

The BUR
The New York State Chapter of the
American Chestnut Foundation Inc.
c/o Buffalo Museum of Science
1020 Humboldt Parkway
Buffalo, NY 14211

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**IF YOU HAVE FRIENDS WHO ARE INTERESTED IN OUR
GOAL OF RESTORING THE AMERICAN CHESTNUT,
PLEASE GIVE THEM THIS APPLICATION.**

Membership Application

Enclosed is my
membership support of:

- Gold leaf, \$1000
- Silver leaf, \$500
- Bronze leaf, \$250
- Green leaf, \$100
- Regular, \$40
- Student, \$15
- Other \$ _____
- Special Gift to NY
State Chapter \$ _____

Total Amount \$ _____

Enclosed is an additional contribution in the amount of
\$_____ in support of the New York State Chapters' activities.

Name: _____

Address: _____

City/State/Zip: _____

Telephone: E-mail: _____

This is a gift membership from: _____

Address: _____

Membership includes subscriptions to The Bark and Journal of the American Chestnut Foundation and enrollment in the New York State Chapter. The Chapter publishes the BUR, helps guide research at CESF, and includes nine Districts for local involvement in maintaining the American chestnut gene pool. Please make check payable to The American Chestnut Foundation, PO Box 4044, Bennington, VT 05201-4044. TACF is a 501(c) (3) non-profit organization. Except for the member services portion of your contribution (valued at \$15), your gift is tax deductible to the full extent allowed by law.

The American Chestnut Foundation, 469 Main Street, PO Box 4044, Bennington, Vermont 05201-4044

THE BUR



Newsletter of the New York State Chapter of the American Chestnut Foundation, Inc.

Volume 14, No.2

Winter 2004-2005

PRESIDENT'S MESSAGE

A milestone has been reached after fifteen years of research at the CESF Syracuse Laboratories, as there are now, actually growing in the laboratory, the very first transgenic embryos which will become seedlings to be planted out by the fall of 2005.

After fifteen long years of waiting we finally have something. Up to this point there has been much effort and hard work. We won't know what we have in the way of resistance to the blight until these plants can be grown big enough to test.

While we are waiting, the three gene construct can be initiated and seedlings grown for testing with hopes of even greater resistance to the blight in the near future.

The pollen transformation work can go into a larger test program in 2005, as the techniques for isolating the transformed pollen have been further improved. The June/July pollination time will be very busy, and hopefully a much larger number of transformed nuts can be collected this fall.

These are exciting times and the next two years should give us a much better look at what we have accomplished and what new challenges might show up. We should all be proud of the progress!

LONG LIVE THE AMERICAN CHESTNUT!

Herbert F. Darling, Jr., President

Many, Many Thanks to all the 2004 volunteers who did so much to make our Annual meeting a success!!

SCIENCE REPORT

RESEARCH BREAKTHROUGH!!

A research breakthrough was the highlight of the reports from the ESF team of Dr. Charles Maynard, Dr. William Powell, and Dr. Danilo Fernando at the 2004 TACFNY annual meeting. Thanks to a grant from Monsanto Corp., one of the world leaders in plant research, they were able to access increased approaches and expertise to bring about transformation of embryos that tested positive for containing blight resistant genes.

Transformed treelets are growing happily in the labs and will be placed in the greenhouse, and in the ground when the soil is accessible. They will be inoculated with blight spores later in the year to determine blight resistance.

The pollen research project conducted by Dr. Fernando was also reported successful in transforming pollen with blight resistance construct developed in Dr. Powell's lab that was also used for the embryos. Some American chestnut mother trees at the college orchard at Heiberg were pollinated with this transformed pollen. The progeny from these trees will also be tested later in the year for blight resistance.

Following is a summary of more detailed information written by the three principal researchers; Dr. Maynard, Dr. Powell, and Dr. Fernando.

We have spent many years putting together gene constructs and testing them in hybrid poplar and Arabidopsis. As we progressed, we have added new genes, new genetic control switches (promoters), and put them together in various combinations to find the best blend for the restoration of the American chestnut tree. Our most advanced gene construct to date is pVspB-OxO and it will be the first "prototype" American chestnut we produce. This construct will add only

three genes to the American chestnut genome. Although we do not know the actual number of genes in American chestnut, it likely falls between the smallest plant genome, Arabidopsis, which has 25,000 genes and a typical larger plant genome, rice, at around 55,000 genes. Therefore, if we estimate it is in the middle, let's say 40,000 genes, then by adding just three genes, we are only changing 0.0075% of the plant. But this small change is targeted at enhancing blight resistance and providing useful tools for the restoration program.

The three genes being added are: 1) a gene that will make an enzyme, oxalate oxidase (OxO), to degrade oxalic acid. Oxalic acid (or oxalate) is produced by the blight fungus, *Cryphonectria parasitica*, in large amounts, which reduces the pH at the margin of a canker to toxic levels, precipitates calcium, and interferes with the lignin formation. The OxO gene we are adding will remove the oxalic acid and therefore protect the tree. We added to this gene a control switch that will only allow the enzyme to be made in the vascular tissues (ie. in the stems) and it enhances the production when the stem is wounded. The oxalate oxidase gene comes from wheat and the control switch comes from soybean. 2) the second gene being added confers tolerance to an herbicide called "Finale." This gene is currently being used in several transgenic crops including corn, soybean, rice, cotton, and beets. This gene is necessary in the first steps of moving our gene constructs into chestnut because it allows us to select the cells that take in the gene construct from the majority that do not. This gene will also help later in the restoration programs for both weed control and for selecting the resistant seedlings that are produced after the crossing with "Mother trees" in the TACFNY orchards. 3) lastly, the

third gene produces a green fluorescent protein (GFP), which has been essential in developing the methods to put genes into the American chestnut. This gene is not luminescent, so we are not producing glowing plants. Instead it is fluorescent and gives off light much like clothing does under a blacklight. The light given off is also very dim and can be seen only under a microscope. This last gene will be in the "prototype" tree, but it might or might not be used in the final tree used for restoration. This will depend on public opinion and the regulatory process. We hope the oxalate gene will be all that is needed for sustainable blight resistance, but because of our research over the years, we have several other genes we can test alone or in combinations if we do not reach the levels of resistance expected. We are confident that a resistant American chestnut tree is on the way to reality.

Pollen Transformation

In the pollen transformation side, a strategy to collect pollen grains from male catkins of American chestnut ideal for invitro culture or germination studies was devised. Cutting mature catkins into segments and rolling them on a culture plate evenly dispersed large amounts of pollen with minimal unwanted accessory floral parts. To optimize pollen viability, the effect of various storage conditions on invitro germination was examined. This study was done last year and repeated again this summer with similar results.

To optimize transformation of American chestnut pollen through biolistics, the effects of parameters such as target distance, target pressure, and pollen developmental stage were established earlier. Statistical analysis showed that bombardment of ungerminated pollen at 1100 psi resulted in the highest percent transient GFP expression (4%). These parameters were used in generating transformed pollen using the pVspB-OxO construct that was developed in Powell's lab and similar results were obtained.

Pollination was done in the field using a combination of strategies, including: 1) use of transformed pollen, and 2) dipping developing American chestnut female flowers in *Agrobacterium* suspension. The *Agrobacterium* contains the pVspB-OxO construct that confers GFP expression and disease resistance. Some seeds were obtained using these

strategies. The seeds have been air-dried for a few days, packed in sawdust, and currently stored in the fridge. Germination test and assay for GFP expression will be done in early spring.

Tissue Culture

The gene transfer in tissue culture and plant regeneration phases of the project have also made great strides this year. A year ago, we were seeing lots of green dots, but they all insisted on either disappearing or growing into little green lumps of callus. By early spring, we had the transformation process well worked out. Nearly every time we did a transformation experiment we would see a carpet of GFP dots covering embryos. Most of the dots would disappear, but enough remained to regenerate dozens to hundreds of growing clumps of cells. Most of these were callus, but scattered among the calli were a few that appeared to be embryogenic. We carefully picked these out and some of these grew into stable, vigorously multiplying clumps of embryos. This was a major milestone in itself. However, it takes a trained eye (and a good microscope) to distinguish between "lumps of callus" and "clumps of embryos," so we very much wanted to push some of these little green clumps as far along as we could before the annual meeting. Linda Polin put some of these embryo clumps onto a fast track toward whole plants. Just two weeks before the annual meeting, Linda announced, "I've got SHOOTs!" As of late October, Linda has 8 GFP-positive shoots growing happily. She is now testing to see if the oxalate oxidase gene is being expressed in the correct tissues. The next step will be to regenerate whole plants from these shoots, put them into field trials and begin screening for blight resistance.

This breakthrough occurred because of the contributions of many graduated and postdoctoral students over more than a decade of painstaking effort. Dr. Zizhuo Xing first established chestnut somatic embryo cell lines in our lab nearly a decade ago. He was also the first to regenerate whole plants from chestnut embryos. Rosy Mukherjee was the first to see multiple transformation events in a single experiment. Sharon LaPierre dramatically improved the rooting and acclimatization process, giving us a clear path from shoots to whole plants

growing in the field. Linda Polin put it all together and was the first person to regenerate transgenic shoots. Bill Powell and Ron Rothrock have now also obtained transgenic shoots, confirming that the technique is repeatable.

The Support Team

Thanks to the support provided by AborGen LLC, The Monsanto Fund, NYS-DEC, and the New York Chapter of the American Chestnut Foundation, and previous funding from New York State's legislature, with special thanks to Sen. Mary Lou Rath, we have reached another major milestone on the path to blight resistant American chestnut.

Thank you all for your support,
Bill, Danny, and Chuck

MEMBERSHIP CAMPAIGN

In the next issue of *The Bur* we would love to print your name and the name(s) of the new member(s) you recruited.

Got an Idea?

If you have some other idea on how to get new members, try them out. If you find a method that works, please share the information with the campaign chair, John Neumann. Call him at 607-865-6497 nine a.m. to noon on weekdays, or anytime on weekends, or write to him at 7143 State Highway 206, Sidney Center, NY 13839-2105.

Thank You!

With your help, we can have a thousand member strong TACFNY. If the future American chestnut forests of our state were able to speak, they would thank you.

Additional Suggestion

In addition to telling your prospect about the free chestnut gift, reinforce this point by taping a piece of paper to the inside of the membership brochure with this information handwritten, typed or photocopied from below:

If you live in New York, and join TACF by April 2005, the Chapter will send you four (4) American chestnut seeds in April with planting and care instructions. FREE.

Fall 2004 offered a bumper crop of seeds.
No guarantee for next year. Join now!

DISTRICT NEWS

District 1 - By Lenny Lampel

Six viable nuts were harvested in Stony Brook and one in Rocky Point. The two trees were cross-pollinated in late July. From there, two of these nuts were planted in the Native America Preserve in Hauppauge. The preserve is owned and operated by former District 1 director, Dr. John Potente. Two nuts were planted in Sunken Meadow State Park in Kings Park. Two fifth grade classes from Cherokee Street Elementary School in Ronkonkoma witnessed the event. The Western Suffolk BOCES Outdoor and



Mud Packing with District 1 Members

Environmental Education Program coordinated the event.

Program of Alt. Comprehensive Education (P.A.C.E.) high school students planted the last three nuts at the Brookville Outdoor Education Center in Nassau County. Both the education center and the P.A.C.E. program are managed by Nassau County BOCES.

18 young chestnut trees were discovered in Blydenburgh County Park in Smithtown thanks to a lead from a former Long Island resident. Also in the area were several large, heavily blighted American chestnut trees that were recently killed. Many of the younger trees are already infected with the blight. **The public was invited to assist in mud packing some of the blighted chestnut trees. The three-hour event was titled *Return of the King - American Chestnut Field Work Day*. The event was publicized through the Seatuck Environmental Association and the Suffolk County Department of Parks, Recreation and Conservation.**

Nine healthy young American chestnut trees, ranging from 10 to 20 feet tall, were located on the wooded campus of Stony

Brook University. Two mature American chestnut trees on the property have been the primary source of viable nuts harvested on Long Island over the past several years.

District 2 – Margaret Collins

Plantings chestnut trees in Central Park, Fort Washington Park, and Inwood Hills Park in Manhattan; Alley Pond Park, Udall's Cove Upland Preserve, and Forest Park in Queens and Prospect Park in Brooklyn. Jean Paliswiat has indicated that the trees for Hich Rock Park in Staten Island were potted out, but will be planted this fall. Ann Wong of the Prospect Park Alliance and Maria Karpfinger the education coordinator for Pelham Bay and Van Cortlandt Park in the Bronx both held back about half of the plants and potted them up in hopes that they will be stronger in the spring (many of the plants had j rooting and although the trees that were planted mostly leafed, they are leafing unevenly.)

Many of the people involved in the Udall's Cove planting were involved in our first planting in April 1996, including Pat St. John (who was involved in a bad accident and now guards our trees, but can no longer plant them) and Bruce Stuart and his sons (who were part of the Cub Scout Troup then but now are grown men).

Sarah Fitzsimmons has put up a map of Central Park with 5 of our larger trees (we have now planted over 100 there) that you can view on http://chestnut.cas.psu.edu/maps/central_park_chestnuts.pdf Tim Wenskus of National Resources Group, an agency within NYC Parks has sent GPS locations for the trees in Pelham Bay Park, Fort Washington Park, Inwood Hill Park and Alley Pond Park.

Overall, it has been an excellent year for the NYC District. We have had several new parks contact us.

District 3 – Frank Munzer

In 1993, Bill White harvested American chestnut seeds from an old American chestnut tree growing along Sawmill Road in Cattaraugus County in the southern tier of New York from District 9. These seeds were given to me in Dutchess County, NY, where they were grown in the Institute of Ecosystems Greenhouse in Millbrook, NY.

The following year they were transplanted

to a protective cover in a fenced in "lath shed." These trees soon became fine specimen and when Margaret Collins from District 2 asked if we had any trees we could spare for the New York City District to put into Central Park, it was decided that these trees in the Lath House would be great there.

With the help of Rollands Nursery who volunteered to dig up the trees and ball them for transportation to the Big Apple and the help from the New York Park Department the trees were picked up, transported, and planted in Central Park's Great Hill where they are now flourishing. It is great to see what teamwork can do. Remember when you are next in NYC to visit the Great Hill in Central Park and check out the growth of these fine trees.

District 4 – Allen Nichols

I will be sending 1,000 nuts to Iceland this spring, for some more trial plantings. I sent about 35 to them last year.

I have purchased some grafting supplies and have been reading up on grafting on the internet. I plan to do some grafting into the top of some of my large nut producing trees to see how long it takes the scions to produce pollen and to also check my grafting success.

This is in anticipation of grafting blight resistant material into the top of my isolated mother trees as soon as it is available. I have about 15 mother trees that are isolated with no pollinators that I hope to pollinate with this procedure.

I also have a friend whom is working on finding a systemic fungicide for the Chestnut Blight. I hope to use this on these mother trees, so that if the grafting is successful I will be able to get resistant nuts from them for many years.

District 6 – Tom Walker, Retired

There is a need in District 6 for a new District Director.

District 8 – Roy Hopke

"Twenty Five Planters showed up at the District 7 plantation at the Rogers Environment center on April 3rd. Fortunately the weather was good and we were able to plant another 25 trees and put up another 30 wire deer protectors."

Every Member Gets A Member Campaign Launched at TACFNY Annual Meeting

If the American Chestnut Foundation New York State Chapter is to meet the challenges of the critical years that lie just ahead, we need a substantially larger membership base. What is at stake is our ability to accomplish our mission. So at our 2004 annual meeting we launched the biggest membership campaign in the history of the chapter.

Our Goal: to double our members in one year.

We Need YOU!

To reach our goal, we really do need YOU to recruit at least one new member between now and the annual meeting next fall.

To help each of our members to do this, a campaign kit was distributed to all who attended last fall's annual meeting and was mailed to every other member.

The kit includes a chapter DVD video *The American Chestnut – Biological Powerhouse*, five membership brochures, a letter from our president, Herb Darling, and a step-by-step guide.

If you did not get your campaign kit, please call the campaign's volunteer chair, John Neumann at 607-865-6497.

If you do not have a DVD player, but have a VCR player, call President Herb Darling at 716-632-1125 and he will send you a VHS copy of the video.

Incentive Awards

The chapter is offering these "Thank you" incentive awards during the period of this campaign. 1) Every member who recruits a new member for our chapter will receive a token memento; a razor blade-aided letter opener, dubbed the 'cutting edge,' symbolic of our cutting edge research programs. (It's yellow with green custom printing: "THE AMERICAN CHESTNUT FOUNDATION Help Restore the American Chestnut 802-447-0110" with Charlie Chestnut artwork and signature. (Please note that the campaign kit stated that the letter opener goes to the new member. This is an error. The letter opener goes to the recruiter. We do have

an incentive for the new member, which is explained later in this article.)

In addition to the letter opener, a member who gets a new member will have his or her name and the name of the new member listed in an issue of *The Bur*.

2) Every member who recruits five or more members will have his or her name in an issue of *The Bur* along with the names of the new members recruited. The member or director will also receive a TACF coffee mug in addition to the letter opener and a thank you letter.

3) Every member who recruits ten or more members will have his or her picture and name in an issue of *The Bur* along with the names of the new members recruited. The member will also receive a Chapter Achievement Award Certificate (presented by President Herb Darling at the 2005 annual meeting – or mailed if not present) along with a TACF cap in addition to the TACF coffee mug, the letter opener and a thank you letter.

You are one of 500 TACFNY members who have been asked to join this Every Member Gets a Member campaign. Our Board of Directors is very enthusiastic about this membership drive and has invested considerable time and funds to make it possible, but **we need YOU to help to make it happen. Imagine what a thousand member strong New York Chapter can do!**

New Member Incentive

In April 2005 the chapter will send a pack of four American chestnut seeds to each new member you have enrolled. Along with the chestnuts, we will send planting and care instructions.

Step by Step Guide to Get a Member

1) View the free DVD chapter video *The American Chestnut – Biological Powerhouse*. If you don't have a DVD player or DVD compatible computer, call 716-632-1135 to get a free VHS copy from the chapter or Call a friend with a DVD player and ask if you can come over to look at a 12 minute DVD you got in the mail from The American Chestnut Foundation. Set up a time when you can view the video together.

2) Neatly print "NY" and your name in the green border on the right edge of the application in each brochure. (This credits you for each member you get. Incentive awards are listed in the previous columns.)

ACTION

1) Invite a friend to view the video with you. (You will be more effective with one, or a couple, rather than a group.)

2) View the video with the friend or couple.

3) Discuss the video. Be sure to say that you are a member.

OPTIONAL: If you have American chestnut trees growing at home, show them. If you have seedlings growing by your window, show them. If you have chestnuts germinating in your refrigerator, show them. If you know of an American chestnut tree in a nearby forest, extend an invitation to go with you and take a look.

4) Offer the friend/couple a brochure. Say you would be proud for him/her/them to become a member(s) too. Point out the application. Explain that New York State residents who join The American Chestnut Foundation automatically become members of our chapter. Mention the special membership incentive from our chapter: All who join receive a gift of four American chestnut seeds with planting and care instructions. (They will be shipped in April 2005.) Be relaxed. Be yourself. Don't pressure.

Repeat steps 1 through 4 until all your brochures have been accepted.

"Strive For Five" Because some friends may accept the brochure but not send in a membership, if you want to insure getting five (or more) new members, call 802-447-0110 to get additional brochures.

Consider gift membership for grown children, siblings, or special friends.

Do It Now!

Don't put this important campaign off to when you "have more time." To overcome "procrastination disease" just do three things:

1) Think about your commitment to the American chestnut.

2) Commit yourself to getting at least one new member this month.

3) Follow the step-by-step guide.

2004 ANNUAL MEETING REPORT

The meeting room was bursting at its seams as members and guests browsed the exhibits and nibbled roasting chestnuts after registering. It was the 14th Annual Meeting of the NY Chapter of TACF held on October 16, 2004, at the Holiday Inn, Cooperstown Oneonta.

The reports from District Directors and committee chairmen telling about happenings of the year throughout the state began the meeting. On the financial side, New York members responded loyally to the campaign to help continue the ESF research program seeking blight resistance in the American chestnut. Funding was needed until grants lost due to 9/11 could be replaced. We were able to provide \$70,000 during 2004 to help maintain the program thanks to our members. In addition, a grant from Monsanto Corp. made it possible to access additional expertise and help, which accelerated the program. (see science report.)

Also fascinating was the historical lore given by featured speaker, Barry Keegan, Director of NYS Historical Society, on Native Americans and the American Chestnut tree. It was surprising to learn of the many aspects in which the tree was integral to their lives; food of course, utensils, shelter, equipment, even hunting – deer were more easily found when they came to eat the chestnuts.

The morning closed with “The Kick-Off.” The program said, “not a football game, not a deep sea dive – but A Challenging Project.” It was the launching of a major Membership Drive developed and chaired by board member, John Neumann. The goal is an ambitious 500 new members, and the theme is “Every Member Get A Member.” New members will receive 4 American chestnuts, sent to them in the spring for their personal planting. The member doing the recruiting will receive a thank you gift of a Charlie Chestnut envelope opener representing the cutting edge of the future restoration. (see membership article)

Our luncheon keynote speaker, John W. Dougherty, Regional Vice President and Director of International Projects, Monsanto Corp., Retired, focused on the impact and future promise of plant science biotechnology. Many examples were cited showing the resulting contributions to the provision of food for hungry populations. Enriched corn, rice, wheat and others in greater production have made a vast difference in many countries. He stated that the continuing biotechnology research is important and will give leverage

to the restoration of the American chestnut.

A field trip led by Host District 4 Al Nichols, took us to a local remaining American chestnut grove that provides many nuts for the Harvest Exchange. (Incidentally, the 2004 Harvest Exchange netted 9538 nuts dispersed throughout the state and other chapters’ planters.) It was also a Mycorrhizae Research site, and intern Kris Dulmer gave an interesting explanation of what was being done and how the research results hopefully will result in helping the American chestnut survive.

Dinnertime found us on a 1896 vintage train winding our way through the scenic, history-laden Susquehanna Valley. Our genial train crew intrigued us throughout the trip with ambiguous conversation challenging us to guess who was doing what to whom with what. Elizabeth White proved to be the most intuitive and won a prize. There was all this, a delicious down-home barbeque, and cheesecake too. The group’s enjoyment was topped off by the raffle being held in the train station when we got back. Generous members donated several items including hand made chestnut wood items, and the winners appreciated all prizes. Back at the Holiday Inn, member sharing continued, and continued, until who knows when.

An open board meeting was held on Sunday morning. Goals and budget items were set for 2005 and a long range planning session to determine strategy for future development in preparation for the restoration was set for June 2005. A resignation from T. Urling Walker, Director of District was regrettably accepted with many thanks for his service. Jack Mansfield announced his retirement as Editor of the BUR, and introduced Bethany White as the new Editor. Among other items, the board enthusiastically voted to accept the ESF invitation to hold the 2005 TACFNY Annual Meeting in Syracuse. Visits to the research labs and possibly a field trip to the orchards will be highlights.

The 14th Annual Meeting of TACFNY was adjourned at 11:30 AM.

**Annual Meeting 2005 Syracuse
here we come!
More details will come soon.
Put the date on your calendar
10/22/05.**

TACFNY 2005 OFFICERS AND DIRECTORS OFFICERS 2005

H. F. Darling, Jr., President
Stanley Wirsig, Vice President
Arlene Wirsig, Treasurer and Secretary
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Jim Donawick, John Ellis,
Adrien Gaudreau, Craig Hibben,
Ted Kozlowski, Leonard Lampel,
Robert Nowack, Dr. John Potente
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Margaret Collins, Wayne Cooper,
Thomas Deacon, Roy Hopke,
Chip Leavy, Frank Munzer,
Allen Nichols, John Neumann,
William Snyder, Bethany White
Dr. Richard Zander

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Douglas Campbell, Herbert F. Darling, Jr.
John Gordon, Jack Mansfield,
John Spagnoli, Arlene Wirsig,
Stanley Wirsig
NOMINATING COMMITTEE
Herbert F. Darling, Jr.
Jack Mansfield, Chairman



*Working as a Team Across Districts
with Sara Fitzsimmons*

District 9 – Bill Snyder

Hopefully members are looking for new members. A few events planned for 2005, include, Plantasia March 31-April 3, 2005 at the Agri-center, Hamburg, NY. Please call Bill Snyder if you could help man the booth at 716-839-5456 or 839-0096. This show is sponsored by the Reg 9 Nurserymen’s Association and will have over 12,000 attendees, people interested in plants. We will also hold our planting day April 30th at the William W. White chestnut Plantation at Zoar Valley. We will follow this with a hot dog roast that afternoon.

In August our region hopes to have a booth at the Erie County Fair, in the conservation building. We need a chairperson to sign up volunteers.