NE-1015 Technical Committee Meeting
Biological Improvement, Habitat Restoration, and Horticultural Development of Chestnut by Management of Populations, Pathogens and Pests

University of Missouri—Columbia, Missouri
September 12-13 2003

Attendance:

California:	Pam Kazmierczak (University of California, Davis)
Connecticut:	Sandra Anagnostakis, John Anderson-Administrative Advisor (Connecticut Agricultural Experiment Station)
Maryland:	Donald Nuss, Angus Dawe, Lynn Geletka, Chris Root (University of Maryland Biotechnology Institute, Center for Biosystems Research)
Michigan:	Dennis Fulbright (Michigan State University)
Missouri:	Michael Gold-NE-1015 Chair, Ken Hunt, Michele Warmund, Julie Rhoads, Johann Bruhn (University of Missouri Center for Agroforestry)
New Jersey:	Bradley Hillman (Rutgers University)
North Carolina:	Paul Sisco (The American Chestnut Foundation)
Pennsylvania:	John Carlson (Pennsylvania State University)
Tennessee:	Scott Schlarbaum (University of Tennessee), Hill Craddock, Mark and Steven Alexander (UT Chattanooga)
Virginia:	Fred Hebard (The American Chestnut Foundation)
West Virginia:	William MacDonald, Mark Double, Brian Bell and William Rittenour (West Virginia University)
Wisconsin:	Albert Ellingboe (University of Wisconsin)

The meeting was called to order by Chairman Gold at 8:00 am on September 12, 2003 at the Columbia Hawthorne Suites Hotel. Dr. Gene Garrett, Interim Director, Missouri University School of Natural Resources and Director, University of Missouri Center for Agroforestry, welcomed the group to Columbia. The Center for Agroforestry began in 1975 in an effort to integrate trees, crops and livestock on Missouri farms. Dr. Garrett was challenged by his director in the mid-1970s to travel the state and develop an applied forestry program to help Missouri citizens. He traveled for a year and decided that one approach to advance agriculture in Missouri was to put trees back on working farms. He focused on nut trees for unproductive farms. A partnership was developed using black walnuts and they also looked at pecans and oaks in an effort to answer the question, 'How do we maximize opportunities?' In 1992, Garrett became interested in chestnut and he put chestnut into some plantings in 1993, but he planted them in the wrong sites. He began reading publications by Sandra Anagnostakis and he invited Anagnostakis to the University of Missouri to give a seminar. As a result, Garrett became more convinced that chestnut should be included in the Agroforestry program. He sees chestnut on family farms in Missouri. Ken Hunt brought his knowledge of chestnut to the program in 1996. Missouri was fortunate to land a $2M grant on floodplain/Agroforestry initiative, and Garrett

hired Michael Gold to run the program. Gold was looking for a research effort and thought chestnut would fit into the Agroforestry initiative. With the help of Hunt and Michele Warmund, Gold has made great strides. Senator Kit Bond (R), senior Senator from Missouri, has been very instrumental in obtaining funding for chestnut; he has chestnut planted on his own property. Garrett encouraged members of NE-1015 to take a close look at the Missouri program as they are very receptive to comments and criticism.

OBJECTIVE 1. To evaluate and integrate multiple approaches for the biological control of the chestnut blight fungus and other pathogens and pests that threaten chestnut, by investigating host/pathogen/parasite relationships from the molecular to the ecological level.

Bradley Hillman, Rutgers University

Reoviruses. Ninety percent of the *C. parasitica* viruses found in nature are the hypovirus type, designated CHV. Reoviruses are very different from the CHV type. Two reoviruses of *C. parasitica,* C-18 and 9-B-2-1, were obtained from the West Virginia University group about ten years ago. While reoviruses are very important for RNA studies, Hillman is not proposing reoviruses as biological control agents.

Reovirus properties are as follows:

- 10-12 dsRNA segments.
- They are found across kingdoms.
- Terminal sequences are conserved.
- Particle is important for replication.
- There is no known nuclear component.

Reoviruses are fundamentally particle-associated viruses. There are double or triple structures containing the core, which is transcriptionally active. The covered core is not transcriptionally active, but it is infectious. Reoviruses contain exactly one segment of each of the 10-12 segments of dsRNA that constitute the viral genome, encapsidated in a single complex virus particle comprised of 6-8 proteins.

All eleven segments of C-18 and 9-B-2-1 are found in one particle. Each core is transcriptionally active and all the enzymes necessary are in the particle itself.

C-18 and 9-B-2-1

- Were isolated in West Virginia about 20 miles apart.
- 11 segments segregate in an all-or-none fashion.
- dsRNA segments of 9-B-2-1 do not cross hybridize.
- Low cross-hybridization with homologous segments of C-18.
- Different effects on virulence and phenotype.
- 9-B-2-1 virus causes more dramatic phenotype changes.
- 9-B-2-1 virus substantially reduces *C. parasitica* virulence; it doesn't grow at all in apples.

Hillman tried to isolate these viruses while working in Nobohiro Suzuki's laboratory in Okayama, Japan. He succeeded in producing a good band in a sucrose gradient and they looked like reovirus particles; they were 80 nanometers, had 11 segments of dsRNA and proteins.

9-B-2-1 genome characterization

- Strategy was to characterize the genome starting with the largest segment.
- Segment-specific cDNA libraries were made.

The largest three segments were homologous. Homology of 9-B-2-1 with other reoviruses (a hypovirulent isolate of *Rosellinia necatrix* and Colorado Tick fever virus) is as follows:

9-B-2-1	R. necatrix W370	CO Tick Fever Virus
1	1	1
2	2	2
3	3	3
4	4	4
5,6	5,6	—
7	—	—
8	—	—
9	11	—
10	—	—
11	—	—

— = blast searches that came up with nothing

Human implications may be to ticks or mites; there is a long, intimate association of mites and *C. parasitica*.

The largest three segments are somewhat understood. These segments contain viral polymerase, methyl transferase and a replication factor. The function of the other segments is still unknown. The complete sequence of 9-B-2-1 is 23,436 bp. Hillman showed relatedness of 9-B-2-1 in clades of reovirus, and it is most closely related to Coltivirus, specifically Colorado Tick Fever Virus. Segment six was related to the Reovirus in the hypovirulent isolate of *Rosellinia,* a fungus in the Xylariaceae family.

Is the virus infectious? Hillman took a sucrose fraction and added particles to protoplasts. He found sectors a few days after plating. The mycelium was thinner. These fungal viruses are difficult to infect from particles, although the technique works well enough to put particles into different backgrounds, Ep 155 for example. He found that 9-B-2-1 increases sporulation and upregulates cryparin production.

These viruses are transmitted via anastomosis (cytoplasmically) but they are not ecologically fit. The fungus may deal with this virus better than the traditional CHV.

There is no evidence of maternal inheritance in either reovirus. Hillman does not see any potential in biological control. He is investigating this on an ecological basis; how did it get into the fungus and what does it do with respect to upregulated products? It seems like an ecological aberration since it is found so rarely.

C-18

- New cDNA libraries made.
- Sequence is about 60% complete.
- Shares about 50% identity with 9-B-2-1.
- Terminal sequences are unknown.
- Particles are infectious.

Hillman is just beginning to look at C-18/9-B-2-1 co-infection. He noticed that C-18 has more aerial hyphae and is less stable than 9-B-2-1.

Mark Double, West Virginia University

West Salem update. Assessment of disease progress, spread of two hypoviruses and canker evaluation continues at the West Salem, WI site. Because of the ever-increasing task of sampling and assessment, twelve plots were established in 2001 in three areas of the stand: Disease Center; Disease Front; and, Beyond the Disease Front. In May 2002, cankers within the plots were sampled and subjectively rated. The bark samples removed from cankers were cultured and assessed for hypovirus content and vegetative compatibility (v-c) type.

Findings for 2002 include:

- Fifty-four percent of the 1330 cankers that exist in the 12 plots were sampled.
- CHV1-Euro 7 continues to be the most commonly identified hypovirus but CHV3- COLI still persists at the site.
- Hypovirus was associated with 87% of the cankers that received hypovirus treatment from 1992-1997.
- Eighty percent of non-treated cankers on trees with treated cankers have acquired hypovirus.
- Non-treated cankers on non-treated trees acquire hypovirus but at a greatly reduced rate.
- Subjective canker ratings show improvement in canker morphology in successive years; this is most notable for trees where hypoviruses were applied.
- Five-hundred, seventy-six isolates were v-c typed. Results were: WS-1 (Bockenhauer) 537; WS-2 (Schomberg), 2; WS-3 (Rhyme 133), 21; WS-4, 2; WS-8, 1; WS-10, 3; mixed infections, 6 and nonreactive, 4.
- To determine if cankers were infected by multiple v-c types, 80 cankers were selected for intensive v-c screening. Eight-to-ten virulent isolates from each canker were paired among themselves on Bromocresol Green Medium. A single v-c type was found in 74 cankers; 6 cankers yielded more than one v-c type per canker.

The CHV1-Euro 7 hypovirus was reintroduced in September 2003 in the eight plots that are located in the 'front' and 'beyond the front' areas. Two deployment methods were used. In one-half of the eight plots, cankers were treated by a combination of punch and scratch inoculations. Hypovirulent inoculum was introduced in a corresponding set of plots by inoculating scratch wounds that were made to healthy bark in an effort to create hypovirulent inoculum sources. One-third of the trees in each of the eight plots was left untreated to serve as trap trees to assess tree-to-tree spread.

Anastomosis/Transfection study. This study is being conducted by graduate student, Jenise Bauman. Transfection is an alternative laboratory method of hypovirus transmission that can be used to create hypovirulent strains. This technique involves the insertion of a synthetic hypovirus dsRNA into fungal spheroplasts by electroporation. The spheroplasts are then regenerated on specialized media and the successfully transfected colonies can be chosen based on phenotypic changes. A transfected strain has the same phenotypic traits as a strain infected by anastomosis. One benefit of this technique is that it overcomes the barriers imposed by vegetative incompatibility. Results from a preliminary study at West Virginia University, in cooperation with Don Nuss at the University of Maryland, indicated that isolates recovered from cankers initiated by CHV1-transfected isolates are significantly more likely to be hypovirulent than those arising from anastomosis. The objective of this research is to compare hypovirus infected isolates obtained via transfection and anastomosis. Six virulent isolates (Ep 155, Euro 7 ssv, Bockenhauer, Schomberg, WR2 and JR10) were infected with either of two CHV1

hypoviruses (Euro 7 and Ep 713) via transfection or anastomosis. A third unrelated hypovirus, CHV3-COLI from County Line, MI, inserted into each virulent isolate via anastomosis only, also was included. Three experiments are underway. The first will reconfirm the growth and sporulation of both isolate types that were tested previously, along with the third set of isolates containing the County Line hypovirus. The second study involves both laboratory and field tests to evaluate the production of hypovirulent inoculum. Conidia produced *in vitro* and *in vivo* will be recovered and single spored to determine the influence of the isolate and the method of hypovirus infection on the production of hypovirulent conidia. In a third experiment, transfected isolates will be evaluated to determine if they can transmit their hypoviruses to a greater number of vegetative compatibility types, in comparison with anatomosed isolates. Results gathered in these studies should provide data on whether transfected isolates are potentially better biological control agents.

Brian Bell, West Virginia University

Chestnut canker treatment procedures for hypovirus introduction. Hypoviruses were introduced into cankers via an array of treatment procedures to determine if techniques employed to treat cankers play a role in the success of biological control. One major barrier to hypovirus introduction is a system of vegetative-incompatibility in *C. parasitica*. This system restricts the transfer of nuclei and cytoplasmic elements during vegetative growth, and can preclude hypovirus transmission. To address the problem of canker treatment methods and vegetative-incompatibility, twelve hypovirus introduction methods were tested. Isolates that were vegetatively compatible or incompatible with the canker inciting strain were employed. Seventy-two trees were inoculated with an orange-pigmented virulent strain of *C. parasitica* (two inoculations per tree). After 11 weeks, the resulting cankers were treated with either a compatible or incompatible CHV1 hypovirulent *C. parasitica* isolate (brown-pigmented) using one of the following methods: 1) a non-invasive treatment where the inoculum was painted onto the canker surface; 2) an invasive treatment where the canker face was wounded with a sharp blade prior to the painting application of the hypoviruses; and, 3) a margin punch treatment where a series of wounds were made around the canker perimeter and filled with inoculum of the hypovirulent strain. Additionally, half the cankers in each treatment were covered with an absorbent pad to protect treatment inoculum from rain and other abiotic factors and to keep treatment inoculum moist. Twelve weeks after hypovirus introduction, twenty-four bark plugs were removed from each canker. Pigmentation and morphology of the isolates isolated from the bark plugs were used to determine if hypoviruses were transmitted to the virulent thallus. Successful transmission of hypoviruses was indicated when lightly pigmented orange isolates were recovered. Recovery of the treatment inoculum was indicated when brown-pigmented isolates were observed. When no hypovirus transmission occurred, orange virulent isolates were recovered. Preliminary results indicate that hypovirus transmission was best when cankers were treated with compatible hypovirulent inoculum. Among the compatible treatments, transmission was best for treatments that were scratched (45%) and least for treatments that were not wounded (25%). In contrast, hypovirus transmission was best for the incompatible treatments when cankers were punched (34%). Again, the least amount of hypovirus transmission occurred in the non-wounded treatments (8%). Covering the cankers had no effect on hypovirus transmission. Wounded cankers have reduced canker expansion in the compatible treatments. Canker expansion in incompatible treatments was relatively uniform. In compatible treatments, there is a positive correlation between canker expansion and orange-hypovirulent recovery. A

second set of samples was removed in late May and currently is being examined to determine if similar relationships are maintained. Asexual spore production also is being assessed to determine if any particular treatments favor production of hypovirulent inoculum.

Pam Kazmierczak, University of California, Davis

Foundation Plant Materials Service. Deborah Golino, Director of Foundation Plant Materials Service, has acquired the necessary permits for chestnut importation. There is still a 5-year quarantine for all chestnut material—held to check for gall wasp, etc. Seeds were collected from 22 cultivars in one U.C. Davis grove as the entire section of property utilized by FPMS is being turned into housing. These seeds will be moved to the USDA germplasm repository. Luciene Grunder donated 30 trees, including 'Colossal' and various Chinese seedlings. Rootstock for scionwood is maintained at the holding location at FPMS; cultivars also will be held at that site.

Intracellular processing and secretion of the fungal hydrophobin, cryparin. Their lab is looking at the biochemical and cellular-level to determine how the virus affects the fungus. The have found three genes that are down-regulated by the virus. Many of these down-regulated products are enclosed in vesicles. These products are: Cryparin, a cell wall hydrophobin, laccase and MF1-1 pheromone. Hydrophobins are ubiquitous, important in aerial mycelium production and very important for spore dispersal. They found that cryparin accumulates outside the cell wall in virulent isolates but in hypvovirulent isolates, cryparin accumulation is inside the cell; it cannot get out. Kazmierczak found cryparin on aerial hyphae and on the outside of fungal pustules. When cryparin was deleted (strain Δ119) pustules were unable to break the surface of the bark of American chestnut; there was no eruption of stromatal pustules. Secretion of cryparin is abundant (20-30% of mRNA is comprised of cryparin) and it is conducted via a Kex2 pathway. (*Note*: The Kex2 protein of the yeast *Saccharomyces cerevisiae* is a membrane- bound, Ca2(+)-dependent serine protease that cleaves the precursors of the mating pheromone alpha-factor and the M1 killer toxin at pairs of basic residues during their transport through the secretory pathway). The hypothesis is that CHV1 disrupts cryparin production by interfering with a Kex2 protein secretory pathway. To test this hypothesis, they created mutants of cryparin that have incorrect signals for signal peptidase and Kex2 recognition. They created GFP (green fluorescent protein) constructs of wild type strains to follow cellular disruption. A cryparin/GFP fusion vector was created to see where GFP is accumulating in the cell. The GFP is losing expression as *C. parasitica* does not hold GFP well. She has gone to liquid shake cultures, which are better than plates when viewing GFP. In her control, the expression of GFP was throughout the mycelium while the mutant expressed GFP only in the vesicles. They hypothesized that the virus affects the system such that accumulation should be in the hyphae. However, the buildup was only seen in the septa and cell wall; this was an unexpected result. The current hypothesis is:

- As CHV1 uses vesicles for regulation, the proteins are not excreted but build up inside the hyphae.
- Proteins build up and do not fold correctly, as with *Trichoderma* sp.

Conclusions:

- Kex2 processing is not necessary for secretion of cryparin.
- Mutating Kex2 processing site does not affect the wettability of the fungus or its ability to break through the bark.

- Cryparin/GFP fusion mutants appear to contain the fusion protein in discrete vesicles within the hyphae.

Angus Dawe, The University of Maryland, Center for Biosystems Research

His efforts involve sequencing efforts in *C. parasitica* using ESTs (expressed sequence tags). The procedure using ESTs is as follows:

- ESTs are derived from mRNA sequences and therefore represent only expressed genes.
- The library is stored as individual bacterial transformants.
- Plasmids are recovered, then the insert is sequenced once, from one end only (single-pass sequencing).
- Sequence data is collected and analyzed.

Why use ESTs?

- Only about 30 different *C. parasitica* genes are in Genebank as of May 2001.
- It is easier to generate than genomic data (smaller DNA fragments).
- They can be used to generate spotted cDNA arrays.
- The disadvantages are, there is no genomic data, redundant sequencing, highly expressed genes are found more often.

The sequencing took place from May 2001 to September 2002. The analysis occurred between October 2001 and February 2003. The procedure was as follows:

- mRNA was isolated and cDNA libraries were made.
- Individual bacterial colonies were generated.
- Cataloged and stored in 96-well microtiter plates.
- "Miniprep" to recover plasmids containing cDNA sequences.
- Sent to sequencing facility.
- BLAST (Basic Local Alignment Search Tool) to compare sequence to data from other organisms.
- Using Linux PC running stand-alone BLAST package, download the entire non-redundant protein database from National Center for Biotechnology Information.
- Compare DNA sequences to all know and predicted protein sequences.

Once the data is put together, putative functions can be assigned. Dawe can take the information of all ESTs and put them into categories of functions or biological properties. To date, Dawe has produced the following:

Minipreps	5000
cDNA sequences returned	4448
Useful sequences	4217
# individual gene products represented	2200
Total base pairs sequenced	2, 703, 587

Microarray Spotting. Todd Allen developed the microarray/spotting. Microarrays are glass slides with 10,080 spots of DNA within a 20 x 56 mm area. The spot spacing is 300 μm and the spot diameter is 100 μm. Allen has spotted 3,864 *C. parasitica* clones. After RNA samples are obtained, fluorescently labeled probes are prepared (i.e. Ep 155 is labeled with green fluorescence and Ep 155 (CHV1-Ep713) is labeled red). Samples are mixed, hybridized, washed and spotted. The data is then analyzed to identify differently expressed genes.

Controls and Validation

- Control spots on chip (glass slide)

- Viral coding regions
- Genes encoding ribosomal proteins
- Non *C. parasitica* sequences

- Multiple RNA preps, reciprocal labeling (dye swap)
- Different expressed clones must be changed in 4 of 6 or 3 of 4 independent hybridizations
- Differently expression verified for a subset of putatively changed sequences using "real time" PCR.

Interpretation

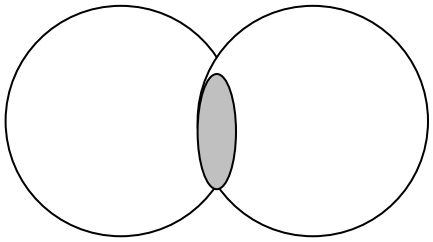
- Gridding (*Spotfinder*)
- Normalization (*MIDAS*)
- Identification of differentially expressed clones
- Association of chip locations to clone ID and BLAST data (*Excel*)

What do we do with a Cryphonectria chip?

Ask the following questions:

—How does infection of Ep 155 with CHV1-Ep 713 hypovirus alter the trascriptional profile? (answer: a lot—295 of 2200 genes (13%) are altered at the transcriptional level; 132 were up-regulated and 163 down-regulated with PCR validity of 93% accuracy)

—How do these changes differ when compared to infection with the milder CHV1-Euro 7 isolate?



713 vs 155
(295 genes)

Euro 7 vs 155
(166 genes)

Overlap (80 genes)

In the overlap, 73 of 80 genes changed in the same direction. Generally, greater magnitude of changed in 713 than in Euro 7.

—Are specific regions (determinants) of viral ORFs responsible for a specific subset of changes?

—Do any of these changes correlate with those seen in G-protein signaling mutants that have certain phenotypic traits in common with hypovirus-infected cultures (e.g. lack of pigment, sporulation, reduced virulence)?

Summary

- The EST library has greatly increased the sequence information available for *C. parasitica.*
- Microarrays provide a useful insight into how the host cell is reprogrammed by the hypovirus.
- 4200 genes = 2200 clones
- Add microarrays = vast amount of data
- We are probably missing 75% of the changes.

Don Nuss, The University of Maryland, Center for Biosystems Research

Nuss planted American chestnut trees in 1987 in northwestern PA, near Meadville. Chestnut blight was first noticed in 2002. Some Chinese chestnuts are interplanted. Nuss sampled the cankers and transfected the isolate with Ep 713. The transfected isolate was used to treat the cankers and he is convinced hypovirulence works because the cankers were shut down.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

Transgenic strains. Anagnostakis reported on the status of the transgenic release in the Meshomesic State Forest, begun in 1997. This study consisted of two plots—one was sprayed with water and the other with transgenic conidia 10^{10}-10^{12} spores/ml). All stems over 1" dbh were measured. A sum of all measurements totaled 47" in the water plot and 84" in the treated plot. She noted many swollen cankers in the treated plot. No samples were taken and she has no plans for further transgenic introduction.

John Carlson, Pennsylvania State University

Silviculture studies. Carlson presented data through 2002 since survey in 2003 has not been conducted. One silviculture study examines the suitability of a range of native forest sites for reforestation of American chestnut in two studies, initiated in 2001 and 2002. Direct seed studies are being conducted at seven sites across central Pennsylvania. The sites vary in soil type, elevation, aspect, and competing ground vegetation. All sites were established in fenced areas that were cleared before planting. Fifty seeds were planted at each site, except one and seed protectors were used to discourage herbivory. Competition at the sites was similar at the start of the two trials. After two years, growth in the 2001 trial was unacceptable at all sites, often due to competition from hay-scented fern and/or deer browse. These trials will be monitored for five years or until they succumb to chestnut blight.

F_1 families for genetic linkage mapping. Crosses were made in an attempt to develop full-sib families for genetic linkage mapping. A large, wild American chestnut from Black Moshannon State Forest was pollinated by *C. mollissima* cv. Mahogany. Seeds from this tree will be collected in October 2003.

Genomic DNA level selection in backcross generations. The goal of this project is to develop an approach based on total genomic DNA to screen backcross generations for individuals with the least amount of *C. mollissima* genetic material. The two approaches are GISH (genomic *in situ* hybridization) and dot blot. GISH examines chromosomes from chestnut roots under the microscope after hybridization with a DNA probe that targets Chinese chestnut DNA. A reliable protocol is still being worked out. In addition to GISH, a colleague at Texas A&M (M. Narul Islam-Faridi) performed FISH, a more general test using fluorescent hybridization. Islam-Faridi experimented with 18S rDNA and 5S rDNA probes. In the dot blot method, a small amount of leaf DNA is isolated. Aliquots of total DNA are fixed to filter membranes as spots. Spots are hybridized with total DNA genomic probes from each parent using ^{32}P. These are then exposed to X-ray film. To be sure if he had enough signal, he combined material form six individuals; he will go back and refine with lesser individuals. Are there elements of DNA that can be picked up?

Library for economic and evolutionary important plants. A library is being created for 100 economically and evolutionarily important plants. The genomic sizes of some North American Fagaceae species are included since none of the Fagaceae have been sized. This work is being done in collaboration with Virginia Mason Institute in Tacoma, WA. Carlson asked

Fred Hebard and Scott Schlarbaum to send chestnut samples. Some genomic sizes are as follows:

Species	Genome Size mbp/1C	DNA content pg/2C
Castanea dentata	805	1.67 ± 0.022
Castanea mollissima	794	1.65 ± 0.015
Castanea sativa	776	1.61 ± 0.013
Castanea crenata	795	1.65 ± 0.016
Castanea seguinii	756	1.57 ± 0.009
Quercus alba	766	1.59 ± 0.070
Quercus rubra	762	1.58 ± 0.050
Quercus veluntina	564	1.17 ± 0.005
Quercus macrocarpa	646	1.34 ± 0.030
Quercus nigra	641	1.33 ± 0.030

Floral genome project. The Fagaceae are not included in this project, but Carlson hopes that data from this project will be applicable to other areas. The goals of this project are to:

- learn about the genes involved in floral meristem development to get an idea of what the original progenitor of flowering plants looked like.
- learn more about the origin, conservation and diversification of the general architecture of flowers.
- collect over 100,000 ESTs from early flower development from 15 major branches of flowering plants.
- identify genes expressed.
- identify the site and timing of gene expression for key regulators.

<u>OBJECTIVE 2.</u> To improve chestnut trees for reestablishment in forest ecosystems, and chestnut cultivars for nut production by selection, breeding and marketing, and determine the cultural criteria of all chestnuts for successful production of nurseries, orchards and/or natural settings.

Dennis Fulbright, Michigan State University

Pollination studies. He has been learning how to establish chestnut orchards in Michigan. He is directing growers away from planing non-grafted seedlings, unless they are planning on grafting. Cultivars offer:

- More production/tree
- Earlier production
- Better nut quality
- Predictable yields

So far however, cultivar-based orchards do not yield as well as trials would suggest. This may be due to a lack of pollen. To date, there is no suggestion of pollen incompatibility.

In a 40-tree trial in Michigan, twenty trees have some production with 12-15 trees producing an average of 10 pound/tree after ten years. Cultivar trials with 'Colossal' suggest

that a tree should produce 10 pounds of nuts after six years. 'Colossal' is the most readily available cultivar in the U.S.; other cultivars are not nearly as available.

Cultivars in a 1995-established variety trial had the following yields in 2003:

'Colossal'	35 pounds	'Orrin'	12 pounds
'Dunstan'	25 pounds	'Mossbarger'	5 pounds
'Revival'	25 pounds	'Willamette'	5 pounds
'Eaton'	15 pounds		

Cultivar orchards are not giving yields like this for growers. 'Colossal' has the flowering potential but the following are still concerns:

- It is not being pollinated.
- Pollinizers winter-kill easier.
- Poor weather at the time of pollination.
- Amount of pollen is limited in young orchards.

For the above reasons, orchard growers are losing the early advantage over seedling orchards. Seedlings grow well but there is no guarantee that they will pollinate.

Fulbright discussed hazelnut cross-compatibility. The compatibility and timing issues are very complex in hazelnut. He doesn't see similarities in chestnut with the hazelnut but he feels that timing is the critical issue.

'Colossal' is male sterile and does not self-pollinate, hence a pollinator variety must be included in any 'Colossal' orchard. In a pollination study conducted in 2001, a 'Colossal' orchard (plated 25 feet apart) was interplanted with 'Nevada' as the pollinizer. Data indicated that 'Colossal' trees closest to 'Nevada' produced the highest yield. A chestnut trial on the East Lansing campus produced similar results.

There are many more burrs available than are being pollinized. We need to know more about pollen, starting with the time of pollination. If we are to find new and better pollinizers, then we need to know when the trees are receptive. To study this, a trial was conducted with the earliest know pollen producer, 'Okei'. 'Colossal' trees were planted at varying distances (25' 45' and 70') from one 'Okei'. Even the trees at 25' were not pollinated well. The timing was correct, but the pollen did not move well.

He showed slides of a pollination trial in which some stigma were pollinated while stigma were removed from other flowers. In the pollinated flowers, he noticed dark brown area; he questioned if those areas represented fungi following the pollen tube.

Peeling study. Using the peeling machine, purchased through Federal funds, Fulbright presented data on the percent pellicle removed.

Test	Variety	% Pellicle Peeled	Average
1	Chinese	81-86%	84%
2	'Colossal'	22-57%	44%
3	'Colossal'	20-80%	54%
4	'Colossal'	61-96%	80%

In the peeling studies, successful pellicle removal depends on:

- Variety
- Growing environment
- Maturity of nut
- Moisture and temperature management after harvest
- Temperature of nut entering peeler

Conditions in the peeler can be adjusted to improve peeling. Those include the temperature of the oven and the residence time of the nuts in the oven.

Fred Hebard, The American Chestnut Foundation

A short review of the backcross breeding method was given.

Parents	Offspring
American x Chinese	F_1
F_1 x F_1	F_2
F_2 x F_2	F_3
F_1 x American	B_1
B_1 x American	B_2
B_2 x American	B_3
B_3 x American	B_4
B_1 x B_1	B_1F_2
B_1F_2 x B_1F_2	B_1F_3
B_2 x B_2	B_2F_2
B_2F_2 x B_2F_2	B_2F_3
B_3 x B_3	B_3F_2
B_3F_2 x B_3F_2	B_3F_3

More than one American parent is necessary for the backcross method and more than one chestnut line is necessary. There are extensive plantings in seven states (ME, MA, PA, IN, KY, NC and TN), with more than 20,000 trees planted by 2003. The PA state chapter has planted B_3F_2s. Hebard wants to know what the crossing pattern is when the B_3 level is reached. How many F_2s should be selected? A B_3F_2 family size needs to be 9-10 to capture most of the alleles. Added observations:

- In relation to self-incompatibility, there may be some genetic variability in the population that allows this phenomenon to occur rarely.
- One can select more than one individual per B_3 family without increasing in-breeding under open population.
- Hebard noted that on his trip to Italy in October 2002, that there are many trees in Italy that never died from chestnut blight. There is blight in many coppice stands.
- With regard to naturally occurring hypovirulence, there are longer periods of survival in clearcuts associated with the release of young sprouts from competition. Clearcuts can be detrimental to survival of chestnut on mesic sites.
- Is resistance holding up in the B_3 population? Hebard tested B_2F_2 from open pollination and several had good levels of resistance. This is evidence that resistance is holding up.
- Al Ellingboe believes that there are more than 2-3 genes responsible for resistance.

Paul Sisco, The American Chestnut Foundation

Sisco feels now is the time to put in an application for serious money. We have political support. TACF just received a grant based mostly on two letters—one from former President Jimmy Carter. Sisco believes that there are people who can be tapped for major donations. This group should think seriously about serious money. Ellingboe stated that the complexity of the group will aid in generating money

Southern breeding program. Sisco gave an update on what is going on with TACF. There are 20,000 trees planted at Meadowview covering more than 70 acres. Expanding plantings throughout the region will help with: (1) genetic diversity and (2) interest by more people. Sisco showed a map of eastern U.S. orchards, mother trees, etc. There are a number of people/institutions that are TACF cooperators. There is a huge amount of activity in PA; they have, in total, almost as many trees planted in PA as in Meadowview. MA and ME also have many trees planted while TN has a program of finding large, surviving American chestnut trees. TACF's objective is to take B_2s from Meadowview, cross with local mother trees and then outplant the nuts.

Molecular markers. A large number of easily and cheaply-scored markers would allow us to:

- Eliminate blight-susceptible trees at the seedling stage.
- Select against Chinese genes other than disease resistance.
- Identify all resistance genes present in Chinese, Japanese and American chestnut.
- Detect and eliminate outcrossing due to pollen contribution.
- Fingerprint the parents in our seed orchards for plant patent protection.

There are several types of markers:

- **RAPD.** This was the first type of marker used. This method looks for random 10 bp primers. The way DNA works, there will be enough amplification that takes place to get a gel with sufficient bands to follow the pedigree. It is a dominant system; you either have it or not. It allows you to follow one allele, but not the other. It offers good, cheap information.
- **RFLP.** This method is more complicated. Certain enzymes recognize certain sequences. This method cuts DNA from Chinese and American chestnut and offers different number of bands. The method is good because you can see both alleles. It is, however, expensive and requires a lot of DNA.
- **AFLP.** This method has precise sequences that take certain DNA sequences that are ligated to a primer. The reaction can be run in both directions and gives vast number of bands. A great deal of information can be gained from this technique.
- **SSR.** The simple sequence repeat method is difficult to develop. You must know your sequence DNA well and you need to know the precise sequence on both sides of the ssr. This technique is cheap to run and give much information on that locus. Sisco had six original alleles in one of their studies and they were able to pick out all six alleles using ssr.

Sisco is trying to get a good genetic map on chestnut. An article published by Tom Kubisiak in 1997 (Phytopathology) had 102 individuals. Chestnut has 12 chromosome pairs. They found 12 linkage groups and it was hoped that each linkage group corresponded to a chromosome. In further mapping studies, some data indicated that linkage groups B and E were on the same chromosome, leaving only 11 chromosomes. At the same time as American chestnut was being investigated, Europeans were examining *Castanea sativa*. They found 12 linkage group in both European chestnut and European oak (*Quercus robur*).

CHROMOSOME	**Linkage Group *C. sativa***	**Linkage *Group Q. robur***
A	1	2
B		

C	8	?
D	10	10
F	7	8
G	3	11
H	6	1
I	5	?
J	12	?
K	2	4
L	9	9

Conclusions:

- Everything linked in European chestnut linked to American chestnut.
- Could not get a common marker for B or E
- For B, there is a marker that is polymorphic in European and *Q. robur*, but it is not in *C. dentata*. Hopefully, we will figure out B and E.
- Do not have *C. sativa* linkage group 4 or 11.
- For resistance loci, it may be on chromosome B or F. There may be more possibilities of resistance loci if they have more trees mapped. Sisco wants to look at chromosomes under microscope with fluorescent markers but he needs very large markers.

ArboGen is a spin-off company funded by MeadWestvaco and International Pulp and Paper to research genetic engineering for improved design and growth of elite tree varieties. ArboGen is looking at genetic transformants with chestnut. Within the realm of forest products, there is debate on genetic engineering; it is difficult for some individuals to make the leap to genetic engineering because they get good results from traditional breeding methods. ArboGen approached TACF to look at transforming chestnut; they granted $50,000 to Scott Merkle (University of Georgia) and William Powell (SUNY, Syracuse ESF). Both researchers are trying to regenerate trees with antifungal properties. It has been easy to use reconstructs from yellow poplar, but much more difficult with chestnut.

Scott Schlarbaum, University of Tennessee

UT Tree Improvement Program—Castanea research. Schlarbaum was looking at Ozark chinquapin (*Castanea ozarkensis*) in the Ouachita National Forest (partly in AR and OK). He found some very large trees, one with a canker. Schlarbaum sent a sample to Sandra Anagnostakis who found dsRNA.. There is natural regeneration of chinquapin occurring in the Ouachita forest. Schlarbaum collected seed from 17 open-pollinated trees (2,00 to 2,500 nuts) and sent them to the Flint River Nursery (GA) for Paul Kormanik, USDA-FS. The chinkapins were planted in 700' beds. Slides were shown of seedlings in June 2003 after planting in December 2002. Some areas of the nursery had too much water; this led to *Phytophthora* problems. He expects most chinkapins to be 5' to 7' tall in October 2003. Flint River also had *Phytophthora* problems in their chestnut beds.

Field Plantings of American Chestnut. Field plots are located in:

- Berea College Forest, KY
- Daniel Boone National Forest
- PA State forests (four locations)
- Mammouth Cave National Park

Berea College Forest planting was established in 2000 on two slopes in an incomplete block design. He planted 367 seedlings; their initial height was 102 cm. This site was heavy clay with no percolation. At the end of the 2000 season, there was 48% mortality. There were 174 trees alive with an average height of 127 cm (25 cm average growth). These trees compete with yellow poplar on good sites in southern states. At the end of the 2002 season, mortality was 59% with only 72 seedlings remaining (average height of 187 cm). The tallest tree was 3 meters. Some trees were lost to ambrosia beetles at this site.

All trees were lost in the Daniel Boone planting. Trees planted in the PA forest plots were 100% American chestnut. These plots were established in 2000 at four locations. The seedlings were protected with tree tubes or fencing to protect from deer browse. Survival is greater than 85%. There is no *Phytophthora* problems on any of the four PA locations.

Mammoth Cave National Park planting was established in 2000 with genetic families of American chestnut from Fred Hebard. About 450 seedlings were planted. There was some *Phytophthora* mortality and he encountered fescue problems.

Fading Forests II. Faith Thompson Campbell and Schlarbaum wrote this document for Congress. It is titled, "Trading Away North America's Natural Heritage". CD copies of the document were given to participants.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

Nutrition, site and genotype versus tree growth. Seeds collected in 1997 were planted, dug, root-pruned, measured and planted at three sites (forest/ridge; forest/level; old field). The seed represented four families:

BC_2 (Japanese) x American, Watertown
BC_2 (Japanese) x American, Roxbury
BC_3 (Chinese) x American, Watertown
BC_3 (Japanese) x American, Roxbury

The height of each seedling is being measured every year. Plot 1 is a forest ridgetop with very thin soil and a pH of 3.9. Plot 2 is a forest clearcut with a little better soil and a pH of 4.5 while Plot 3 is a field previously planted to tobacco; it has a much higher pH, 5.5. No soil supplements were added. The average growth is similar among all plots. Anagnostakis analyzed soil minerals. To collect soil, an "X" was made across each plot and soil samples were taken over the entire plot. Plot 3 (old agricultural field) had very little soil nitrogen. However, when leaves were analyzed, there is plenty of leaf nitrogen; it may be coming from mycorrhizae although she has not yet investigated mycorrhizae. Other minerals were much the same but there is more aluminum in the forest than in the agricultural field.

Anagnostakis commented that Japanese backcross trees have grown better than Chinese backcross. Chinese hybrids are all male sterile while Japanese hybrids are all male fertile.

Soil calcium. A Portuguese woman reported that European chestnut trees with high calcium have less chestnut blight. Anagnostakis tested calcium in the three field plots and in the greenhouse. She found much more calcium in the agricultural field than in the forest; there was also more calcium in the leaves. In the greenhouse, she planted seedlings in fiberglass cubes and watered with either normal nutrients or with high calcium. Growth was not as good with high calcium. The seedlings were inoculated with the WK isolate (less pathogenic than Ep 155). In general, the trees watered with high levels of calcium were more susceptible to blight than the ones watered with normal nutrients. Anagnostakis concluded that calcium does not offer any

protection and the Portuguese researcher was incorrect, at least in terms of American chestnuts, under Connecticut conditions.

Nut grafting. Anagnostakis started using this technique several years ago since traditional grafting in the field is very difficult. She did nut grafting in the spring, with the following procedure:

- A fat nut was used.
- The tip was cut off with a razor blade.
- A slice was made through the cotyledon and radical.
- Scion wood was sharpened to a point.
- The scion was pushed into the radical.
- Pro-mix was used for potting.
- Nuts formed roots.
- The scion grafted itself to the roots produced by the nut.
- After they have grown a while, as much of the nut as possible was removed; an attempt was made to leave as many roots as possible.

Anagnostakis is worried about overwintering. The grafts will be mulched to assist in cold protection. After that, care is very critical. Hill Craddock stated that many nut grafts just sit for 3 years and languish for several years after that. He indicated that Jaynes called this technique "nurse tree cutting". Scion cambium and petiole form the graft union. Roots are adventitious from callus. These types of roots are very weak and thus difficult to establish.

Anagnostakis informed the group of a new publication by the Northern Nut Growers Association, *A Guide to Nut Tree Culture in North America*, edited by Dennis Fulbright.

Hill Craddock, University of Tennessee Chattanooga

Craddock showed an entirely pistillate catkin from a tree in the northern Alps, as he has an interest in such phenomena.

Chattanooga chestnut project. The productivity of Craddock's program has increased greatly as a result of the Chattanooga chestnut project. A grafting workshop brought people from all over TN, GA and NC. Chestnut is rare in western TN. GIS mapping has been conducted to correlate soil typing; a direct consequence of this mapping was abundant data on flowering American chestnut.

Breeding update. Craddock began his breeding program in 1997. He has made some crosses. One of his Chinese chestnut parents was a TVA tree (Friendship Forest). Unfortunately, the crosses are not blight resistant; they have just begun screening this year. In screening his backcross progeny, he has noticed a root rot problem. He has not identified *Phytophthora*, but he continues to lose trees every year. Craddock has gone to dry pollen for breeding; he is using glass vials to store pollen. A population of fifty B_2F_2 is being screened via inoculation with Ep 155 and isogenic Ep 155 (Ep 713) and Ep 155 (Euro 7). These trees, planted in 1996-1997 were from Meadowview.

Biological control. In order to control chestnut blight on useful trees, Craddock has collected bark samples, obtained pure cultures of *C. parasitica* and then sent them to Sandra Anagnostakis for conversion. Anagnostakis has sent 3-4 isolates containing French and Italian dsRNA. A former student, Pearl Hwang, converted some local isolates for field inoculations and she used a few isolates in a slurry mixture. Craddock removes the face of a canker prior to treatment. He noted that some trees that were blighted in 1997 are still living and available for

breeding. He does not conduct vegetative compatibility testing; he uses blind inoculations. He also has inoculated uninfected trees as a prophylactic dose. Often, Craddock uses hypovirulent isolates from both Anagnostakis and Hwang.

Host pathogen-interaction study. In order to study host-pathogen interaction, Craddock needed a clonal orchard. In his first attempt, he had two tree plots containing B_2F_2, American controls and Chinese controls. He realized that two plots were not sufficient, so he planted 400 more trees this year and 400 more planned for 2004.

Silviculture notes. Craddock noticed that chestnuts require light. He removes the overstory from mid-story plots. From now on, his plantings will be in forest clearcuts. Craddock has a 4-acre site at Dollywood. He is trying to revegetate the site with chestnut trees; chestnut is growing well there.

Cultivar test. Craddock has a cultivar test in northern Hamilton County. He will plant chestnuts on 10-acres of land. In designing the trial, he went to commercial nurseries in search of 20 cultivars. He found commercial sources for about half of the cultivars. He will plant 4 complete blocks, using a 20 x 20 design (5' x 10' spacing). There will be five tree plots with 20 cultivars x 20 trees of each cultivar. Craddock is trying to replicate Ken Hunt's cultivar planting and the trees were planted with an auger. Craddock noted that survival of container-grown stock after one year was far better than bare root stock. This orchard will be plumbed and will contain a well house to pump water. Craddock received a matching grant to put in the irrigation system.

Pollination study. Christina Bock conducted a pollen study and found that most of the pollen goes straight onto the forest floor. Some pollen was found 50' to 300' from the source but that was not common. The take-home message—the closer the pollen source, the better.

Architypal tree type. Craddock showed slides of some very large chestnut trees in Italy. He then talked about the importance of the book written by J. Russell Smith. In the early 1900s, Smith studied and taught economics at the University of Pennsylvania and its Wharton School of Business (1903–1919), and then moved on to Columbia University's Business School where he chaired the school's economic geography program, a field that he largely initiated, defined, and developed. He remained at Columbia until 1944, publishing several books on economic geography. *Tree Crops: A Permanent Agriculture* by J. Russell Smith is the classic work on tree crops. First published in 1929, this blueprint for the development of high-yield tree crops proves that vast, untapped food sources can be harvested from common species of North American trees. Smith's philosophy is based on the idea that agriculture must be "adapted to physical conditions," that "farming should fit the land." He observed worldwide the catastrophe of hill agriculture whose one-time cycle he described so accurately as "forest -- field -- plow -- desert."

Smith described Corsica and its tree development. Craddock noted that there are grafted chestnut cultivars in almost all of Corsica and chestnut is truly a cradle-to-grave species for the people in Corsica, as it gave food and shelter to early Italians. In fact, during the Roman Empire, tributes were paid to Caesar with chestnuts. Despite two global wars, the depression and chestnut blight, there are some large chestnuts. Craddock has seen superficial cankers in Corsica. The Italians make a commercial hypovirulent paste used in treating chestnut cankers. The application is via gouge wounds in the canker face. Five different vegetative compatibility groups are included in the paste. Once cankers begin to heal, the dead wood is pruned out and the healing cankers are left. There are enormous orchards that are recuperating in Italy and these orchards are all grafted chestnut. These orchards are treated with the help of local co-ops.

Arborists are trained in blight canker distinction and they leave healing cankers. Some orchards have been continuously harvested since Roman times. These same orchards have also been used for Bolete production. Large European chestnuts have a tourism appeal. They are a very valuable part of the landscape. 'Bouche de Betizec' is Europe's #1 cultivar.

Steven Alexander, University of Tennessee Chattanooga

Alexander is studying host-pathogen interaction with a segregated population of B_2F_2 hybrids of *C. dentata* and other chestnut species. He is looking at inoculations with virulent and hypovirulent-containing strains. He observed that biological control works some times and not others. He want to know why. His hypothesis is that host resistance is a significant factor. His prediction is that some trees in a B_2F_2 population will have good biological control and others will not.

Alexander screened his B_2F_2 population with Ep 155, and isogenic lines of Ep 155 containing CHV1-Ep 713 or CHV1-Euro 7. In addition to the B_2F_2 population, he also inoculated *C. dentata*, *C. sativa* and *C. pumila*. Ep 155 was inoculated 20 cm from the ground. Ep 155 (CHV1-Euro 7) was inoculated 15 cm above the Ep 155 inoculation and Ep 155 (CHV1-Ep 713) was inoculated 15 cm above the Euro 7 inoculation site. The trees were inoculate on June 2-3, measured on June 23-24, July 29-30 and September 4-5. Since the trees are valuable, the Ep 155 cankers were treated on September 5 in an attempt to produce a converted canker and stop canker expansion. Alexander used the cork borer method of application. He had problems measuring the cankers because some cankers produced very corky bark. The Ep 155 and Euro 7 cankers were much deeper and had more necrosis while the Ep 713 cankers were much more superficial.

As predicted, the B_2F_2 population had a variation in susceptibility. The cankers on *C. denata* and *C. sativa* performed as predicted; Ep 155 produced the largest cankers followed by Euro 7 and Ep 713. Cankers on *C. mollissima* had smaller cankers overall. Alexander's conclusions were as follows:

- B_2F_2 population did segregate.
- *C. sativa* was not more resistant than *C. dentata* or *C. pumila*.
- *C. mollissima* was highly resistant.
- Ep 155 and Euro 7 appeared similar after 3 months, therefore Euro 7 may be used effectively for resistance screening.

Ken Hunt, University of Missouri Center for Agroforestry

It is hoped that chestnut can be a good orchard crop in Missouri. To test this, he is conducting cultivar selection trials. The objective is to evaluate and characterize available chestnut cultivars for marketable traits. Study #1 is the chestnut repository, planted in 1996. It will test: cold hardiness; nut quality characteristics; phenology; and, growth habit, precocity and resistance to diseases and insects. A replicated chestnut cultivar trial was established in 1999. Five trees of each cultivar have been planted on sites similar to peach plantings, hilltop sites that are well-drained. Trees in this study include:

- NC-8 (European x Chinese) from Doug Campbell
- 'Willamette', Dunstan hybrid from Bob Wallace

- ‘Revival’, Dunstan hybrid from Bob Wallace
- ‘Colossal’
- ‘OK Kwang’, Chinese hybrid
- ‘Auburn Homestead’, Chinese hybrid
- ‘Easton’, Chinese hybrid from Greg Miller
- ‘Mossbarger’, Chinese hybrid from Kentucky
- ‘Amy’, Chinese hybrid from Greg Miller
- ‘Sleeping Giant’, a Chinese x Japanese x American hybrid
- ‘Peach’, Chinese hybrid from Greg Miller
- “Qing’, a discovery from western KY with high quality fruit

These trees were planted on a 27’ x 27’ spacing.

One of the goals of this planting is technology transfer, so new orchardists can be given a framework for decision making when establishing new orchards.

A nut production orchard was established in the fall of 2001. The goal is one ton/acre. Three cultivars, ‘Peach’, ‘Qing’ and’ Willamette’ were planted on a 27’ x 13.5’ spacing. ‘Qing’ has a shiny, mahogany color. The nuts are medium size, store well and they have an excellent, sweet flavor. ‘Willamette’ is a Dunstant/Chinese American hybrid. It has large nuts and upright branching habit and it has mid-season to late maturity. ‘Peach’ is a cultivar from Greg Miller. These nuts are medium size. The tree has an upright branching pattern with mid-season maturity. There are two pruning types planned for the orchard—peach-style and hedgerow (open center). Pruning will be performed on an annual basis. The goal is to maximize the sunlight into the tree canopy. This will take annual training and pruning. The orchard has been pruned to 5’ for sweeping in the fall. This will help with deer control as well. Roundup herbicide is sprayed 3-4 times per year. The trees have been staked since they are hard pruning. The trees put out 4-5’ growth this summer. The central leader will be dehorned after several years to open up the interior of the crown.

‘Colossal’ is male sterile. Other trees in the Missouri repository are male sterile but they produce plenty of nuts.

Michele Warmund, University of Missouri Center for Agroforestry

She is concerned that some of the more precocious trees are still producing catkins in late summer. She is concerned since this is wasted energy by the tree. This energy, if controlled, would be better spent on fruit production. She is studying the effects of second flush flowering to:

- Identify cultivars that produce secondary burrs
- Determine effects of secondary burr removal on nut weight
- Determine the effects of thinning

To accomplish the above, she has three treatments:

- Removal of the secondary burrs
- Leaving secondary burrs intact
- Tagging the terminals with secondary catkins (but no secondary burrs), and tagging the terminals without secondary catkins and identify cultivars that produce secondary burrs

She may use NAA for chemical drop.

Michael Gold, University of Missouri Center for Agroforestry

The establishment of a viable chestnut industry depends on the following factors:

- Consumer market development
- Nutritional information and recipes
- Increased production of chestnut acreage to meet demand
- Use of grafted chestnut cultivars
- This should have consistent yields, have well-characterized fruit quality and good agronomic traits
- Establish and adhere to quality standards
- Have adequate prices for the grower.

The market "pull" strategy is to get more people to want chestnuts. Consumer market development efforts at the University of Missouri Center for Agroforestry include: (1) a chestnut roast to familiarize the public with chestnuts, and (2) work with local chefs. The Center for Agroforestry is also looking at every possible niche for chestnuts. For example, what do growers do with smaller chestnuts; value will be added to their product if they can find a market for chestnuts too small to sell to the public. He showed examples from a local grower's market—gourmet pepper jelly with pecans and black walnuts. He wants the manufacturer to try chestnuts. Gold also had chestnut honey; Europeans use chestnut honey as a spice.

The Food and Drug Administration came out with data in 2003 that nuts contain healthy fat.

Business Meeting

John Anderson, Administrative Advisor, thanked Michael Gold and Julie Rhoads for hosting the meeting. Many members of this group have been with NE-140 a long time; there are field workers, geneticists, molecular biologists and breeders, all with a passion for reinstating American chestnut in the ecosystem. He indicated that this group is comprised of a good mix of institutions and researchers. Our Federal representative is Robert Noweirski, National Program Leader-Biobased Pest Management, USDA-CSREES-PAS. Noweirski had intentions of attending the meeting, but he did not have sufficient funds.

Anderson indicated that, as a group, this project has wonderful minutes. The annual report has to be sent to Anderson within 60 days of the meeting. These are then sent to the Northeast Executive Director.

Bradley Hillman nominated John Carlson as chair-elect. Sandra Anagnostakis seconded the motion. This position filled a vacancy created when Chuck Rhoades, formerly of the University of Kentucky, left for a Forest Service position in Colorado. Pam Kazmierczak nominated Alice Churchill as secretary. Sandra Anagnostakis seconded the motion. Both nominations were approved unanimously. John Carlson will host the 2004 meeting in Pennsylvania, either at State College or at the Mount Alto station at a date of his choosing in September 2004.

The meeting was adjourned at 6:00 pm on September 13, 2003.

Respectfully submitted,

Mark Double
October 2003