Emerging Sassafras Wilt in Indiana. Olivia Bigham Planting Trees for Resilient Woodlands. Lenny Farlee Recognizing Native vs Invasive Trees. Lenny Farlee - To be posted soon

#### **PROGRAMS**

These programs shared the latest HTIRC information to landowners and/or natural resources professionals in a mix of online and in-person formats:

- available at https://rngr.net/resources/webinars/2024-butternut-workshop/.
- Landowners Conservation Tree Planting Workshops
- Forest Management for the Private Woodland Owner courses
- Walnut Council Field Days
- Forest Pesticide Applicators Continuing Education Program
- IFWOA/Tree Farm Landowners Field Days

Woodland Management Moment series: This series covers various woodland management topics in short

Woodland Stewardship for Landowners video series: Videos from this collaborative series between Purdue Forestry and Natural Resources and the Indiana Department of Natural Resources address management

• The Planting and Care of Fine Hardwood Seedlings publications provide practical information to landowners and managers for the establishment and management of hardwood trees in plantations and native forests.

• Conservation Tree Planting Webinar covering the steps to success for conservation tree plantings.

 September Butternut workshop brought together researchers from Canada and the United States working to conserve American butternut. Researchers are examining both the tree and the disease to develop strategies for conservation and restoration of the species. Recordings of the presentations from the workshop are

- Training for U.S. Department of Agriculture, Department of Natural Resources, Soil and Water Conservation Districts and other agencies on conservation practices
- Invasive Species field days and presentations
- Presentations at the Walnut Council annual meeting •
- Presentations at Green Industry continuing education programs
- Natural Resources Teacher Institute educator training program
- Society of American Foresters Indiana and national meetings

We actively engage with our partners and many other groups, agencies and organizations with similar goals and interests to understand management and information needs and facilitate distribution of research-based tree and forest management information to appropriate audiences.

### EDUCATION

Developing future researchers and practitioners with expertise in the science and application of tree improvement, management and protection of hardwood forests is fundamental to the HTIRC. This year, through our project-based funding model, we supported four master's students, eight PhD students, eight postdoctoral researchers and six undergraduate research technicians.

#### **HTIRC STUDENTS WHO GRADUATED IN 2024**



#### Aishwarya Chandrasekaran, PhD

Thesis title: "Broadleaf Tree Species Classification Using UAS and Satellite Imagery" Advisor: Guofan Shao



#### **Elias Bowers Gaffney, MS**

Thesis title: "Evaluating Artificial White Oak (Quercus alba) Regeneration Along Light Competition Gradients" Advisor: Michael Saunders



#### **Brianne Innusa, MS**

Thesis title: "Evaluating Resource Competition of Live Oak (Quercus Virginiana) Regeneration to Support Maritime Forest Restoration" Advisor: Douglass Jacobs



#### Sylvia (Minjee) Park, PhD

Thesis title: "Hyperspectral Characterization of Forest Health" Advisors: John Couture and Douglass Jacobs

### 2024 TREE IMPROVEMENT REPORT

#### Author: Caleb Kell, operational tree breeder, Hardwood Tree Improvement and Regeneration Center (ckell@purdue.edu)

For more than 25 years the HTIRC has sought to improve the economic value and resilience of forests within Indiana and the Central Hardwood Forest Region via population improvement for high-value hardwood species and development of refined regeneration approaches. 2024 was a productive year for the HTIRC's tree improvement program, with the complete measurement of 13 black walnut and two white oak test plots, the grafting of 102 new selections, the establishment of four new genetic tests and the harvest of more than 110,000 seeds for research and stakeholder needs. The HTIRC's new commercialization agreement with Tree Pro will further the HTIRC's ability to make a positive impact by distributing the HTIRC's best select seedlings beyond Indiana.

#### **BREEDING SUMMARIES BY SPECIES**

#### **American Chestnut**

Development of a chestnut blight (Cryphonectria parasitica)-resistant American chestnut population is an ongoing endeavor at the HTIRC, with steady progress being made with both the transgenic and hybrid resistance breeding models. Breeding a blight-resistant American chestnut requires a diverse breeding pool of wild-type American chestnuts, and the HTIRC continues to maintain a grafted collection of American chestnut germplasm. Four new clones and six existing clones were grafted in 2024, bringing the HTIRC's count of American chestnut clones to 52.

For the transgenic breeding effort, the second replicate of the HTIRC's Darling 54 transgenic American chestnut screening trial was inoculated with the chestnut blight pathogen in July, with canker and growth data recorded in December 2024. Like the first replicate of crosses made in 2019, canker resistance and growth rate were highly variable in transgene-positive trees. A significantly lower level of canker size and severity in transgenic trees compared to wild-type controls also was consistent with the first replicate. Out of the 400 inoculated trees, 29 displayed a combination of strong resistance and vigorous growth and were selected for grafting into a nextgeneration breeding orchard.

The creation of new F1 American x Chinese crosses continues to be the focus of the HTIRC hybrid chestnut breeding effort. With time, the goal of these crosses is to create F2 crosses for selection of hybrid trees with both excellent timber form and strong blight resistance. Intercrossing of these F2 selections into the backcross breeding program will produce a population of hybrid trees diverse and blight-resistant enough to use for restoration purposes.

#### **Black Cherry**

A major black cherry project for 2024 was fencing a mixed black cherry progeny test on Indiana Nature Conservancy land in Union County. Established in 2021, this test was planted on good soils with a mixture of black cherry, black walnut, butternut, northern red oak and white oak. Survival at the planting is better than 90%, but excessive deer browse prevented the planted trees from growing above the browse line. Now with deer protection, a coppice in spring 2025 should restore the potential of this planting as a genetic test.

Black cherry orchards had a modest crop this year once again, primarily at the Lugar Forestry Farm, with most of the 22,000 seeds harvested split between the Indiana Division of Forestry and Tree Pro. Seed production at the Martell orchards continues to be inconsistent, an issue that hopefully will be relieved by a more aggressive spraying and fertilization program in 2025.

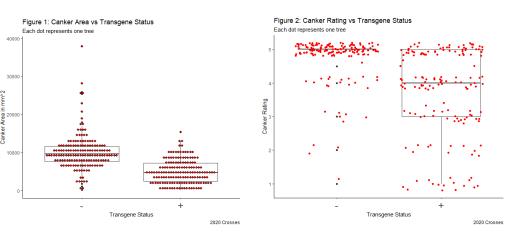
#### **Black Walnut**

Measurements of 13 HTIRC walnut progeny tests were completed by January 2024, giving a trove of new performance data for roughly 100 walnut families. From the new data, 44 new second-generation selections were made, with roughly 60 remaining to be grafted.

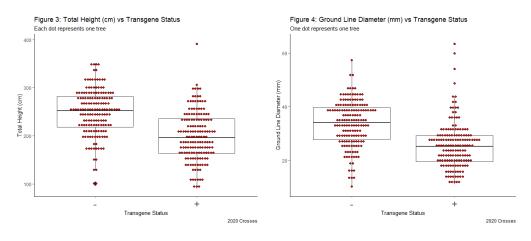
A new mixed silvicultural study and progeny test for black walnut and butternut was planted in May. Half butternut and half black walnut, these tests are designed to directly compare the growth performance of these

two walnut species and explore the potential of using butternut as a trainer tree in black walnut plantations at 8 foot x 8 foot and 8 foot x 6 foot spacings. The two 2024 replicates are a delayed-planting design, with butternuts planted one year earlier than the walnuts. The 2025 replicates will see the walnuts and butternuts planted at the same time.

After 2023's bumper crop, the black walnut crop at the HTIRC's orchards was light. All seed harvest efforts were directed to collecting seed for the Indiana Division of Forestry or Tree Pro except for some unique clones. A new oversized sizing chain for the HTIRC's Savage seed harvester arrived in September and greatly increased the HTIRC's collection capacity for bulk seed. Overall, the HTIRC was able to supply the Indiana Division of Forestry with 50,000 bedrun seeds and 6,000 select black walnut seeds



American chestnuts that tested positive for the oxalic acid oxalase (OxO) transgene displayed significantly smaller cankers (Figure 1) and a lower (Figure 2) canker rating than transgene-negative trees. OxO-positive trees continue to display a significantly lower growth rate than wild-type trees (Figures 3,4).



#### Butternut

The HTIRC continues to be a leading institution for butternut research and conservation. With the world's largest butternut germplasm collection and multiple grafted orchards, the HTIRC's goal of butternut restoration is well within reach. In September, the Butternut Working Group (or BUTRI) convened at Martell Forest. A diverse consortium of researchers, foresters and interested landowners



Three of the 44 new second-generation walnut selections grafted in 2024.

from the United States and Canada discussed the latest advances in butternut research and ongoing restoration efforts for the first time since 2019. The HTIRC was well represented, with research presentations given by HTIRC researchers and the operational tree breeder. Interest in butternut is growing appreciably, but there is still a pressing need for resistance breeding, increased orchard capacity and more nurseries to grow and distribute butternut seedlings.

To facilitate restoration efforts in Indiana and beyond, the HTIRC partnered with Tree Pro to distribute both butternut canker disease-resistant hybrids and susceptible pure butternut seedlings. The new Buckeye State Nursery in Ohio also received a mixed lot of pure and hybrid seed for distribution. Combined with the HTIRC's ongoing partnership with the Indiana Division of Forestry, these three nurseries are each

The HTIRC's population of screened resistant pure and hybrid butternut continues to expand and diversify, with 24 new clones added in 2024. These clones represent the conclusion of a 2005 hybrid butternut progeny test established at the Eli Lilly-Evonik plant in Tippecanoe County as well as the first year of selections from the Martell 2011 Butternut IV screening block. The evaluation of new butternut germplasm also continues with the establishment of a new butternut screening block at the Jasper-Pulaski State Nursery. Most of the seedlings in this new trial are from wholly untested clones and seedling families, ensuring the discovery of new disease-resistant butternut selections for years to come.

The resistant butternut seed orchard at Pinney Purdue Agricultural Center produced a substantially lower crop than in 2023, while seedlings at the Martell Butternut IV screening block produced their first significant crop. Most of the seed harvested from these seedlings was sent to Tree Pro and the Buckeye State Nursery, but a sample was kept from each seedling to facilitate a new FNR undergraduate research project evaluating the nut morphology of pure and hybrid butternut. Overall, a mix of 6,300 susceptible pure and untested hybrid butternut seeds was sent to the Ohio DNR's Buckeye State Nursery, while 2,500 seeds from resistant trees were provided to Tree Pro. Despite a large showing by Hoosier National Forest staff, the pure butternut seed orchard in Huntingburg yielded only about 15,000 seeds, far less than last year's harvest.

#### **Northern Red Oak**

Thinning was completed in the 2008 and 2009 Martell progeny tests to facilitate seed production, but no further breeding steps were taken in 2024. Seed collection of the Martell progeny tests was hampered by excessive vertebrate and invertebrate seed predation, and only 20% of the seed harvested from the Martell orchards was viable. The grafted red oak subline at the Jasper-Pulaski State Nursery had a strong crop, and lightweight "catch nets" were used to capture and hold acorns as they dropped. These nets substantially increased the amount of seed harvested in a relatively remote area, while reducing the re-entry interval to only twice per week.



The acquisition of an oversized sizing chain (left) for the HTIRC's Savage pecan harvester considerably increased the speed of bulk harvests of black walnut, especially in seed orchards like the grafted select black walnut orchard at the Jasper-Pulaski state tree nursery (right).

contributing toward the goal of bringing the butternut back to the Central Hardwood Region.

#### White Oak

In January 2024, measurements of the first two HTIRC white oak progeny tests planted in 2012 were completed. While both tests were small in size, strong marcescence in some families posed a challenge not encountered with other HTIRC target species, as the

retained leaves obscured stems and hampered accurate measurements of merchantable height and stem straightness. Accurate measurements of white oak might require collecting data during the spring, after bud swell pushes last year's leaves off.

Collection of new white oak germplasm is ongoing. Twentyone new plus-tree selections were collected and grafted in 2024. These selections are frequently the highest-quality white oaks in a tract, with emphasis on trees with clear boles and little to no epicormic sprouting. Nine new elite selections were pulled from white oak plantations in southern Indiana, where only the best few trees in each plantation are selected. A new clone bank of grafted plus-tree



Two excellent white oak plus-tree selections from Yellowwood State Forest.

selections was established at the Richard S. Lugar Forestry Farm, with enough expansion room to accommodate hundreds of white oak clones at orchard spacing.

Progeny testing of existing HTIRC white oak orchards restarted in 2024, with a new test established in May at a former HTIRC black walnut progeny test site in Union County, Indiana. This planting was the first white oak progeny test to use 2-0 stock. Use of the larger 2-0 seedlings alleviated the size issues encountered with 1-0 white oak stock, but also created new issues as some seedlings were too large to fit through the shoe of the tree planter and had to be planted by hand.

The normally prolific precocious white oak orchard at Martell suffered a nearly complete crop failure. While last year's heavy crop may have contributed, the same seed predation issues that plaqued the adjacent northern red oak orchards also consumed many acorns from the precocious white oak orchard as well. Conversely, the elite white oak orchard at the Lugar Forestry Farm yielded more than 18,000 acorns. Seed was also collected from wild trees throughout Indiana to serve as genetic checks for progeny testing and to support Dana Nelson's HTIRC-funded grant for white oak. Overall, 7,400 elite and bedrun seeds were sent to the Vallonia State Nursery; 5,900 seeds from elite sources were sent to Tree Pro; and 4,000 seeds from 10 mother trees were furnished to the HTIRC-funded grant. Nurseries were also sown for white oak progeny tests in 2026 and 2027.

## CREATING A DIRECTORY, DEPOSITORY AND DATABASE FOR HISTORICAL GENETIC AND TREE IMPROVEMENT TRIALS

Author: Katie Grong, research associate, Forestry and Natural Resources, Purdue University (kgrong@purdue.edu)

Throughout the mid-to-late 20th century, federal, state and tribal governments established thousands of genetic and tree improvement field trials across the United States. These studies were established primarily for hardwood and conifer tree species with commercial value to study genetic variation within and among different populations. Over time, however, many of these plantings have been abandoned or forgotten due to the retirement of key scientists, lack of funding or a shift in funding priorities. Some plantings have been misplaced, but many plantings still exist on the landscape along with hard copies of data residing in offices and storage facilities, even though the plantings themselves were abandoned.

This untapped resource is in demand by 21st-century scientists to help overcome seed shortages and to better understand the effects of assisted migration on genotypes and provenances. Genetics trials also may be useful in the quest to locate seed sources with improved resistance to invasive pests. Thus, locating these plantings, and their corresponding metadata, is essential to help answer questions about the adaptability of our tree species to future conditions.

In 2022, the HTIRC began an effort to preserve historical documents and data from genetic and tree improvement trials to be archived in a centralized public directory through a cooperative agreement with Purdue and the United States Forest Service. Purdue Forestry and Natural Resources (FNR) professor Songlin Fei and Forest Service regeneration specialist Carolyn Pike have guided this project. FNR research associate Katie Grong has worked under the direction of both to gather and digitize the historical planting data for inclusion in the directory and public dashboard.

#### **Methods**

- Researchers used "A Guide to Forest Tree Collections of Known Source or Parentage" by Raymond Guries, Susanne Brown and John Kress; "1981 Directory of Forest Tree Seed Orchards in the United States" by the USDA Forest Service; and "A Guide to Forest Genetics Field Trials at North Central Forest Experiment Station" by Jerry Van Cleve, Don Riemenschneider and George Rink; and the HTIRC/Forest Service plantings database organized by James Warren to create a list of historical genetic tree plantings.
- Data and information were received from the Tree Regeneration Center at Michigan State University; Fernow Experimental Forest in Parsons, West Virginia; Daniel Boone National Forest in Winchester, Kentucky; and the Ohio Agricultural Research and Development Center at Ohio State University.
- Missouri; the USDA Forest Service Region 9 Office in Milwaukee, Wisconsin; Cloquet Forestry Regeneration Center at Michigan State University in East Lansing, Michigan, to digitally scan and physically collect the data of historical genetic tree plantings to digitize in an electronic format.
- A website was created for the directory, depository and database for currently verified plantings and research purposes or communicate about planting information that could be added to the database
- Researchers developed a catalog that inventories all scanned documents and tags them based on common attributes of interest (survival/health, measurements, genetics/genealogy/seed, correspondence, plans/maps)

#### **Key Findings and Progress to Date**

• Using the above approach, the existence of 2,183 plantings has been confirmed across 48 states. Of the 2,183, a total of 503 plantings were verified to be active and live plantings; 268 were verified to confirmed to be active but researchers are awaiting location information to verify them.

Visits were made to Vallonia State Nursery in Vallonia, Indiana; the University of Missouri in Columbia, Center in Cloquet, Minnesota; the Northern Research Station in Rhinelander, Wisconsin; and the Tree available data that can be accessed by interested researchers using Weebly and ESRI products. The website includes a request and contribute data feature that enables users to view and request data for

not be recoverable; 48 were verified to be abandoned but not removed (inactive); and 355 have been

- Out of the 2,168 plantings that have been confirmed, only 629 have an accompanying dataset. Some of the data that are available are very limited, but approximately 13% do include extensive data measurements and accompanying documents.
- Researchers have digitized approximately 7% of collected data into a workable format for statistical programs.

#### 2024 Progress

- Katie Grong continued to make progress on expanding the project connections, data acquisition
  and planting confirmations with emphasis on New England and expanding into the southern region.
  Documents have been shared by Nebraska, New York and Maine that are in the confirmation
  process. Grong also traveled to the University of Minnesota's Cloquet Forestry Center to finish
  scanning physical records stored there. Documents across 89 studies/plantations were digitally
  recorded.
- Researchers generated a standardized cataloging and tagging system for scanned documents.
- Original document sources were digitized to increase ease and accuracy of inquiries for planting data.
- Relevant planting data from 2,796 pages spanning nine studies also were digitized.
- A catalog was developed that inventories and tags scanned documents. Records from Wisconsin are the primary focus. Thus far, 390 records spanning 35 studies have been cataloged. Of those,198 have been tagged.
- Fifteen plantings were added to the master list, thanks to the addition of plantings in a new state, and all were confirmed.
- Plantings within the scanned documents collected this year are in the process of being added to the master list and confirmed. Their addition to the confirmation database is awaiting the finalization of automation and reorganization of the system for planting metadata.
- Physical files, from 15 boxes stored at the John S. Wright Center at Purdue, were re-filed and organized into more permanent, safer storage with the help of undergraduate student research assistants.



## HTIRC PARTNERS WITH TREE PRO TO DISTRIBUTE HARDWOOD SEEDLINGS

The Purdue Hardwood Tree Improvement and Regeneration Center has signed a commercial partnership agreement with Tree Pro to distribute select hardwood seedlings from its breeding program beginning in March 2025.

select hardwood seedlings from its breeding program beginning in March 2025. The partnership, which will mark the HTIRC's first commercial release of seedlings in its 25-year history, will see West Lafayette, Indiana-based Tree Pro distribute bareroot seedlings of six species across its timber select, conservation and wildlife lines. Timber select varieties are black walnut, black cherry, northern red oak, white oak and pure and hybrid butternut. Conversation species are pure butternut and American chestnut. The wildlife line features select precocious white oak.

For more than five decades, Purdue University and the HTIRC have assembled, tested and selected populations of Indiana's most valuable hardwood tree species (black cherry, black walnut, northern red oak and white oak) for deployment across the Midwest's Central Hardwood Forest region. Populations of all four species have been selected for stem straightness and growth rate and are under continuous improvement as breeders remove underperforming clones and seedlings from seed production areas.

Today, as a product of the HTIRC's efforts, numerous progeny tests, grafted clone banks and orchards exist at Purdue properties across Indiana and are now producing commercial quantities of seed for distribution. A commercial partnership with Tree Pro gives the HTIRC an outlet to distribute its hardwood trees to landowners throughout the Central Hardwood Forest Region.

"Tree Pro's established reputation with conservation groups, private landowners and foresters makes them an ideal distributor of the HTIRC's select material," said Matt Ginzel, director of the HTIRC. "We want to make it clear that this partnership will not take away from our existing relationship to provide seeds and seedlings to the Indiana Department of Natural Resources. This simply gives us another avenue to get our material into the hands of the public and to achieve our goal of increasing the value and resilience of woodlands by enriching the diversity of seedlings available for reforestation efforts."

Tree Pro has been in business for 37 years and is known for its tree protection products (Miracle Tube) and lines of fruit and nut trees oriented to hunters and wildlife enthusiasts.

"When we were approached about the opportunity to distribute genetically diverse select hardwood seedlings from the HTIRC, we were instantly excited," said Tommy Mills, co-owner of Tree Pro. "It brings our start as a company with black walnuts full circle."

In addition to making selections on four major hardwood species, the HTIRC has been working to preserve and develop disease-resistant populations of two endangered native fine hardwood species — butternut and American chestnut.



An overhead shot of the HTIRC's Elite white oak orchard at the Lugar Farm.



A bagged and caged American chestnut bur from the HTIRC's American chestnut resistance breeding program.

After more than 20 years of work, the HTIRC now possesses the most diverse collection of butternut in the United States and has made significant advances in breeding butternuts resistant to the deadly butternut canker fungus. The HTIRC's canker-resistant butternut program utilizes both pure butternut and hybrids between the butternut (Juglans cinerea) and the Japanese walnut (Juglans ailantifolia), with a program goal of decreasing the proportion of Japanese walnut genetics in the population while increasing overall canker resistance. A collection of pure, disease-susceptible butternuts also is maintained for research purposes and provides valuable seed for organizations and individuals that prefer pure butternut over hvbrid butternut.

The American chestnut, once the backbone of timber production for all uses in the eastern United States and a prolific producer of nuts for animals and humans alike, is now functionally extinct due to the accidental introduction of the chestnut blight fungus in 1904. In collaboration with the American Chestnut Foundation and other entities, the HTIRC has assembled an American chestnut collection that represents pure American chestnuts from Indiana and neighboring states. It stands as one of the few grafted American chestnut collections in the country. While currently unavailable for public release, the HTIRC's ongoing efforts to breed a blight-resistant American chestnut encompasses the use of both interspecific hybrid breeding and biotechnology to restore a long-lost forest species.

"The mother trees for HTIRC's Timber Select lines were all selected for straight stems with few defects, like low forks, that can negatively impact standing timber value. The Timber Select butternuts were selected for their resistance to butternut canker disease," said Caleb Kell, operational tree breeder for the HTIRC. "Landowners have a good chance of getting similar characteristics out of their seedlings if they are planted on suitable soils with deer protection, which is one of Tree Pro's specialties."



#### 1. HTIRC Timber Select Black Walnut (Juglans nigra):

HTIRC's black walnut clone bank at Martell Forest.

These seedlings are the result of more than 50 years of black walnut breeding at Purdue University. They are grown from seed from the best performing parent trees in dedicated seed orchards. Seedlings from these orchards frequently outperform seedlings from other sources in numerous Purdue test plantings and represent outstanding trees from across Indiana with a high degree of genetic diversity. Just recently, second-generation selections along with orchard-sourced trees of the best first-generation selections are being mixed and exceed 50 unique genotypes to maximize genetic diversity.

#### 2. HTIRC Timber Select Black Cherry (Prunus serotina):

The parent trees for these seedlings were collected from veneer-grade trees throughout Indiana, Michigan and the Allegheny Plateau of Pennsylvania, where there is a concentration of high-guality black cherry veneer trees. Assembled into dedicated seed orchards, seedlings from these parent trees frequently outperform seedlings from other sources in Purdue test plantings and have shown the most genetic gain in selection. This collection consists of roughly 60% Pennsylvania and 40% Indiana trees, with some originating from Michigan. Our research has discovered that not all PA trees are adapted to Indiana and the upper Midwest, and those are being rogued out.

### 3. HTIRC Timber Select Northern Red Oak (Q. rubra):

These second-generation seedlings are from the best performing trees at Purdue in test plantings of over 70 elite parents selected from forests and plantations. Only trees with above-average growth and timber form are selected. Thinning of poorly growing families and individuals is ongoing. The families come from a wide geographic range and offer a very high level of genetic diversity.

#### 4. HTIRC Timber Select White Oak (Quercus alba):

HTIRC Timber Select white oak is from an isolated grafted white oak orchard at Purdue University. Parent trees for these orchards were selected from test plantings across Indiana for above-average height growth and timber form. New selections are discovered and added every year to maximize diversity.

#### 5. HTIRC Timber Select Pure and Hybrid Butternut (J. cinerea and ([J. ailantifolia × J. cinerea] × J. cinerea)):

Seedlings sourced from a HTIRC breeding orchard of grafted hybrid and pure butternut trees, approximately 50:50 (half pure and half hybrid clones) selected for high butternut canker disease resistance in screening trials at Purdue. The hybrids contain Japanese walnut (J. a heavy walnut crop. ailantifolia), which add to and enhance the limited resistance of pure butternut. Hybrid selections are largely three-guarter *J. cinerea* and one-guarter J. ailantifolia as determined through DNA and morphological analyses. Formal HTIRC studies have additionally revealed that these butternut hybrids perform nearly identically to pure butternut when planted in the wild.

#### 6. HTIRC Conservation Pure Butternut (J. cinerea):

These are pure butternut seedlings sourced from a grafted orchard established by the Hoosier National Forest and the HTIRC. Parents consist of genetically tested pure butternuts sourced from southern Indiana, Ohio and northern Kentucky. Designed to provide seed for reforestation, this pure J. cinerea orchard has begun to produce more seed than the Hoosier NF can plant. Additional sources and backgrounds will be added as new HTIRC butternut conservation orchards mature.

### 7. HTIRC Conservation American Chestnut (Castanea dentata):

Pure American chestnut is a keystone species of the Eastern Deciduous Forest that was decimated by the chestnut blight fungus introduced from Asia in 1904. Seed is sourced from grafted pure American chestnut orchards and rare wild survivors from our Indiana chapter of The American Chestnut Foundation (TACF) cooperators. The HTIRC provides material in support of ongoing breeding and research needs first. Excess seed is then distributed to the public. Seed for this line is collected only from isolated places without other chestnut species to hybridize with. All of these American chestnuts are highly susceptible to chestnut blight and have no genetic resistance and are best used as seed trees for conservation and wildlife attraction.

### 8. HTIRC Wildlife Select Precocious White Oak (Q. alba):

Seedlings sourced from a grafted orchard of white oaks selected for early flowering starting at 9 years of age. Additionally, many of these trees bear large and regular crops of acorns. This option is a valuable addition for those wanting to add white oak acorns to their wildlife food plots. The timber form and growth of these selections is average at best, as parents were selected only for precocity and acorn yield.



A good example of a black walnut with

### **HTIRC PUBLICATIONS 2024**

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- Thomas, A.M.; Coggeshall, M.V.; O'Connor, P.A.; Nelson, C.D.; 2024. "Climate adaptation in white oak (Quercus alba, L.): A forty-year study of growth and phenology." Forests 15(3), 520.
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### **ENGAGE WITH HTIRC**

#### Did you know?

HTIRC breeding program plant materials are available to the public from two sources starting in spring 2025. Some of this material was selected for stem straightness and growth rate and is under continuous improvement as breeders remove underperforming clones and seedlings from seed production areas. The HTIRC has also been working to preserve and develop disease-resistant populations of two endangered native fine hardwood species - butternut and American chestnut.

Seedlings can be ordered from:

cherry. DNR: Forestry: Tree Seedling Nurseries



 Tree Pro distributes bare root seedlings of six species across its timber select, conservation and American chestnut. The wildlife line will feature select precocious white oak. Purdue HTIRC Timber Select Seedlings - Tree Pro.



### How Can I Get Involved with HTIRC?

- Do you have property you would like to donate or to allow HTIRC to utilize for research?
- Do you have ideas for research projects that could help advance the science and application of tree improvement, management and protection of hardwood forests, especially as they related to tree breeding, tree nursery practices, tree plantation establishment and management and silvicultural systems in the Central Hardwood Forest Region?

If so, let us know at htirc@htirc.org or 765-496-7251.

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Check out our webpage: htirc.org

Give a tax-deductible donation to our research fund.



 Indiana DNR Division of Forestry tree seedling nursery sells bare root select tree seedlings for conservation plantings in Indiana. Species include black walnut, red oak, white oak, and black

and wildlife lines. Timber select varieties will include black walnut, black cherry, northern red oak, white oak and pure and hybrid butternut. Conservation species include pure butternut

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# LEARN MORE ABOUT THE HTIRC

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