



THE BUR

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Newsletter of the New York State Chapter of The American Chestnut Foundation

Spring 2020

Part IV: The Long and Difficult Road to the World's First Blight-Tolerant Chestnut

ESF / NY-TACF Partnership Produces Science Breakthroughs

By John Neumann, NY-TACF Secretary & Vice President for Education

In the first three parts of this article (appearing in the Fall 2018, Spring 2019, and Fall 2019 issues of this newsletter), we reviewed the history of why and how our chapter and the State University of New York College of Environmental Science and Forestry (ESF) American Chestnut Research and Restoration Project were formed, and the people who caused it to happen, along with a description of the research itself. Part IV (below) concludes this article.

From Darling 58 Success to the Present

At first glance, it may seem that once the success of the transgenic Darling 58 blight-tolerant American chestnut was announced, the research was completed and all efforts would immediately switch to restoration. Wrong. Research at the ESF American Chestnut Research and Restoration Project continues on several fronts, and in fact will need to continue during years of American chestnut restoration. Examples include the transformations done with American chestnut trees from southern states as well as transformations done with the oxalate oxidase (OxO) gene driven by a wound inducible promoter, a switch that turns the gene on only when the tree is wounded.

Importantly, the ESF chestnut team has expanded research related to gaining the required approval by three federal agencies, U.S. Department of Agriculture, Food and Drug Administration, and Environmental Protection Agency, so that public distribution of our transgenic materials (seedlings, pollen, and scions) may proceed. Andy Newhouse, who has been leading the

ESF effort to get non-regulatory status of the Darling 58, describes the process as “petition, revision, submission, permission?” Some of the research, such as with tadpoles, fungi, and bumble-bees, was reported in the Spring 2018 issue of *The Bur*.

Current members of the ESF American Chestnut Research and Restoration Project carry on traditions of determined science excellence and collaboration with NY-TACF that was instituted thirty years ago by Dr. Charles Maynard and Dr. William Powell. Present-day team members include Erik Carlson, Dakota Mathews, Linda McGuigan, Josh Mott, Andy Newhouse, Allison Oakes, Hannah Pilkey, Kristen Russell-Stewart, Sean Satchwell, and Jeff Zarnowski.

Special mention must be made about Linda McGuigan. She is not only a long time lab manager for the chestnut project, who advanced the science of transformations by assisting with the insertion of the OxO gene into the American chestnut, but also as a member of NY-TACF is the editor of *The Bur*, helps organize annual chapter meetings, and serves on our board of directors.

Looking to the Future

At our chapter’s last annual meeting, held at the ESF Heiberg Memorial Forest in Tully, NY, Dr. Bill Powell announced that ESF has designated approximately 8 acres to become the nation’s first American Chestnut Restoration Demonstration Forest. Best practices and ESF research related to American chestnut restoration will be primary objectives.

Continued on page 5



Darling 58 transgenic American chestnut

In this issue:

The Long Road (Part IV)	1 & 5
President’s Message	2
District Reports	3
ESF Chestnut \$3.2 M Grant	4
Reward Program	4
Matt Nichols Photo TACF Winner..	4
Darling 58 Petition	5
Annual Meeting Highlights	6
How Darling 58 was Developed ...	7



New York State Chapter
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Founded in 1990, the New York State Chapter (NY-TACF) is the oldest chapter of The American Chestnut Foundation, Inc., a non-profit 501(c)(3) membership organization. NY-TACF, in partnership with the State University of New York College of Environmental Science and Forestry, is working to restore the American chestnut tree to our eastern forests by developing truly blight-tolerant American chestnut trees through biotechnology. Membership information may be found on the back page of *The Bur*.

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Linda McGuigan – *The Bur* Editor

President’s Message



Looking back, 2019 was a great year for the New York chapter and 2020 looks to be just as exciting. First, I want to thank everyone who contributed to our fundraiser to finance an orchard/greenhouse manager at the State University of New York College of Environmental Science and Forestry (ESF). We needed \$50,000 to cover the 2019 expenses and so we set a goal of \$75,000. You far surpassed our expectations with the fund reaching over \$100,000 by the end of the year!!! In November, ESF asked if we could finance the orchard/greenhouse manager once again in 2020. We agreed to do it as we collected enough from our fundraiser to cover the cost for both years. Thank you again for your generosity and dedication to this historic restoration project!

2020 looks to be a very exciting and busy year. One question I constantly hear is “When will the blight-tolerant Darling 58 be approved for distribution?” I know it has been longer than anticipated, but not all has been standing still during this wait. ESF has been working to produce seedlings for distribution and has develop pollen collection and storage methods. Many of the blight-tolerant seedlings will be older and larger when approved for distribution. In conjunction with that, mother tree orchards that members have planted are more mature, many with female flowers ready for pollen from the blight-tolerant seedlings. Also, over 100 new members have requested nuts to start their own mother tree orchard. Part of the process for approval to distribute material from the Darling 58 tree will be a 60 day comment period by the public. We expect that could start anywhere from two weeks to two months from now and we encourage all members to comment on why they would like to have the blight-tolerant Darling 58 approved for distribution. We will be sending out information on when and how to make comments, when the comment period starts.

Allen Nichols
President, NY-TACF
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District Reports

Niko Nantsis, District 1

Hi everyone! I hope all of you enjoyed the holiday season. The past few months have been full of very exciting events! I recently showed one of the large American chestnut trees at Blydenburgh County Park to members of the Suffolk County Parks and Recreation Department and I have been working in tandem with them and Save The Great South Bay to start planting American chestnut seedlings from Long Island. Frank Piccinnini, one of the directors from Save The Great South Bay, and I are getting a proposal ready to submit to Suffolk County in order to plant more American Chestnut trees and do cruddy bark experiments on wild trees in Suffolk county. We are also expanding our tree searching expeditions to find more American chestnut trees. I hope to go to Shelter Island and see if any trees are still around and I plan to create a map of relative abundance of American Chestnut trees on Long Island. I am still woodworking as well and am in the works of creating new walking sticks. Can't to grow more Chestnut trees!

Dale Travis, District 2

We planted a mother tree orchard last year at the Queens Farm Museum and I planted another one in District 3 three years ago at the Harlem Valley Rail Trail. The NYC Parks department continues to expand its interest in chestnut restoration. We will have another lecture for prospective park rangers this month and they want to plant an orchard in Inwood Park in Manhattan, growing their own seedlings from our nuts. Urban forestry is real!

Allen Nichols, District 4

I gave several presentations locally and have a few more lined up for this spring. We've had a large increase in membership this January, with 42 new members, and I am expecting similar numbers for February. The big jump was related to two new articles recently published, one in the Woodstock Times, <https://hudsonvalleyone.com/2020/01/13/the-american-chestnut-can-bloom-again-with-your-help/> and the other by Quality Deer Management Association, which is a national publication. This has kept me extremely busy answering phone calls and emails. I have responded back with information on our restoration program as well as putting information into a spread sheet regarding nuts being sent and germination dates. I've had over 180 people requesting nuts, many of them being new members. It is very gratifying to see the excitement people have about our restoration program and their desire to participate and plant trees. Any member wanting to plant more nuts for mother trees, contact me to get on the spring list.

District 1 – Niko Nantsis, nikolaos.nantsis@gmail.com and Enrico Nardone, EGNardone@Seatuck.org

District 2 – Dale L. Travis, dale@daletravis.com

District 3 – Bill Munzer, billmunzer@gmail.com

District 4 – Allen Nichols, fajknichols.75@Gmail.com

District 5 – Emmett Hoops, emmett.hoops@gmail.com

District 6 – T. Urling Walker, watnrotary@gisco.net

District 7 – Roy Hopke, SnowHawke1@gmail.com

District 8 – Paul Ackerman, trapnman1@netzero.net

District 9 – William A. Snyder, wasnyderhort@gmail.com

Emmett Hoops, District 5

I've had twelve requests for seedlings, ten of whom thought the blight-tolerant American chestnuts were already available. After being informed of the status of chestnut restoration, only five are still interested. These five, however, are considering large plantings of several acres. Following my presentation before the Essex County Master Gardeners' meeting in October, I've been asked to follow up with a spring meeting of area farmers and those who may wish to plant chestnuts commercially. I've been asked to return to Paul Smith's College to speak to the American Forestry Association in early March. The 4H of Essex and of Clinton counties are preparing public education presentations about the chestnut for use at county fairs. I would like to spread this idea through other 4H organizations in District 5.

Roy Hopke, District 7

On March 3rd, I presented to the North Fenton United Methodist senior citizens, and on March 5th, I gave a presentation at the Sherburne plantation to students from the Invasive Course at SUNY Morrisville. I plan to do some planting at Sherburne orchard come spring.

Bill Snyder, District 9

I will be giving a talk on the American chestnut restoration to the Erie County Master Gardeners on April 7th.



Mark Your Calendars!



Pollination Workshop:

July 11, 2020 in Syracuse, NY

Learn to use blight-tolerant pollen developed at ESF to pollinate your Mother Trees. Class is limited to 20 people and preference is given to people who have not attended before. More information will be posted at www.acf.org/ny.



Annual NY-TACF meeting:

Oct. 17, 2020 in Batavia, NY

We have 15 rooms reserved for the fall meeting at the La Quinta in Batavia, NY. (<https://www.wyndhamhotels.com/laquinta/batavia-new-york/la-quinta-batavia/overview>). These rooms will be held until September 15, 2020. To reserve a room for \$94 plus tax, please call (585) 344-7000 and reference "The Chestnut Group" to receive your discount.

ESF's American Chestnut Research and Restoration Project Receives \$3.2 Million Gift from Templeton Foundation

ESF has received a \$3.2 million grant from the Templeton World Charity Foundation to support the American Chestnut Research and Restoration Project over the next three years. This grant will allow for the establishment of a new chestnut/oak/hickory demonstration forest, with paths and signage to facilitate public tours; the testing of two shelterwood restoration sites using the blight-tolerant American chestnut trees; and the expansion of current chestnut research plots to continually make improvements to American chestnut and other threatened tree species. There will also be agroforestry demonstrations with chestnut trees mixed with grape vines as well as American chestnut and American/European hybrid chestnut orchards.

In addition to the programs mentioned above, the gift will support the completion of the regulatory review (see page 5 for more information); establishment of production orchards for public distribution of the blight-tolerant trees; production of transgenic trees for use in larger-scale forest restoration; establishment of small educational plantings at botanical gardens, arboretums, parks, historical sites, and other public venues; and the start of distribution to the public.

The blight-tolerant American chestnuts developed at ESF can withstand the invasive blight that killed four to six billion of the economically and culturally important trees in the early 20th century. The oxalate oxidase gene from wheat allows the tree to detoxify the oxalic acid produced by the fungus. Lab tests have shown that subsequent generations of trees are also blight-tolerant and produce gluten-free chestnuts. Research shows the genetically engineered trees are not significantly different from wild-type American chestnut trees in regard to their effect on insects and wood frog tadpoles that feed on leaves, leaf decomposition, seed bank germination in leaf litter, or beneficial mycorrhizal fungi colonization of roots.

The Templeton World Charity funds scientific breakthroughs and development of practical tools relating to the search for meaning, purpose and truth. The charity serves as "a global philanthropic catalyst for discoveries relating to Big Questions of life and the universe, in areas of science, theology, philosophy, and human society." For the last several years, the chestnut project at ESF has been supported primarily by philanthropic funds.

For more information, go to www.esf.edu/chestnut

Large Chestnut in Cooperstown



Matt Nichols, nephew of New York chapter president, Allen Nichols, took this amazing drone photo of a flowering American chestnut last July (see photo above).

It won first place in TACF's 2019 Chestnut

Photo Contest. Congratulations Matt! This tree was one of three discovered while Matt and Allen were flying with NY-TACF member Jon Scott. After locating the tree from the air, Matt went back the next day and took pictures with his drone. Allen went back to that tree, measured it (photo on right) and another tree from the ground, took pictures of them, and entered them into the TreeSnap app (<https://treesnap.org/>). While looking for the second tree, which measured 14" DBH, Allen found six other trees in the area, with two of them producing catkins. However, he was not able to locate the third tree.



\$200 Reward for Largest American Chestnut Tree Reported in 2020

We are offering a \$200 reward for the largest American chestnut tree found each year. For 2019, the \$200 reward has not been finalized, as we are still waiting for verification of the two largest trees reported. One was submitted as 22" DBH and the other as 14" DBH, however, we are still waiting for a sample from the 22" DBH tree and verification of measurements. The tree that won the photo contest was measured as 15.6 DBH, although we did not enter it into the reward contest. If anyone owns an airplane, or knows a friend who does, we would love for you to locate any flowering trees. They were easy to see when we were flying around on July 13th in the Oneonta area last year, but that may vary by a week in other areas of the state.

The Long and Difficult Road

Continued from page 1



ESF's American Chestnut Research and Restoration Project team. Left to Right: Vern Coffey, Chuck Maynard, Kristen Russell, Bill Powell, Andrew Teller, Allison Oakes, Tyler Desmarais, Andy Newhouse, Dakota Matthews, Yoks Bathula, and Linda McGuigan

NY-TACF President Allen Nichols has announced a goal of having a chapter managed American chestnut orchard in each of our nine service districts. This would open opportunities for chapter members to volunteer “orchard work” closer to where they live. These chapter managed orchards would be on privately or publicly owned land, in cooperation with the owners.

Allen Nichols is promoting mother tree orchards of 4-8 mother trees with a Darling tree planted in the middle, when available. This will insure that the blight tolerant tree will have ample pollinators and the nuts will have plenty of genetic diversity, having been pollinated by multiple mother trees.

Meaning of the “Long and Difficult Road”

The story of the “long and difficult road” is the story of our New York State Chapter and the story of the ESF American Chestnut Research and Restoration Project. It is the story of their productive partnership leading to the world’s first blight-tolerant American chestnut trees. It is the story of cutting edge research in biotechnology pioneering in transgenic research and application. It is the story of both professional scientists and citizen scientists working toward a common goal. It is the story of planning, fund-raising, field work, lab work, and education. It is the story of our four dedicated chapter founders, who more than 30 years ago believed an alternative pathway to American chestnut restoration was needed. It is the story of these people, and those who came after, who so loved the American chestnut that they gladly dedicated themselves to its

restoration. It is the story of the restoration yet to come. The work is not finished.

The research means that the century old quest to find the way to restore the American chestnut has culminated in a science breakthrough of historic proportions. This has been achieved through a partnership of dedicated scientists and citizen volunteers who love the tree. Through dedicated pioneering research and the application of innovative biotechnology, a long and difficult quest has been achieved. A blight-tolerant American chestnut tree is now a reality through genetic science. The restoration of the American chestnut will now begin in earnest.

There is more to this story. The chestnut blight was discovered in New York City in 1904, and from that, the unstoppable blight and the death of American chestnut spread from New York State through the tree’s natural range. Now, more than a century later, a science-based proven way to restore the tree using ‘Darling’ transgenic American chestnut, may also spread from New York State to wherever people want to bring back this iconic tree.

The transgenic blight-tolerant American chestnut tree is a victory for dedicated scientists united with devoted citizens to achieve a noble goal over a long period of time, with some not living long enough to see that goal accomplished. As during the long and difficult research period, the restoration will depend on future generations of dedicated people who will love the American chestnut. The story continues...

Darling 58 Petition

The ESF Research and Restoration Project has filed a “Petition for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut (*Castanea dentata*)” with the USDA’s office of Animal and Plant Health Inspection Service (APHIS). This is one part of the U.S. government’s coordinated framework that also includes the EPA and the FDA. Approval of the petition would be a critical step toward planting this blight-tolerant transgenic tree in unrestricted areas as part of restoration programs.

Public comments play an important role in the review process of USDA-APHIS because they demonstrate broad public support for our efforts. We need your help to show support for this groundbreaking opportunity to save the American chestnut. Anyone can submit a comment. The case of the Darling 58 American chestnut is unique, as it will be the first time a petition has been evaluated for non-profit use in environmental restoration, so we think it is especially important that the USDA knows many members of the public want to plant these trees. In order to be the most persuasive, comments should be original and focus on the scientific arguments for accepting the petition, not a copied and pasted note or a form letter. Your comment can report on scientific evidence that supports the theory behind approving nonregulated status for the Darling 58 transgenic tree. Regardless of your scientific training, it is also helpful to tell your own story, your relationship to the American chestnut, and why you personally want to plant these trees.

The comment period will begin soon. When we have the date, we will post it along with where to submit the comments at www.acf.edu/ny or www.esf.edu/chestnut.

The review process usually takes 18 months for a new crop, however, the American chestnut is unique and may take longer. We appreciate your patients and support throughout this process!

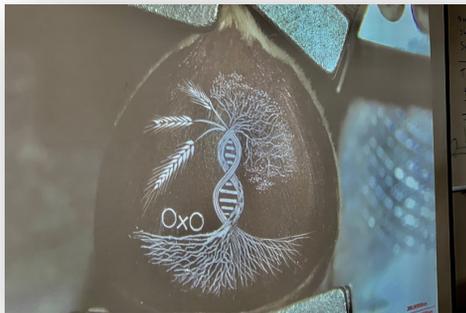
2019 Annual Meeting is a “Future Oriented” Success!



Our chapter’s 29th annual meeting was held in Tully, New York on October 25 & 26, beginning on Friday evening with our chestnut harvest exchange. Saturday’s



Sergey Jivetin etches an image relating to the American Chestnut Project onto an American chestnut.



A close-up image of the etching on the American chestnut.



A NY-TACF member wears an American chestnut T-shirt supporting the project.

program began at the ESF Heiberg Memorial Forest in Tully starting at 8 AM with meeting registration, coffee, tea, and donuts. Silent auction items were set out for display and attendees could also get tickets for the 50/50 drawing. The chestnut harvest exchange continued.

President Allen Nichols welcomed the attendees at 9 AM and then gave his president’s report. Next came our district directors reports. They spoke about local activities that involved chapter members, others who are working on a variety of local projects, the mother tree plantings, and also on future needs and plans.

At 9:45 AM the science reports by the ESF Chestnut Project staff and students began. First up was Dr. Allison Oakes, who spoke on her research to restore the American elm tree. Much of what was learned from the American Chestnut Project is helping this effort. Next, Jeff Zarnowski gave a report on his work at mother tree seed production orchards and the holding plots. Then Hannah Pilkey reported on her work with honey bees as possible pollen collectors for American chestnut breeding.

After a break, the science reports resumed. Eric Carlson reported on another method of OxO expression in transgenic American chestnut, driven by a wound inducible promotor from poplar trees. Andy Newhouse then reported on the ESF effort for non-regulated status of the transgenic Darling 58. He described the process as "Petition, Revision, Submission, Permission?" Stay tuned... Dr. Bill Powell finished the science reports with the announcement of planting the first American Chestnut Restoration Demonstration forest on ESF property in Syracuse and Tully, NY.

At noon, we took a break to have a group photo taken outside. We then enjoyed a very nice lunch catered by Cathy’s Corner Cafe. This was followed at 1 PM with an interesting demonstration of chestnut etching by artist Sergey Jivetin. Because the work was so small, a camera enlarged the image as it was being etched.

At 2 PM, President Allen Nichols opened the annual business meeting. Secretary John Neumann presented the minutes of the 2018 Annual Meeting. They were unanimously approved by the members as written. The annual financial report was presented by Treasurer Fran Nichols. The annual financial report was unanimously approved by the members. Nominating chair John Neumann, reported that two long time directors, Frank Munzer and Bethany Ruane are retiring after many years of faithful service. The other five of our current directors, who are completing their three year terms, are willing to serve again. They are Wayne Cooper, Tom Deacon, Alec Newlands, John Neumann, and Bill Snyder. Two new candidates for director were Tom Huff and Niko Nantsis. There were no nominations from the floor. All seven nominees were elected unanimously.

After the business meeting, winners to the silent auction and the 50/50 drawing were announced. Linda McGuigan reviewed the afternoon and evening activities: the tour of the ESF Demonstration Forest and the ESF Field Station, Apple Picking at Navarino Valley, and dinner at The Loft at Vesper Hills Golf Club. Chuck Maynard, co-director emeritus of the American Chestnut Research and Restoration Program, joined us for dinner. President Allen Nichols gave some closing remarks.

The American Chestnut Research and Restoration Project

From Seed to Seed: The Development of a Transgenic American Chestnut Tree

By Linda McGuigan, ESF Plant Tissue Culture Lab Manager

The process begins with immature nuts gathered approximately four weeks after pollination. Because of a low success rate, the more nuts collected, the better. The nuts are brought to the Plant Tissue Culture lab at ESF, soaked in 70% ethanol for 30 seconds, immersed in 50% bleach for 5 minutes, and finally rinsed with sterile distilled water three times. After that, the nuts are brought to a laminar flow hood, which is a cabinet designed to blow sterile air towards the front opening. The hood is equipped with a HEPA filter (think of a home air purifier), which stops microbes larger than 0.5 microns from entering the work area. In the hood, using a stereo microscope, the nuts are cut in half horizontally. The pointed half is carefully cut opened exposing 10 to 12 premature zygotic embryos (Fig 1). These embryos are placed on a nutrient rich medium and stored in the dark until new embryos grow. Unfortunately, most will either die, become covered in fungus or bacteria, or turn into callus (undifferentiated cells). Only approximately 1% of the embryos will multiply into more embryos. These new embryos are clonal and called somatic embryos because they formed from cells not normally involved in embryo development.

Once a somatic embryo cell line is developed it can be transformed with the gene of interest, in this case, the oxalate oxidase (OxO) gene. The transformation is done using *Agrobacterium*, a bacterium that is a natural genetic engineer and was involved in genetically modifying sweet potatoes over 8,000 years ago. The bacterium is grown in a liquid nutrient medium and then mixed with the American chestnut somatic embryos. After one hour of mixing, the embryos are transferred to a desiccation plate (Fig 2), which is a Petri dish containing a slightly moistened filter paper, and then placed in the dark for two days. Afterward, they are transferred to a semi-solid medium containing antibiotics, which will remove the *Agrobacterium*.

Two weeks later, they are transferred to a temporary immersion system that covers the tissue every four hours with a liquid medium containing antibiotics to get rid of cells that were not transformed. The embryos stay in the temporary immersion system for four to six weeks, with the medium being changed every two weeks. Surviving embryos that grow and multiply are considered individual events and are checked by Polymerase Chain Reaction to confirm that the new gene was incorporated into the chestnut's genome.

The next step is to regenerate the event(s) into shoots. This is done by transferring them to three different nutrient rich media during a three to six-month time period. Once a shoot emerges (Fig 3), it is multiplied and tested for relative copy number and expression levels. Only events with low copy numbers and medium to high expression levels will be kept. These events are multiplied in a semi-solid medium and then rooted *ex vitro* (out of culture). The rooted plantlets are placed in a growth chamber until new leaves emerge, then they are transferred to a greenhouse for two to three months, and finally are planted in the field.

It can take 3 to 4 years for field planted trees to produce pollen. To speed up the process, 2 to 3 month old plantlets from the greenhouse can be brought to a high light growth chamber instead of the field. In many cases, pollen is produced in less than one year. The pollen is collected and used to pollinate Mother Trees in the field. Nuts (Fig 4) are collected roughly eight weeks later, completing the cycle. They are tested for the OxO gene, which is expected to be inherited by approximately half the nuts. Those that test positive for the gene are planted in either pots or the field to get ready for distribution or testing.

The process takes a large number of people many years to complete. See page 1 for a list of the current team members.



For more information about the American Chestnut Research and Restoration Project, visit: www.esf.edu/chestnut

or join our Facebook group: www.facebook.com/groups/esfchestnut



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 50 North Merrimon Avenue, Suite 115, Asheville, NC 28804

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 A total of \$ _____ is enclosed.

All memberships to TACF include TACF publications, a car decal, membership to one of the state chapters as well as opportunities to participate in local chestnut activities. Visit www.acf.org or call (828) 281-0047 for more information.

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NY Chapter membership includes the Newsletter *The Bur*. The NY Chapter helps guide research at ESF and maintains plantings to keep the American Chestnut gene pool. TACF & NY-TACF are 501 (c) (3) non-profit organizations. Except for the membership services portion of your contribution (valued at \$15) your gift is tax deductible to the full extent allowed by law.