Marylnd Chapter of TACF
Winter Meeting at Fox Haven
Sunday, February 8, 2004

Forty members of the Maryland Chapter of The American Chestnut Foundation met on a beautiful afternoon to hear a University of Maryland scientist describe his research into genetic engineering of hypoviruses, to see Fox Haven Farms and hear about and discuss plans to establish a breeding orchard in partnership with the Maryland Chapter, and to discuss the upcoming chapter events for the remainder of the year.

Welcome

The meeting opened with a welcome from Harriett Crosby, owner of Fox Haven Farms, and an expression of her goals of creating an environmental learning center, developing a sustainable permaculture farm operation, and reforesting riparian buffers..

Introductions

Dr, Doug Boucher, the President of the Maryland Chapter then took the floor and introduced Marshal Case, the President and CEO of the national TACF organization, who had come from Vermont for this meeting. Then he introduced Brad Yohe, Science Coordinator for the Carroll County Public Schools, who was responsible for distributing 300 of the American chestnut seedlings from the Sugarloaf Mountain nursery to 11 middle and high schools in Carroll County. In describing their program, Dr. Boucher said that Mr. Yohe and his faculty have developed a curriculum starting with genetics in 8th grade and continuing through independent science research in 12th grade, using American chestnuts as a theme. He reported that Robert Foor-Hogue, head of the Science Research Center at South Carroll High School, has just received an award as the Outstanding Teacher Environmentalist from the Chesapeake Bay Trust.

Then, Dr. Boucher introduced the speaker, Dr. Donald L. Nuss from the Center for Biosystems Research at the University of Maryland. He explained that Dr. Nuss had authored a paper in 1992 that demonstrated that the effects of hypovirulence, observed in some blighted American chestnut trees, is actually caused by a virus that attacks the blight fungus.
Presentation

Dr. Nuss' topic was "Prospects for Engineering Hypoviruses for enhanced Biological Control of Chestnut Blight." He began by explaining that hypovirulence was first observed in Genoa, Italy, in 1951, and went on to say that since there was very low vegetative compatibility diversity in Europe, the naturally occurring virus was able to curtail the destruction of the European chestnut forests.

In order for the virus to infect the fungus, there must be a breakdown of the cell walls separating a fungal strain containing the virus and the fungal strain that receives the virus so that actual fusion of the two fungal strains occurs, and cytoplasm is exchanged between them.

This fusion can only take place between two fungal strains when they are of the same vegetative compatibility (VC) type. If they are of different VC types, then the cell walls do not break down but rather form a barrier to fusion.

There are six different genes in the fungus that determine the ability to fuse, and there are two alleles for each, so there are 2 to the 6th power possible vegetative compatibility types or 64 VC types.

In contrast to Europe, where there are few VC types present, in the United States there can be 40-50 different VC groups in a forest. In Europe, chestnut trees in orchards continue to be successfully

treated by annually introducing a mixture of viruses representing only five VC types. Additionally, natural hypovirulent strains spread unaided through the forests.

Dr. Nuss then described the process of treating an infected chestnut tree with viruses-infected fungal strains tailored to match the VC types present in the blight cankers on the tree. He first did this on his father-in-law's property in Pennsylvania, and the cankers healed.

A handout, "Outline for constructing hypovirulent C. parasitica strains for tailored biocontrol of chestnut blight on individual trees." described the steps to be followed.

Step 1. Recover C. parasitica from individual cankers.

On January 13 of this year, Dr. Nuss and his research assistants, Chris Root and Lynn Geletka, visited the property of Barbara Knapp in Germantown where the champion American chestnut in Montgomery County is located. With the help of a volunteer, Tommy Tamarkin, four samples were taken for each of six cankers identified on the champion tree.

Dr. Nuss showed pictures of Tommy gathering blight samples high on an extension ladder and above. He showed the bone biopsy tool used to collect four fungus samples from each canker, resulting in 24 samples stored in a standard 96-well plate.

Back at the laboratory, Dr. Nuss' team sterilized the bark plug samples and put them in a growing medium. Fortunately, the blight fungus, C. parasitica
grows faster than other fungi that are commonly present, so they can be isolated and transferred to another growing medium.

Step 2. Determine the number of VC types represented.

To do this, the isolated blight fungi from the same and from different cankers are paired to see whether they will fuse, indicating that both fungi in the pair are of the same VC type, or whether a dark line, or barrage, will form between the two paired samples, indicating that their VC types are incompatible.

He noted that it is not necessary to determine which of the 64 possible VC types are present. Rather, it is only necessary to determine how many different VC types are present and where on the tree.

In the 24 samples grown from the cankers on Barbara Knapp's tree, there are three different VC types. Of the six cankers on the champion tree, the highest one and the lowest one were each unique, whereas the four intermediate cankers contained fungus of the same VC type. Dr. Nuss passed around petri dishes showing the pairings of fungus samples, and it was easy to recognize those that were of the same VC type.

Step 3. Prepare spheroplasts from isolates of each VC type.

Enzymes are added to each isolated fungus to break down the cell walls so that spheroplasts can be isolated. This process takes a full day.

Step 4. Introduce hypovirus RNA into spheroplasts by transfection.

A portion of the hypovirus DNA is synthesized as an RNA strand and combined with the isolated spheroplasts. Then an electric current is applied briefly, and 90% of the spheroplasts are killed. However, the remaining 10% of the spheroplasts contain the virus in RNA form.

These surviving spheroplasts are then placed in a regeneration medium, and "as the cell walls regenerate, the introduced viral RNA initiates a replication cycle and begins to spread" through the regenerating fungus. The infected regenerated cells are then transferred from the edge of the regenerating colony to a favorable growing medium and tested.

These blight fungus cells are genetically identical to those cells in the isolated sample before their cell walls were broken down. Therefore, since there has been no genetic modification, no permissions are needed to introduce the infected virus into the wild.

In contrast, if the cDNA form of the virus were combined with the fungus isolates, the resulting infected cells would have been genetically modified by introducing the virus cDNA, and permission would have to be obtained from the US Department of Agriculture and from the Environmental Protection Agency in order to use this hypovirulent strain outside of a laboratory.

Step 5. Apply hypovirulent strains of the appropriate VC background to canker margins.

Returning to the tree from which the blight samples were taken, the research team will use a cork bore to remove a bark plug from each canker margin and insert a plug containing the matching tailored hypovirulent strain of fungus. The wound is covered with masking tape.

Dr. Nuss plans to return to treat the champion Montgomery County tree in April, before the leaves come out on the tree. Treatment will have to be repeated annually, but the prepared hypovirulent strain can be retained for repeated applications.

Limits to Spread of Hypovirulence

This labor-intensive treatment is feasible for specimen trees, but it would be desirable to have the infected blight fungus spread naturally. Dr. Nuss said that the problem is that the infected fungus acts to prevent the production of spores, so even though the virus infects the spores, there are few spores produced by the canker, once it is treated with a tailored hypovirulent strain. He said that there needs to be a balance between the virulence of the virus used to infect the fungus and the ability of the infected fungus to reproduce and colonize and spread to other sites.

Fox Haven Breeding Orchard Project

After an interesting question and discussion session with Dr. Nuss, Dr. Boucher turned the meeting over to Ms. Kathy Marmet, a Member of the Fox Haven Council, to describe the recently developed plans for a breeding orchard on the property.

In 2003, Fox Haven planted American chestnut nuts and also received 50 seedlings from the Sugarloaf Mountain nursery in November. These initial orchards will be augmented by adding a fenced breeding orchard of approximately 3 acres.

Fox Haven has signed a Germplasm Agreement with TACF so that nuts resulting from controlled pollination using backcross pollen, as well as backcross seedlings can both be accommodated on their property.

Kathy distributed a handout, presenting a budget of nearly $4,000 for fencing the orchard. Their plan

is to raise money this year and have the area ready for planting in the spring of 2005. Kathy introduced Sara Fitzsimmons, a TACF staff member at Penn State, who was present and had helped put together the budget.

Dr. Boucher led some discussion of the project and then the Chapter members voted to undertake the necessary fundraising and support. Kathy said that she and Harriett Crosby would personally match the first $500 gifts, and several people presented checks to the Chapter Treasurer, Jim Coleman.

Preview of 2004 MD Chapter Events

Dr. Boucher briefly described the plans for the coming year with our next event being the planting of 3,000 nuts in the Sugarloaf Mountain nursery, tentatively scheduled for Saturday, March 13.

Those trees that are mature enough in the TACF backcross orchard at ThorpeWood will be inoculated in early June. Sara Fitzsimmons will provide the inoculants and will help in the process.

Dr. Boucher said that we would pollinate selected Maryland trees with backcross pollen again this June, harvest and process nuts in October, and distribute seedlings from the Sugarloaf Mountain nursery in November.

Chapter Business

Dr. Boucher asked for volunteers to sign up for a workshop he would lead to teach people to distinguish American chestnut trees from other types. Especially with the extensive publicity we have recently gotten, there are numerous requests to identify trees as being American chestnut, and having several people who could make a first determination and take leaf and twig samples would save time for Dr. Boucher and TACF researchers at Meadowview who are asked to assess samples.

The need for a Maryland Chapter of TACF website was then discussed and an informal team of Kathy Marmet, Essie Burnworth, Robert Strasser, Stephen Dodge, and Doug Boucher volunteered.

Closing Remarks

Marshal Case came forward to congratulate the group and applaud our enthusiasm and efforts. He spoke briefly about a recent grant of $247,000 from the US Forest Service to be used in the southern Appalachians, and he encouraged us to maintain our momentum to keep the chapter growing.

Notes prepared by Essie Burnworth, February 12, 2004
Revised February 17, 2004 to reflect comments from
Dr. Nuss and Kathy Marmet