NE-140 Technical Committee Meeting
Biological Improvement of Chestnut (*Castanea* spp.) and Management of Pests

Incarnation Center, Ivoryton, CT
October 18-20, 2001

Attendance:

Alabama:	James Maddox (Tennessee Valley Authority)
California:	Pam Kazmierczak, NE-140 Chair-Elect (University of California-Davis)
Connecticut:	Sandra Anagnostakis, NE-140 Chair, Pam Sletten, John Anderson, NE-140 Administrative Advisor (Connecticut Agricultural Experiment Station), Phillip Gordon (NY Botanical Garden)
Kentucky:	Chuck Rhoades (University of Kentucky)
Maryland:	Donald Nuss, Lynn Geletka, Chris Root (University of Maryland Biotechnology Institute)
Massachusetts:	Terry Tattar, Timothy McKechnie (University of Massachusetts)
Michigan:	Dennis Fulbright, Andy Jarosz, Carmen Medina-Mora (Michigan State University)
Missouri:	Michael Gold, NE-140 Secretary (University of Missouri, Center for Agroforestry)
New Jersey:	Bradley Hillman (Rutgers University)
New York:	William Powell (SUNY-ESF), Steven Jakobi (Alfred State University)
Ontario:	Colin McKeen (Canadian Chestnut Council), Adam Dale (University of Guelph)
Pennsylvania:	John Carlson (Penn State University)
Virginia:	Fred Hebard (The American Chestnut Foundation), Clarissa Balbalian (Virginia Department of Agriculture)
West Virginia:	William MacDonald, Mark Double (West Virginia University)

An evening gathering with an Elderhostel group was held at the Incarnation Center on October 17. The meeting was called to order at 9:00 am on October 18, 2001 by Chairman Anagnostakis. Objective 2 was discussed prior to Objective 1.

Objective 2: To better understand the interactions and ecology of the host/pathogen/parasite system at the molecular, organismal and environmental levels in order to develop effective biological controls for chestnut blight.

Colin McKeen (Canadian Chestnut Council)

McKeen is interested in hypovirulence and how it is operating in Ontario. He raised the question, "Can it restore American chestnut in southern Ontario?" The original host range of *Castanea dentata* extended northward into Ontario; a band of clay soil kept

chestnut from expanding north and east. This clay soil relegated chestnut to an area in Ontario just north of Lake Erie.

McKeen has found eight chestnut sites in Ontario and all sites contain dsRNA with the exception of the Arner site. He knew of the Arner tree as far back as 1966; it was 13" dbh at that point. He was in Ottawa for many years and did not return to the Arner site until 1983 when he noticed the Arner tree had many infections. He guessed that the tree first became infected around 1975. Despite infections, this tree continued to grow and he isolated from cankers on this tree. Although the isolates from this tree did not contain dsRNA, he inoculated other trees with the Arner isolate. He showed slides of moderate canker control after inoculation with the Arner isolate.

A slide of the largest American chestnut in Ontario was shown to the group. Unfortunately, this tree died in 2000, possibly from ink disease (*Phytophthora infestans*).

McKeen reported that Greg Boyland (University of Guelph) found several cankers from southern Ontario that contain the same type of hypovirulence GH2 (MI), but he was not able to use these isolates to convert virulent types. Boyland convinced McKeen to get into a breeding program and they interested Adam Dale to enter into the program as he is a small-fruits breeder at the University of Guelph. (Dale's report is listed in these minutes).

The status of hypovirulence in Ontario is such that it does not prolong the life of chestnut trees. He believes that hypovirulence has the capability for success, but the right hv strain must be used at the appropriate time and often enough for control to be realized.

Dennis Fulbright, Michigan State University

Genetic elements in the mitochondria of C. parasitica strains. Work has focused on mitochondrial hypovirulence involving mutations of the mictochondrial DNA (mtDNA), because it is seen frequently. It's role, however, is still uncertain. There is no dsRNA and the hypovirulent phenotype is maternally inherited. Strains have been categorized based on the following:

- Reduced sporulation with hyphal growth and abnormal colony morphology
- Attenuated virulence
- Alterations on mitochondrial DNA
- Induced alternative oxidase activity
- Cytoplasmically transmissible elements
- Some elements are maternally inherited

These mitochondrial mutations include deletions or insertions (InC9) or the presence of mitochondrial genetic elements such as plasmids (pCRY1).

Mitochondrial DNA

	Characteristics	***Effects***
•*C. parasitica* mutants	alternative oxidase pathway cytochrome deficiencies abnormal mtDNA	reduced growth abnormal growth reduced conidia
•Insert InC9	973 base pair sequence within s-r DNA gene incompatible strains	reduced sporulation enhanced senescence

•plasmid pCRY1	4234 base pair within microchondria incompatible strains	attenuated virulence

Fulbright looked at McKeen's Arner isolate and found nothing abnormal with the mito.

Bark pathogenicity tests. Cultures can be severely debilitated by the insert, InC9 and by the plasmid, pCRY1. The plasmid is specific to strains in which it resides, as some isolates with pCRY1 produce large cankers; others produce small cankers.

Carmen Medina-Mora, Michigan State University

She has three objectives as part of her Ph.D. program:

- To characterize *C. parasitica* strains based on the presence of mitochondrial elements, InC9 and/or pCRY1;
- To determine if InC9- and/or pCRY1-containing strains are dispersed on diseased chestnut trees in Michigan, Connecticut and Wisconsin; and,
- To compare DNA sequence of InC9 insert from selected strains from Michigan, Connecticut and Wisconsin.

In 1978, all cankers observed at the Lockwood Farm in Connecticut were treated with a fungal suspension of eight dsRNA-containing strains from Italy, France and Michigan. In 1996, 46 strains of *C. parasitica* were isolated from four cankers in this plantation. In 2001, 68 cankers were collected from 17 non-lethal appearing cankers and another 26 from lethal-appearing cankers at the Meshomasic Forest in CT. In 2000, 35 isolates were collected from the Rockford, MI stand, the original source of the Michigan strains used as treatment strains at the Lockwood planting in 1978. Strains from West Salem, WI were also used in this study. The number of strains examined can be found in the table below.

Mitochondrial insert, InC9. She used PCR primers, within and outside the exon of the small rRNA. If the insert was present, there should be an increase by ~1000 bp on the PCR fragment. She wanted to compare the insert in various strains using PCR amplification and sequencing. She showed a partial nucleotide sequence of the InC9 element. From the isolates examined so far, InC9 is present in every population with the exception of Meshomasic Forest. She has examined several strains from each of the above-listed sites and found that all strains are identical within a site, except Lockwood. One of her objectives is to see how identical InC9-containing strains are from various locations.

InC9 looks like an intron, but it does not have characteristics of transposable elements. The insert is transferred from one strain to another via anastomosis and some of the flanking regions of the insert are transferred as well.

Mitochondrial plasmid, pCRY1. The mitochondrial plasmid, pCRY1, was present in isolates from the 1996 and 2001 isolates from Lockwood Farm and from the Meshomasic Forest, but not from Rockford and West Salem.

The following table summarizes her detection of the insert and plasmid, to date.

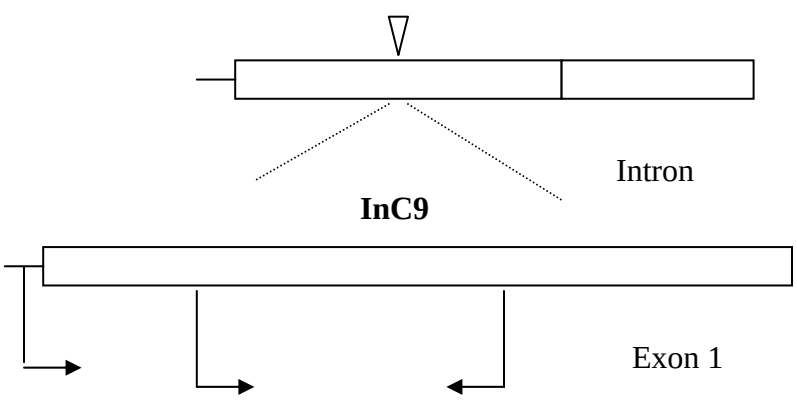
Location	# Isolates	# Processed	%InC9	%pCRY1
Lockwood 1996	46	44	95%	41%

Lockwood 2001	68	42	81%	60%
Meshomasic	26	14	0%	7%
Rockford	35	31	74%	0%
West Salem	46	21	38%	0%

Conclusions:

- The incidence of InC9 and pCRY1 on Lockwood, CT is high (81-95% and 60-41%, respectively).
- InC9 could have originated from Rockford, MI where InC9 incidence is high (74%).
- Incidence of InC9 on West Salem, WI is less than 50%.
- Origin of pCRY1 at Lockwood is still unknown.
- pCRY1 is absent at two locations, Rockford and West Salem.

She hopes to put InC9 elements from Bockenhauer (WI) and Rockford (MI) into other strains.



DF50 HB276 DF
44 bp 356 bp 97 bp

Terry Tattar, University of Massachusetts

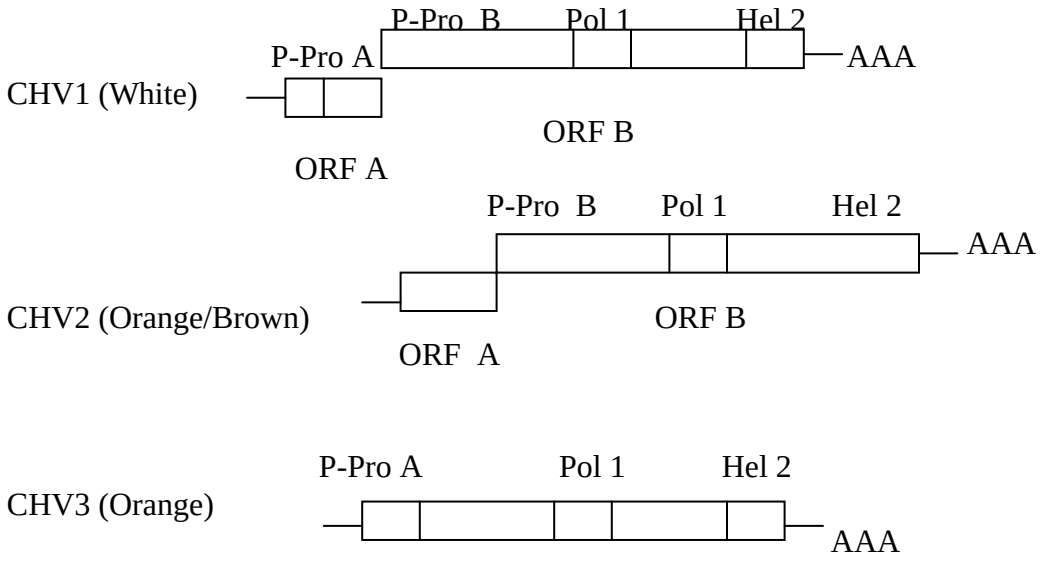
Antagonistic microorganisms against C. parasitica. Tattar was initially interested in *Trichoderma* sp. that live on chestnut bark. There was some difficulty in trying to prove *Trichoderma* is antagonistic to *C. parasitica* since it is found so often. Their research goal is to determine the presence of highly antagonistic strains of *Trichoderma* on the bark of American chestnut trees and assess their biocontrol potential. The DNA of various *Trichoderma* sp. are being examined currently. Segments of DNA from *Trichoderma atroviridae* and other *Trichoderma* species from *in vitro* cultures have been identified using existing fungal DNA libraries.

Bacillus megaterium. An orchard plot of 80-90 trees (from Bear Creek Nursery), planted 8-9 years ago, is being maintained on a 0.25 hectare plot in South Deerfield, MA. The trees were planted on 8-foot centers and the goal is to keep them alive to test the effectiveness of *B. megaterium*. One *B. megaterium* spray, consisting of 3 antagonistic isolates, was sprayed on the trees in July 2001. The bacterium has been shown to survive.

There are two apple orchardists who are interested in chestnut. Tattar has supplied the orchardists with several hundred American chestnut from Wexford County (MI). These trees do get conventional chestnut blight cankers and the trees are kept alive with mudpacks. Many of the infections occur at the soil line. One strategy is to "hill up" soil over the basal cankers. Cankers higher up on the stem are controlled with a slurry of soil and endospores of *B. megaterium.* The slurry is applied with a masonary brush. The trees are sprayed prophylactically with *B. megaterium* and the slurry is applied to any cankers that form. The success rate is not 100% but many of the trees are still surviving.

Bradley Hillman (Rutgers University)

Hillman explained the genetic elements of *C. parasitica* as follows:



He is currently investigating:

- Viruses: (CHV4-SR2) (?)
- Transposons
  - Crypt 1
  - Crypt 2
- *Cryphonectria nitschkei*

	Virulent	**CHV1**		**CHV2**	**CHV3**		**CHV4**			**Reoviridae**	
Character	Ep 155	Ep 713	Euro 7	NB58	GH2	D2	SR2	HM3	SH4	C18	9B21
Virulence	++	-	+	-	-	++	++	++	++	-	-
Pigment	++	-	+	+	++	++	++	++	+	+	++
Conidia	++	-	+	+	++	++	++	++	+	+	++

There can be additional RNA complexity with a particular hypovirus.

RNA1 (9.8 kb)

RNA 2 (3.6 kb)

RNA3 (1.9 kb)

Accessory RNAs that may be present

or absent in a virus strain.

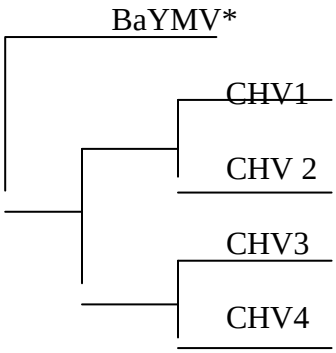
RNA 4 (0.9 kb)

dsRNA for strain SR2 (Savage River, MD)

- SR2 is very close in virulence to wild type strains.
- No cross-hybridization between SR2 and CHV1, CHV2 or CHV3 dsRNAs.
- Most closely related to CHV3-GH2 dsRNA.
- Less dsRNA associated with SR2 and similar viruses than from other hypoviruses examined.
- May be most common *Cryphonectria* virus species in North America.

Working with SR2 is difficult as there is not definitive phenotype, plus much more mycelium is needed as the titer is low. This type of hypovirus has been found all over North America, so it is very fit. Differences in hypoviruses and a phylogenetic tree are listed below:

CHV1 contains Protease 1 and Protease 2
CHV2 contains Protease 2
CHV3 contains Protease 1
CHV4 contains ?



*BaYMV (barley yellow mosaic virus) is the most closely related virus to the Hypoviridae.

There is very little homology of the first portion of the SR2 dsRNA from the 5' end on. It looks like the non-translated region and may be very short (200 bp or less).

Crypt1, a Class II transposon of the hAT (Ac-like) family.

- Identified as moderately repetitive probe by Milgroom in the early 1990s.
- 8-20 copies in most *C. parasitica* isolates.
- Active, but at low levels.
- Some defective copy found, ?

There is a large population of *C. parasitica* isolates from southern China that has only a single copy of the Crypt1 transposon. Isolates from northern China and Japan have 7-8 copies. The first transposon they identified is also Barbara McClintock's element. Crypt1 is interrupted by Crypt2. There are differences in the size of direct repeats. Crypt2 is a smaller element. Hillman thought this can be used as a historical marker, but it turns out not to be as useful as he had hoped.

Crypt2, a Class III transposon of the fot1 family.

- More copies than Crypt1, in most *C. parasitica* isolates.
- Defective copies may be more prevalent than Crypt1.

- Activity is unknown.
- Identified in many *C. nitschkei* isolates.
- Many copies in all *C. parasitica* isolates examined, worldwide.
- No copy found in *C. radicalis*.
- Fewer copies in the *C. nitschkei* isolates examined.

Cryphonectria nitschkei, a species found in the same habitat as C. parasitica.

- Grows on, but does not cause disease on chestnut.
- Contains some of the same genetic elements as *C. parasitica*.
- More distantly related to *C. parasitica* than is *C. radicalis*; similar to *C. cubensis* in distance.
- System set up to examine conditions for Crypt1 transposon in *C. parasitica.*
- Is easily identified by its ITS region.

Cryphonectria Characteristics						
	Ep 713	9505 (CN)	JA (JA75)	JA (VB1)	JA (CD28)	JA (YM2)
Crypt1	++	-	-	-	-	+
Crypt1/2	+	+	+	-	-	-
Crypt2	++	++	++	+	+	+
Virus	CHV1	CHV21	Yes	None	CHV1	None
ITS	parasitica	parasitica	parasitica	nitschkei	nitschkei	nitschkei
C.p. Mat1/2	+	+	+	-	-	-

Summary:
An approximate 7kb of sequence of dsRNA from *C. parasitica* strain SR2 shows that it is most likely associated with CHV3-GH2.

William Powell (SUNY-ESF)

Powell has been working on genetically engineering chestnut for 12 years. Two labs are working on this project at SUNY-ESF: Powell's lab is working on resistance transgene design and testing, while the tissue culture work is done in the lab of Chuck Maynard.

What genes are on-hand to put into the tree?

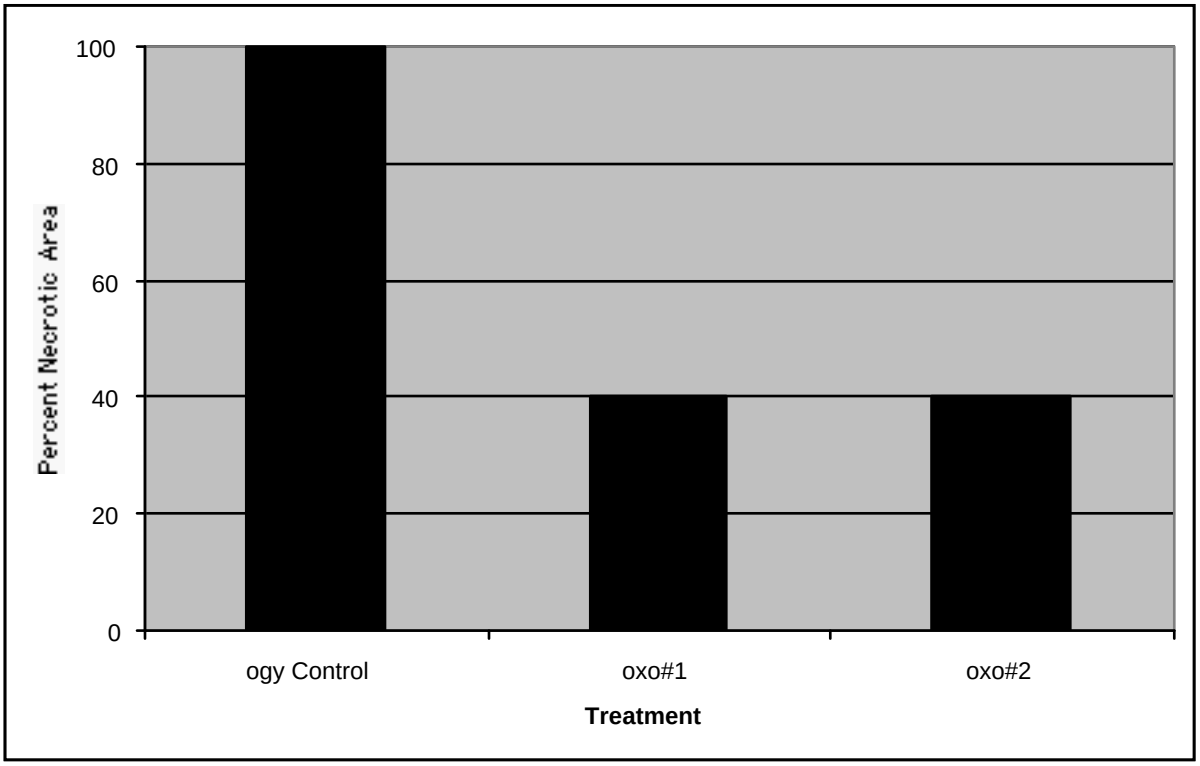
- ESF synthetic antimicrobial peptide designs
    - ESF 12 and ESF 9 (magainin-like peptides)
    - ACAMP1.2 (analog of Amaranth seed coat AMP)
- Chitinases
    - *Trichoderma*
    - Poplar
- Wheat oxalate oxidase gene
- American and Chinese chestnut cystatin
- NIa proteinase from TEV (tobacco etch virus)
- Transcription factors (genes that control other genes)

Poplar as a model:

- It is easy to transform.
- 7-9 months to go from leaf to whole plant.

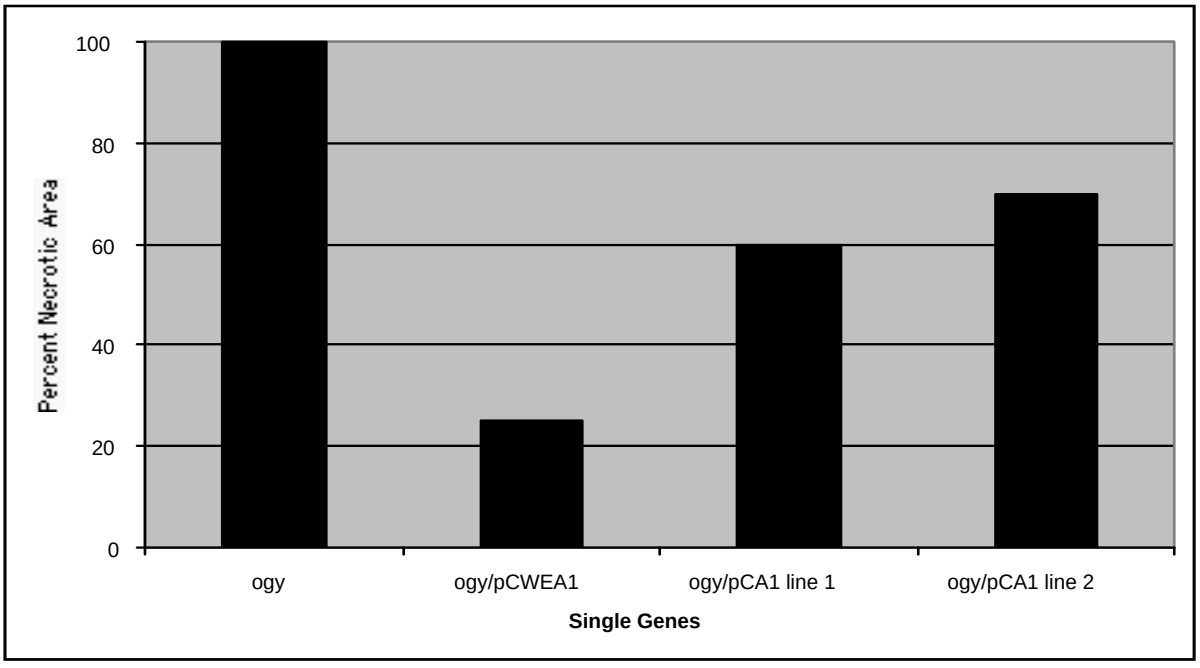
- It has a nice stem canker (*Septoria musiva*).
- Nice leaf disk assay already developed for Poplar and *Septoria.*

One of the first genes they inserted was wheat oxalate oxidase gene. They asked the question, "Will wheat oxalate oxidase actually enhance resistance?" The associated



graph shows the results that they do obtain enhanced resistance.

Poplar Field Trial. A field trial was first planted in Spring, 2000. The trees are growing well and 23/24 have maintained expression of the gene. Results from other gene constructs are shown in the following graph.

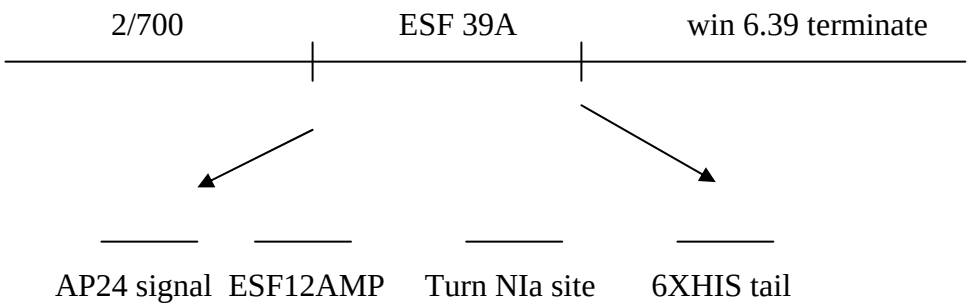


Powell's group is also looking at single promoters to drive several genes. They have a construct with chitinase, upstream of NIa which is upstream of oxox. This is being transformed into *Arabidopsis*. To date, there is confirmed presence of NTP2, chitinase and proteinase.

American Chestnut Stem ESTs (expression sequence tags). They are looking at ESTs in stem tissue. Fifty-five ESTs have been sequenced and submitted to GenBank. Several full-length clones have been examined; they plan to examine one in more detail. They are conducting RT-PCR of cystatin gene; this gene is expressed in many tissues such as stem, canker and margin. They are continuing EST work to go after promoters. The promoters they have on hand are:

- CaMV35S
- Win 3.19
- Three stem-predominant promoters (one stem promoter is mostly expressed in vascular system)

A promoter has been added to ESF39A



American chestnut tissue culture.

- They have developed a micropropogation system.
- Two vigorous somatic embryo cell lines have been established.
- An acclimatization system has been developed.

Somatic embryo cultures are maintained for four years but they have to be transferred every two weeks or they die. From shoots to roots to plantlet to field plant--less than 1% regenerate to a whole plant. They found that tissue culture plantlets do not grow as fast as a seedling from a nut. The root systems are different; the seedling root system is much more fibrous. The next step is to optimize soil mix, fertilization and growth conditions. Transformation is being added to the protocol, but this adds many complications. They have two transgenic shoots but they both died during the multiplication process.

Alternative Transformation Methods.

- Pollen transformation via electroporation
- Hypocotyl, epicotyl and zygotic embryo transformation; this is *Agrobacterium*-mediated
- Shoot meristem transformation

Fred Hebard, The American Chestnut Foundation

He presented a brief summary:

- AFLP mapping is completed. It did not help resolve the problems with the original map. The AFLP work was done by Catherine Clark from North Carolina.
- TACF has a grant program. Steve Rogstad at the University of Cincinnati works with mini satellites (DNTR) and has developed a new method of extracting DNA using polygalacturonase. This data should be published soon.

Assorted chestnut diseases.

- Leaf blight. This was noticed on trees lower than 3000' elevation. Stunted leaves are due to necrosis; it seriously impacts flowering. The causal fungus has not been isolated.
- Crown thinning associated with stem canker, *Pseudovalsa*. Anaganostakis believe it is *Cryptodiaporthe castaneae*. Stem inoculations provided the following data:

Type of Chestnut Tree	Uncankered	Cankered
American	51	1
Chinese	114	12
Chinese X American	100	2

- Naturally occurring hypovirulence.
    - Chestnut blight is rare on sprouts in mature forest, but becomes epidemic after clearcutting and the incidence of blight approaches 100%.
    - Incidence of blight in mature forest is 20% and 75-100% in a 9-year-old clearcut. Epidemics occur due to a 10-fold increase in sporulation per canker caused by the larger size of trees in young clearcuts than in mature forests.
    - Toward the end of the epidemic, a few trees survive blight longer than usual.
    - Longer periods of survival in clearcuts are associated with release of young sprouts form competition as proposed by Griffin. Hebard releases trees to promote flowering which will increase with added exposure to sunlight.
    - The pattern of survival of chestnut in the eastern U.S. when it occurs, is similar to that reported in Italy by Mittempergher.
        - It does not appear until 10-15 years after initial infection.
        - It is associated with the second wave of sprouting and infection.
        - The U.S. pattern is dissimilar from the Italian in that it does not occur at all sites which undergo a second wave of sprouting.
        - Much more so in the U.S. that in Europe, the etiology of extended survival is unclear. In the eastern U.S., it apparently is not incited by European hypoviruses and perhaps not any series of hypoviruses.
        - At those sites in the U.S. where disease remission occurs, its ultimate level may turn out to be similar to the level in Europe.

Sandra Anagnostakis (Connecticut Agricultural Experiment Station)

Anagnostakis reported on a CT chestnut plot that was treated aggressively from 1983-1986 with Ep 747, an Italian CHV1 hypovirulent strain. No hypovirulent introductions have been made since 1986. Young cankers (< 2 years old) were sampled in 1999-2000. There are 109 sprout clumps in the plot and 45 small cankers were sampled. Twenty-one isolates contained no dsRNA but 24 isolates were found with CHV1-type dsRNA. Many of the larger chestnuts in this plot have died, as a result of the

drought of 1999-2000. Her next goal is to show that the hypovirulent isolates recoverd in during sampling in 1999-2000 are genetically similar to Ep 747.

Don Nuss (University of Maryland Biotechnology Institute)

Nuss updated the group on field trials and laboratory work.

Field testing transgenic hypoviruses.

The question was posed, "Is the additional mode of transmission, via ascospores, an additive effect for chestnut tree survival?" To answer this question, a field trial, using transgenic Ep 713, was set up in the Husatonic Forest in Connecticut in 1993. APHIS was very helpful in setting up this initial release experiment. They have shown that:

- Virus did not persist.
- The release was very small.
- Little risk associated with the release.
- The forest was a closed canopy.

This led to intensive deployment in Meshomasic State Forest in CT in 1997 to conduct risk assessment. The clearcut of the forest was conducted in 1994. Three indigenous field strains were transformed. Hygromycin-resistant markers were added to follow virus movement. Risk assessment and biocontrol efficacy were evaluated. Trees were small at the onset of the experiment. Plots were 28 m x 28 m with a buffer zone between the control plot and treatment plot. A mixture of spores ($10^{10\text{-}12}$ spores/ml) of three transgenic strains were sprayed onto the trees several times during the summer. A data summary follows:

	1998	**1999**	**2000**	**2001**
# Treatments	4	6	4	0
Cankers sampled	20 external 5 internal 32 isolates	0 external 92 internal 114 isolates	0 external 180 internal 367 isolates	60 external 266 internal 581 isolates
From insects	N/A	185 insects 0 isolates	3300 insects 156 isolates,1Hyg^R	N/A
Strains with input virus	0	5	12	13
Transgenic Ascospores	N/A	9/13	3/25	N/A
VC Results	25	4 discrete groups 9 clusters		

One area in which they need help with with vegetative compatibility testing. They have found some isolates that look compatible with more than one isolate.

With few Hyg^R isolates, there does not seem to be much of an impact on the disease. Some of the trees have single events and some have multiple events.

Conclusions of field studies at Meshomasic State Forest:

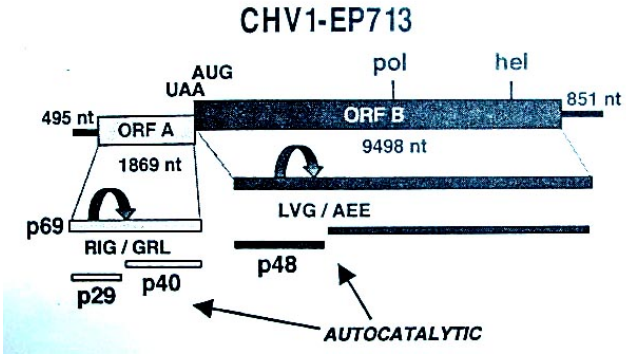
- After 3 years of spraying transgenic conidia, there is good evidence for transmission of cDNA-derived hypovirus RNA, independent of input transgenic strains and for transmission of viral cDNA.
- No evidence of spread of transgenic strains or derived viral RNA to control plot or buffer zone.
- Insects do not appear to be major vectors.
- Some transgenic *C. parasitica* isolates with atypical morphology were recovered.

Future plans for field studies:

- Complete molecular characterization of transgenic strains with atypical morphology.
- Follow tree condition over time at the Meshomasic State Forest site.
- Test performance of more ecologically fit transgenic hypovirus *C. parasitica* strains, transformed with CHV1-Euro 7 cDNA.

He showed slides of virulence of field isolates of *C. parasitica.* Ep 713 produced very small cankers and Euro 7 produced a moderate size canker. This was in contrast to Ep 155 and Euro 7 (virulent form) that produced large cankers on excised stems. Euro 7 typically produces a larger canker than Ep 713, but it shuts down and does not expand further.

Chimeras were made using Ep 713 and Euro 7 to look at sporulation and canker size. Nobuhiro Suzuki found the first 24 codons are required for virus replication. A map of CHV1-Ep713 is shown below:



Symptom determining domains for CHV1-Euor7 are:

- p 29 (aa 25-73)—suppression of pigment and conidiation
- p40—required for RNA accumulation
- p48—canker morphology
- nts 3575-5310 controls colony morphology

GTP subunits (CPG-1 and CPG-2)

Heterotrimeric G-proteins are a large and growing family of proteins that play an essential role in response to environmental stimuli in all eukaryotic cells. Two genes from *C. parasitica*, *cpg-1* and *cpg-2* have been cloned. These genes are members of the large and diverse Gα subunit family. Deletion of either *cpg-1* or *cpg-2* resulted in reduction in growth and altered colony morphology, but the changes for the Δ*cpg-1* strain were much more severe with this mutant proving avirulent on chestnut while Δ*cpg-2* was only slightly less invasive than the wild type. Additional characteristics of the Δ*cpg-1* strain included reduced pigmentation, loss of conidiation, female infertility and a reduction in laccase production. Members of the Giα family are generally believed to

function as negative regulators of adenylate cyclase. Nuss indicated that pathways interact in very intricate ways; studies continue with *cpg-1* and *cpg-2*.

Characterization of South African C. cubensis isolates infected with C. parasitica hypovirus. Nuss is working with Mike Wingfield in Pretoria, South Africa to infect *C. cubensis*, a serious canker disease of *Eucalyptus* spp. with *C. parasitica* hypovirus. Wingfield successfully used electroporation to transfect a synthetic RNA transcript corresponding to the full-length coding strand of CHV1-Ep713 hypovirus. This transfection resulted in pronounced morphological changes that included a striking increase in yellow-orange pigment production, a reduction in mycelial growth rate and reduced sporulation. Colleagues in Japan are working with a similar pXH9 system, using *Phomopsis* and *Valsa*, pathogens of fruit trees.

Andrew Jarosz (Michigan State University)

Jarosz reported on the evaluation of the *C. parasitica* epidemic and hypovirus introductions at West Salem, WI. A brief history of the stand is as follows:

- Largest stand of healthy American chestnut in the U.S. (> 4000 trees with dbh > 1 in).
- First infection noticed in 1986.
- COLI hypovirus was introduced between 1992-94.
- Euro 7 hypovirus was introduced between 1995-97.
- Trees were GPS mapped in 1999.
- Goals for this study include:
  - Monitor the spread and v-c diversity of the blight.
  - Evaluate the degree of recovery due to hypovirus spread.
  - Evaluate community dynamics.

Problem: The epidemic has progressed to the point where it is impossible to sample cankers from all infected trees.

Solution: A series of plots in different areas of the population were established in 2001. These areas include:

- Old area where initial infections were found.
- Front area where blight has spread in the past 5-8 years.
- Uninfected area where trees are still uninfected or became infected in the past 2 years.

There are four plots in each of the three areas for a total of 12 plots. For each tree in a plot, all cankers are sampled, the dbh is measured and the number of stems (epicormic shoots) are recorded and the tree is subjectively rated for evidence of recovery.

Area	Avg dbh (in)	Avg #stems/tree	Disease incidence	Proportion of diseased trees with healing cankers
Old	9.4	7.3	96%	78%
Front	5.2	2.5	32%	42%
Uninfected	5.7	1.9	24%	13%

Averages	6.7	3.8	40%	54%

How the data will be used:

- Monitor spread of the pathogen into plots, within plots, within trees and within cankers.
- Monitor spread of hypovirus.
- Evaluate recovery based on the tree growth patterns over time.

Bill MacDonald, West Virginia University

In 2002, the national meeting of the American Phytopathological Society will sponsor a symposium on West Salem. This meeting will be held in Milwaukee, WI with a field trip to the West Salem site.

West Salem study. MacDonald continued the report on West Salem, WI. The American Chestnut Foundation convinced the owners of the stand (Ron and Sue Bockenhauer and Carl and Dolores Ryme) to continue the study. TACF is reimbursing the owners for not cutting dead timber.

Assessment of disease progress and the spread of two hypoviruses continues at the West Salem site. In 2001, a sub-sampling scheme was established because of the increasing number of infections. The plot design described above by Jarosz includes three groups of plots: those where cankers were treated and hypoviruses are established, those plots at the advancing disease front and plots where disease is not yet present. The average number of stems in each of 12 plots is 32.

As of mid-October, approximately 80% of the bark samples removed from cankers during May 2001 have been cultured and assessed for hypovirus content and vegetative compatibility. New findings for 2001 include:

- Approximately 650 of the 1100 cankers that exist in the 12 plots were sampled.
- CHV1 (Euro 7) continues to be the most commonly identified hypovirus but CHV3(COLI11-1) still persists at the site.
- Hypovirus was associated with over 80% of the cankers that received hypovirus treatment from 1992-1997.
- Non-treated cankers on trees with treated cankers readily acquire 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 but this is most notable for trees where hypoviruses were applied.
- Additional vegetative compatibility types were discovered in 2001 but at very low frequency.

Mark Double, West Virginia University

Long-term dissemination studies in cleared and on-cleared cut-over areas. In 1988, a series of plots was established in Pocahontas County, WV to evaluate the effect of periodic removal of competing vegetation on chestnut population. Further, hypoviruses were introduced into these sites to determine if hypovirus introduction would help diminish the effects of *C. parasitica* and thus improve chestnut survival. The plots that were established that were either repeatedly cleared or not cleared. Hypovirus introductions were made to both types of plots using isolates infected by CHV1-type hypoviruses. Plots were visited twice annually to assess tree survival, to sample infections and introduce hypoviruses. After 1997, new stems were added to cleared plots. As of Spring 2001, almost 1,800 cankers have been sampled and the following generalizations can be made:

- The rate of tree mortality of the original sprouts has been similar among all treatments until 1996 when mortality in the cleared plots slowed.
- The number of cankers form which hypovirulent bark samples have been removed was always greated from trees that received hv treatment. Further, the recovery of hypovirulent isolates increased as sets of cankers were sampled after one or two years.
- Cankers on non-inoculated trees have acquired hypovirus but at much lower rates.
- In plots where a clearing regime was not maintained, little or no sprouting occurred and all stems are now dead.
- Hypovirus recovery was best on trees in non-cleared plots that received hv inoculum.

The influence of time of growing season and infection age on hypovirus transmission to Cryphonectria parasitica. This study examined the influence of canker age and time of hypovirus introduction on the development of hypoviruses in *C. parasitica* cankers. An orange-pigmented strain of *C. parasitica* was used to initiate 160 cankers on forty American chestnuts; four cankers were established on each tree. Cankers were initiated in either May or July of 1999 and outlined with a permanent marker at eight-week intervals (excluding dormant season) after their initiation. Cankers were treated with vegetatively compatible isolates containing one of two hypoviruses (CHV1 [Euro-7] or CHV3 [COLI 11-1]). Sets of cankers were challenged in July, September and November, 1999 and April and July 2000. Each hypovirus was introduced into the advancing canker margin (top and bottom) of eight replicate cankers/date. The outermost ring of each canker was sampled at periodic intervals after challenge.

One year after challenge, all growth increments were sampled. The extent of hypovirus colonization was determined by removing bark plugs and evaluating whether the resultant colonies represented virulent, hypovirulent or non-*Cryphonectria* plugs. As of this date, the following results can be reported:

- Cankers continued to expand following introduction of hypovirulent inoculum, but the CHV3(COLI11-1) hypovirus reduced canker growth more than CHV1(Euro7), regardless of the date of challenge.
- Cankers initiated in May-99 and treated in April-00 showed the greatest reduction in growth.
- When the outermost growth ring of cankers was sampled, the greatest recovery of hypovirulent isolates occurred in April for either hypovirus.
- When the entire canker was sampled one year after hypovirus introduction, the greatest recovery of hypovirulent isolates occurred in April for either hypovirus.

Pam Kazmierczak (University of California at Davis)

The UC-Davis lab is looking at biochemical and cellular level problems. They know the virus is inhibiting protein secretion, sexual reproduction and pigment production. They have found three genes that are down-regulated by the virus. Many of these down-regulated products are enclosed in vesicles. The genes that are Kex-2 processed are:

- Cryparin (cell wall hydrophobin)—when it is deleted, virulence is not reduced, but stromotal pustules do not break the bark surface.
- Laccase
- MF1-1 pheromone

The hypovirulent phenotype can be mimiced by a Kex-2 inhibitor. They are in the process of deleting Kex-2 to see what it does to phenotype. Is there any feedback going on? They have found that the cargo (proteins) builds up and signals the genes to stop transcribing.

Their group is continuing to characterize vesicles. They have found that vesicles are contained in both virulent and hypovirulent isolates but they proliferate in hypovirulent strains. The clatherin-coated vesicles go to the membrane and the clatherin targets the vesicles to go to the surface (outer membrane) of the cell to be secreted. The virus is affecting the vesicles so they are no longer secreted.

Kazmierczak reported on the deletion of MF1-1 pheromone from *C. parasitica*, work done by Massimo Turina, Antonio Prodi and Neal Van Alfen. They have:

- Constructed a deletion plasmid.
- Screened transformants by southern and northern blots.
- Shown biological effects.

The big upshot of deleting MF1-1 is it creates male sterility in the fungus. Turina found that most expression is in lag phase, so he looked at various media:

- Minimal media
- Minimal media supplemented with wood, bark and wood and bark
- Complete media

Turina concluded that sex is probably a starvation-induced process. While effect of yeast extract on the expression of MF1-1 is not significant on pheromone production, the difference is qualitative. Turina looked at the inoculum age on pheromone expression. He examined leading-edge mycelium, center-of-culture mycelium and isolations taken from the middle of a colony. He found the older the mycelium, the more inducement of pheromone expression.

Conclusions:

- Deletion of MF1-1 results in male sterility.
- Older inoculum produces more pheromone expression and faster expression.
- Minimal media induces pheromone expression sooner.
- There is no expression of MF1-1 in conidia.

Kazmierczak showed slides of chestnut plantations in California. She also showed slides of groundhog control in chestnut plantations. Pure oxygen was pumped into groundhog holes and then ignited.

<u>Objective 1.</u> To improve chestnut trees for timber and nut production, and determine the cultural requirements of chestnut seedlings in nursery and natural settings.

Michael Gold, University of Missouri Center for Agroforestry

Gold stated that the establishment of a chestnut industry in the State of Missouri is to enhance the viability of small farms. He presented work in conjunction with: Ken Hunt (University of Missouri Center for Agroforestry), Michele Warmund (University of Missouri, Horticulture), William Reid (Kansas State University) and Sandra Anagnostakis (CAES). Much of the work this group has conducted has been funded by USDA-ARS for the Agroforestry Small Family Farm and Floodplain Program.

Objectives:

- To evaluate and characterize available chestnut cultivars for marketable traits.
- To establish replicated chestnut cultivar reseach/demonstration orchard trials in commercial areas in Missouri and Kansas.

Establishment of a viable chestnut industry depends on:

- The use of clonally propagated chestnut trees.
- Well characterized fruit quality.

Some of the chestnut trials are planted on the Hark Farm, a 640-tract of land. The farm was begun in 1953 and Agroforestry began on the site in 1993. Chestnuts were planted on a 27 x 27' spacing with five replicates. For the past few years, data has been collected on all cultivars in the repository. Cultivars that look quite good are, 'Willamette' and 'Qing.' So far, size is a major character of chestnuts. Also, first-to-market chestnuts get the premium price, so part of the study examines ripening dates.

Cultivars are also examined for tolerance to chestnut blight. A new orchard was just established in October, 2001 at New Franklin, MO. The spacing is 27' x 13.5' with three cultivars, 'Peach,' 'Qing' and 'Willamette.' There are eight replicates with six trees/replicate. The plan to examine: pruning treatments, irrigation regimes and fertilizer regimes.

James Maddox, Tennessee Valley Authority, Environmental Research Center

He reported that Sandra Anaganostakis came to Alabama to assist with the establishment an Alabama chapter of TACF. While there, they toured the Bankhead National Forest where they looked at *Castanea henryii* that was planted in the 1950's The trees are of good size and diversity.

In 1994-95, Maddox began looking at mycorrhizae inoculum in relation to survivability of American chestnut in field situations. There is a good deal of conservation control land that incorporated chestnut as a wildlife planting. He commented that after 6-8 years, they are ready to make some changes in their approach. To prepare chestnuts, they set up three sets of artificial, ventilated beds, 25' long. The rows are 18" deep X 2' wide and drip irrigation is supplied by the Tennessee River. The soils in this area are high in phosphorous as the TVA has used this area of the nursery for the past 30 years. Mycorrhizal inoculants were supplied by Joseph Morton (West Virginia University) and David Sylvia (Univesity of Floria). Maddox began experiments to determine if mycorrhizae could alter the root architecture of American chestnut and aid in survivability. He examined root length of chestnut after planting in Minimum/Maximum tillage systems. Plantings were in Decatur Silt Loam soil; maximum tillage was rototilled to 8" while minimum tillage was 4.6". The nursery beds were fertilized with 15-15-10 @ 150 lbs/acres. In 1997, the seedlings were hand-dug, measured and outplanted into Little Bear Creek Watershed, a TVA property that is being reforested. There was virtually no difference in root length, based on tillage depth. Tree height was also negligible. There was, however, a difference in root width, as the Maximum tillage produce wider roots. Further data are:

Effect of Soil Tillage on Root Dimension and Seedling Height

	Root Dimension		
Tillage	**Length**	**Width**	**Height**
Minimun	21.8	32	48.8
Maximum	22.3	16	49.8
Significance	NS	*	NS

Conclusions:

- Tillage has no significant difference on tree survival after four years.
- Tillage has no significant difference on tree height.
- Maximum tillage provided seedlings with wider roots but not no significant difference on root length.

Field Cover Crop Study

- 1998 fall cover crop-vernal alfalfa, crimson clover and overseeded again in fall 1999.
- four plots, two replicates, 16 trees.

- 10’ X 10’ spacing.
- one-half of the hole locations were perimeter seeded to yellow sweet clover.
- 1999 planted 32 ‘Lundy’ chestnut seedlings.
- 8 1-year seedlings reared in the nursery with following amendments to yellow sweet clover:
    - none
    - TVA/VAM (*Glomus etunicatum*)
    - *Azospirillum*
    - *Azospirillum* + TVA/VAM

Effect of Cover Crop on Tree Survival

Cover Crop	1999	2000	2001
Alfalfa	12	7	7
Cr. Clover	15	12	11
Significance	NS	*	*

Effect of Tree Growth (Height in Inches)

Cover Crop	1999	2000	2001
Alfalfa	43	56	87
Cr. Clover	47	67	92
Significance	NS	*	*

Effect of Yellow Sweet Clover 2-Foot-Perimeter Planting on Tree Survival (Number)

Sweet Clover	1999	2000	2001
Yes	11	9	9
No	16	11	10
Significance	*	NS	NS

Similar data was found for tree growth. The conclusion is that a cover crop helps while a companion crop is detrimental.

Effect of Nursery Microbial Inoculant and Perimeter Sweet Clover on Tree Survival

	1999		2000		2001	
Inoculant	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
None	4	3	2	2	2	2
TVA/VAM	4	3	3	3	2	3
Azospirillum	4	2	3	1	3	1
Azo+TVA/VAM	4	3	3	3	3	3

Summary:

- Survival and growth is better with Crimson clover than alfalfa when used as a cover crop.
- Yellow seet clover grown as a companion crop decreased survival and growth.
- Nursery source generally did not affect survival on growth after three years.
- *Azospirillum* may have interacted with sweet clover to reduce survival and growth in the presence of sweet clover as a companion crop and increase tree growth without a companion crop.

Ink Disease. Maddox worked on chestnut that was planted in a field that had corn; this area has been in continuous corn for 2 centuries. Some of the trees have died, possibly due to ink disease (*Phytophthora infestans*). Chestnut on this site is interplanted with pine.

Miscellaneous:

- Maddox discussed fire ants. He does not see them on trees without disease.
- He showed slide of chestnut damaged by 17-year cicada; the trees had tremendous callusing where the insects created wounds via ovipositioning.

Chuch Rhoades, University of Kentucky

Rhoades is looking at the effect of canopy manipulation and aspect on seedling establishment in Kentucky forests. His work is in conjunction with Jeff Lewis and David Loftis, USFS.

Objectives of his study:

- Silvicultural requirements for artificial regeneration in forest settings.
- Soil and site requirements
- Develop site selection and silvicultural guidelines for future plantings
- Ecosystem processes

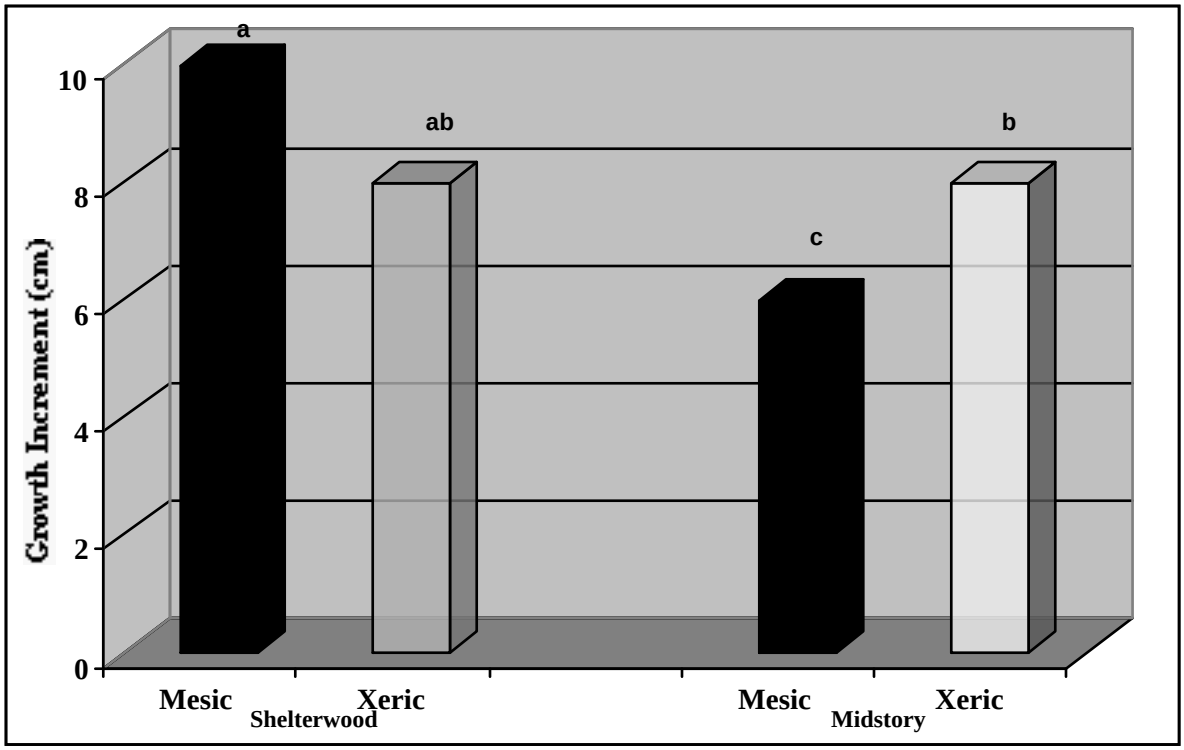
Work is being conducted in the Cumberland Plateau (eastern Kentucky) at the following sites:

- University of Kentucky Robinson Forest (north of Hazard)
- Tygart State Forest (northeast of Morehead)
- Berea College Forest (southeast of Berea)

His original plan was to set out five plantation sites, however, two were lost to administrative reasons; this is a barrier to working with the U.S. Forest Service. The basic study is to examine how to get chestnut started in clearcuts where there is competition. Two types of plots were chosen: shelterwood (20 foot-square residual basal area) and mid-story removal (intermediate and suppressed crowns). Two aspects were compared: xeric, south-facing slopes that are dry and mesic, north-facing slopes that are moist. The objective was to look at sites where competition is different.

	Tygart Forest	Berea Forest
Mesic Site	%Basal Area	%Basal Area
Sugar Maple	33	53
White Oak	24	
Chestnut Oak	15	
Shagbark Hickory	5	
Yellow Poplar	5	10
Ash		7
Xeric Site		
Chesnut Oak	36	35
White Oak	26	47
Black Oak	10	2
Sugar Maple	7	
American Beech	5	
Scarlet Oak	5	9
Shagbark Hickory	5	
Sourwood	5	

Canopy Manipulation Effect



Insect herbivory. This was heavy in midstory (both mesic and xeric sites) compared to shelterwood. Rhoades is interested in looking at leaf compounds to see if there is a connection to insect palatability.

Other aspect attributes:

- Aspect differs with soil nitrogen availability.
- More NH_4-nitrogen in mesic sites than xeric sites. Same for NO_3-nitrogen.
- Nitrates levels are higher in mesic sites, based on litter composition and species differences.
- In addition to looking at nitrogen pools, they are also looking at changes in nitrate levels, nitrification and mineralization.
  - High nitrification in mesic sites
  - Zero nitrification in xeric sites
- There are soil temperature differences.
  - Shelterwood is much higher than midstory for both mesic and xeric sites (24.5º C for sheterwood compared to 21º C for mistory).
- Similarly, it is moister in mesic sites--less canopy interception and more rain hits the ground.
- Higher pH in mesic versus xeric sites and higher potassium in mesic sites.

Site differences

Site	Survival
Berea Forest	93%
Tygarts Forest	90%
Robinson 1	77%
Robinson 2	66%

Greenhouse and field studies. In terms of site differences, Rhoades combined a greenhouse study with a field study. Seedlings were grown in soils form all three sites under three regimes: control, 100 kg/hectare nitrogen and ECM (ectomycorrhizae, *Scleroderma* and *Pisolithus*). Results were: (1) nitrogen treatment had the highest seedlings in all soils; (2) ECM treatment had higher seedlings at Robinson and Tygarts but not at Berea (compared to the control); and, (3) they found very low infection of ECM on roots.

Next steps:

- Competition effects
- Foliar nutrients, chemistry and herbivory
- Growth and disease incidence

John Carlson (Penn State University)

Carlson is working on an American chestnut silviculture study in conjunction with Tim Phelps and Kim Steiner. Their objective is to examine the suitability of a range of native forest sites and various silvicultural methods for reforestation of American chestnut for the eventual planting of blight resistant seedlings in a forest setting.

- Project has been undeway since 1997 with a plantation of 150 seed at Stone Valley Experimental Forest to test the effect of different planting techniques on early height growth.
- The study was replicated in 1998 and expanded in 1999.
- The 1997 and 1998 studies were planted in 5-foot tree shelters, both vented and unvented and seed planted without tree shelter protection.
  - Data indicate there is an advantage to tree shelters in the early years of growth.
  - There was no difference in the type of shelter.
- Seven new sites were added in 2001. These sites varied in soil type, elevation, aspect and ground vegetation. All sites were established in fenced areas.
- Results from 1999 and 2000 were disappointing as drought had a significant impact on the study.

Germination rates and minimum, maximum, and mean heights (cm) measured in September 2001 at seven sites established in 2001.

	Aspect	Germination Rate		Height				
Site		**n**	**%**	**Min.**	**Max.**	**Mean**	**Vegetation**	**Deer**
Deep Hollow	S	26	68 a,b	3	20	9.4 d	-	-
Galbraith Gap	S	30	60 b,c	3	18	8.8 d	+	+
Owl Gap	N	40	80 a,b	3	31	14.9 a,b	+	-
Pine Swamp Rd.	E	32	64 a,b,c	2	25	12.8 c,b	+	+
Spruce Mt.	S	41	82 a	4	30	17.0 a	-	-
Dead End Rd.	E	24	48 c	3	9	4.4 e	+	+
Eby Ridge	E	37	74 a,b	3	24	11.2 c,d	-	-

Genomic in situ hybridization for selection against Chinese chestnut genetic background in the TACF breeding program. The objective of this study is to develop a cytological procedure for screening trees or seedlings in back-cross generations for the amount of American versus Chinese genetic background (chromosomes) that they carry. Immediate objectives include:

- Develop a procedure for obtaining high mitotic index in root-tip squashes from germinating chestnuts.
- Optimize FISH (fluorescent *in situ* hybridization) protocol for American chestnut.
- Develop GISH (genomic *in situ* hybridization) protocol using Chinese chestnut genomic DNA as a probe against hybrids and back-cross individuals.
- Determine the limit of detection of GISH for visualizing Chinese chromosome fragments in chromosomes of advanced back-cross populations.

After a GISH protocol is developed for chestnuts, they will begin to test the effectiveness to directly select for the smallest amounts of the Chinese genome in progeny of the BC_3 generation.

Adam Dale, University of Guelph

Dale gave an overview of the chestnut situation in southwest Ontario. His is stationed at Simco, Ontario, approximately 70 miles south of Guelph. He stated that the area around Simco had 170,000 acres in tobacco in the 1970s. This acreage has dropped to 70,000 acres in 2000, so there is a lot of good farmland available for planting nut trees. There are currently about 2,000 acres of nut crops in the Canadian Provinces.

Restoring chestnut. Dale is working with Greg Boyland to develop a restoration program for chestnut. They are investigating:

- Breeding program
- Hypovirulence
- A survey of chestnut in southern Ontario

They have found much more hybridization than antidicatped, so there is less pure American chestnut than expected. Dale is a small-fruit's breeder and his goal is to:

- Breed blight resistant American chestnut within 20 years.
- Get a high 'Canadian' content within two generations.

He is using B_3 and B_4 pollen crossed to Ontario trees. Since he does not want to end up with germplasm from just one tree, he has pollinated 40 trees. Canadian chestnut will be used as females. He will then go to F_2s. His goal is 75% Canadian content. In 15

years, he should have good resistance in a Canadian background. This means he will need a large population of trees.

To date, Dale pursuaded the Trillium Foundation for money for three years for a technician. They were able to cross 12 trees and obtain 400 nuts; pollen was obtained from Sandra Anagnostakis.

Phillip Gordon, New York Botanical Garden

Gordon's thesis is that the American chestnut will recover from chestnut blight and evolve into the present Eastern forest ecosystem in a manner very similar to the position it held before chestnut blight decimated it as a forest tree. The means by which this process occurs will not involve present research methodologies. Simply stated: American chestnut will recover on its own. Gordon reached this position from an ongoing review of the history and the science used during the past century in combating the effects of the blight. "I had a little nut tree, nothing would it bear but a silver apple and a golden pear." This jingle came to Gordon while he sat under a Japanese chestnut while its chestnuts were dropping. He correlates chestnut to the silver apple and chestnut wood to the golden pear. Gordon was brought to chestnut by Professor Charles Burnham back in the 1970s. Gordon found Burnham to be a wonderful and inspiring teacher and he regarded Burnham with awe and admiration. Gordon, however, turned a deaf ear (in the 1980s) when Burnham tried to get him interested in the blight of the American chestnut. Gordon finally began to understand that Burnham was using his background in corn genetics as a biological methaphor for the devastation of the American chestnut in the earliest years of the 20th Century. There is a great deal of similarity between the growth and development of maize and that of American chestnut and Gordon found it very useful to correlate plant growth of these two species although they are very different biologically. Corn is an annual monocot which can be inbred or outcrossed. Chestnut is a forest tree, a long-lived dicot and an obligate outcrosser. It is not a comparison of how the two species grow that might be revealing. It is how the two species respond to disease processes that may be enlightening, how they may point to other ways to restore the chestnut as a forest tree in its original range. Gordon is referring to methodologies to restore chestnut that are not presently being considered seriously as useful research. Perhaps by looking at the interaction between the disease and the chestnut from the differing points of view, we might see other perspectives for restoring the tree. As a result of these considerations, Gordon is persuaded that much of our present research will not result in the restoration of chestnut as a forest tree and that there are better ways to achieve this through research. Gordon's thesis is that the American chestnut will recover if:

- We leave the American chestnut alone (Graves, Stewart).
- We institute programs involving the widespread planting of American chestnut (USDA-riparian river buffer trees and shrubs).

We are considering blighted American chestnut from the point of view of the:

TREE	**DISEASE**
Forest Tree Sciences	Phytopathology and Mycology
In the forest or stand	Sick trees in forest or orchard

RESISTANCE	**VIRULENCE**
Immunity of the trees	Treatment of the disease

Immune system , as Gordon sees it, is something within the organism which perceives the disease for the first time and reacts to it positively (develops resistance or immunity).

He suggested the members of NE-140 review the following articles:

Graves, A.H. 1926. The cause of the persistent development of basal shoots from blighted chestnut trees. Phytopathology 16:615-621. (Includes a good discussion and a photo of an uprooted chestnut shrub with multiple new roots).

Stewart, F.A.S. 1915. Report of the Pennsylvania Chestnut Tree Blight Commission for 1911-1913. (Includes lively opinion of why American chestnut should be left to its own survival devices).

Anagnostakis, S.L. 2001. American chestnut sprout survival with biological control of the chestnut blight fungus population. Forest Ecology and Management 152:225-233. (Contains many leading references form early in the 20th Century to more recent papers).

Gordon hopes to publish an article in *Economic Botany* in the near future.

Note: Colin McKeen asked to address the group following Gordon's remarks. McKeen stated that he knew what Burnham did in corn breeding. About a year ago, McKeen wrote an article for the Canadian Chestnut Council on wheat breeding and he sees similarities between wheat and chestnut. In WW I, when there was a need for food in Europe, a group of scientists gathered in Minnesota to wrestle with the wheat/rust problem. The wheat breeders decided to put maximum energy into solving the rust problem. Rust research labs were set up around the country to look at resistance. Many good scientists put their heads together to find out about sex in rust. Renowned scientists Stakeman and Wetzel spearheaded the movement. If their discoveries hadn't occurred, our knowledge of rusts today would be sorely lacking. It was their focus on the disease that led the way and McKeen does not want the NE-140 group to forget about the pathogen. "Let's not forget about the host, but continue work on the pathogen."

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

Hybrid chestnuts. Hybrids were planted in two forest clearcuts in central Connecticut (Prospect) and in a nursery (Windsor). Two additional plots were established in 2001; both sites have a mature canopy. BC_2, BC_3, American and Japanese chestnuts were dug up and the roots measured. The seedlings were then planted in the aforementioned sites. She reported good growth, even at the Prospect Ridge site that has a pH of 3.6. A sixth plot will be established in 2002 in a partially harvested forest area.

Manganese. She has been looking at manganese as a correlation with blight resistance. To date, she has found no correlation.

Calcium /Nitrogen ratios. A Portuguese woman reported that chestnut bark with higher Ca/N ratios seem to be more resistant to chestnut blight disease. The Ca reported in Portugal is a magnitude higher than that found in CT.

Business Meeting

John Anderson, NE-140 Administrative Advisor, stated that NE-140 is entering its 3rd year of the current proposal. The project began in 1982 and it is time to begin thinking if a renewal will be written. Anderson suggested that the chair set up a committee to look into the renewal re-write. He commended the group for their basic research to bring back chestnut to North America. He mentioned that the milestones that were listed for 2001 in the previous proposal are as follows:

- Additional forest site test begun.
- Characterization of putative genetically transformed American chestnut embryogenic cultures completed.
- Field evaluation of confirmed transgenic chestnut trees containing single-transgenic contructs.

The two goals for 2002 are:

- Sampling of 60 chestnut trees from each of 25 sites within 135-mile grid completed, to assess the level of diversity in wild American chestnut populations and to determine the geographic component of such diversity.
- Evaluation of transgenic hypovirulent strains of *C. parasitica* for biological control of chestnut blight.

Anderson noted that it will be necessary to complete the SAES 422 form; it is due 60 days from October 21, 2001. He encouraged NE-140 to write good impact statements and keep the milestones listed. Anderson noted that the SAES (State Agricultural Experiment Stations) contributors are: CT, MA, MD, NJ, NY-I and WV. Partners are: GA, MI and TX. Participating Federal Agencies are: USDA Southern Institute of Forest Genetics and agencies listed as 'others' include: UT, SUNT-ESF and TACF.

Anagnostakis noted that next year's annual TACF meeting is scheduled for October 17-20, 2002 and she suggested that NE-140's meeting not coincide with TACF. Next year's NE-140 meeting will be hosted by Pam Kazmierczak at UC-Davis. The consensus on a date was October 24-27, 2002.

Anagnostakis suggested that Bill Powell assist her with writing the goals. It must be stated how each goal has been accomplished. A committee to assist with rewriting the new proposal is comprised of: Michael Gold, Bradley Hillman, Bill MacDonald, Fred Hebard and Don Nuss. Anagnostakis suggested that the project will need a "new twist" if it is to pass.

Anderson said the current project terminates September 30, 2003. The new project has to be approved by the NE Directors. Anderson noted that the major opponent to the chestnut work is not longer on the board. It was suggested by Anderson to have major objectives that are important to stakeholders and also have cutting-edge research. The NE Directors meet in February or March, 2003, but the new project proposal must be sent out for review prior to the February/March meeting. It was suggested that the new proposal be completed by October, 2002 for review. The project should not exceed 15 pages of text; this does not include the bibliography.

Fulbright asked if orchardists could be included for practicality. Anderson commented that the "practical people" are already present within the group. Hebard questioned if we need to be discussing cultivar varieties.

The first step, however, is the submission of a request to write a proposal. This request is not to exeed 4,000 characters. This should be done as soon as possible and sent to Anderson. He reiterated:

- The need for research as indicated by stakeholders.
- Importance of work and what the consequences are if the work is not done.
- Technical feasibility.
- Advantages of doing work as a multi-state effort.
- What the likely impacts will be from successfully completing this work.

Fulbright added that the NE-140 group conducts cooperative as opposed to individual projects; this area needs to be highlighted. Anagnostakis suggested we each go back to our labs and look at how and where we cooperate and why we could not complete our work without cooperation. This list goes to Michael Gold. Hillman suggested a genomic section be added to the new project. Anagnostakis mentioned the NE-140 Home Page, done by Rubie Mize at the University of Maryland. David MacKenzie is Executive Director of the NE Directors and Rubie works for MacKenzie. She has developed a system that all project work will be on the web—submissions, reviews, etc. The URL for the NE-140 Home Page is: http://www.agnr.umd.edu/userforms/nera/projects

MacDonald reported on the deaths of two former NE-140 members, Dale F. Hindal (West Virginia University) and John E. Elliston (Connecticut Agricultural Experiment Station).

The meeting was adjourned at 5:30 pm on October 20, 2001. Following the meeting, Anagnostakis conducted a tour of the Lockwood chestnut site in Hamden, CT.