THE AMERICAN CHESTNUT FOUNDATION®

2015 Annual Report

The Mission of The American Chestnut Foundation is to restore the American chestnut tree to its native eastern woodlands for the benefit of our environment, our wildlife, and our society.

Red Admiral on chestnut catkins in Rimersburg, PA. Photo by Mark Moore.

Dear Friends

In several important ways, 2015 has been a benchmark year for The American Chestnut Foundation (TACF). Our new President and CEO, Lisa Thomson, is off to a strong and energetic start. Our new quantitative geneticist, Jared Westbrook, is delivering encouraging news about the breeding program. We have successfully transitioned to a hopefully more effective board structure without, I believe, losing the support of some of our most important volunteers. And the tremendously successful fall meeting in State College, Pennsylvania, was full of exciting news from many scientists about their current research on chestnut genetics. I am truly very pleased with the state of the organization, and I believe the coming years are going to be very good ones for us.

The dedication and passion of our chapters, volunteers, and partners is large part of the foundation's success. In the past year, our chapters planted more then 52,000 chestnut trees at 101 separate locations throughout the range. More than 12,000 Restoration Chestnut 1.0 trees have been planted in national forests in Tennessee, North Carolina, Georgia, Virginia, West Virginia, Pennsylvania, Indiana, Ohio, Maine, and Vermont. With the annual progress at our research farms in Meadowview, Virginia, the genetic qualities of the Restoration Chestnut Trees 1.0 continue to improve.

Your essential partnership with TACF, through the generous sharing of your resources and energy, allows our research and development to continue and brings us closer to success in our mission. This publication is not simply an accounting of the foundation's work. It is a tribute to you and others who have made the achievements of TACF possible. As an organization, TACF thrives on the support of its members, volunteers, donors, and staff.

Thank you for your loyalty and dedication to the restoration of this American icon.

Sincerely,

Kim Steiner, Ph.D.
Chair, Board of Directors

THE AMERICAN CHESTNUT FOUNDATION®

By The Numbers

(July 1, 2014 – June 30, 2015)

Inoculate

354

F_1 trees inoculated with 125 fungal strains, five strains per tree, to study the pathogenicity of the fungus

2187

B_3F_3 trees inoculated in progeny tests

2171

B_3F_3 trees inoculated in Meadowview seed orchards

481

large surviving American chestnut breeding orchard trees inoculated

DNA

sequence data obtained for

680

B_3F_2 trees to predict disease resistance

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93 acres of pollinator-friendly plots

37 controlled pollinations on mother trees in Meadowview orchards

40%
increase in
Facebook followers

35%
increase in
Twitter followers

In Perspective

By Lisa Thomson

As my first year of service comes to a close, I continue to be moved and humbled by our very unique vision: that a self-sustaining American chestnut will once again be a dominant tree in our forests. It is the overriding motivation for so many of us, and I am struck by the breadth of passion behind our mission. Thanks to all of you for such a warm welcome; I am especially grateful for the patient guidance of our spectacular board of directors, led by Dr. Kim Steiner.

Along with their sage advice, I also learned that some of the best mentors are all of you: the steadfast supporters and volunteers who give of their time, treasure, and talent, growing season after growing season. From planting and maintaining orchards, to public outreach, to hosting events, you do it all, and we could not do it without you. Our volunteer corps is 6,000 strong and we now have more than 500 distinct plantings throughout the chestnut's original range. I have had the pleasure to meet many of you at the Annual Meeting, local Chapter meetings or special events, and I deeply appreciate your work on behalf of our favorite tree!

Our many and varied partners are a tremendous source of growth and continuity as we refine our science and breeding programs. We are grateful for the dozens of federal, state, and municipal agencies as well as our invaluable academic partners. They stand with us to save the iconic American chestnut by providing research, expertise, and much-needed resources. We also have a growing cadre of private landowners willing to help us plant orchards near and far to ensure the genetic diversity is captured for future generations of blight-resistant trees.

None of these relationships would be possible without the tireless hard work of our talented TACF staff. From the groundbreaking work of my CEO predecessors, to the current staff members who carry the passion for our mission with endless enthusiasm, curiosity, and optimism, you should rest assured that TACF is in very good hands. If you have not met your region's Science Coordinator, toured Meadowview Research Farms, or visited us at the Asheville national office, please do so. We love meeting our members and updating them on the progress of our restoration and breeding programs. The American chestnut will return, and we are spreading the word as far and wide as we can. We are delighted you are with us on this journey!

Photos 1-12 courtesy of Lisa Thomson. Lisa's travel diary includes: (1) education specialists tour Tim Shields chestnut orchard in Gettysburg; (2) the Dinmits family in Highlands, NC; (3) posing with George and Rad Thompson in Plains, VA; (4) with Richard Will in front of Don Willeke's Legacy tree at Meadowview Research Farms (photo by Jeff Donahue); (5) with Doug Levin presenting prizes at the 6th Annual Chestnut Restoration Celebration; (6) with Lois Melican at the New England Regional Annual Meeting; (7) with Katherine Macdonald and Wayne Mezitt, officers of the Massachusetts Horticultural Society, at the presentation of the Jackson Dawson award to TACF; (8) planting a Restoration Chestnut 1.0 with Joe Nassif and Robert Sypolt at the 8th Annual West Virginia Chestnut Festival (photo by Mark Double; (9) with Smokey the Bear in Washington, D.C. while meeting with TACF partners the USFS and NRCS photo by Bruce Moltzan); (10) participating in a very meaningful planting experience at the Flight 93 Memorial (photo by Michael French); (11) with Chuck Leavell at Charlane Plantation in Dry Branch, GA during filming of TACF's first public service announcement (photo by Rod Murphy); (12) along with Michael French, more than 500 volunteers planted 22,000 seedlings across 32 acres at the Flight 93 Memorial.

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SMOKEY
SMOKEY

Planting Chestnuts

at the Flight 93 National Memorial

On April 17-18, The American Chestnut Foundation (TACF) was honored to participate in "Plant-a-Tree at Flight 93" for its fourth year in a row. This annual event is organized by the National Park Service, the Friends of Flight 93, and the National Park Foundation as part of a major reforestation effort that will ultimately result in large areas of new forest at the Flight 93 National Memorial.

The Flight 93 National Memorial is a national park created to commemorate the passengers and crew of Flight 93 who, on September 11, 2001, courageously gave their lives by thwarting a planned attack on our nation's capital. The memorial is near Shanksville, Pennsylvania, where Flight 93 crashed, resulting in the loss of its 40 passengers and crew.

More than 500 volunteers helped prepare and plant 22,000 seedlings, including 1,500 Restoration Chestnuts 1.0 on 32 acres of reclaimed mined land which is part of the Memorial. This year, there was an added focus on removing invasive plants that are threatening trees planted in past years. Volunteers included friends and family members of the victims of the terrorist attack, college students and professors, forestry professionals, and the general public.

TACF Forester Michael French helped organize the chestnut plantings, working with the Appalachian Regional Reforestation Initiative (ARRI), Green Forests Work (GFW), and many other partners. French says of the event, "The Flight 93 National Memorial Reforestation effort is always a highlight of the planting season. The National Park Service staff and the volunteers are wonderful to work with, and it's rewarding to return each year to see the growth of seedlings from the plantings of previous years."

TACF's President & CEO, Lisa Thomson, joined the planting efforts this year: "This was my first volunteer planting as President of TACF, and one I won't ever forget. I was moved by the setting and the dedication of the National Park Service and their partners to honor the memories of the heroes of Flight 93."

The Flight 93 National Memorial is the largest mixed hardwood/American chestnut reforestation effort that TACF has been involved with to date. Since 2012, TACF has planted more than 3,400 chestnuts across more than 100 acres. A project of this scope heightens the visibility of American chestnut restoration. Symbolically, the addition of TACF's potentially blight-resistant Restoration Chestnuts 1.0 to the site is a powerful statement of renewal and hope. We are proud to be able to provide trees for this purpose and look forward to participating in years to come. TACF is grateful to the Richard King Mellon Foundation for generously providing funding for this project.

For more information, visit their website at: flight93friends.org

TACF Completes

Multi-year Conservation Innovation Grant

In 2011, The American Chestnut Foundation (TACF) was awarded a national Conservation Innovation Grant (CIG) by the USDA – Natural Resources Conservation Service (NRCS). This was an exciting opportunity that had multiple objectives to help TACF forward the Foundation's mission of restoring American chestnuts to eastern forests, while also working toward NRCS's mission of putting private lands into conservation to benefit society and wildlife.

Project objectives included: ❶ the establishment of diverse, mixed hardwood/American chestnut forests on reclaimed mine lands in Kentucky, Ohio, Pennsylvania, Virginia, and West Virginia; ❷ the creation of workshops, models, and a manual to aid landowners who wish to reforest mined lands or establish chestnut plantings; and ❸ support for TACF's online trees database. TACF worked closely with the Appalachian Regional Reforestation Initiative (ARRI) and Green Forests Work (GFW) to implement this grant and has successfully completed all of the project objectives.

From 2012 through 2015 (when the CIG was being implemented), this NRCS grant allowed TACF to assist in the planting of more than a million trees across 1,692 acres in eight states (AL, TN, KY, VA, WV, OH, PA, and MD).


1


2

MINED LAND PLANTINGS

A major objective of the CIG was to demonstrate large-scale reforestation of reclaimed surface mined lands using ARRI's most recent reclamation recommendations, known as the Forestry Reclamation Approach (FRA), while also demonstrating the blight-resistance and competitive ability of TACF's most advanced generation of chestnuts. In all, 12 plantings were established on coal surface mines: two in Kentucky, two in Ohio, two in Pennsylvania, four in Virginia, and two in West Virginia.

Each of the 12 plantings included a one-acre progeny test for TACF's Restoration Chestnuts 1.0, which will help TACF determine and demonstrate which families carry higher levels of blight resistance. Progeny test trees were direct-seeded (i.e. planted as seed); sheltered with two-foot tree shelters to protect the seeds and young seedlings from rodents, and locations of the individual trees were mapped at the time of planting. A randomized complete block design was used for the progeny tests to allow for later analysis of differences in blight resistance, growth, and other characteristics between the families.

The progeny tests are surrounded by larger reforestation areas, approximately 30 acres in size, and planted to create a mixed hardwood/chestnut forest type, which has been virtually absent from Appalachian forests for more than 60 years. The mixed hardwood reforestation areas will demonstrate how chestnuts compete against other species in a mixed hardwood setting.

Although the CIG called for approximately 360 acres to be reforested for the 12 projects, TACF and many partnering organizations and individuals managed to apply the FRA to slightly more than 425 acres, resulting in the planting of 294,588 trees. Several of the CIG projects included working with active mining operations to implement the FRA for the first time, and some of those companies were so happy with the projects that they intend to implement the FRA on future reclamation projects. Unfortunately, not all 12 plantings can be described in this report, so two have been selected to highlight.

SCHUYLKILL COUNTY, PENNSYLVANIA (2012)

This 22-acre site was established on an active coal mine within the Chesapeake Bay Watershed. Residents downstream from the site were attributing flash flooding and sedimentation issues to the mining operation, which had been reclaiming the land by compacting it, seeding it with grasses, and planting trees. A mix of trees including white oak, chestnut oak, black cherry, sugar maple, Restoration Chestnuts 1.0, hazelnut, eastern redbud, and others were then re-planted. Survival of planted seedlings across the site has been very good and native species such as aspen and birch have been colonizing the site.

COSHOCTON COUNTY, OHIO (2014)

This 30-acre reforestation project occurred on a surface mine reclaimed as hay/pastureland on a property owned by Tom Brannon, a long-time TACF volunteer who contacted TACF when the CIG was announced. TACF, ARRI, GFW, and the Brannons worked with NRCS and local volunteers to plant the progeny test. The 30-acre reforestation area was planted with more than 21,000 trees from 20 different species.

These reforestation projects will result in cleaner air and water, increased carbon sequestration, future timber production, and better wildlife habitat for numerous species. However, many exhibit additional benefits for pollinators immediately after ripping, the process by which compacted mined lands are mechanically loosened to prepare for plantings. Following the herbicide application and ripping, or ripping alone, several sites showed a noticeable increase in native wildflowers, both in terms of diversity and in the percentage of total groundcover.

Tools for landowners and cooperators

CIG, TACF, ARRI, and GFW developed two state-and-transition models to help natural resources professionals understand and describe the existing conditions of mined lands, and to recommend methods to help them achieve success in reforesting mined lands. We also developed a technical manual that gives general guidelines for helping landowners establish and maintain different types of chestnut plantings. Although only 12 workshops were required, the partners hosted 25 training workshops held throughout the Appalachian region. Each of these was designed to educate landowners, mining regulators, and the public about mined land reforestation using the Forestry Reclamation Approach and chestnut restoration efforts.

Online Trees Database

As TACF's restoration effort grows, and the number of plantings increases, the creation of a universal database for tracking will be essential to monitor and record chestnut plantings. The CIG provided funding to develop an online database to help TACF staff, members, landowners, and natural resource professionals to store, share, and track data on American chestnut plantings.

Photo 1: NRCS staff (l-r) District Conservationist Dennis DiOrio, Public Affairs Specialist Molly McDonough, State Biologist Barry Isaacs, and State Conservationist Denise Coleman help to plant the progeny test in Schuylkill County, PA.

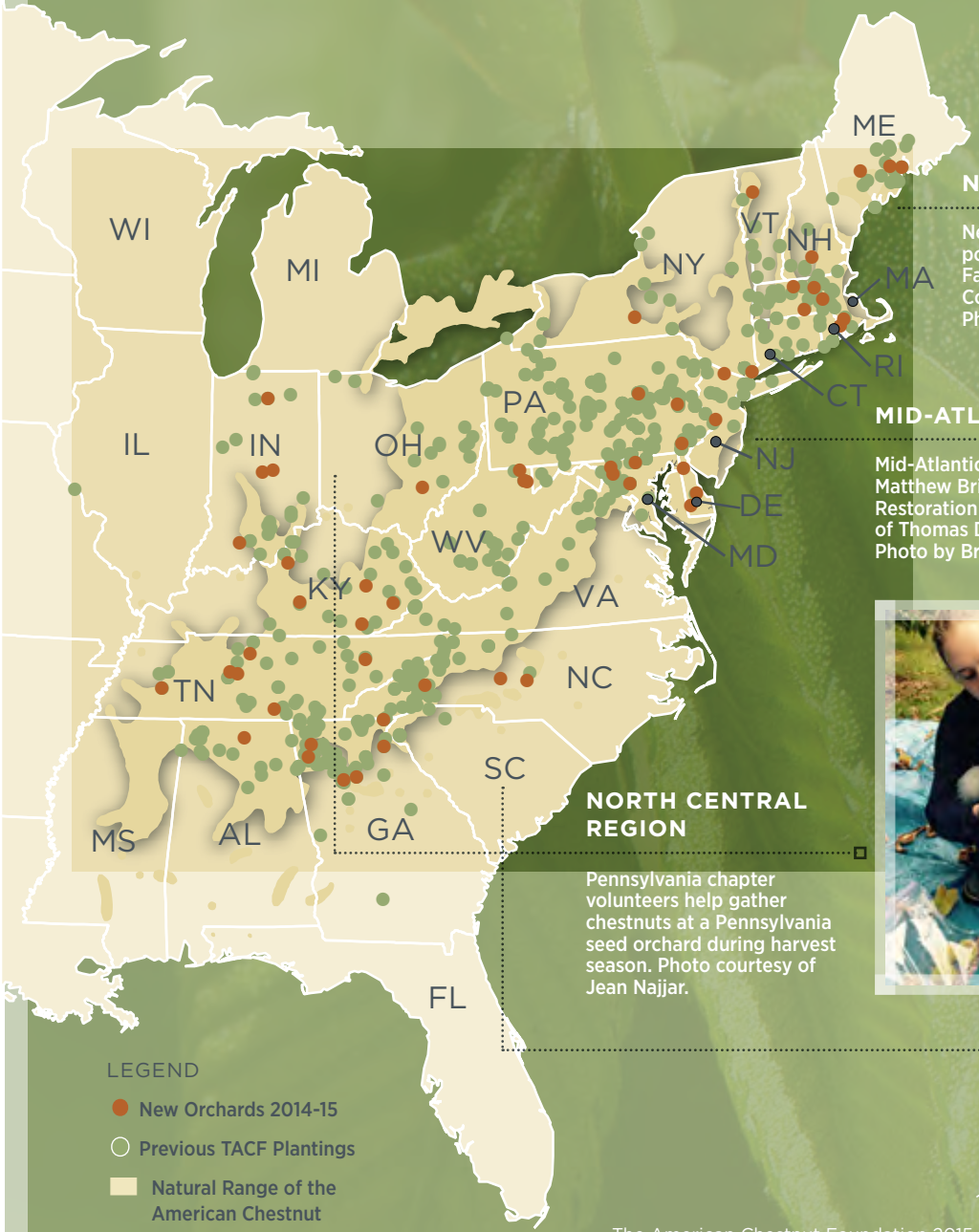
Photo 2: During the first CIG workshop in 2012, Bill Reichert, Schuylkill Headwaters Association, discusses how the FRA benefits trees, water, and soil.

Photo 3: Bob Brannon and NRCS District Conservationist Chuck Reynolds planting and recording chestnuts in Coshocton County.

Photo 4: Two chestnuts in the progeny test emerge from the native wildflowers. Photo by Michael French.

Photo 5: Pennsylvania Wildlife Habitat Unlimited and volunteers help plant seedlings on the ripped slope in Elk County. Photos by Michael French.

Regional Accomplishments



NEW ENGLAND REGION

New Hampshire volunteers assess potential planting areas at Old Gurdy Farm with Regional Science Coordinator Kendra Collins. Photo by Janet Roberston.

MID-ATLANTIC REGION

Mid-Atlantic Regional Science Coordinator Matthew Brinckman plants a ceremonial Restoration Chestnut 1.0 under the instruction of Thomas Dierauf, retired research forester. Photo by Brian Smith.

NORTH CENTRAL REGION

Pennsylvania chapter volunteers help gather chestnuts at a Pennsylvania seed orchard during harvest season. Photo courtesy of Jean Najjar.

SOUTHERN REGION

The Tennessee chapter has added *Phytophthora* Root Rot (PRR) resistance to its breeding objectives. Chapter volunteers use pollen from PRR-resistant F_1s to cross into blight-resistant BC_3 selections at the Tennessee Tech University backcross orchard in Cookeville, Tennessee. Photo by Dr. J Hill Craddock.

The American Chestnut Foundation consists of sixteen state chapters, six restoration branches, and more than 5,000 volunteers, all of whom are committed to the restoration of a foundation species. Each year, regional volunteers dedicate thousands of hours to new plantings, orchard maintenance, and outreach activities to ensure that future generations will experience the splendor of the American chestnut.

NEW ENGLAND REGION

State Chapters: Connecticut, Massachusetts/Rhode Island, Maine, Vermont/New Hampshire

Regional Stats:

- Approximately 35,000 chestnuts harvested
- Approximately 10,000 chestnuts planted
- 33 new plantings
- 37 outreach activities, serving approximately 100,000 individuals
- 118 new members enrolled

Notable Accomplishments:

- The Massachusetts Horticultural Society Gold Medal for Excellence in Hybridization was awarded to TACF;
- The Connecticut chapter planted a 1.5-acre American chestnut sanctuary in collaboration with the Greenwich Land Trust. More than 350 Restoration Chestnut 1.0 seedlings were planted as a part of the 14 acre forest preserve;
- The Maine chapter organized the first aerial flights to locate surviving chestnuts by using true color and inferred GPS;
- The Vermont/New Hampshire chapter established two American chestnut reintroduction trials using Restoration Chestnuts 1.0 – one at UVM's Jericho Research Forest and another at the New Hampshire Division of Forests and Lands' Vincent State Forest.

NORTH CENTRAL REGION

State Chapters: Indiana, New York, Ohio, Pennsylvania/New Jersey

Regional Stats:

- Approximately 41,250 chestnuts harvested
- Approximately 36,875 chestnuts planted
- 9 new plantings
- 45 outreach activities, serving approximately 17,500 individuals
- 189 new members enrolled

Notable Accomplishments:

- The Indiana chapter created and implemented the new Southern Hills Restoration Branch;
- The Pennsylvania/New Jersey chapter established a research planting that holds more than 27,500 chestnut trees in New Tripoli, Pennsylvania;
- A large-scale chestnut education plot at the 4H center that serves more than 6,000 people annually in Camp Palmer, Ohio was established;
- More than 2,000 "mother" tree chestnuts were distributed state-wide to New York chapter members.

MID-ATLANTIC REGION

State Chapters: Maryland, Virginia, West Virginia

Regional Stats:

- Approximately 15,000 chestnuts harvested
- Approximately 1,200 chestnuts planted
- 16 new plantings
- 57 outreach activities, serving approximately 3,500 individuals
- 209 new members enrolled

Notable Accomplishments:

- The Virginia chapter established an online database to track all Restoration Chestnut 1.0 ceremonial plantings, with more than 300 recorded;
- The Maryland chapter signed an MOU with the Central Maryland Research and Education Center of the University of Maryland to establish a second seed orchard for the state;
- The West Virginia chapter planted a 100 chestnut seedling demonstration orchard at the West Virginia University Research Forest.

SOUTHERN REGION

State Chapters: Alabama, Carolinas, Georgia, Kentucky, Tennessee

Regional Stats:

- Approximately 14,000 chestnuts harvested
- Approximately 4,000 chestnuts planted
- 43 new plantings
- 50 outreach activities, serving approximately 8,600 individuals
- 157 new members enrolled

Notable Accomplishments:

- More than 300 chestnuts were harvested and control pollinated to further the Carolinas chapter research on *Phytophthora* root rot resistance;
- The Georgia chapter established two new, high-profile plantings at the Carter Center and Governor's mansion;
- The Kentucky chapter developed a collaborative program with Eastern Kentucky University which will include a seed orchard in 2016, several education programs, and demonstration plantings at two natural areas;
- The Alabama chapter helped implement a new restoration branch;
- The Tennessee chapter tracked and recorded more than 3,000 surviving American chestnut trees.

Appalachian Trail

MEGA-Transect Chestnut Project

TACF Stanback Intern Sarah Hagan shows MEGA-Transect Project volunteers how to identify an American chestnut leaf. Photos by Matthew Brinckman.

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The Appalachian Trail MEGA-Transect Chestnut Project trains volunteers to collect data on American chestnut trees along the Appalachian Trail (AT). It is part of the Appalachian Trail Conservancy's AT Mega-Transect Project, which seeks to engage the public in citizen-science efforts involving the collection of data along the AT. This important effort raises awareness of threats to the environmental health of the Appalachian Region.

The MEGA-Transect Chestnut Project is a long-term effort that provides critical information about trends and characteristics of locations that support chestnut survival. It began in 2008 and continues to be in a piloting phase. As of 2014, TACF volunteers and partner organizations have hiked nearly 1,300 miles of the AT, making an outstanding contribution to this important initiative.

Two types of data are collected: (1) total number of American chestnut trees three feet or taller in height within 15 feet on either side of the trail and (2) location and description of large individual trees 13 inches or greater in circumference at 4.5 feet above ground. Data on large individual trees with the potential to produce flowers assists the Foundation in increasing the genetic diversity of its backcross breeding program. Data collection takes place along the Appalachian National Scenic Trail between May 10 and October 31 to ensure that the trees have leaves on them.

Training opportunities are provided each year, led by scientist volunteers and other experts. Training includes an introduction to the American chestnut restoration efforts of The American Chestnut Foundation, instruction in techniques for data collection, and recordation using the project protocol. Training participants are encouraged to collect data in teams in the afternoon following the training to provide practice in the identification process and prompt feedback on the training and data collection process.

Project information and data are available on TACF's AT MEGA-Transect Chestnut Project web page at http://ecosystems.psu.edu/research/chestnut/reports/mega-transect/data-files.

FY 2015 STATS

Trees Counted

More than **8,800** trees were counted in 2014, bringing the total trees counted to more than **32,500** living trees within fifteen feet of the AT.

Large Trees

Data collectors recorded observations on an additional **24** "large trees" of thirteen or more inches in circumference along the AT, bringing the total number of large trees to more than **200**.

Miles Counted

Data collectors hiked and collected data on **225** miles of trail in 2014. In all years, a total of more than **1,125** miles of trail have been hiked while collecting data and more than **900** of the roughly **2,000** miles of the AT has been counted.

Training

Three trainings were offered during the 2014 data collection season in Damascus and Washington, Virginia, and Slatington, Pennsylvania.

Education

The American Chestnut Foundation is committed to providing educational opportunities for our nation's youth, and to strengthening the bond between communities and forests. Pictured here are some of the Foundation's educational initiatives from this past fiscal year.

Students extract chestnut DNA as a part of the Olympic High School B3 Program. This is a unique summer camp and Saturday science enrichment program designed for high school students enrolled in one of the Olympic Community of Schools of Charlotte, North Carolina. Photo by Jennifer Weller.

At Winters Mill High School in Westminster, MD, seniors Zachary Peters and Ryan Cunningham work in their school's newly remediated chestnut orchard. This project was implemented through the Science, Technology, Engineering and Mathematics (STEM) program to incorporate hands-on, scientific chestnut tree explorations, civic tree plantings, project-based learning, and environmental activities.
Photo by Jim Peters.

Twenty-one American Chestnut Learning Boxes were distributed to schools and community organizations during the fiscal year. Students are able to learn about the Foundation's goal to restore the American chestnut to its native woodlands and about the responsibility of forest stewardship through various biological samples, photos, graphics, and activities provided in the box. The Learning Box brings together a collection of natural objects to facilitate discussion in an environment where it would not normally occur in the natural world.
Photo by Ruth Goodridge.

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Restoring American Chestnuts

to our National Forests

The American Chestnut Foundation has partnered with the USDA Forest Service for the past 26 years. As one of the Foundation's largest funders and longest partners, the Forest Service provides the use of national forest land and personnel to help restore the American chestnut.

To date, more than 12,000 Restoration Chestnut 1.0 trees have been planted in national forests in Tennessee, North Carolina, Georgia, Virginia, West Virginia, Pennsylvania, Indiana, Ohio, Maine, and Vermont. These plantings allow TACF to test and evaluate the Restoration Chestnuts 1.0 in real forest environments and also fulfill an important goal of the USDA Forest Service to restore native trees to our forests.

Currently, the majority of the Restoration Chestnuts 1.0 have grown as fast as American chestnut, not slower like Chinese chestnut. This critical result indicates we have achieved one of the two key objectives of our breeding program, producing trees that look and grow like American chestnut.

Where feasible, The American Chestnut Foundation invites the public to participate in these progeny test plantings. Through hands-on events, partially funded by the National Forest Foundation, citizens develop a deeper connection to our national forests while participating in a historic restoration project.

More than 2,000 Restoration Chestnuts 1.0 were planted in national forests this past year. Restoring the American chestnut to our national forests benefits forest health by providing ample, nutritious food for wildlife and creating more diverse ecosystems.

Cub scouts and their siblings from Pack 117 in Meadowview, Virginia, assist with site preparation for the seedlings planting. Photo by Krystal Lee.

Accelerating Selection

for Disease Resistance with Genomics

Selection is the major bottleneck when producing blight and *Phytophthora* root rot (PRR) resistant American chestnut backcross hybrids. At the TACF seed orchards in Meadowview, VA, there are approximately 10,000 trees. Of these, 500 to 1,000 final selections must be made of the most disease-resistant parents. The selected trees will be a seed source for species restoration and for future generations of breeding.

Aerial view of Duncan Farm, 2011.

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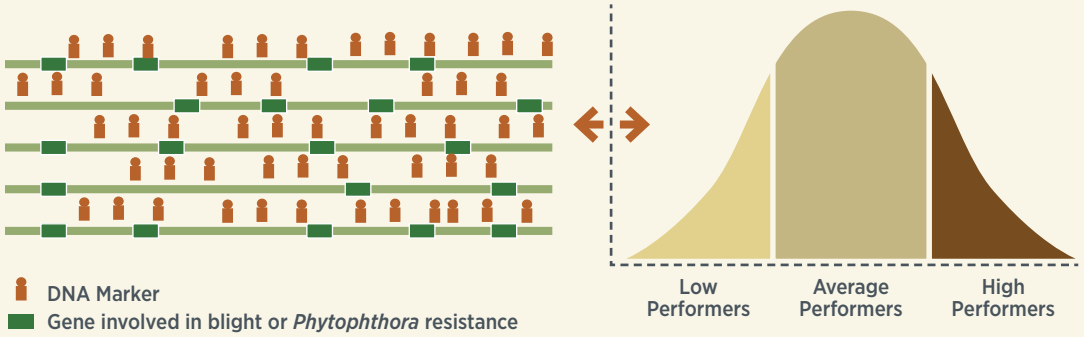
A B_3F_2 seed orchard for the Clapper source of resistance is planted at the Duncan Farm in Meadowview, VA (photo, p. 16). Seed orchard plots of 150 trees can be distinguished in the photo. Selection in seed orchards will be complete when all but one to two of the blight or PRR-resistant individuals from each plot have been culled. Genomic selection will accelerate and increase the accuracy of selection in seed orchards by predicting the genetic resistance from DNA sequence. Once selection is complete, open pollination among the most disease resistant B_3F_2s will generate disease resistant B_3F_2 seed for restoration.



GENOMIC PREDICTION MODELS FOR BLIGHT AND PRR RESISTANCE will be developed from the correlations between DNA sequence variants and genetic variation in disease resistance among trees that have been progeny tested. The disease resistance of trees in seed orchards that have not been progeny tested will be predicted from the summed effect of DNA sequence variants in proximity to genes that control blight or PRR resistance.

The first round of selection for blight resistance has been made by inoculating stems with the blight fungus and removing individuals with significant canker growth. Monitoring natural blight infection has informed further selection; however, this method may not be accurate enough to make the final selections. Environmental factors such as competition from neighboring trees may obscure genetic variation in blight resistance.

Genetic variation in blight and PRR resistance is being assessed with progeny tests. Progeny tests estimate the relative disease resistance of trees in seed orchards from the average blight canker growth or PRR symptoms of their progeny. The first trees were planted in Meadowview seed orchards in 2002, and since then, 500 trees have been progeny tested for blight resistance, and 200 trees have been progeny tested for PRR resistance. It is simply not feasible to progeny test the thousands of trees remaining at Meadowview, let alone the several hundred thousand trees currently being planted in seed orchards by TACF's state chapters.

Using a technique called genomic selection, the genetic component of blight and PRR resistance may be predicted from DNA sequence, potentially enabling TACF to finish selections at Meadowview within five years. Genomic selection consists of sequencing DNA markers throughout the genome in a training population of trees that have been progeny tested for blight or PRR resistance.

A prediction model is developed from correlations between DNA sequence variants and genetic variation in disease resistance that is estimated from progeny tests (**see figure**). The genetic resistance of trees that have not been progeny tested is predicted from DNA sequence of markers in close proximity to genes that control blight or PRR resistance.

With funding from the USDA and Forest Health Initiative, scientists from TACF and Virginia Tech are collaborating to develop genomic prediction models for blight and *Phytophthora* root rot resistance in the 'Graves' and 'Clapper' sources of resistance. These prediction models are expected to increase the accuracy and speed of selection in TACF's Meadowview and state chapter seed orchards.

Phytophthora

Root Rot Screening

Phytophthora root rot (PRR), caused by the soil borne pathogen *Phytophthora cinnamomi*, infects the roots of American chestnuts, and obstructs water transport to the stem. The disease was imported to North America from Eurasia in the 1800s and eradicated American chestnuts from the coastal plain of the Southeastern United States prior to the introduction of chestnut blight.

Chinese chestnuts (*Castanea mollissima*), which are sources of blight resistance in TACF's breeding program, are also resistant to PRR. Collaborators at Clemson University (Drs. Joe James and Steven Jeffers) and North Carolina State University (Dr. John Frampton) have screened more than 200 B_3F_3 families that originated from Meadowview seed orchards. Due to variation in the inheritance of PRR resistance, gene families vary from highly susceptible (100% mortality within families) to highly resistant (less than 20% mortality).

Genomic selection is a method to predict a tree's resistance to PRR without having to infect its progeny with the disease. Genomic selection entails first discovering regions of the chestnut genome that are correlated with PRR resistance. The DNA from remaining trees are sequenced in these regions to detect the presence or absence of genes responsible for PRR resistance. The sequencing of DNA will be used to identify and select trees that inherited genes for resistance from both parents, and will be selected based on their DNA sequence. The genes that control PRR resistance are different from those that confer blight resistance. Therefore, to combine blight and *Phytophthora* resistance, it will be necessary to breed the most blight resistant individuals with the most PRR resistant individuals. The progeny of these crosses are expected to have intermediate levels of resistance to both blight and PRR. To generate progeny that are as resistant to blight and PRR as Chinese chestnut, one or two generations of breeding and selection will be required to enhance resistance to both diseases.

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Week 1

TACF B_3F_3 chestnut seedlings four weeks after inoculation with *Phytophthora cinnamoni* in a trial at N.C. State University evaluating family differences in resistance.

Week 2

The same B_3F_3 chestnut seedlings eight weeks after inoculation.

Re-inoculating seedlings surviving after eight weeks to reduce escapes and ensure that surviving seedlings are *Phytophthora*-resistant.

All photos by Anne Margaret Braham.

Research & Restoration Project

at State University of New York College of Environmental Science and Forestry (SUNY-ESF)

2015 SUNY-ESF CHESTNUT PROJECT TEAM: Co-Directors: Dr. William Powell and Dr. Charles Maynard • Managers & Technicians: Linda McGuigan, Kathleen Baier, and Andy Newhouse
Graduate students: Allison Oakes (PhD), Tyler Desmarais (MS), and Dakota Matthews (MS) • Visiting Scientist: Qingqin Cao (Professor from China) • Visiting Scholar: Christie-Anne Lovat (Doctoral candidate from McGill University in Quebec) • Lab Assistants: Allison DeSario and Natasha Kacoroski (undergraduates); Justin McMillan (graduate student) • Field Assistant: Eric Antosh (undergraduate)

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Highlights

LEAD EVENTS BEING PREPARED FOR REGULATORY REVIEW

One of the most exciting things the SUNY-ESF research team is working on is preparation for a federal regulatory review. According to Dr. William Powell, "Well, the review isn't exciting, but it means we will be getting closer to being able to distribute the 'Darling' line of American chestnut trees to the public. Currently, the trees we have developed can only be planted on USDA permitted sites, but with federal approval, that will change."

In previous years the American Chestnut Research & Restoration Project team demonstrated that it is possible to enhance chestnut blight resistance using the tools of genetic engineering (Zhang et al. 2013, Newhouse et al. 2014) even to levels as high as Chinese chestnut controls as seen in leaf and small stem assays (**Photo 1**). The transgenic events (i.e. lines of trees) produced to this point had two extra marker genes that were added to develop the techniques they use to add genes that potentially increase blight resistance. These marker genes (GFP and BAR) are no longer needed, so this year researchers are producing new transgenic events without them to simplify the federal regulatory review. The final events will only have the oxalate detoxifying enzyme gene (OxO) and marker gene (NPT2, already deregulated) added to select trees that contain the OxO gene. To produce these new events, the research team goes through a lot of testing and then culling, similar to the culling of less desirable trees in the breeding program, but with the transgenic trees, most of this is done before the trees are put into the field. After screening hundreds of events, they have chosen Darling 58 to be the "lead" event and Darling 54 to be the back-up event to be submitted for review by the federal regulatory agencies of the EPA, USDA, and FDA. Because they are using these newer Darling events containing fewer added genes, several of the tests performed on the older events, such as mycorrhizal colonization of the roots (D'Amico et al. 2015), are being repeated to ensure similar results where they have seen no differences to wild type American chestnut trees. This year they have been engaged with the regulators to determine what is needed for a review. Their goal is to have enough data collected to begin the regulatory review possibly before the end of this year, but if not, within the next year.


2

RESEARCH STILL CONTINUES

Although the SUNY-ESF researchers found that the oxalate detoxifying enzyme is very effective at enhancing blight resistance, they don't want to put all their eggs in one basket. They are also looking at more than 30 other "candidate" genes that might be added to bolster the blight resistance or to enhance *Phytophthora* (Ink disease) resistance. Twenty-seven of these genes come from

Chinese chestnut and the others come from additional plants. Using leaf assays, they have discovered eight promising genes, seven from Chinese chestnut and one from grape. The Chinese chestnut genes may be involved in natural Asian resistance being used in the breeding program, or they might just be chestnut genes that when over expressed (making more enzyme) adds to blight resistance. These genes are still in the early stages of study and none provide as high a level of blight resistance as the OxO gene. But if they continue to give good results, they could be added by genetic engineering or by breeding to the OxO to create an even more durable resistance. The most interesting one of these genes so far comes from Chinese chestnut and makes an enzyme called acid phosphatase. The normal function of this type of enzyme is to help the plant take up phosphorus from the soil. The initial hypothesis is that this may simply be making the tree healthier and therefore more resistant to the blight fungus. Learning more about this gene and others will improve the prospects for the American chestnut restoration and possibly also help other trees.

PUBLIC OUTREACH

A very important aspect of American chestnut restoration is reaching out to the public. This is crucial to the breeding program, and probably even more vital for the biotech program because it can take people a while to feel comfortable with new technologies. As a result, SUNY-ESF researchers have been very active in outreach with many popular press articles (links can be found here: http://www.esf.edu/chestnut/) and more than 30 public presentations this past year. The team also hosts high school interns who help with the research and go on to prestigious colleges. They are also involved in many public outreach events including a Camp Fire Club of American Youth Conservation Day, where families helped plant a demonstration planting of transgenic American chestnut trees; a Relay For Life event for cancer survivors where they distributed wild type American chestnut "mother" trees; and the Great New York State Fair, where they talked with fair goers about the American chestnut.

Photo 1: This is an example of a small stem, blight resistant assay. Ellis 1 is a New York wild type, blight susceptible, clonal line of trees used as the starting material for producing the transgenic Darling 215 and Darling 311 line of chestnut trees. Qing is the blight-resistant Chinese chestnut control. All were stem inoculated with the virulent Cryphonectria parasitica strain. Photo by William Powell.

Photo 2: A group of volunteers from The Camp Fire Club of America plant transgenic American chestnut trees on Youth Conservation Day. Photo by Preston Bruenn.

Photo 3: High School interns work in the SUNY-ESF lab and learned various genomic sequencing techniques. Photo by William Powell.

Photo 4: Pure American chestnut seedlings are handed out to registered cancer survivors at the 11th Annual Madison County Relay for Life event. Photo courtesy of Kristen Russell-Stewart.

Photo 5: SUNY-ESF volunteers discuss the restoration of the American chestnut at the Great New York State Fair. Photo by William Powell.

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Research Funded

through The American Chestnut Foundation

The American Chestnut Foundation funds research projects that provide critical knowledge to help understand the complex ecology of the chestnut and its interactions with the natural world. In October 2014, the Foundation awarded $19,900 in grants to external organizations to conduct chestnut research.

RESEARCH PROJECTS	ORGANIZATIONS
Investigating the decomposition of disease-resistant transgenic American chestnut (*Castanea dentata*) leaf litter and the colonization of the litter by ectomycorrhizal fungi	State University of New York College of Environmental Science and Forestry
Secondary microorganisms in chestnut blight cankers: Can they reduce blight severity and be used as biological control agents?	Michigan State University; West Virginia University
Identification of different ecotypes and centers of adaptive genetic diversity in American chestnut	Michigan Technological University; Ohio University; USDA Forest Service, Southern Research Station
Integration of Host Resistance and Hypovirus	West Virginia University
Mapping of resistance to *Phytophthora cinnamomi* in interspecific American/Chinese chestnut population	Clemson University; University of Kentucky; USDA Forest Service, Southern Research Station

This image shows the initial chestnut shoot phenological stages of TACF- funded project: "Assessing phenological differences among American chestnut sources in a rangewide progeny planting in Vermont." This project established an American chestnut planting near the northern limit of the species' range to assess how genetics and silvicultural treatment influence growth and physiology in this region. Preliminary analyses indicated that both genetic source and silvicultural treatment affect growth and shoot winter injury. Photo by Kendra Collins.

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A Legacy Tree

The Promise of a Hundred Future Forests

Photo by Paul Franklin. Inset photos by Lisa Thomson.

The American Chestnut Foundation's Legacy Tree orchard began in 2002 using 200 of Meadowview Research Farm's most blight-resistant chestnut seedlings. Legacy Trees are approximately 15/16 American chestnut and 1/16 Chinese chestnut. Each tree is sponsored by an individual(s) – either in his/her own name, the name of a company or organization, or in honor of a friend or relative – and the sponsorship is acknowledged by an individual plaque placed by the named tree.

The Foundation's Legacy Tree sponsors have made a significant contribution to help fund the scientific research of this foundation species. The Restoration Chestnut 1.0 seeds produced in the orchard contain the greatest level of American chestnut characteristics and the most blight-resistance to date.

Legacy Trees are truly a living legacy of TACF's scientists, staff, state chapters, and volunteers. This magnificent orchard is only the beginning of the Foundation's restoration efforts. Scientific rigor, diligent support, and ongoing patience will bring our breeding strategy to fruition over the next 100 years.

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Photo by Catherine Farist.

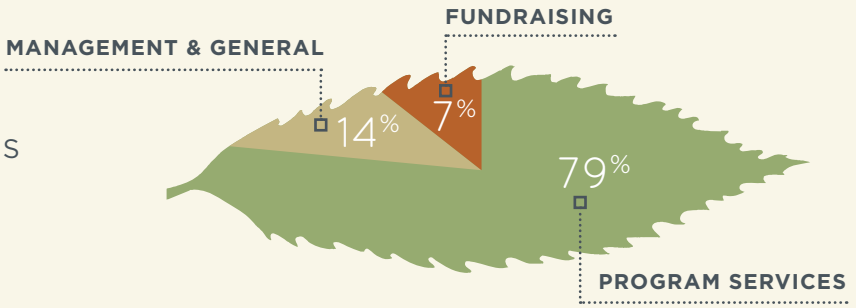
Financials

(July 1, 2014 – June 30, 2015)

STATEMENT OF ACTIVITIES AND CHANGES IN NET ASSETS

	Unrestricted	Permanently Restricted	Total
Public Support and Revenue			
Contributions and foundation grants	$1,983,835		$1,983,835
Federal grants	260,876		$260,876
Membership dues	337,997		$337,997
Investment income (loss)	885	(1,249)	$(364)
Merchandise sales (net of cost of $10,265)	13,646		$13,646
Donated services	263,500		$263,500
Other support and revenue	6,369		$6,369
Total Public Support and Revenue	2,867,108	-1,249	2,865,859
Expenses			
Program services	2,169,669		2,169,669
Management and general	392,782		392,782
Fundraising	183,419		183,419
Total Expenses	2,745,870		2,745,870
Change in Net Assets	121,238	(1,249)	119,989
Net Assets, beginning of year	5,012,213	25,966	5,038,179
Net Assets, end of year	$5,133,451	$24,717	$5,158,168

EXPENDITURES



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