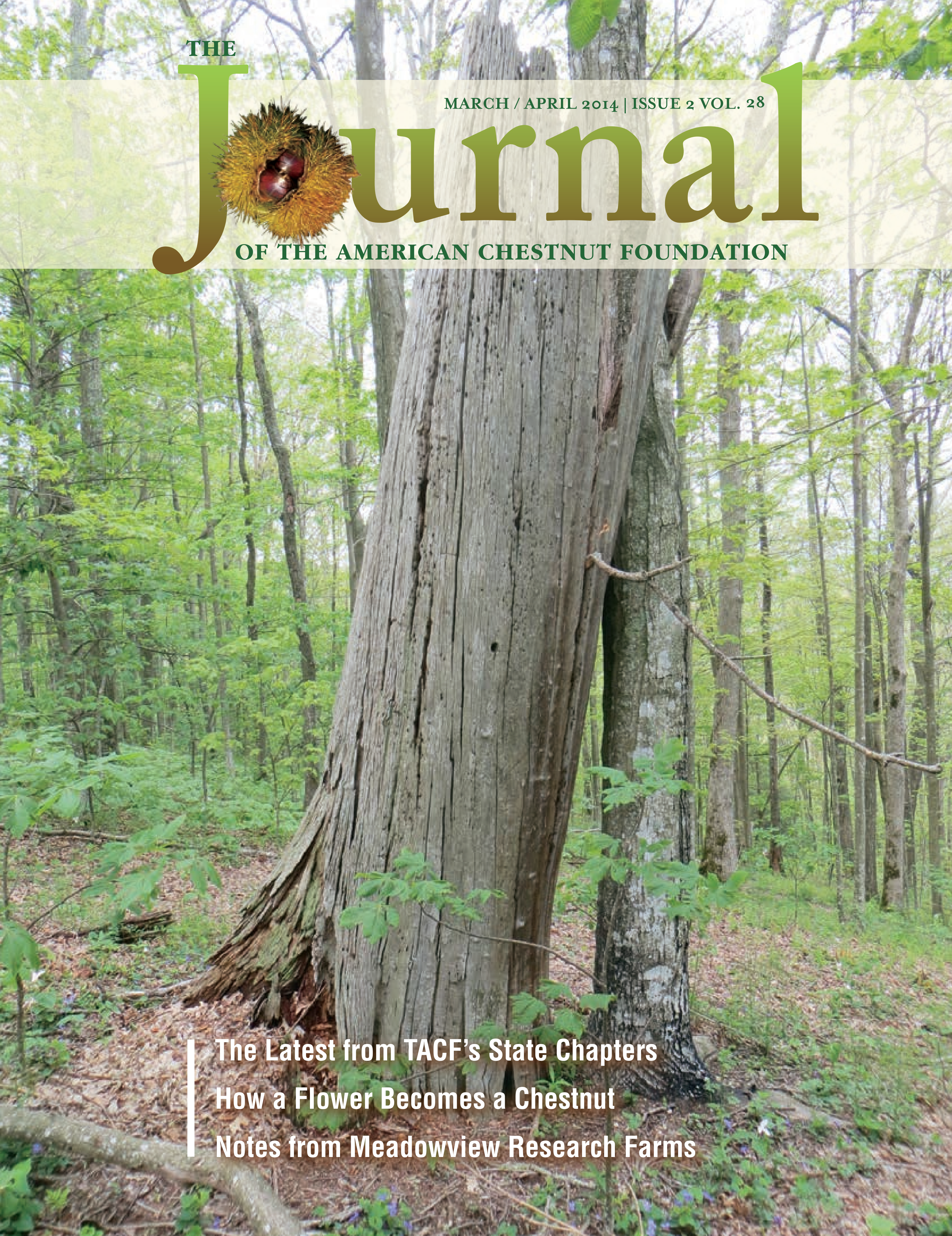
THE Journal OF THE AMERICAN CHESTNUT FOUNDATION

MARCH / APRIL 2014 | ISSUE 2 VOL. 28



The Latest from TACF's State Chapters

How a Flower Becomes a Chestnut

Notes from Meadowview Research Farms

Join Us this Fall in Front Royal, Virginia for TACF's 31st Annual Meeting

October 17 - 19, 2014

at the Northern Virginia 4-H
Educational Conference Center

Look for details in the next issue of *The Journal*

Northern Virginia 4-H
Educational Conference Center

The Center is situated on 229 acres of land nestled in the Blue Ridge Mountains, sitting at the gateway to Shenandoah National Park and is only minutes away from historic Front Royal. For more information about the venue, visit www.nova4h.com.

THE AMERICAN CHESTNUT FOUNDATION®

The Mission of The American Chestnut Foundation

Restore the American chestnut tree to our eastern woodlands to benefit our environment, our wildlife, and our society.

We harvested our first potentially blight-resistant nuts suitable for widespread testing in 2005, and the Foundation is beginning reforestation trials with potentially blight-resistant American-type trees. The return of the American chestnut to its former range in the Appalachian hardwood forest ecosystem is a major restoration project that requires a multi-faceted effort involving 6,000 members and volunteers, research, sustained funding, and most important, a sense of the past and a hope for the future.

About Our Cover Image

This issue's cover photo was taken by Jon Taylor of Asheville, North Carolina, a Carolinas Chapter volunteer who dedicates many of his weekends to counting American chestnuts along the Appalachian Trail (AT) for the AT MEGA-Transect Chestnut Project. Jon came across this ghost chestnut while hiking in North Carolina. The photo earned first place in TACF's 2013 Photo Contest. Stay tuned this summer for the opportunity to submit your chestnut photos to our 2014 Photo Contest.

THE
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INSIDE THIS ISSUE

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An American chestnut sapling reaches toward the sun.
Photo by Mathew Morse

An American chestnut in Fitzgerald, Maine.

A Time for Growth

By Bryan Burhans, TACF President & CEO

As winter finally relinquishes its chilling grip, our thoughts turn towards the wonders of spring. Instead of seemingly endless days of snow, ice, and dreary skies, we all welcome the warmth of an arriving spring. For TACF volunteers, this season is a busy time of year as they plant chestnuts from Maine to Alabama.

The work of our volunteers and cooperators is truly amazing. Although many of us are not able to get out in the field and contribute our time in the orchards or trial plantings, there is a way in which we can all support the restoration of the American chestnut: donate to our Spring Appeal!

You will receive our Spring Appeal in the mail very soon. However, you don't have to wait for the letter to arrive. Instead, you can visit our website today and make your contribution online (www.acf.org) or fill out the donation form enclosed in this issue of *The Journal*. No matter the gift size, please know that it will make a difference.

It is thanks to your support of our past appeals that we have been able to continue the important work at Meadowview Research Farms. Each year the number of trees planted and the intensity of management of our existing orchards continue to grow. Our staff face ever-increasing workloads and it is thanks to you that we can continue to make progress.

Although TACF's success has been astounding, it comes at a real cost. Everything from labor at our research farms to supplies, such as aluminum cylinders to protect seed, weed mats, and numerous other materials, are needed to keep our dream alive.

We face some other challenges this year. We need to purchase an additional 15 acres of land near our Meadowview Research facility for our third seed orchard. The fact that we have finally reached the point that we can plant a third orchard is a testament to the organization's success.

In addition to purchasing the land, we hope to replace two of our aging pickup trucks, buy a fertilizer spreader, and acquire an industrial-quality zero-turn mower to allow us to more effectively maintain the orchards at Meadowview. Not to mention, several pieces of scientific equipment are needed to further the work we are doing at our Glenn C. Price Research Laboratory.

Your support of the Spring Appeal extends beyond our work at Meadowview. Your gift also helps our efforts to develop a tree resistant to ink disease and provides funding for critical long-term research.

Although our organization is based on the sweat and sacrifice of the volunteers who implement our programs throughout the country, we must also meet the dramatic costs involved in moving our programs forward.

So, today, I ask for your help; please give generously so we can continue our work to restore the American chestnut.

MA/RI Chapter Gift to Have a Place of Honor at New TACF National Office

TACF President & CEO Bryan Burhans and Vice President of Operations Betsy Gamber display the painting by John F. Francis.

This spring, TACF's National Office will move to a new location approximately five minutes north of our current location in Asheville, NC. We've outgrown our offices in the US Forest Service building and are excited to transition into a spacious, storefront location nearby. When we move into the new space, we will find a place of honor for a beautiful gift presented to us by the MA/RI Chapter. The gift is a print, titled *Still Life with Apples and Chestnuts*, by John F. Francis, and surrounded with a hand-crafted chestnut frame made by Chapter board member Brian Clark.

"It's our hope that its beauty and significance will always inspire members, volunteers, donors, partners, and visitors who come to your office," wrote Denis and Lois Melican of the MA/RI Chapter, in a beautifully articulated letter to TACF. "And that with help, TACF can restore the chestnut to its rightful place on America's dinner tables as well as in its eastern forests."

Tom Hunter, center, is honored by TACF during his retirement ceremony in February. Hunter's wife, Phyllis, is pictured left and Appalachian Regional Commission Chief of Staff, Guy Land, right.
Photo by Keith Witt

Post-Retirement, Appalachian Regional Commission Executive Director Initiates Membership in TACF

The executive director of the Appalachian Regional Commission (ARC), Thomas Hunter, retired in February after 20 years of distinguished service to the Commission and the people of the Appalachian Region. As he leaves a career of service, he has made a commitment to American chestnut restoration by joining TACF and choosing to direct his retirement gifts to TACF.

ARC is a regional economic development agency that represents a partnership of federal, state, and local governments. Established by an act of Congress in 1965, ARC is composed of the governors of the 13 Appalachian states and a federal co-chair. At Hunter's retirement ceremony, the governors cited him for modernizing the Commission, setting an example of integrity and professionalism, and showing a profound care and concern for the region that inspired all those who worked with him.

Also during the reception, TACF, ARC, and Green Forests Work presented Hunter with two Restoration Chestnuts 1.0 and two pure American chestnuts to honor his leadership in the reforestation of the Appalachian Region.

"I have always seen the restoration of the American chestnut as a symbol of rejuvenation of the Appalachian region," said Hunter. "It would be a huge economic boom for the region: a nutritious nut crop, superior wildlife habitat and food, and strong, long-lasting timber." As a member of TACF, he plans to propagate American chestnut trees on his family's homestead near Nashville, Tennessee, and assist with local restoration efforts. We are pleased to welcome Hunter into the TACF family.

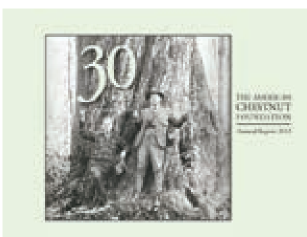
Explore American Chestnut Restoration with Road Scholar

Elderhostel, Inc. is a not-for-profit world leader in lifelong learning since 1975. The organization's Road Scholar programs offer adults and youth the opportunity to travel and take educational programs on a variety of topics. For more than 15 years, road scholars have been going to the epicenter of American chestnut restoration —TACF Meadowview Research Farms in Meadowview, Virginia—to experience the work firsthand and personally participate in the tree's amazing comeback story.

Road Scholar's "Restoration of the American Chestnut Tree" program is hosted by the Southwest Virginia 4-H Educational Center in nearby Abingdon, Virginia. During their week stay, scholars assist in performing disease inoculations on backcross American chestnut trees at Meadowview Research Farms, enjoy entertainment at Barter Theatre, go sight-seeing around historic Abingdon, and stroll down the Virginia Creeper Trail.

Last summer, Craig and Betsy Sheldon from Alabama participated in the program for the first time. They were very excited to spend time on the research farm, meet the scientists, and work with trees at different stages in the breeding program. They recommend this project to other TACF members for the opportunity to see their membership put to work.

For summer 2014, the dates for this program are June 1-6. If you would like to learn more about participating in the Road Scholar program in Meadowview, visit http://www.roadscholar.org and search for keyword "American chestnut."



2013 Annual Report

TACF's 2013 Annual Report is hot off the press! Covering fiscal year 2013 (July 2012 to June 2013), the report is designed to give readers a fast, easy-to-read overview of TACF's projects, goals, and progress for the year. You can obtain a digital copy online at www.acf.org/annual.php. If you would like a print copy of the report, please call the National Office at 828-281-0047.

In Memory of and In Honor of Our TACF Members January-February 2014

In Memory of

Mark Blackburn
Lee Levine

Robert Crowley
Carol Wallace

Norman Hochella
Virginia Hochella
Pricilla Howker

Anne Kerelak
Shelley Packard

Dane Mitchell
Candance Mitchell

William Parana
Ellen Goldfarb
Susan Pare

John Rick
Robert Rick

In Honor of

Thomas M. Hunter
Denise Ambrose and Andrew Meng III
Jim and Beverly Byard
Brooxie Carlton
Linda Gandara
William Grant and Melissa Lord
David R. Hughes
Charles Howard and Sakina Susan Thompson
Guy Land
Julie Lawhorn
James Lester
P.E. Lovett
Julie Marshall
Barbara Moreland
Daniel Neff
E. William Rine
William and Cynthia Shelton
Kostas Skordas
Hubert and Mary Ann Sparks
Eric Stockton
Candace E. Stribling
Donna Suber
Margaret Theobald
Jason Wang and Rui He
Kenneth and Kathleen Wester
Kathryn Whiteman
Jill Wilmoth

Sister Marie Julie
Barbara Welch

Dorothy and Eliot Silverman
Peter Silverman

Phyllis A Meyer
Jackie and Glenda Anderson
John Davis and Susan Dye
Lynne Detrick
Linda and Michael Ferrara
John and Ann Kohler
David and Linda Melgaard
Herbert Meyer
Janet Rawlings

ALABAMA

Planting chestnuts at Jacksonville State University. Back row, left to right: Will Calhoun, Mac Phillippi, Pat Nelson, Don Nelson, BJ Johnson, Mary Shew, and Dave Swinford. Front row, left to right: Tim Chesnut and Tom Saielli. Photo by Jack Agricola

Volunteers Plant Trees at Jacksonville State University's Little River Canyon Center

The Alabama Chapter officially became an essential component of one of the nation's largest and most significant landscape projects when Restoration Chestnuts 1.0 were planted on the grounds of the Jacksonville State University's Little River Canyon Center, near Ft. Payne, Alabama. Considered a crown jewel in Northeast Alabama, adjoining and in partnership with the Little River Canyon National Preserve, the Canyon Center served as host site for a Chapter board meeting and classroom experience on March 1, 2014.

Located in the population triangle between Atlanta, Birmingham, and Chattanooga, the Canyon Center offers more than 200 programs annually including interpretive hikes, nature camps, K-12 field trips, lectures, demonstrations, and festivals. The Canyon Center capitalizes on its unique location offering outdoor opportunities that draw upon large public holdings such as Little River Canyon National Preserve, Dugger Mountain National Wilderness, Mountain Longleaf National Wildlife Refuge, Talladega National Forest, and Mount Cheaha National Wilderness. The building itself is an educational experience as it is a LEED (Leadership for Energy and Environmental Design) Silver Certified structure that utilizes geothermal heating and cooling, recycled materials, added insulation, and many other innovative and sustainable design elements.

Under the direction of regional science coordinator Tom Saielli, board members and guests carefully dug holes around the maze of geothermal pipes to accomplish the inclusion of TACF's Restoration Chestnuts 1.0 in the campus scene. In time, interpretive signage will be installed to facilitate and educate the public on the chestnut's return to this natural corridor.

- Submitted by Jack Agricola

CAROLINAS

Jayden Walsh is one of the students from Olympic High School in Charlotte, NC, participating in the summer biotechnology research program. Photo courtesy of Jayden Walsh

Students Learn Lab Techniques Using American Chestnut Leaves

Participants in a summer biotechnology research program at Olympic High School in Charlotte, North Carolina, are learning to identify genetic markers for the American chestnut tree. The Carolinas Chapter assisted University of North Carolina at Charlotte (UNCC) Associate Professor Jennifer Weller and Olympic teachers Jeanne Smith and Erica Putnam in developing the program. Each summer, the class travels to North Carolina forests to collect leaf samples and later extracts DNA in the lab. Students perform restriction digestion and polymerase chain reaction assays that yield DNA markers on the samples.

Students also learn how chestnut blight drastically affected the biodiversity of eastern forests and the lives of people dependent upon the tree's bounty. The program incorporates scientific inquiry, laboratory techniques for manipulating DNA, math skills, communications skills, and computer technology. Students interact with scientists and researchers to learn how they conduct their investigations and apply their expertise in field work. Students develop biotechnology laboratory skills and are introduced to the emerging fields of genomics and bioinformatics. Involvement in the program stimulates student interest in further education in the sciences, biotechnology, and health studies.

continued

Carolinas *continued*

During the 2012-13 school year, Jayden Walsh, a rising senior at Olympic, completed an internship with Dr. Weller. The program gave him an opportunity to learn in a setting not available anywhere else. He was intrigued that his lab work with leaf assays might possibly save a threatened tree. This coming summer, Dr. Weller is again hosting students in her research lab at UNCC to provide them with more hands-on experience and the opportunity to work with more advanced molecular biology equipment.

- Submitted by Doug Gillis

CONNECTICUT

Ellery Sinclair, right, leads a tour of the Great Mountain Forest Orchard for the 2013 Housatonic Heritage Walk last fall. Photo courtesy of Ellery Sinclair

Housatonic Heritage Walk Explores the Connecticut Chapter's Great Mountain Forest Orchard

The annual Housatonic Heritage Walk, initiated in 2008, is a program developed by the Upper Housatonic Valley National Heritage Area in partnership with the National Park Service to showcase the historical, cultural, and environmental resources of the area. The fall 2013 presentation, "American Chestnuts Lost and Found," was the sixth at the Great Mountain Forest Orchard in Falls Village.

Each year, Ellery Sinclair, who manages the Great Mountain Forest Orchard, outlines the history and the extraordinary value of the American chestnut, and describes the impact of the loss of the tree on the forests, the wildlife, and human welfare and economy. With photographs and through simulating the pollination process with dried catkins and flowers, Sinclair demonstrates TACF's restoration efforts and the role of this orchard in that effort. He also discusses the local high school agriculture students' contribution to this project.

For refreshments, Ellery's wife, Mary Lu, offers American chestnut cookies to accompany the sweet cider served off the truck tailgate. The group later adjourns to a brief walk behind the Sinclair's house to visit a cabin, built in the 1930s of fallen American chestnut trees that had succumbed to the blight's devastation.

- Submitted by Ellery Sinclair

INDIANA

In April, the Indiana Chapter will plant two progeny tests in state forests within the original range of the American chestnut. Photo by Ben Finegan

Indiana Expands Progeny Test Sites

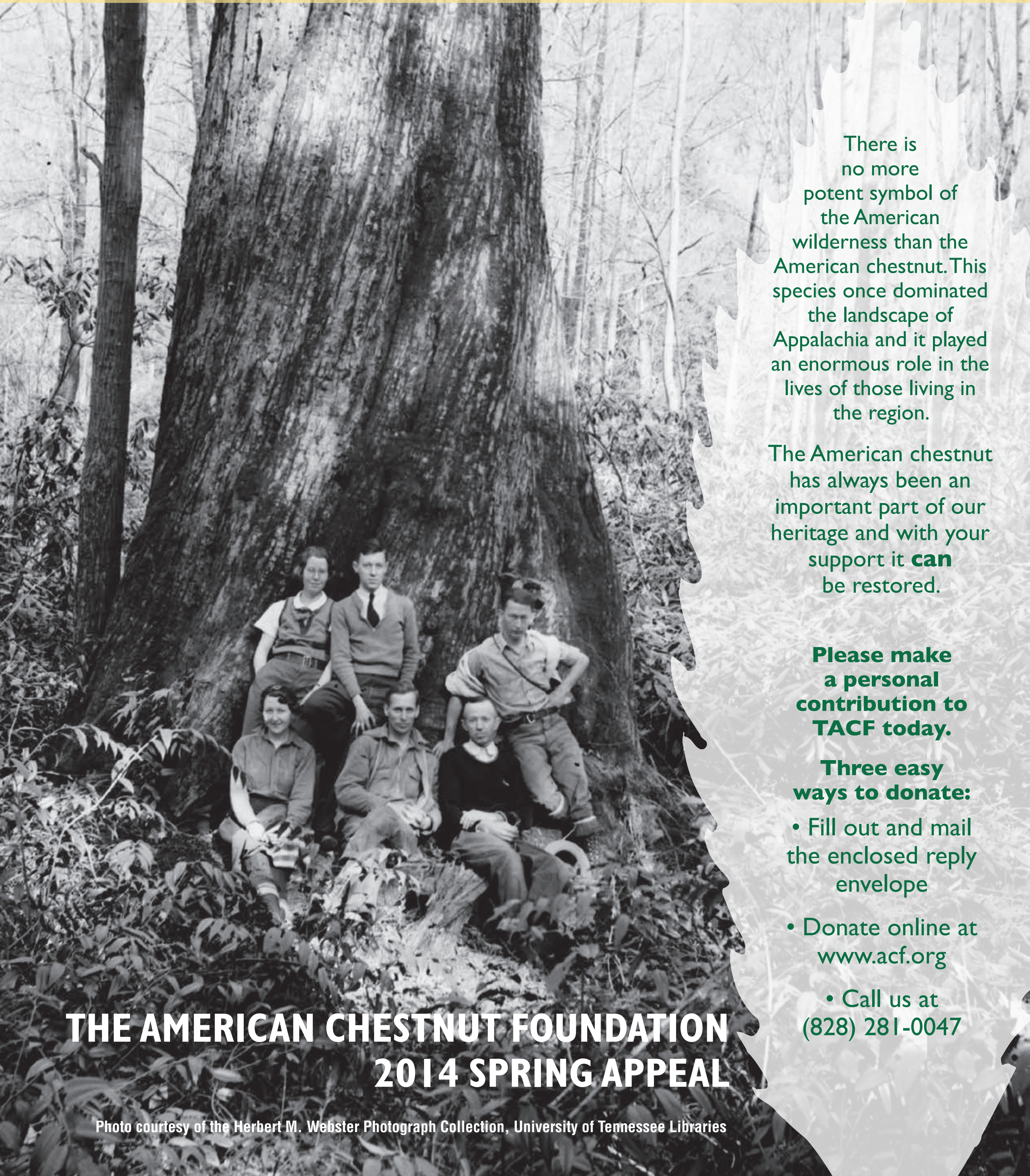
The Indiana Chapter will be planting two Restoration Chestnut 1.0 progeny tests in state forests this April. A total of about 400 seedlings will be planted at the Harrison-Crawford and Jackson-Washington State Forests, both of which are part of the original range of the American chestnut in Indiana. The Harrison-Crawford Forest is also playing host to research on chestnuts and food preference with the endangered Allegheny wood rat. Volunteers interested in helping with these plantings are welcome to contact Chapter President Ben Finegan.

The Indiana Society of American Foresters met at the Southern Indiana Purdue Agricultural Center in October, 2013. The event, hosted by Ron Rathfon, included a tour of one of Indiana's B_3F_2 breeding orchards. Compared with some other locations in the state, American chestnut trees tend to thrive on the soils there. The offspring of these trees will produce Indiana's first generation of regionally-adapted Restoration Chestnut 1.0 trees. About 50 state and private foresters attended the meeting.

- Submitted by Ben Finegan

THE AMERICAN CHESTNUT FOUNDATION®

The power of ordinary Americans working in common purpose to achieve a worthy and visionary goal



There is no more potent symbol of the American wilderness than the American chestnut. This species once dominated the landscape of Appalachia and it played an enormous role in the lives of those living in the region.

The American chestnut has always been an important part of our heritage and with your support it **can** be restored.

Please make a personal contribution to TACF today.

Three easy ways to donate:

- Fill out and mail the enclosed reply envelope
- Donate online at www.acf.org
- Call us at (828) 281-0047

THE AMERICAN CHESTNUT FOUNDATION 2014 SPRING APPEAL

Photo courtesy of the Herbert M. Webster Photograph Collection, University of Tennessee Libraries

KENTUCKY

Rex Mann, right, presents a clock made of reclaimed American chestnut wood to Bill Morton. Photo by Lynn Garrison

William P. Morton Honored by the Kentucky Chapter

The Kentucky Chapter recently honored William "Bill" P. Morton for his long-term commitment to restoring the American chestnut. Bill has served tirelessly as a member of the KY-TACF Board of Directors since our beginning, and was instrumental in getting the Chapter to where we are today.

Two Restoration Chestnut 1.0 seedlings were planted in Bill's honor at the W. P. Morton Farm in Leslie County, which is listed in the Federal Register of Historic Places and is the oldest known standing structure in the county by more than 20 years. The Chapter also presented Bill with a clock mounted on a piece of American chestnut wood in the shape of the state of Kentucky.

In other news, the Kentucky Division of Forestry (KDF) is nearing completion on rebuilding its facilities at the Morgan County Nursery, due to destruction caused by a tornado on March 2, 2012. The nursery is the site for several of the Kentucky Chapter's mother tree and backcross orchards, playing a vital role in their backcross breeding program. The locale is poised to fill the niche as the mass producer of blight-resistant American chestnut seedlings for Kentuckians when that day arrives.

In addition to the Morgan County Nursery, KDF staff have been key players in our programs by helping to find mother trees, conduct pollinations, and assist with plantings. Their efforts will hasten the day when we will be able to restore the American chestnut to its former role in Kentucky's forests.

- *Submitted by Lynn Garrison and Tim Sheehan*

MAINE

Maine Chapter Campaign Reaches Goal

An important fund-raiser that took place during the Maine Chapter's campaign was the Freeport Restoration Branch event. Dr. Ray "Bucky" Owen is pictured addressing attendees. Photo by Larry Totten

In February, the Maine Chapter met its goal of raising $120,000 for its seed orchards. More than 100 donors contributed to the campaign, which was led by Chapter board members Dr. Ray "Bucky" Owen and Ann Rea. The campaign lasted three years and included Restoration Branch gatherings hosted by Richard and Elizabeth Warren in Bangor and Joe and Carol Wishcamper in Freeport.

Funds raised through this campaign will be used for the Chapter's eighteen seed orchards in Hartland, Phippsburg, Searsport, Stetson, and Winthrop. Part of the success of the fund-raising efforts, according to TACF President & CEO Bryan Burhans, has been this specific and limited purpose.

"It's easy to raise money for The American Chestnut Foundation," says Owen. "When people learn about what we're doing and what we've accomplished so far, they're glad to contribute." Rea adds, "We're incredibly grateful to all of our donors. Having their support makes the Chapter's work so much easier." Rea also noted that special thanks are due to the Spellissey Foundation for their continuing support of the Maine Chapter over the years. "The Spellissey's donations were not part of our capital campaign, but their help kept our Chapter going when we had little other income."

- *Submitted by Ann Rea*

MASSACHUSETTS/RHODE ISLAND

Pictured is just a portion of the chestnut buffet laid out by MA/RI Chapter members for their 2013 Annual Meeting. Chestnut pumpkin soup, chestnut and sausage casserole, and roasted chestnuts were among the delicacies served. Photo by Kathy Desjardin

Chestnut Potlucks Enhance MA/RI Chapter Meetings

Chapter Annual Meetings are something that we plan and look forward to all year. We always have stellar guest speakers and engaging programs, and we have something else – a fabulous chestnut potluck!

A few years ago we began focusing on chestnut dishes to enhance the program and entice members to attend. We started simply by serving roasted chestnuts, then a crockpot of chestnut pumpkin soup. Lois and Denis Melican soon created their famous chestnut hermits, and now we've expanded to include some other really fine chestnut dishes.

Our members really step up to the plate for the chestnut potluck! Now, along with hot roasted chestnuts, we offer a variety of appetizers, salads, casseroles, and desserts. Past president Brad Smith always brings a delicious chestnut and sausage casserole; our Chapter secretary, Kathy Desjardin, brings a wonderfully healthy three bean and chestnut salad, our president Yvonne Federowicz can be counted on to bring something decadently chocolate and loaded with chestnuts. These are only a few of the delicious chestnut treats that we've offered in the past. All of these dishes, along with heirloom apples from Brian Clark, make a meal fit for royalty that recharges our idealism and rededicates us to our work each year.

- Submitted by Yvonne Federowicz

NEW YORK

The SUNY-ESF team is moving their plant tissue culture lab to the Central New York Biotechnology Accelerator Building in Syracuse, NY. Photo by Chuck Maynard

ESF Starts up a New Plant Tissue Culture Laboratory at the Central New York Biotechnology Accelerator

The SUNY-ESF team in Syracuse, New York, is moving most of the production of blight-resistant chestnut trees to a new lab in the Central New York Biotechnology Accelerator. Dr. William Powell explained the need for the new lab: "It will give us more space for production as we gear up for deregulation and distribution." You can watch a video of American chestnut trees with enhanced resistance at www.esf.edu/chestnut/resistance.htm#.UxcvhNztH7U.

The new lab came with a start-up equipment budget that covered two additional plant growth chambers and a water purification system. Dr. Charles Maynard remarked, "Now, we just need the funds for personnel and supplies, and we will be off and running." The chestnut project was one of three laboratories selected by the college to showcase applied biotechnology in the new state-of-the-art building.

- Submitted by Chuck Maynard

PENNSYLVANIA/NEW JERSEY

Dr. Laura Guertin (left), from Penn State Brandywine, and Kristine Averill (right), a Penn State graduate student, were both speakers for the PA/NJ member meeting at Tyler Arboretum. Dr. Guertin discussed the history of the Tyler Arboretum and Averill talked about deer herbivory in northern forests. Photo by Stephanie Dempsey

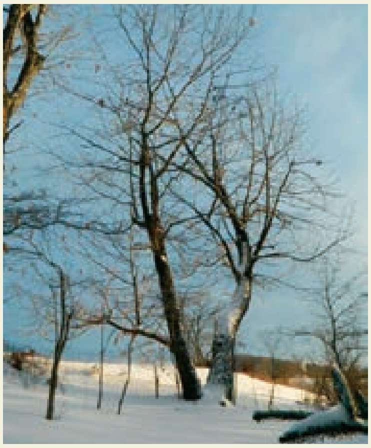
PA/NJ Chapter Gears Up for Another Growing Season

It's hard to believe that another growing season is right around the corner. Despite the bitter cold temperatures and onslaught of snow, it doesn't really feel like we ever slowed down here in Pennsylvania. We marked the close of last years' growing season with a member meeting at Tyler Arboretum in Media, Pennsylvania, on November 2. Participants enjoyed a tour around the arboretum following the meeting, as well as an opportunity to visit the American chestnut orchard there. Pennsylvania and New Jersey volunteers have been busy manning booths at events over the winter, such as the Pennsylvania Farm Show in Harrisburg, while the staff and board have been busy with board and planning meetings, and ushering in a new cycle for our five-year strategic plan.

Our new year started off with a generous donation made by the family of John Rick, a Pennsylvania native and chestnut enthusiast who passed 50 years ago this year. These funds have greatly helped us to initiate much needed updates and improvements to our current efforts in outreach and education. By summer, we should have a new and updated display, logo, and handouts. We are also working on developing materials for Project Learning Tree, an award-winning environmental education curriculum utilized by teachers in Pennsylvania and throughout the country. We are also scouting a location for a memorial orchard to honor Mr. John Rick, a man who gave out thousands of chestnut trees in his life. Today, his efforts have become a part of our efforts in the restoration of this beautiful, historical, and significant tree.

- Submitted by Stephanie Dempsey

VIRGINIA



This American chestnut tree on Jack LaMonica's property in Marshall, Virginia, measures 26 inches in diameter at breast height (dbh). The photo was taken with eight inches of snow on the ground in March, 2014. Photo by Jack LaMonica

Winter Planning and Outreach Projects Keep Virginia Volunteers Busy

The Virginia Chapter is busy monitoring and measuring orchards, thanks to coordinators Deborah Fialka and Katy McCune. Determining locations for our last few backcross orchards and planning for our first seed orchard are top priorities. The Rockley Foundation orchard will be planted with nuts from a Fairfax tree's 180 backcross nuts. American nuts were collected by Dr. Harmony J. Dalgleish from College of William and Mary for a graduate research project, and by Dan Miles of Lynchburg College's Claytor Nature Study Center. Kathy Marmet, Cathy Mayes, and Leslie Ziegler have spearheaded efforts to monitor ambrosia beetle infestations, with the help of students from Highland School in Warrenton, VA.

TACF's Annual Meeting last October featured a well-attended reception and tree tour to the property of Chapter President Jack LaMonica, where several large American chestnuts can be found. This year, we hosted a teacher's workshop in Reston. The Frontier Culture Museum in Staunton scheduled a four-speaker series on American chestnut. A seminar at the Virginia State Arboretum will take place in May. Demonstration plantings are also planned throughout the commonwealth.

A committee headed by Warren Laws will help incorporate strategic planning recommendations into the Chapter structure. Emphasis is also being placed on developing Restoration Branch events, encouraged by the success and diligence of the Southwest Virginia Branch, and Dick Olson, respectively.

- Submitted by Jack LaMonica

VERMONT/NEW HAMPSHIRE

Tom Thiel crafted the soundboards of these two mountain dulcimers in 1974 from American Chestnut wood he salvaged. Thiel is one of several luthiers interested in making musical instruments from the Berlin chestnut tree. Photo courtesy of Tom Thiel

New Educational Partnerships Strengthen the VT/NH Chapter

Over the fall and winter, VT/NH Chapter members continued outreach and education efforts and prepared for the spring planting season. An educational event in New Hampshire netted several woodworkers and luthiers interested in making musical instruments and other items from the Berlin chestnut tree that was salvaged in November 2012.

The Chapter has approved funding for and is embarking on an exciting education partnership project with Mount Anthony Union Middle School (MAUMS) in Bennington, Vermont. MAUMS students will be preparing soils, removing invasive species, laying out a planting design, and planting Restoration Chestnuts 1.0 in the spring of 2014. With the help of volunteers, students will be required to do basic research and partake in the fieldwork necessary to grow chestnut trees. In addition to capturing the "big picture" for students, faculty, and the community, a three-panel kiosk telling the American chestnut story will be built and put in place at the high-visibility entrance to the planting area that looks south to Mount Anthony and Mount Greylock in the Massachusetts Berkshires. The entire kiosk will be built from American chestnut beams that came from an old barn in Cornwall, Connecticut, originally dismantled by a Connecticut Chapter member.

- Submitted by Yurij Bihun

WEST VIRGINIA

Pictured are some of the members of the American Chestnut Club at Preston County High School. Photo courtesy of the West Virginia Chapter

Volunteerism and Donations Advance West Virginia's Mission

Volunteerism and significant donations are furthering the mission of The American Chestnut Foundation in Preston County, West Virginia. Robert Sypolt has volunteered numerous hours raising awareness, working with educators, and finding surviving trees in Preston County. His efforts and two significant donations by Duane Waddell, a former resident of the county, helped to spur the formation an American Chestnut Club at the Preston County High School. In 2013, members of Laah Wolford's greenhouse class planted 20 American chestnut trees in an orchard on campus. In 2014, the club plans to plant a full demonstration orchard including local surviving trees.

Waddell's donation will support the demonstration orchard, provide memberships for new members, purchase learning boxes, and support other plantings in the county. He stated: "When I first heard about the existence of The American Chestnut Foundation and the efforts to bring back this magnificent Appalachian tree with all its benefits, I became a member and looked for ways to support the movement. Then I read in the *Preston County Journal* about the West Virginia Chestnut Festival and I knew I wanted to support Preston County chestnut projects. I'm pleased and gratified a chestnut orchard is to be developed in Preston County. It is much more than I hoped for at this time and I look forward to supporting its future growth."

- Submitted by Brian Perkins

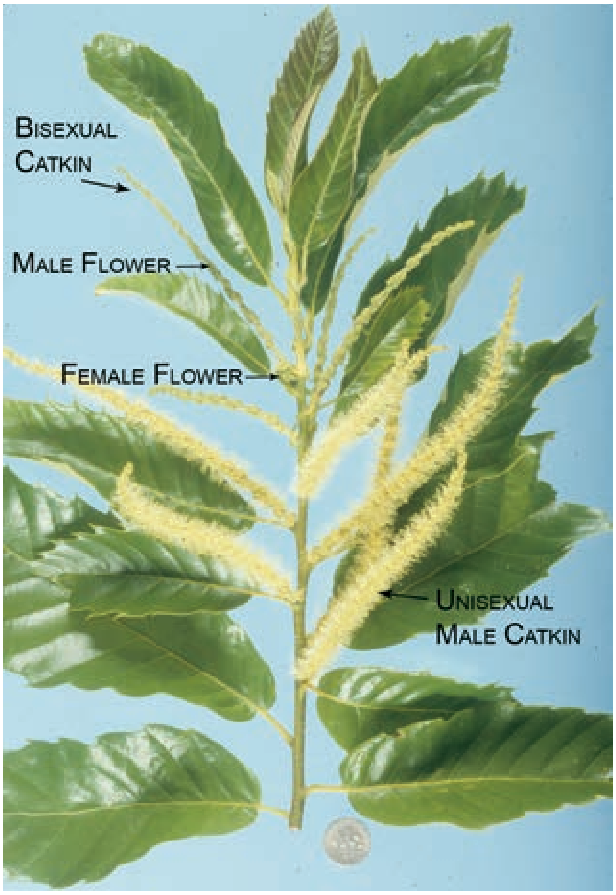


Figure 1. Twig with catkins at the onset of flowering

How a Flower Becomes a Chestnut:
Morphological Development of Chinese Chestnuts (*Castanea mollissima*)

Amy Miller,[1] Diane D. Miller,[2] and Paula M. Pijut[3]

[1]Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907,
[2]The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691, and
[3]USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907

*This article is a variation of Miller, A., Miller, D.D., and Pijut, P.M. 2013. How a flower becomes a chestnut: Morphological development of Chinese chestnuts (*Castanea mollissima*). 103rd Annual Report of the Northern Nut Growers Association (Sept.): 27-33.*

Chestnuts, members of the genus *Castanea*, family Fagaceae, are popular worldwide and consist of three sections with at least seven distinct species, but may include up to 12 species (Bounous and Marinoni 2005). All species have noteworthy ecological, economic, and cultural importance in southern Europe, Anatolia, the Caucasus Mountains, temperate eastern Asia, and eastern North America (Conedera et al. 2004; Davis 2006). Chestnut species, which regularly bear sweet, nutritious nuts that are high in carbohydrate, but low in fat (Bounous and Marinoni 2005; McCarthy and Meredith 1988; Senter et al. 1994), have historically been an important food source for people in remote, mountainous areas, and are highly valued in the cuisine of several cultures around the world. The nuts are also an important food source for wildlife (Burke 2013; Paillet 2006).

Of the seven distinct species, three chestnut species--Chinese chestnut (*C. mollissima* Blume), Chinese chinquapin (*C. henryi* (Skan.) Rehder and E.H. Wilson), and Seguin chestnut (*C. seguinii* Dode.)--are native to China; Japanese chestnut (*C. crenata* Siebold and Zucc.) is native to Japan and Korea; European or Sweet chestnut (*C. sativa* Mill.) is found in Europe, Anatolia, and the Caucasus; and American chestnut (*C. dentata*) and the chinkapin (*C. pumila* (L.) Mill.) are native to North America (Mellano et al. 2012). Despite separation by seas and continents, chestnut species are similar in terms of their site requirements and climatic limits (Hunt et al. 2012; Fitzsimmons 2006; Fei et al. 2012). General biological traits of these species are also similar, including reproductive strategies and morphological development (Bounous and Marinoni 2005), and they easily interbreed when cultivated together. In their native forests, most chestnut species are canopy trees with upright growth forms, while the chinkapin is a large shrub restricted to forest edges. There is great interest in chestnuts for several reasons: forest and timber restoration of the American chestnut, reforestation and reclamation of strip mine lands, and commercial cultivation and production of other *Castanea* spp. (namely, Chinese chestnut) and hybrids. It is important to understand reproductive development in order to design effective breeding programs (Shi and Stösser 2005; Shi and Xia 2010).

We have assembled a collection of photos that show the sequence of morphological development of Chinese chestnut from flowers to mature fruit, and these photos illustrate growth of the structures that are the components of yield. The developmental sequence of flower and fruit affects gene expression, which has implications for commercial crop yields, selecting components to target for crop improvement, the mechanics of artificial pollination, and inheritance studies. These specimens were photographed from orchard-grown Chinese chestnuts in Carrollton, Ohio, USA. The timing of flower development reflects climatic conditions for that area in eastern North America.

Chinese chestnut flowers in mid-summer, with flowers typically reaching anthesis from late June through mid-July in Ohio. Flowers are borne on catkins arising from the leaf axils of current season's growth (Figure 1). Two types of catkins occur: bisexual catkins that bear one or more female flowers at the base and male flowers toward the tips, and unisexual male catkins, also called staminate catkins. Bisexual flowers occur toward the apex of the twig and tend to reach maturity later than the unisexual flowers, which occur toward the base of the twig (Bounous et al. 1992).

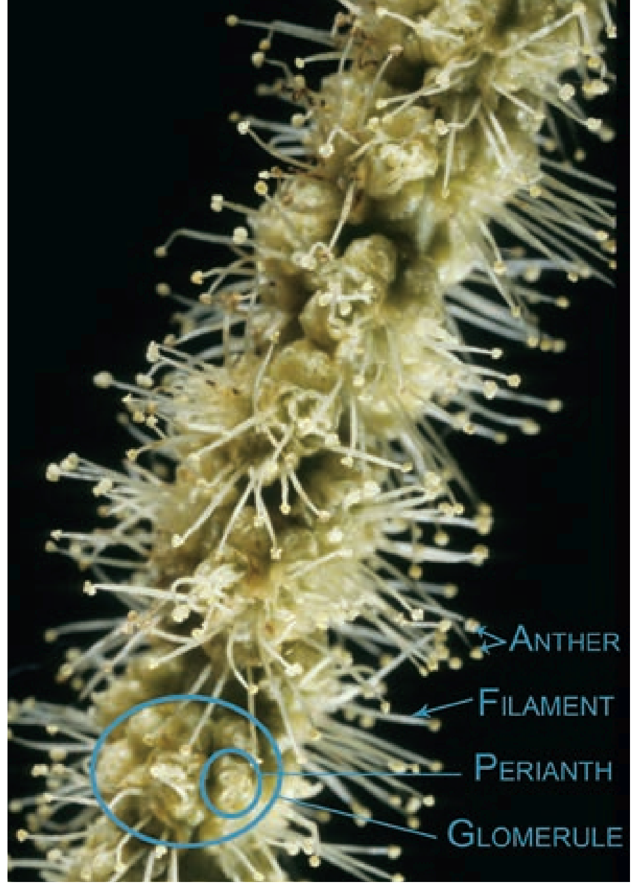


Figure 2. Fertile catkin

The male catkins occur in abundance and can produce a large quantity of pollen, which is typically wind-dispersed. Upon each catkin are glomerules, clusters of staminate flowers (Figure 2). Each staminate flower is composed of a perianth and multiple stamens, with each stamen composed of a filament and anther. These

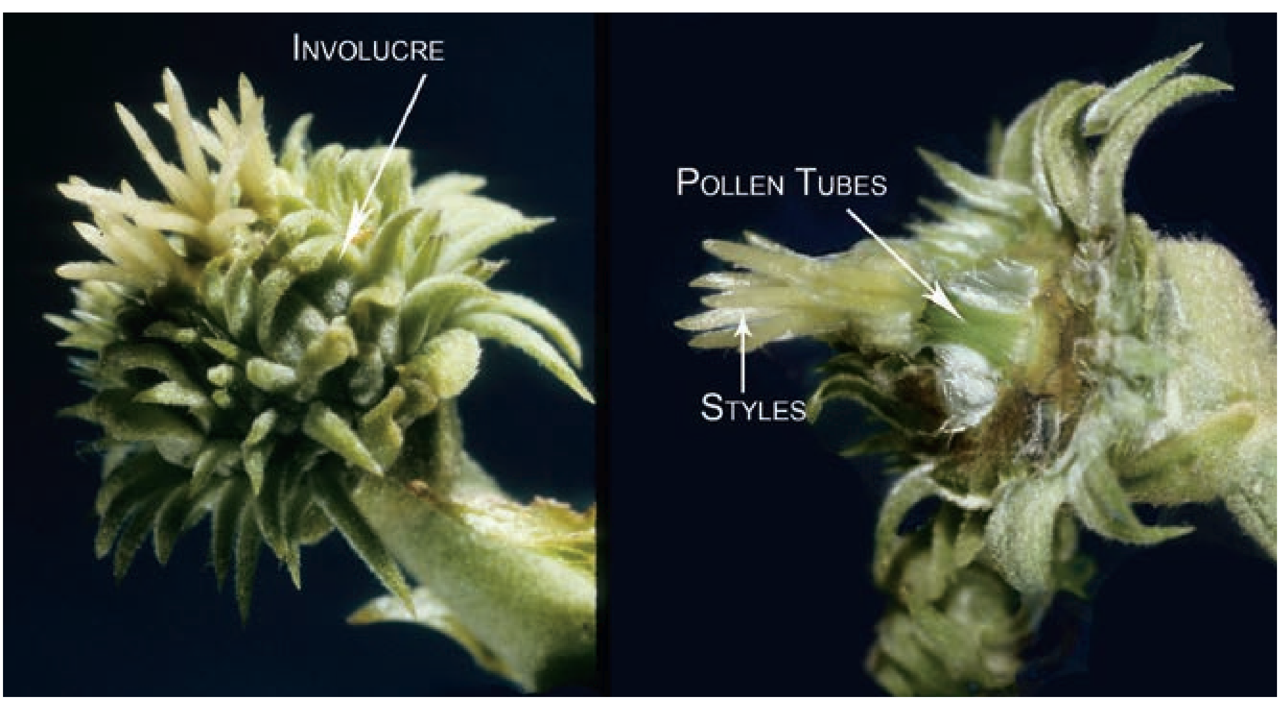


Figure 3. Involucre: outside (left) and inside (right)

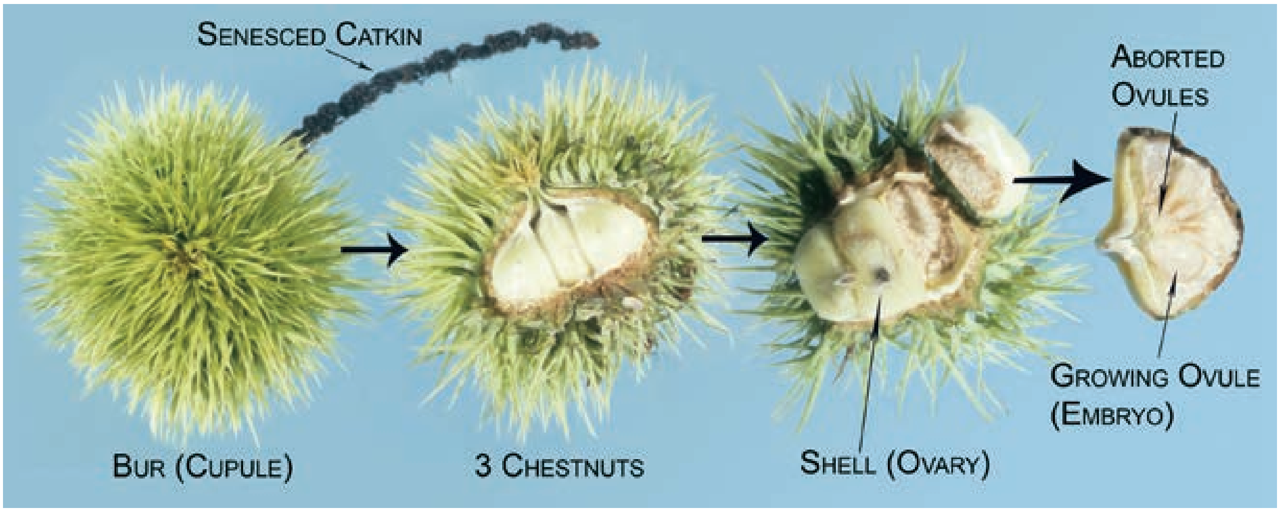


Figure 4: Layers inside the expanding bur

filaments and anthers give the catkins their characteristic fuzzy appearance, as well as their distinctive pungent odor.

At the base of each bisexual catkin is one or more pistillate inflorescence, also referred to as the involucre (Figure 3), which typically contains three female flowers (three pistils per involucre). Each pistil comprises an ovary and multiple rigid, pubescent styles, each with a glabrous stigma at the tip. Peak pollen receptivity of the three flowers is staggered in time, with the central flower becoming receptive several days before the adjacent ones. As each flower becomes receptive, the cluster of styles becomes visible. *Castanea* is generally not self-fertile; therefore, cross-pollination is needed for effective fertilization.

Roughly three weeks after the start of bloom, typically in mid-July in Ohio, pollen production ceases, coinciding with senescence, browning, and drop of male catkins and the male portions of bisexual catkins. Simultaneously, female flower stigmas cease receptivity, the styles begin to darken, and the fertilized ovaries begin to grow. The soft, green involucre containing three female flowers now becomes the recognizable green, spiny cupule (the chestnut bur) containing the three growing ovaries (nut shells). Approximately two months after pollination, three separate nuts are visible nestled side by side (Figure 4). Removing the shell from one of the nuts reveals the ovules. Each ovary contains 12-18 ovules, which remain equal in size until approximately one month after peak pollination.

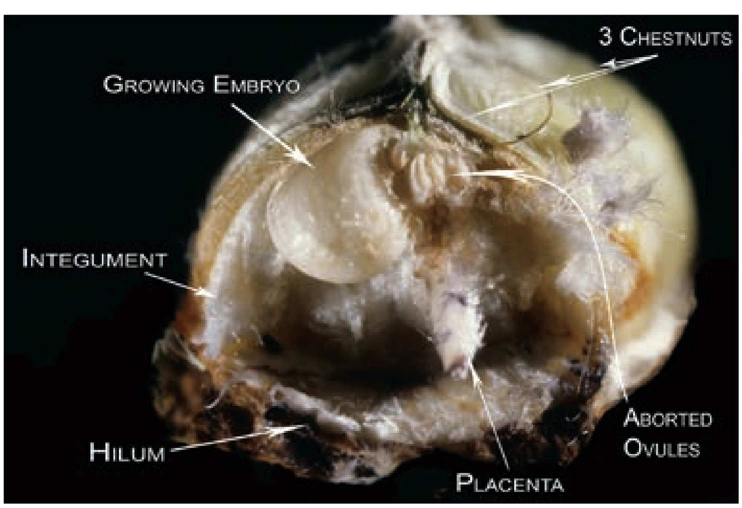


Figure 5: Internal anatomy of a developing chestnut

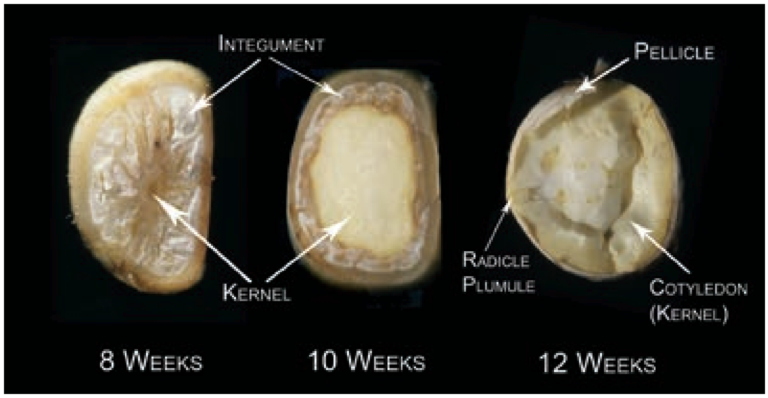


Figure 6: Expansion of the kernel inside the shell

A close-up view of the embryo and the internal structure of a nut, when the bur and shell are peeled away, reveals that one embryo develops into the kernel while the others abort (Fig. 5). The developing embryo, attached at the tip to a placenta, connects the tip of the nut to the base of the shell (the hilum). The hilum is the point of attachment of each nut to the inside of the bur and allows the transfer of carbohydrates and nutrients into the developing kernel from the bur and shoot. The embryo fills from the tip and expands downward, eventually filling the entire space inside the shell. As the kernel expands, the placenta is pushed to the side and eventually fits tightly between the kernel and the nutshell. The shell and bur size up before the kernel grows to capacity.

Approximately eight weeks after pollination, the kernel is very small and most of the inside of the shell is filled with the integument, appearing fuzzy and white (Figure 6). By 10 weeks, the yellow kernel has expanded significantly, but not yet filled the shell. Approximately 12 weeks after pollination, the yellow kernel has fully expanded to fill the inside of the shell, and the integument has become a skin-like seed coat, brown and white in color (the pellicle). The ripe kernel is composed of two cotyledons and an embryonic axis with a radicle and plumule, which will form the first root, shoot, and leaves during germination.

A mature Chinese chestnut comprises three layers: the shell, the pellicle, and the kernel. The shell is typically brown, light to moderately pubescent, with a white tuft of stylar remnants at the tip, and the lighter-brown, rougher-textured hilum at the base. Shells are somewhat soft and pliable, unlike the hard shells of other tree nuts. Peeling back the shell reveals the skin-like pellicle,

Figure 7: Layers of a ripe chestnut

Figure 8: Maturation of the shells inside the bur

the light brown seed coat covering the kernel (Figure 7). The distinct groove seen in the kernel is residual evidence of the placenta's location between the kernel and the shell. Removing the pellicle reveals the bright yellow kernel composed of two cotyledons and the radicle plumule.

Approximately six weeks after pollination, the shells appear green and fuzzy (Figure 8). After 10 weeks, the shells have grown to mature size and have become much shinier and more glabrous; however, the shells are white in color and very soft. At time of ripening, the shells have changed from shiny white to the familiar shiny brown. The brown coloration coincides with the hardening of the hilum and dehiscence of the ripe nuts from the bur. When ripe, Chinese chestnut burs usually open while still attached to the tree and nuts fall individually to the ground. Harvest occurs approximately 12 to 14 weeks after pollination, typically ranging from mid-September to mid-October in Ohio.

In contrast to many other tree nuts, chestnut kernels are mostly composed of carbohydrates (mainly starch) with smaller amounts of fat and protein. Chestnut kernels continue to undergo chemical and physiological changes after they ripen and fall from the tree, including the conversion of much of the starch to sucrose, making the nuts sweeter and more delectable post-harvest. The extent and nature of post-harvest changes in chestnut kernels vary greatly depending on environmental (storage) conditions (Miller 2006; Tzortzakis and Metzidakis 2012).

In conclusion, noteworthy attributes of chestnut development illustrated by this series of photos are as follows:

1. Chestnuts produce separate male and female flowers (monoecy) with an overwhelming preponderance of male flowers.

2. Each ovary (which becomes a chestnut) houses a large number of ovules at pollination time, but only one (sometimes two or three) develops into a kernel.

3. Growth of the bur and ovary (chestnut shell) precedes growth of the enclosed embryo (kernel).

4. Half of the kernel's mass is acquired during the last 2 weeks before nut drop.

The same sequence of development and maturation occurs wherever chestnuts occur, although the timing of bloom varies with geographic location (earlier in the south, later in the north). However, these geographic differences are ameliorated during the season such that the time of harvest is not as variable as the time of bloom.

The genus *Castanea* has long been useful in the ecology and economy of the human environment, and with a strong knowledge base and proper cultivation these species can be improved and utilized to help meet resource demands of the future.

ACKNOWLEDGMENTS
The authors gratefully acknowledge Drs. J. Hill Craddock and Greg Miller for their constructive review and suggestions for the improvement of this manuscript. Empire Chestnut Company, owned and operated by Dr. Miller, was the source of the photographed specimens. The authors also thank Kenneth D. Chamberlain of the Ohio Agricultural Research and Development Center of Ohio State University for enabling the professional quality of the photographs.

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The Wagner Farm 'Graves' seed orchard in late winter, located in Meadowview, Virginia. Photo by Fred Hebard

Meadowview Notes 2012-2013

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The American Chestnut Foundation Research Farms, Meadowview, Virginia

Meadowview experienced good rainfall in 2012 and 2013. Emergence of seeds in 2013 was good to excellent, and we were spared a killing frost in mid- or late spring in both years. Most of the summer of 2013 was wet and cool. Wet, cool weather favors the chestnut tree over the blight fungus, and many cankers had minimal expansion.

Tree and Seed Inventory

Table 1 presents the current holdings of trees and planted nuts as of May, 2013, at TACF's Meadowview Research Farms, and the changes from the previous year. The first few lines of the table serve also as a glossary of TACF breeding terminology. For instance, what we call a B_1 is the backcross of a Chinese x American F_1 interspecific hybrid to an American chestnut. TACF uses a modified backcross method in that we do not backcross to the same American chestnut tree that was the parent of the F_1 hybrid, in order to avoid inbreeding. Likewise, we try to use a different American chestnut tree as the recurrent parent in each subsequent backcross.

Like 2012, 2013 saw a decline in the number of trees at the farm by 6,962 to total 41,870 standing chestnut trees. But this is a good thing! It reflects progress in thinning the Legacy Tree seed orchards that are producing our Restoration Chestnut 1.0 seeds. That progress is indicated by the B_3F_2 line in Table 1, of which there were 15,056 in May, 2013, when the data for the table were assembled. This was a decline of 10,305 B_3F_2s, despite planting 2,852 more. Subsequent thinning in 2013 has reduced the census of B_3F_2s to about 10,000. The ultimate plan is to reduce the number of B_3F_2 trees for the 'Clapper' and 'Graves' sources of blight resistance at Meadowview to about 500 trees total. As we approach that goal, the blight resistance of the progeny produced by the remaining trees should increase, as explained in Meadowview Notes for 2009-2011 (*The Journal of TACF* 26[1]: 8-16).

In 2013, we added 5,285 B_3F_3 Restoration Chestnut 1.0 trees (Table 1). This increase reflects continued planting of progeny tests in orchards at Meadowview. The purpose of the orchard progeny tests is to make final selections for blight resistance among their B_3F_2 parents.

Table 1. Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms, May, 2013. Number of sources of disease resistance, number of American lines in the breeding stock, and changes between May, 2012 and May, 2013.

Type of Tree	Number in 2013 of			Difference from 2012 to 2013		
	Nuts or Trees	Sources of Resistance	American Lines	Nuts or Trees	Sources of Resistance	American Lines
American	1389		61	-3		-3
Chinese	634	27		261	-7	
Chinese x American: F_1	1571	18	56	-28	-1	37
American x (Chinese x American): B_1	590	11	22	-56	0	0
American x [American x (Chinese x American)]: B_2	1053	17	62	-566	1	-5
American x {American x [American x (Chinese x American)]}: B_3	1971	11	96	-56	1	6
Am x (Am x {Am x [Am x (Ch x Am)]}): B_4	574	5	16	-315	1	0
Am x [Am x (Am x {Am x [Am x (Ch x Am)]})]: B_5	66	1	1	66	1	1
(Ch x Am) x (Ch x Am): F_2	213	5	6	0	0	1
[(Ch x Am) x (Ch x Am)] x [(Ch x Am) x (Ch x Am)]: F_3	5	1	1	0	0	0
[Am x (Ch x Am)] x [Am x (Ch x Am)]: B_1F_2	626	7	10	0	0	0
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}: B_2F_2	590	9	13	0	0	1
B_2F_3	31	1	1	0	0	0
(Am x {Am x [Am x (Ch x Am)]}) x (Am x {Am x [Am x (Ch x Am)]}): B_3F_2	15056	4	57	-10305	2	3
B_3F_3	11043	2	35	5285	0	8
Clapper B_3 x Graves B_3: B_3I_1	110	1	9	0	0	0
Chinese x [American x (Chinese x American)]: Chinese x B_1	167	3	7	0	0	0
Ch x {Am x [Am x (Ch x Am)]}: Chinese x B_2	72	1	2	0	0	0
Ch x (Am x {Am x [Am x (Ch x Am)]}): Chinese Test Suite x B_3	286	5	16	0	0	0
Chinese Test Suite x Chinese	1284	88		-25	21	
Chinese Test Suite x Japanese	46	2		0	0	
Chinese Test Suite x European	43	1		0	0	
Chinese Test Suite x Large, Surviving American	139	7		-10	0	
European x American: F_1	2	1	1	0	0	0
Japanese	1	1		-2	0	
Japanese x American: F_1	8	1	1	0	0	0
[(Japanese x American) x American]: B_1	5	1	1	0	0	0
{[(Japanese x American) x American] x American}: B_2	142	1	1	0	0	0
Japanese x European	80	1	1	0	0	0
Japanese x Large, Surviving American	27	5	5	0	0	0
Castanea ozarkensis	21	1		0	0	
Castanea pumila	44	3		0	0	
Castanea seguinii	48	3		0	0	
Seguin x American: F_1	34	2	2	0	0	0
Large Surviving American: F_1	267	10	22	-219	-2	10
Large Surviving American: B_1	188	7	12	-318	-1	-3
Large Surviving American: B_2	82	4	5	0	0	0
Large Surviving American: B_3	161	1	1	0	0	0
Large Surviving American: F_2	180	11		-86	-2	
Large Surviving American: F_3	270	1		0	0	
Large Surviving American: I_1	759	28		-907	-4	
Large Surviving American: I_2	332	12		-72	-1	
Large Surviving American: I_3	119	3		15	1	
Large Surviving American advanced: F_1	1538	17	60	445	1	41
Other	3			0		
Total	**41870**			**-6962**		

Preliminary selections of the B_3F_2 parents are made by inoculating them with the blight fungus, selecting for small canker size, followed by assessing the severity of naturally occurring cankers on the remaining trees as they age. The results from the first 3 years of orchard progeny testing will be presented and discussed in a subsequent section of this report.

With this report, we are including data for the 2013 harvest as well as the 2012 harvest. Next year's "Notes" will only have the 2014 harvest. Moving the date of publication of "Meadowview Notes" back from the January to the March edition of the Journal provides sufficient time before publication for verification of fall canker measurements and completion of the harvest inventory. The 2012 nut harvest saw an increase of B_3F_3 nuts from 22,752 to 72,690 (Table 2). In 2013, 45,069 B_3F_3 nuts were harvested (Table 3). The decrease in the B_3F_3 nut harvest from 2012 to 2013 was due in part to fewer trees harvested in 2013 and in part to fewer nuts per bur. The decrease in number of harvested trees was due primarily to culling of reject trees. The yield of nuts per bur for the 'Graves' orchard declined from 2.7 to 1.9 between 2012 and 2013, although the number of

Table 2. The American Chestnut Foundation Meadowview Farms 2012 nut harvest from controlled pollinations and selected open pollinations.

Nut Type*	Female Parent Cross Type & Source of Blight Resistance	Pollen Parent Cross Type & Source of Blight Resistance	Pollinated			Unpollinated Checks			No. of Crosses
			nuts	bags	burs	nuts	bags	burs	
B_1	Mahogany F_1	American	25	18	37	0	2	3	1
B_2	American	B_1 mollissima11	189	356	441	1	44	56	15
B_3	American	B2 Nanking	585	393	539	1	38	46	15
B_3	B_2 Nanking	American	0	79	150	1	9	21	2
B_4	American	B_3 Meiling	163	69	154	1	6	11	1
B_5	American	B_4 R1T7	66	34	53	0	5	5	4
B_3F_2	B_3 Clapper	open pollinated	7924		5545				48
B_3F_2	B_3 Graves	open pollinated	2880		2139				33
B_3F_3	B_3F_2 Clapper	B_3F_2 Clapper	167		169	12**		29**	5
B_3F_3	B_3F_2 Clapper	B_3F_2 Graves	317		186	12**		29**	7
B_3F_3	B_3F_2 Graves	B_3F_2 Clapper	208		121				4
B_3F_3	B_3F_2 Graves	B_3F_2 Graves	4		11				1
B_3F_3	B_3F_2 op Clapper	open pollinated	43733		20249				352
B_3F_3	B_3F_2 op Graves	open pollinated	28261		10951				202
F_1	Nanking Chinese	American	6	32	55	0	3	5	1
Japn B_3	American	Japanese B_2 PI#104016	495	392	611	2	44	56	19
LSA B_2	American	LSA B_1 DaresBeach	40	54	108	0	6	8	3
LSA I_1 B_1	American	LSA I_1F_1 opWeekly	230	79	232	0	10	11	5
LSA I_1 F_1	American	LSA I_1 Amherst;Ort	222	168	224	0	20	20	2
LSA I_1 F_1	American	LSA I_1 Amherst;ScientistsCliffs	350	237	509	0	28	55	4
LSA I_1 F_1	American	LSA I_1 ScientistsCliffs;Weekly	105	295	505	0	32	53	11
LSA I_1 F_1	American	LSA I_1F1 opWeekly	0	6	6	0	1	0	1
LSA I_2	LSA B_1 DaresBeach	LSA I_1 Amherst;ScientistsCliffs	18	48	82	0	6	6	1
LSA I_2	LSA I_1 Amherst;Ort	LSA I_1 Amherst;Ort	61	160	148	4	13	72	2
LSA I_2	LSA I_1 Amherst;ScientistsCliffs	LSA B_1 DaresBeach	58	90	306	0	12	8	1
LSA I_3	LSA I_1 Amherst;ScientistsCliffs	LSA I_2 Gault;NCChamp;ScientistsCliffs	7	15	19	0	2	0	2
LSA I_3	LSA I_2 Amherst;ScientistsCliffs	LSA I_2 Gault;NCChamp;ScientistsCliffs	44	64	124	6	8	24	1
LSA I_3	LSA I_2 DaresBeach;opWeekly	LSA I_1 ScientistsCliffs;Weekly	0	79	117	0	7	5	1
Total Controlled Pollinations			**3360**	**2668**	**4907**	**28**	**296**	**494**	**109**

*LSA denotes Large, Surviving American, defined as an American chestnut over 13 inches in diameter at breast height (54 inches) that has blight but has survived it longer than approximately 10 years.

**The controls were shared between the two pollen types for the same female type.

Table 3. The American Chestnut Foundation Meadowview Farms 2013 nut harvest from controlled pollinations and selected open pollinations.

Nut Type*	Female Parent Cross Type & Source of Blight Resistance	Pollen Parent Cross Type & Source of Blight Resistance	Pollinated			Unpollinated Checks			No. of Crosses
			nuts	bags	burs	nuts	bags	burs	
B_1	F_1 Mahogany	American	276	253	390	4	25	41	2
B_2	American	B_1 mollissima11	46	172	226	0	14	12	11
B_2	American	B_1 mollissima12	1	40	56	0	5	7	3
B_2	American	B_1F_2 MusickChin;MusickChin	17	69	100	0	6	10	1
B_2	B_1 mollissima11	American	143	86	160	2	8	18	2
B_2	B_1 mollissima12	B1 mollissima12	13	173	286	0	14	20	2
B_2	B_1F_2 MusickChin;MusickChin	American	86	98	148	0	9	12	2
$B_{2\text{-}3}F_2$	B_2 Mahogany	B_3 Graves	38	21	21	0	2	3	1
B_3	American	B_2 Nanking	328	218	428	13	28	30	10
B_3	B_2 Nanking	American	30	45	56	0	7	8	4
B_3 x C	Nanking Chinese	B_3 Nanking	182	146	227	0	15	31	3
B_5	American	B_4 R1T7	36	50	47	6	6	3	3
B_3F_2	B_3 Clapper	open pollinated	4926		3681				13
B_3F_2	B_3 Graves	open pollinated	5569		5150				7
B_3F_2	B_3 Nanking	open pollinated	165		149				12
B_3F_3	B_3F_2 Clapper	B_3F_2 Clapper	27	44	101	0**	6**	4**	2
B_3F_3	B_3F_2 Clapper	B_3F_2 Graves	143	95	237	0**	6**	4**	5
B_3F_3	B_3F_2 Graves	B_3F_2 Clapper	76	96	247		2**	0**	6
B_3F_3	B_3F_2 Graves	B_3F_2 Graves	46	80	191		2**	0**	8
B_3F_3	B_3F_2 op Clapper	open pollinated	28580		12986				134
B_3F_3	B_3F_2 op Graves	open pollinated	16197		8525				135
F_1	Chinese Vanuxem	American	123	124	283	6	24	40	3
F_1	Nanking Chinese	American	79	399	668	0	31	112	2
F_1	Nanking Chinese	Nanking Chinese	22	96	184	0	16		1
Jap B_2F_2	Japanese B_2 PI#104016	Japanese B_2 PI#104016	52	135	271	0	14	14	2
LSA B_2	LSA B_1 DaresBeach	American	18	76	72	0	1	5	1
LSA F_2B_1	American	LSA F_2F_1 Ort;Ort	101	48	67	0	5	5	2
LSA F_2B_1	LSA F_2F_1 Ort:Ort	American	141	53	14	1	7	10	2
LSA I_1F_1	American	LSA I_1 Adair;NCChamp	39	66	91	0	5	4	3
LSA I_1F_1	American	LSA I_1 Amherst;Ort	38	15	31	0	1	2	1
LSA I_1F_1	American	LSA I_1 Amherst;ScientistsCliffs	38	26	38	0	3	5	1
LSA I_1F_1	American	LSA I_1 BH1Hyp;NCChamp	21	10	20	7	1	0	2
LSA I_1F_1	LSA I_1 Amherst;ScientistsCliffs	American	57	44	100	0	6	19	2
LSA I_1F_1	LSA I_1 BH1Hyp;NCChamp	American	36	32	24	0	2	2	1
LSA I_1F_1	LSA I_1 ScientistsCliffs;Weekly	American	14	52	124	1	6	10	1
LSA I_2	LSA I_1 Ort;ScientistsCliffs	LSA I_1 BH1Hyp;NCChamp	26	11	28		1	0	1
LSA I_2F_1	American	LSA I_2 BH1Hyp;Gault;ScientistsCliffs	61	29	36		3	3	1
LSA	Assorted	open pollinated	1269		767				27
Total Controlled Pollinations			**2354**	**2902**	**4972**	**40**	**265**	**426**	**91**

*LSA denotes Large, Surviving American, defined as an American chestnut over 13 inches in diameter at breast height (54 inches) that has blight but has survived it longer than approximately 10 years.

**The controls were shared between the two pollen types for the same female types.

nuts per bur at the 'Clapper' seed orchard held steady at 2.2 and 2.3, respectively. The decline suggests pollination conditions may have been more favorable in the 'Graves' orchard in 2012 than in 2013. There are differences in phenology between 'Clapper' and 'Graves' backcrosses that might allow weather to affect pollination success differentially.

Of note in the 2013 harvest was our first crop of B_3F_2 trees from the 'Nanking' source of blight resistance. These will be the start of a 'Nanking' seed orchard for the third major source of blight resistance we have been adding at Meadowview since 1989. Additionally, we harvested our first crop of nuts from open-pollinated large, surviving American (LSA) chestnut trees. These LSA trees are another potential source of blight resistance. In developing this source we have been following the breeding plan outlined by Burnham in 1989 (*The Journal of TACF* 4[1]:43-45).

Number of Breeding Units throughout TACF, at Both Chapters and Meadowview

Table 4 presents the number of lines by Chapter for four known sources of blight resistance plus an "other" category, with the date seed orchards are expected to start being established by the chapters. There are a total of about 20 potential seed orchards with about 20 or more American background lines that the Foundation is planning to install by 2016. (These seed orchards are defined here as having 9 replicate blocks. Many of the chapters are splitting their replicate blocks into multiple locations, but combining the seed orchards by Chapter makes summary statistics easier.)

These considerations enable us to estimate nut production when these orchards are in full production. Per tree, we estimate a production of 1000 nuts, based on the rate of production of B_2F_2s, one backcross generation earlier, which reached this level of production at an age of 11 years. Our worst case assumption is 200

Table 4. Number of American chestnut background lines for various sources of disease resistance and the date seed orchards were first planted or are estimated to be first planted by TACF's State Chapters and Meadowview.

Chapter*	Source of Disease Resistance									
	Clapper		Graves		Nanking		MusickChinese		Other	
	Lines	Date	Lines	Date	Lines	Date	Lines	Date	Lines	Date
Maine	20	2012	20	2012						
MA/RI	20	2012	20	2014	10	unknown				
VT/NH			20	2016						
Connecticut	17	2016								
PA/NJ	20	2002	20	2011					42	unknown
Ohio					4	unknown				
Indiana	20	2008								
Maryland	31	2012					11	2014		
Virginia			22	2015	8	unknown				
Meadowview	30	2002	24	2004	28	2014			**	2009
Carolinas	20	2012	6	2017						
Kentucky			14	2014						
Tennessee	18	2013								
Georgia			11	2015						
Alabama	14	2014								

* This table is sorted in a roughly North-South order to illustrate the alternate allocation of the 'Clapper' and 'Graves' sources of disease resistance to chapters subsequent to the start of the PA/NJ, IN, ME, & MA Chapter programs. The New York and West Virginia State Chapters do not currently have breeding orchards from which seed orchards could be established, so are not listed in this table.

** In Meadowview, this is a seed orchard for large, surviving American chestnut trees. The number of lines has not been calculated yet.

nuts per tree, which we are getting now at our 'Clapper' seed orchard, with a best case of 2000 nuts per tree. Each seed orchard will contain about 180 trees. The 20 seed orchards would yield 3.6 million nuts per year at 1000 nuts per tree. Currently, the two seed orchards in production at Meadowview should continue to ramp up to about 450,000 nuts per year by 2018 or 2020.

Effective Population Size of TACF's Breeding Population

Chapter breeding does two things for the TACF program. First, the American chestnut trees used by the chapters for backcrossing provide adaptation to the environments local to the chapters. Second, each additional American background line enhances the overall genetic diversity of TACF's breeding population. The inbreeding effective population size of the population depicted in Table 4 will be about 200 at B_3F_4, under fairly conservative assumptions. Our overall target is 500.

Along with genetic diversity on the American side, TACF has always realized that it needed genetic diversity among its sources of blight resistance (Burnham, 1989, *The Journal of TACF* 4[1]:43-45). We estimate that 20 different sources of blight resistance are needed. These should provide a broad enough base of resistance genes that the tree will be able to adapt by natural selection should the blight fungus evolve a means of overcoming one source of blight resistance. They also could raise the overall effective population size to the target of 500.

The reason we did not start adding these sources earlier, through the Chapter breeding program rather than adding more lines from 'Clapper' and 'Graves', was that we did not believe we had suitable materials available. Previously, Hebard (2004, *The Journal of TACF* 18[2]:13-19 and http://www.acf.org/pdfs/tenyear.pdf) proposed breeding backcross F_2s reasonably homozygous for blight resistance and sending them to chapters for two generations of backcrossing onto 20 American chestnut background lines. In practice it has proven very difficult to breed those backcross F_2s. In response, Dr. Kim Steiner suggested that a more gradual increase in the number of lines starting from one tree might be a more efficient procedure, and indeed, he was right!

Most Efficient Procedure for Adding New Sources of Blight Resistance to TACF's Breeding Population

It is necessary at this point to outline some of the breeding designs TACF is using in order to explain Dr. Steiner's suggestion. Figure 1 depicts the original design by Burnham and Inman as finalized by Hebard (1994, *The Journal of TACF* 8[1]:21-28). One Chinese chestnut tree is "fanned out" by crossing it with 20 different American chestnut trees to produce 20 first hybrids. These are then progressively backcrossed to additional American chestnut trees. This design has been largely followed for the 'Nanking' source of blight resistance.

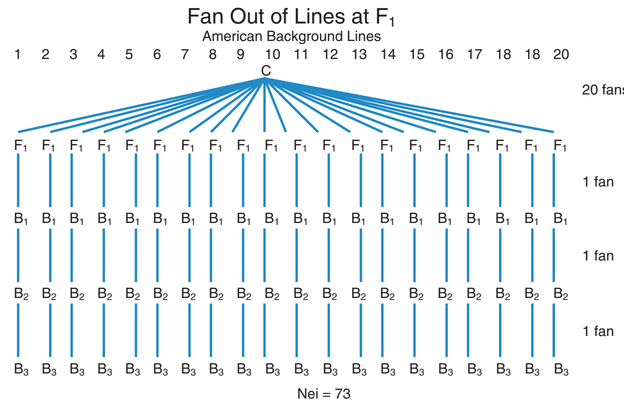

Figure 1. Diagrammatic depiction of chestnut backcross breeding design where one Chinese chestnut tree (C) is crossed with 20 different American chestnut trees to make 20 F_1 interspecific hybrids. These are each backcrossed 3 times to 20 American chestnut trees at each backcross. That cross of 1 tree with 20 is called a "fan out," since it resembles the original paper and wood object. The final inbreeding effective population size (Nei) of this population, if the B_3s were intercrossed in all combinations for three generations to make a B_3F_4 population, would be 73. Eighty crosses are required to make this population. In the TACF breeding program, each backcross would be represented by 100 nuts, or 8000 total backcross nuts. To a large extent, this scheme was followed with TACF's 'Nanking' source of blight resistance.

Figure 2 depicts the design for the 'Clapper' and 'Graves' sources of blight resistance that TACF used to jumpstart its breeding program. 'Clapper' and 'Graves' were existing first backcrosses produced by the original US Department of Agriculture and Connecticut Agricultural Experiment Station programs, respectively. This design is more efficient than that used for 'Nanking' but decreases the inbreeding effective population size from 73 to 27 for the 'Graves' source of blight resistance, because half first cousins are intercrossed at B_3 to produce the B_3F_2 generation rather than half third cousins for 'Nanking.'

Figure 3 depicts the more efficient scheme proposed by Dr. Steiner, where progressively more American chestnut trees are used for recurrent parents with each backcross. The particular design in Figure 3 has a slightly larger inbreeding effective population size (Nei) than

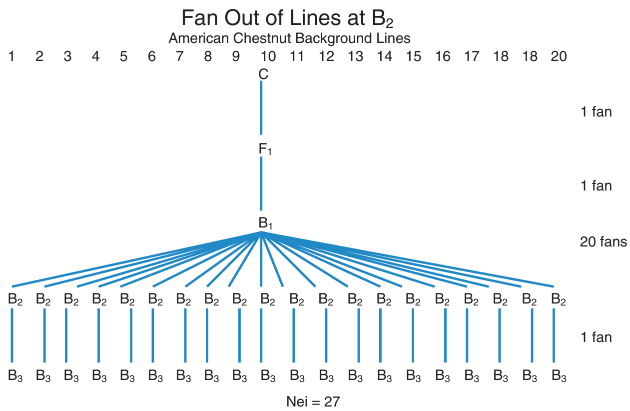

Figure 2. Diagrammatic depiction of chestnut backcross breeding design where one Chinese chestnut tree (C) is crossed with one American chestnut tree to make one F_1 interspecific hybrid, which is then backcrossed to another, single American chestnut tree to make one first backcross (B_1). The B_1 is then backcrossed to 20 different American chestnut trees to make 20 B_2s. These are each backcrossed to 20 more American chestnut trees to make 20 B_3s. The fan out occurs between B_1 and B_2. The final inbreeding effective population size (Nei) of this population, if the B_3s were intercrossed in all combinations for three generations to make a B_3F_4 population, would be 27. If the backcrossing continued to B_4, Nei would be 46. Forty crosses are required to make this population. In the TACF breeding program, each backcross would be represented by 100 nuts, or 4000 total backcross nuts. This is the scheme that was followed for TACF's 'Clapper' and 'Graves' sources of blight resistance. The chapters either produced B_3s using Meadowview B_2s or used Meadowview B_3s to produce B_4s.

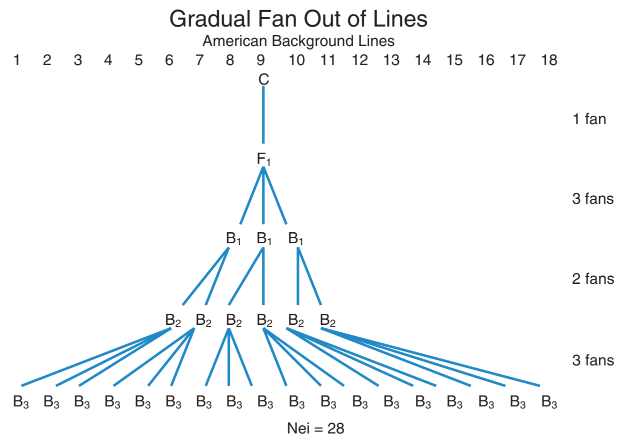

Figure 3. Diagrammatic depiction of chestnut backcross breeding design where one Chinese chestnut tree (C) is crossed with one American chestnut tree to make one F_1 interspecific hybrid, which is then backcrossed to three American chestnut trees to make three first backcrosses (B_1). Each B_1 is then backcrossed to 2 different American chestnut trees to make 6 B_2s. These are each backcrossed to 3 more American chestnut trees to make 18 B_3s. The fan out occurs gradually between B_1 and B_3. The final inbreeding effective population size (Nei) of this population, if the B_3s were intercrossed in all combinations for three generations to make a B_3F_4 population, would be 28. Twenty-seven crosses are required to make this population. In the TACF breeding program, each backcross would be represented by 100 nuts, or 2700 total backcross nuts. Fortuitously, some variant of this scheme has been followed as sources of resistance other than 'Graves,' 'Clapper,' or 'Nanking' were advanced at Meadowview, up to what is illustrated as a B_2 generation in the diagram.

the design in Figure 2 (28 versus 27, respectively), even though only 27 crosses are required rather than 40. Equally important, only six crosses are required to produce the B_2s. This is a fictional design, used to illustrate the process. In the real world, four new sources of blight resistance at Meadowview are estimated, preliminarily, to have Neis ranging from 31 to 46. There are 8 additional sources of resistance at Meadowview that appear ready. If most chapters were to add one of these sources of blight resistance, we would be close to 20 sources and our inbreeding effective population size well above 500.

A major delay in implementing Dr. Steiner's "gradual fan out" principle (illustrated in Figure 3) was calculating Nei for designs conforming to it. It was necessary to try many designs to find optimal ones and our existing method of calculation was too cumbersome, requiring tedious reprogramming and long computer runs. The existing method also did not account for inbreeding arising from only backcrossing for two generations, such as in Figure 2, rather than four (counting the F_1s), such as in Figure 1. That inbreeding reduces Nei from 73 for the design in Figure 1 to 27 for the design in Figure 2. Accounting for that inbreeding is an integral part of finding optimal designs that follow the "gradual fan out" principle. The new method is implemented in ordinary spreadsheets and calculation is instantaneous. It is not as flexible as direct simulation, so cannot simulate arbitrary designs, but the speed is essential for this task.

Forest Breeding

Since we have such a large effective population size, the question arises as to how do we add new sources of disease resistance to the population. That addition would be close to impossible using the orchard techniques we have employed to date, requiring too many controlled crosses to achieve and too many trees to grow. It might be possible to improve the efficiency

Table 5. Number of Restoration Chestnut 1.0 trees and number of lines established in forest progeny tests* that will not be artificially inoculated with the blight fungus, by crop year, state and whether or not located on land owned by the US Forest Service.

Crop Year	Number of	State													
		GA	IN USFS	NC	NC USFS	NH	NJ	PA	TN	TN USFS	VA	VA USFS	VT USFS	WV	WV USFS
2007	Trees				182					182		182			
	Lines				6					6		6			
2008	Trees				250							250			
	Lines				8							8			
2009	Trees		450	380	600		870	1150		600	660	600		440	1395
	Lines		13	15	10		27	41		10	25	10		17	46
2011	Trees				1013	450		550	550	592	550	887		3001	1330
	Lines				21	7		10	10	11	13	13		70	16
2012	Trees	600	400	1150				504	575		550		780	550	
	Lines	20	11	27				12	6		10		10	10	

* For any entry, number of tests equaled the number of trees divided by 800 rounded up to the nearest whole number; so entries with less than 800 trees represented one test.

of screening by starting at a younger age in the greenhouse, using faster techniques or molecular markers, but the barrier of making the desired number of crosses and progeny is almost insurmountable. Our goal is to restore the species, where it can evolve again on its own without our help. So why not let this introgression progress by itself in the wild, perhaps with some assistance from people? Using orchard techniques, we have elevated blight resistance in American chestnut to the point that it can resume evolving. Now, we can allow further improvement to occur in the forest.

If a stand of chestnut trees has one source of disease resistance, another source could be added by planting next to or among the existing stand. Likewise, we need to mix breeding populations from different chapters, to at least some degree. The additional source of disease resistance would need to be in a sufficiently broad genetic base that it would not degrade the diversity of the existing stand, especially if it contained a gene that could come under strong selection, such as a disease resistance gene. Preliminary analysis indicates 20 lines should be sufficient for the new source.

Existing sources of blight resistance such as ‘Clapper’ and ‘Graves’ might benefit from additional rounds of selection for blight resistance at B_3F_3 or even B_3F_4, depending upon the success of selection at B_3F_2. That breeding again would best be accomplished in the forest rather than the orchard.

The exact particulars of which source of disease resistance or genetic diversity and which generation of breeding to mix at a location and how to mix them will require careful attention to the population dynamics of the tree and its pests and the methods of stand establishment and maintenance. There should be enough to do to keep TACF busy for the rest of the century.

Current Forest Progeny Tests

Table 5 presents the number of forest progeny tests of Restoration Chestnut 1.0 trees established in the eastern United States. Some of them were established in open fields rather than forests, but they will grow up into forests, with minimal cultivation, and the chestnut trees will not be artificially inoculated with the blight fungus or other organisms. The primary purpose of forest progeny tests as outlined in our Testing Guidelines (*The Journal of TACF* 18[1]:7-11, http://www.acf.org/pdfs/testing_task_force_protocol.pdf) is to compare the performance of half-sib families of Restoration Chestnut 1.0 and pure species controls for their ability to compete with each other and with other species in our native woodlands. We also wish to compare the families and controls for the severity of blight that develops on them and to correlate that with their performance in orchard progeny tests. The goal of the forest progeny tests is not so much to pick out the best B_3F_2 parents in seed orchards as it is to evaluate the bulked cultivar known as the Restoration Chestnut 1.0. Part of that evaluation process is to differentiate among the individual families comprising the bulked cultivar, in order to determine whether or not selection should be undertaken among those families. Selection would be indicated for traits that vary strongly enough between families to impair performance of the bulked cultivar. Hopefully the forest progeny tests will provide that information.

Blight is only starting to develop in the oldest tests, established in 2009, so we will have to wait longer for those results. Preliminary data on diameter and height indicate that Restoration Chestnut 1.0 trees from most families are growing as well as American chestnut and other species of trees. That is exciting news as, once confirmed and moved from the "preliminary data" stage to the "published in a peer-reviewed journal stage," it would indicate we have recovered enough American type in three backcross generations for our trees to grow like the American chestnut. Now, how much blight resistance do they have?

Blight Resistance in Orchard Progeny Tests from 2011 to 2013

Unlike forest progeny tests, orchard progeny tests are intensely cultivated by mowing, weeding, fertilizing, irrigating and spraying as appropriate to maximize growth or other desired phenotypic characteristics. Their chestnut trees are artificially inoculated with the blight fungus. The purpose of orchard progeny tests is to compare the blight resistance of Restoration Chestnut 1.0 trees and controls as accurately, quickly and efficiently as possible in order to detect B_3F_2 parents reasonably homozygous for blight resistance. The most homozygous B_3F_2 parents would be selected. We expect the family blight resistance to correlate strongly between orchard and forest progeny tests.

Table 6 presents results for the first three years of orchard progeny testing. At this stage of orchard progeny testing, we are concentrating on testing as many families as possible in order to cull B_3F_2 seed orchards as rapidly as possible. As needed, tests of individual families will be repeated to refine selections. With sufficient replication and repetition, more sophisticated analyses than those presented in Table 6 would be appropriate, such as using generalized linear models to deal with the heteroscedasticity and multigenerational analysis to establish selection thresholds. At present, over three years, our B_3F_3 Restoration Chestnut 1.0 trees have significantly shorter cankers incited by SG2-3 than American chestnut (Table 6). The ranking of cross types has been consistent over the past 3 years, with the B_3F_3s intermediate in blight resistance.

That difference for strain Ep155 was not significant. Reasons for such difference between strains have been discussed at length in the last two Meadowview Notes (*The Journal of TACF* 26[1]:8-16 and 27[1]:19-25) and need not be repeated here. As discussed previously in Meadowview Notes, we expect resistance to improve as we continue to cull the B_3F_2 Legacy Tree seed orchards. Already, 8% over 3 years have tested as blight resistant as the best Chinese chestnut trees, and 12% as resistant as the average Chinese chestnut tree. With these levels of blight resistance, many of these trees will be able to fruit continuously for many years.

Lab Activities

Molecular markers and genes

In 2013, we used molecular markers for a number of purposes. First, we verified the cultivar identities of a number of grafted trees growing at Meadowview and confirmed the parentage of an F_1 tree. Those investigations used simple sequence repeat (SSR) markers whose alleles could be resolved on mini polyacrylamide gels. Second, SSR and single nucleotide polymorphism (SNP) markers indicated that some trees in a small collection of B_3F_2s resulted from previously undetected pollen contamination. Third, genotyping of selected B_3 parents of B_3F_2 seed orchard trees with SSRs and SNPs linked to blight resistance loci showed inconsistent association with observed blight resistance.

The inconsistent association of markers with blight resistance may have occurred because the SSR markers had become dissociated from the resistance loci over the generations of backcrossing leading to the B_3 parents, perhaps because they were not closely linked to resistance loci. Alternatively, it may be difficult to maintain favorable alleles for the same trait at multiple loci during backcrossing using phenotypic selection. (If confirmed, this second alternative would indicate a fundamental problem with the breeding program; we do not believe it to be the case, currently.) This result illuminated a need for markers more tightly linked to blight resistance loci. Markers tightly linked to blight resistance loci also should allow for detection of B_3F_2 trees homozygous for blight resistance, which would greatly lessen the burden of orchard progeny testing for state chapters.

Identification of markers more tightly linked to blight resistance loci is the goal of ongoing genetic mapping and quantitative trait analysis of an expanded F_2

Table 6. Least Squares Mean, standard deviation and distribution of canker size classes (length in cm) for cankers incited by two strains of the blight fungus on cross types of American and Chinese chestnut inoculated separately in 2011, 2012, and 2013.

Cross Type	Fungal Strain	Number of Families	N	Least Squares Mean*	Standard Deviation	Length Class					
						0-5	5-10	10-15	15-20	20-25	25-
American	Ep155	7	46	13.5 A	4.8		9	18	13	5	1
B_3	Ep155	5	54	12.7 A	4.4	3	7	34	5	4	1
B_4	Ep155	2	16	11.3 A	3.7	1	3	11	1		
Japanese B_2	Ep155	3	22	11.4 A	4.6	2	6	11	2	1	
$B_{2\text{-}3}F_2$	Ep155	5	98	10.8 A	2.7		32	60	6		
B_3F_3	Ep155	177	1975	11.2 A	4.4	158	509	977	272	56	3
B_2F_2	Ep155	4	95	10.4 A	3.3	6	32	51	6		
$B_{1\text{-}2}F_2$	Ep155	2	12	11.6 A	7.3	3	1	5	2		1
B_2	Ep155	9	101	12.2 A	4.3	8	18	53	20	2	
B_1	Ep155	2	24	9.6 AB	4.7		10	9	4	1	
B_2F_3	Ep155	1	20	10.4 A	1.9		7	13			
B_1F_2	Ep155	5	168	10.8 A	6.0	35	23	70	30	10	
F_1	Ep155	6	37	10.3 A	3.0	1	17	17	2		
B_1xC	Ep155	6	39	8.8 A	5.0	12	9	16	2		
Chinese	Ep155	7	78	4.0 B	2.4	50	27	1			
American	SG2-3	7	43	9.4 A	3.6	7	16	20			
B_3	SG2-3	5	52	8.2 AB	3.3	3	23	23	3		
B_4	SG2-3	2	16	8.1 ABC	3.0	1	10	5			
Japanese B_2	SG2-3	3	22	7.5 ABC	3.0	4	11	7			
$B_{2\text{-}3}F_2$	SG2-3	5	97	7.4 ABC	3.0	32	52	12	1		
B_3F_3	SG2-3	177	1983	6.5 BC	3.7	874	748	338	23		
B_2F_2	SG2-3	4	92	6.5 ABC	3.2	39	36	15	2		
$B_{1\text{-}2}F_2$	SG2-3	2	13	6.1 ABC	5.1	8	3	1	1		
B_2	SG2-3	9	102	5.6 BC	4.0	57	30	13	2		
B_1	SG2-3	2	22	5.0 ABCD	4.5	7	9	3	3		
B_2F_3	SG2-3	1	20	4.8 BCD	2.4	7	12	1			
B_1F_2	SG2-3	5	169	4.5 BCD	4.0	123	26	18	2		
F_1	SG2-3	6	34	4.0 CD	2.3	25	9				
B_1xC	SG2-3	6	39	3.1 CD	2.6	35	3	1			
Chinese	SG2-3	7	80	2.3 D	0.9	78	2				

* Means followed by the same letter are not significantly different at $P < 0.05$ by a Tukey HSD test. The declarations are suspect for strain SG2-3 due to heteroscedasticity.

population. In collaboration with researchers at Clemson University and the US Forest Service, subsets of 16 susceptible and 16 resistant individuals from the F_2 population have been selected for bulk sequence analysis to further define the resistance loci. Furthermore, since resistance loci may vary among different sources of resistance, we also are continuing to investigate the inheritance of resistance in additional sources. We have extracted DNA from a Nanking B_1F_2, and have identified several additional families to study.

We are looking for markers that can be used to follow not only the inheritance of disease-resistance loci but also the recovery of the American chestnut genetic background. Using the program suite "Galaxy Online," we searched DNA sequences of expressed chestnut genes from the National Science Foundation project, "Genomic Tools for the Fagaceae." This search turned up 267 genes that contain one or more candidate SNPs specific to species. We now have to test them against a larger panel of parents. Interestingly, the DNA sequence datasets from genes expressed in chestnut blight cankers contain sequences of over 1000 fungal genes, in addition to sequences of chestnut genes.

In 2014, we plan to inoculate Chinese x American F_1

trees planted in 2012. They will be inoculated with progeny of crosses between the fungal strains SG2-3 and Ep155. SG2-3 and Ep155 differ strongly in pathogenicity to chestnut, although both are virulent. This study is part of an investigation of the genetic basis of the difference in pathogenicity between these two strains of the blight fungus.

Grafting

To finish converting the Meadowview Research Farm's Wagner Farm to a seed orchard for production of Restoration Chestnuts 1.0, we need to remove the older trees still growing there. In 2013 we began "moving" the trees to the Price Farm by grafting their buds onto epicotyls (sprouts) of germinated Chinese chestnuts. We had a success rate of 80%, and over 100 of the seedlings we budded last spring are currently in the ground at the Price Farm. We hope to extend the grafting stock to germinated American chestnuts in 2014. A more comprehensive report on this grafting was published in the last issue of the TACF Journal (*The Journal of TACF* 28[1]:20-23).

Ethylene

In collaboration with Dr. Laura Hainsworth of Emory & Henry College, we have continued to investigate ethylene, a plant hormone involved in defense responses, research pioneered by Hebard and Shain in 1988 (*Phytopathology* 78: 841-845). In a set of F_2 hybrid chestnut trees, we have found a significant ($P < 0.01$) negative correlation between the size of blight cankers on stems and ethylene released from twig segments exposed to *C. parasitica*. The method is not fast enough for high-throughput screening of thousands of samples, but would be quite feasible for hundreds, suggesting that measurements of ethylene production in small pieces of tissue exposed to the blight fungus might be useful for preliminary screening of American chestnut somatic embryos transformed with candidate genes for blight resistance.

Acknowledgements

This work was supported, in part, by funding from the US Forest Service and by the many other contributors to The American Chestnut Foundation. We would like to acknowledge the technical contributions of D. Slack, G. Sykes, L. Sykes, R. Scarborough, J. Lee, L. Mann, L. Cottrell, and of the numerous volunteers throughout TACF.

TACF staff Robert Kling and Sarah Dee harvest chestnuts at Meadowview Research Farms. Photo by Jeff Donahue

Why Doesn't A Chestnut Tree Self-Pollinate (Very Often)?

By Dr. Paul H. Sisco

Why does it take at least two chestnut trees planted close together to produce seed, when a single tree has both male and female flowers? Why can't a chestnut tree pollinate itself to produce seed? The answer in scientific terms is gametophytic self-incompatibility. In plain English, this means that a female chestnut flower recognizes and rejects pollen coming from the same tree, what is called "self pollen." But what evidence do we have that a female chestnut flower can indeed recognize self pollen?

More than 70 years ago, John W. McKay of the U.S. Horticultural Station in Beltsville, MD, published the best early study of self-sterility in chestnut (1942). For his experiment, he used an isolated Chinese chestnut tree (*Castanea mollissima*) that was known to set seed on only two to three percent of its female flowers. In the year he conducted his experiment, he bagged female flowers before they were receptive to pollen. He then divided the flowers into three groups: one-third were pollinated with self pollen, one-third with pollen from other Chinese chestnut trees, and one-third were not pollinated at all. Every week, he would make stained sections of each type of flower for examination under the microscope. He also made stained sections from open-pollinated flowers. From these studies, McKay observed that both cross pollen and self pollen germinated on the stigmatic surface of female flowers and both types produced pollen tubes. The growth of self pollen tubes, however, stopped before the tube reached the egg, while pollen tubes of cross pollen successfully penetrated the egg. This type of self-incompatibility in plants is called gametophytic self-incompatibility in contrast to sporophytic self-incompatibility, where self pollen fails to germinate at all (Fig. 1). Sears (1937) had previously described both types of incompatible reactions in plants.

In more recent years, the availability of inexpensive DNA sequencing techniques allowed a group of Japanese researchers (Hasegawa et al., 2009)[1] to do a more detailed study of the source of pollen and seed in the natural setting of a forest of Japanese chestnut trees (*C. crenata*). They analyzed pollen grains and seed from three chestnut trees in one corner of a 14-acre plot in the Ippitsu Forest Reserve. They assumed the pollen on the female flowers of these three trees would be coming either from the trees themselves (self pollen) or from one of the other chestnut trees in the plot. To determine the source of each pollen grain on each female flower, they isolated and analyzed DNA from a representative sample of pollen grains from male flowers (catkins) from each of the 281 trees and from pollen on the stigmatic surface of female flowers from the three experimental trees. The female flowers on the experimental trees were not covered, so the pollination and seed set was completely natural.

What did they find? First, an average of 90% of the pollen on the stigmatic surface of each female flower was self pollen. Think of this! Almost all of the pollen on a female flower came from catkins on the same tree. But less than 1% of the seed came from self pollen – only 0.3% on average. This was strikingly clear evidence of the ability of chestnut flowers to recognize and reject self pollen, even though the vast majority of the pollen tubes growing down the style toward the egg cell were from self pollen.

Because they knew the location and DNA profile of each of the 281 trees in the plot, Hasegawa et al. were also able to determine which of the other trees was the male parent of each seed produced from cross pollen. Here they came up with an unexpected result. Their DNA analysis showed that trees growing closer together in the plot tended to be more closely related. Since chestnut has a clear mechanism for rejecting self pollen, they assumed that the female flowers on the three experimental chestnut trees would also recognize and tend to reject pollen from more closely-related trees. But that was not the case. More seed were produced by pollen from trees that were closer to the experimental trees, even though these trees were also more closely related to the experimental trees. In other words, chestnut trees may reject self pollen, but they do not reject pollen from close relatives. Inbreeding does occur in a natural setting.

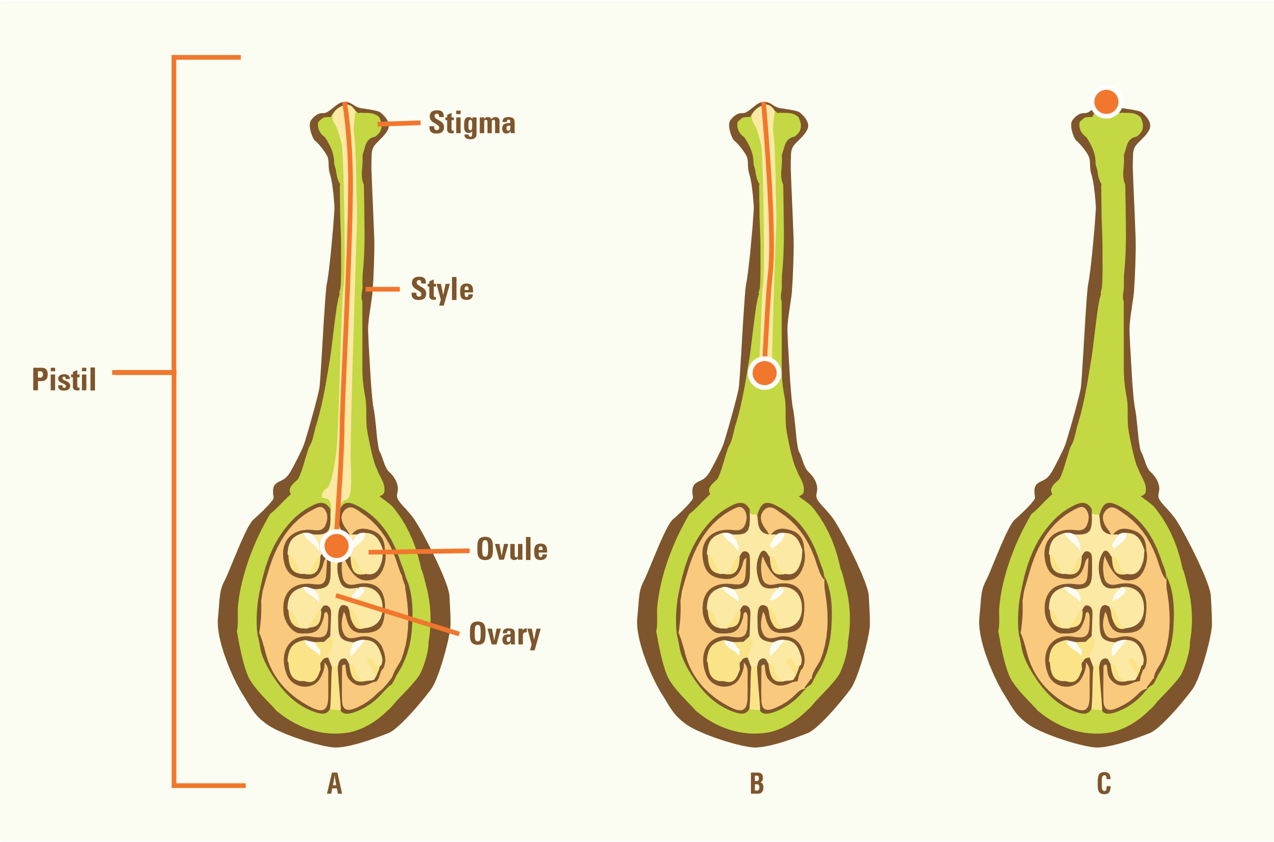

Fig. 1. (A) **Normal pollination**: Pollen germinates on the stigmatic surface of the female flower, grows through the style, and penetrates the egg cell, resulting in successful cross-pollination. (B) **Gametophytic self-incompatibility**: Self pollen germinates normally, but the growth of the pollen tube is arrested in the style, preventing the pollen nucleus from penetrating the egg cell. (C) **Sporophytic self-incompatibility**: Self pollen fails to germinate on the stigmatic surface. No pollen tube is produced and no pollination occurs.

What the researchers could not test was whether seed produced from more distant relatives resulted in seedlings with a greater "fitness," i.e., a greater ability to grow and survive to produce offspring. This is a clear and reasonable possibility, since chestnut has a strong mechanism to prevent self-pollination. However, the fact that researchers found that trees closer together spatially in the plot tended to be more closely related shows that seed produced from close relatives can survive.

REFERENCES

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ENDNOTES

[1] The author would like to thank Dr. Anne Myers Bobigian of TACF's Kentucky Chapter for bringing this recent article to his attention.

RECIPE

Warm Chestnut and Apple Salad

Recipe by Susan Herrmann Loomis, internationally recognized expert on food and award-winning author of *Nuts in the Kitchen* (Harper Collins, 2010)

Photo by Kelly Lytle

Makes 8 servings

Ingredients

6 cups (packed) arugula

6 cups (packed) coarsely torn curly endive

3 tablespoons extra-virgin olive oil, divided

1 1/2 medium Granny Smith apples, peeled, cored, cut into 1/2-inch dice

3/4 cup thinly sliced shallots

1 1/2 cups steamed chestnuts (you can buy chestnuts steamed, or steam them yourself [see below]), coarsely chopped

3 tablespoons red wine vinegar

4 1/2 tablespoons walnut oil

Directions

Toss arugula and endive in large bowl. This step can be done 6 hours ahead. Cover with damp kitchen towel; chill.

Heat 1 1/2 tablespoons olive oil in large skillet over medium-high heat. Add apples and shallots; sauté 5 minutes. Add chestnuts; sauté 1 minute. Stir in vinegar, scraping up any browned bits. Remove from heat; stir in walnut oil and remaining 1 1/2 tablespoons olive oil. Add salt and pepper to taste. Pour chestnut mixture over arugula mixture; toss.

Divide salad among 8 plates.

HOW TO STEAM CHESTNUTS

Cut fresh chestnuts still in their shell in half. A hand pruner garden tool works much better than attempting to cut them in half with a knife. Place cut chestnuts in a collapsible steaming basket placed in a pan with approximately 1/2 inch of water. Bring water to a boil and steam the chestnuts until the meats separate from the shell. The chestnuts will be slightly cooked but still crunchy at this point. You can continue steaming the nuts to reach the desired level of crunchiness or until they are a soft consistency.

Chestnut Moments

A chestnut split rail fence at the Virginia Chapter Office in Marshall, Virginia
Photo by Jack LaMonica

Chestnut trees have been around
just about as long as the sacred ground
of the Chippewa and the Cherokee.
Let`s hope they last eternally.

It`s a handsome wood for furniture
and strong for structures that have endured
in the simple beauty of a split-rail fence
or in walls that shield us from the elements.

Their nuts that rained down in the fall
had nourishment for one and all—
Bears and turkeys and deer and grouse
and bread on the table of every house.

Then their cousin was brought from the Orient.
And although there was no harm meant
Chestnut trees could barely withstand
the blight that spread in our eastern land.

In a few short years our magnificent king
was forced to survive as a mere sapling.
But, those saplings are the source of strength
And whatever it takes we`ll go the full length.

To restore Chestnut trees to their rightful place
in all the forests that they once graced.
Now, scientists, foresters, and people of the land
are working together, giving Chestnuts a hand.

Chief Seattle said, "All things are connected.
Whatever befalls the earth befalls the children
of the earth."

By Dave Waldrop, Webster, NC

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http://www.fs.fed.us/r8/chestnut/