NE-140 Technical Committee Meeting
Biological Improvement of Chestnut (*Castanea* spp.)
University of Georgia Continuing Education Center, Athens, GA
October 31–November 1, 1994

Attendance:

Connecticut:	Sandra Anagnostakis, Philip Gordon, John Anderson
Georgia:	Scott Merkle, John Cairney, Joni Lawrence, Jim Maddox
Kentucky:	Lou Shain
Massachusetts:	Terry Tattar, Mark Mount
Michigan:	Andrew Jarosz
New Jersey:	Bradley Hillman, Donald Nuss, Shaojian Gao
New York:	Michael Milgroom, Stan Wirsig
Tennessee:	Scott Schlarbaum
Virginia:	Fred Hebard
West Virginia:	William MacDonald, Mark Double
USDA–CSREES:	Jack Barnes

The meeting was called to order at 8:00 am October 31, 1993 by Chairman Merkle. Dean Arnett C. Mace, Dean of the D.B. Warnell School of Forest Resources gave a welcome address. He noted that forestry in Georgia is a $13M industry. There are 23M acres of commercial forest land in Georgia, primarily in loblolly/slash pine. There is a move toward hardwoods such as tulip poplar, sweet gum and cottonwood. The problem with Georgia forestry is that demand exceeds supply by 18%, and consequently, a shorter rotation time for hardwoods is being considered to meet the demand for wood.

A letter from former President Jimmy Carter was read. It read as follows:

> *To the Scientists and Participants of the NE-140 Technical Committee on the Biological Improvement of Chestnut:*
>
> *I would like to extend my best wishes to all of you working toward restoring the American chestnut and participating in the meeting at the Georgia Center on October 31 and November 1, 1994.*
>
> *The American chestnut was once a majestic tree of great beauty that provided food for people and wildlife in addition to being one of Appalachian's finest hardwoods. Research and experimentation with breeding of the American chestnut is vital to the preservation of this national treasure.*
>
> *I support the research and breeding efforts of your respective organizations and hope that they will soon succeed in restoring this beautiful tree in the orchards and, ultimately, to the eastern hardwood forests.*
>
> *Sincerely,*
> *Jimmy Carter*

The data presentations are organized by Regional Project objectives and presented by station.

OBJECTIVE 1. To investigate the genetic determinants of hypovirulence (H) and their effects on the establishment and dissemination of the pathogen in the forest ecosystem.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

In June 1994, Anagnostakis, MacDonald and Nuss received official permission from USDA/APHIS/BBEP to test Nuss/ recombinant hv strains of *C. parasitica* in the forest to see if they can: a) survive; b) effect a biological control of chestnut; and, c) spread more efficiently than other hv strains. A large number of *C. dentata* were located in Sharon, CT in the Housatonic State Forest, a typical CT woodland, dense with an open understory.

Two plots were established with 12 trees (six pairs of trees) in each. In late June, three pairs in each plot were inoculated with strain 154 (MAT1-1, v-c 39) and three pairs were inoculated with strain 392 (MAT1-2, v-c 39). The treatment combinations are as follows:

Canker Strain	Treatment Strain
154 (MAT1-1, v-c 39)	146 [pXH9] (MAT1-2, v-c 9)
392 (MAT1-2, v-c 39)	155 [pXH9] (MAT1-1, v-c 40)

When the cankers had grown for 21 days, they were measured and plugs of recombinant strains were inoculated into the bark at top- and two-edges of one canker in each pair. These were measured again after 77 days of growth. Treatment in the second plot was by painting water-suspensions of conidia (10^7–10^8) from the recombinant strains onto the surface of one canker in each pair. Cankers were first measured and treated after 35 days of growth and again after 70 days. At that time, one canker (strain 392) with obvious perithecia was sampled by removing a strip of bark about 2 mm wide and 10 mm long. Twenty-seven perithecia were removed from this bark strip, and the ascospores included both orange and white phenotypes. The ascospores were plated out; 343 white morphology and 237 orange morphology. All of the white isolates and some of the orange isolates were transferred to hygromycin media. All but one of the white isolates was resistant to hygromycin while 213 or the orange were sensitive and 24 resistant to hygromycin. The mass isolate that resulted from the bark strip was orange. The cankers will be measured again and stroma examined the second week of November, 1994.

Donald Nuss, Roche Institute of Molecular Biology

1. Engineered Strain. He reported on the recombinant hv strains that he produced for field testing in CT and WV. The transformed strains have a plasmid that confers resistance to hygromycin B. The viral information is passed through mating and therefore, allows for virus transmission into many v-c groups.

2. Hypovirus Infection of Other Fungi. He also reported on a transfection system using electroporation to force RNA into spheroplasts. Transcripts corresponding to the viral RNA coding strand were synthesized from a full-length cDNA copy of CHV1-713 L-dsRNA in plasmid pLDST. Electroporation of spheroplasts derived from *C. parasitica* strain Ep 155 with linearized pLDST DNA failed to yield virus-containing transfectants. In contrast, electroporation with plasmid and viral transcripts, or DNase-treated transcripts, yielded mycelia that contained cytoplasmic-replicating l-dsRNA. These results demonstrate that a synthetic transcript corresponding to the coding strand of a mycovirus dsRNA can initiate an infection when introduced into fungal spheroplasts. Hypovirus infections were established readily in *C. parasitica* and in related fungal species not previously reported to harbor viruses. The fungi infected were: *C. cubensis* the causal agent of a canker disease of *Eucalyptus; C. havanensis*, also a pathogen of *Eucalyptus*; *C. radicalis*, a nonpathogenic saprophyte; and, *Endothia gyrosa*, a canker pathogen of *Quercus* spp. In addition to profoundly altering the phenotypic expression of the fungi, the

hypovirus also effected virulence. Infected and noninfected strains were inoculated into excised stems of pin oak and red oak. The following is a table of the virulence assay.

Stem Number	Pin Oak		Red Oak	
	Uninfected *E. gyrosa*	Hypovirus-infected *E. gyrosa*	Uninfected *E. gyrosa*	Hypovirus-infected *E. gyrosa*
1	6.89	0.60	22.28	2.96
2	6.01	0.94	19.82	2.81
3	5.67	0.55	24.92	2.47
4	7.94	0.84	20.43	2.01
5	4.58	0.61	26.66	2.40
Mean	6.22 ± 1.27	0.71 ± 0.17	22.76 ± 2.82	2.53 ± 0.37

3. GTP Binding Protein. GTP binding proteins are instrumental in causing increases and decreases of messengers. The accumulation of heterotrimeric GTP-binding protein α subunit of the G_1 class was found to be reduced in hypovirus-containing *C. parasitica* strains. A number of signal transduction and α sub-units (cpg-1 and cpg-2) were cloned. He looked at transformants and saw no difference in antisense constructs, but there were differences in the sense transformants. The results of the virulence assay, done on dormant chestnut stems for cpg-1 sense and antisense transformants is in the following table.

Strain or Transformant	Mean Canker Area (cm^2)
Ep 155	7.13
Ep 713	0.61
1310	0.56
1318	0.44
141	6.02
142	6.17

Data presented above as mean canker areas were based on six replicates. Transformants 1310 and 1318 contained transgenes while transformants 141 and 142 contained antisense transgenes. Transgenic co-suppression reduced the accumulation of this α subunit in virus-free fungal strains. Significantly, the resulting transgenic fungal strains also were hypovirulent. These results indicate a crucial role for G protein linked signal transduction in fungal pathogenesis and suggest a molecular basis for virus-mediated attenuation of fungal virulence.

Brad Hillman, Rutgers University

1. Taxonomy. Taxonomy for Hypovirus (family Hypoviridae) was approved last year by the ICTV. This is the first and only virus family that has no protein coat. The Hypoviridae encompasses three genera: CHV1-713; CHV1-NB58; and, CHV1-GH2.

2. Ep 747 (Matt Brown's work). Brown is studying the differences between Ep 747 and Ep 713. Ep 747 is phenotypically different from Ep 713 as it is yellower and smaller in culture. The virulence between Ep 747 and Ep 713 is similar. Brown compared the first 4,000 nucleotides and found them to be 88% identical. By sequencing and transformation, Brown found that there is virtually no difference in orfA and the first part of orfB. He still does not know what produces the difference between Ep 747 and Ep 713. Taxonomically, Ep 747 falls in a subset of Ep 713.

3. Small autonomous dsRNA (Jim Poloshock's work). Poloshock is working with three members of the Hypoviridae:

- typical 12.5 kb isolates
- C-18, a multicomponent genome with unique segments
- small mitochondrial DNA (2.7 kb) that is quite distance from other Hypoviridae

A comparison between NB58 and NB631 yields the following data:

	NB58 (12,507 kb)	NB631 (2728 kb)
Location	cytosol	mitochondria
Transmission	anastomosis, conidia	anastom., conidia, ascospores
Closest Relative	plant potyviruses	yeast T &W elements and coliphages

NB631 dsRNA is localized to the mitochondria. dsRNA can be isolated from mitochondria and no plasmids have been found associated with mitochondria. Transmission of dsRNA is very efficient through conidia, ascospores and mycelium.

Transmission of *C. parasitica* dsRNA elements through spores.

Isolate	% Through Conidia	% Through Ascospores
NB58	2-5	0
Ep 713	60-80	0
C-18	~30	?
NB631	100	50*

*only if NB631 is the female parent

Transmission of NB631 dsRNA by anastomosis is associated with mitochondrial recombination. Recombined mitochondria are stably inherited through conidia.

4. NB58F. NB58 produced a sector that is uninfectible with NB58. Using single ascospore progeny of a NB58 derivative that was infected with NB631, it could be infected by mating. At this point, the reason for the NB58F phenotype is not known. From CHEF gels, it can be shown that NB58 has an extra small chromosome.

OBJECTIVE 2: To study the ecology and physiology of *Castanea dentata* and ecology and dissemination of the pathogen, *Cryphonectria parasitica*.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

She reported on four items related to objective 2.

1. Cultivar test. Seventy-four grafted cultivars were planted in May 1993. The cultivars were representatives of local nurseries, planted with the hope that recommendations could be made to local citizens of the best cultivars for Connecticut. The trees were planted in a random design in blocks of four. Fifty-two of the 74 trees were lost due to the harsh winter conditions. Other trees, planted at the same time in tree tubes, exhibited no mortality. Tree tubes are lifted several inches in September to allow for air circulation and then the tubes are repositioned in the ground before winter.

2. Gall Wasp. A pest alert on gall wasp was issued by Sandra and Jerry Payne. Gall wasp has been reported in Tennessee, north of Chattanooga, in North Carolina, north of Brevard, NC, and near Asheville, NC; it has also been reported in Alabama. She and Scott Schlarbaum are beginning a breeding program to select for gall wasp-resistant trees. Sandra is focusing on chinquapin (*C. pumila ozarkensis*) and Schlarbaum is working with Japanese chestnut. The resistance trials will be outplanted at three sites: a TVA site in Tennessee, Bent Creek near Asheville, NC and Washington, GA.

3. Vegetative-Compatibility Types. Ursula Heiniger and Daniel Rigling from the Swiss Institute for Forest, Snow and Landscape Research in Birminsdorf, Switzerland sent Sandra their 12 v-c type. They are not compatible with any of the 93 v-c types at the Connecticut Agricultural Experiment Station.

4. Mating of *C. parasitica*. In order to answer the question, "When do cankers generally mate to produce perithecia?", she began Ep 155 virulent cankers on 13 May and spermatized every 2 weeks from 5 August to 5 October with either Ep 393 (cream) or Ep 146 (brown). On 9 November, all stems were cut and stored in cold conditions until perithecia could be examined.

Perithecia were produced on cankers spermatized from 5 August to 22 September, but no perithecia were found on cankers spermatized after 22 September. The experiment was repeated in 1994, with spermatization occurring from 6 June to 9 September.

Phil Gordon, New York Botanical Garden

His interest has been in survival mechanisms of organisms, wounding and healing. A survey of American chestnuts in the forests of Connecticut was begun in 1989 and by 1992, two million (50,000 fruiting population) had been found. American chestnut grows better on acid soil than any other eastern hardwood. American chestnut can be distinguished by the trichomes on the undersurface of the sun leaves. He is working on the survival mechanism of the whole organism.

He is growing chestnut seedlings at a nursery in Fenwick , CT.

Year 1 (1991)	He planted 3 rows x 95 chestnuts/row, 22" apart. Only a few chestnuts came up. He discovered he was planting his trees in a outwash glacial plane that does not hold water–germination was very poor.
Year 2 (1992)	It rained copiously in the spring and he observed that all the chestnut had come up. He discovered that chestnut seed becomes dormant when there is little moisture. Strange coloration (red, purple) is an indication the plants require fertilizer.
Year 3 (1993)	He trimmed all trees back to a single leader and the deer ate all the seedlings two week later.

With regard to nuts, they must be preserved at refrigerator temperatures, not frozen. In nature, nuts are subjected to rising and falling temperatures in the late fall, early winter. Sugar content and texture change over the winter.

Andrew Jarosz, Michigan State University

He is developing a mathematical model aimed at determining conditions that allow dsRNAs to spread within *C. parasitica* populations and predict evolutionary dynamics of dsRNAs infecting fungal populations. The model assumes:

- Pathogen infections increase plant death rate by some factor (e).
- The increase in plant death rate (e) due to infection(s) is positively correlated with the pathogen's transmission rate (β).
- The rate at which virulent infections are converted to hv (s) is related to the diversity of vegetative-compatibility groups in the pathogen populations.
- Plants will have only a singe pathogen infection (e.g. a single canker) that can be infected with dsRNA.
- Resistance to the plant will reduce the pathogen's transmission rate (β) but will not effect the rate of conversion from virulent to hv infections (s).

The model:

C = healthy chestnut
V = chestnuts infected without hv
H = chestnuts infected with hv
e = factor for plant death rate increased by infections

Rate of change for healthy plants:

$$dC/dt = (a_0 - a_1 [C + V + H](C + V + H)) - dC - \beta_V CV - \beta_H CH$$

Rate of change for plants infected with the pathogen but not dsRNA:

$$dV/dt = \beta_V CV - dV - e_V V - s\beta_H VH$$

Rate of change for plants infected with pathogen and dsRNA:

$$dH/dt = \beta_H CH + s\beta_H VH - dH - e_H H$$

The model suggests:

- The size of the tree population affects the probability that dsRNA will spread; the larger the tree population the easier it is to establish dsRNA.
- dsRNAs are more likely to become established in populations of slightly resistant trees.
- For biocontrol to work effectively, a highly debilitated fungus in not the answer because a low sporulating fungus must have a very high conversion rate to succeed.

Bill MacDonald, West Virginia University

He reported on the American chestnut trees at West Salem, Wisconsin. From 1992-94, 186 cankers have been treated at the West Salem site with a hv isolate (Wisc. 25-1/COLI 11-1) that was created by introducing a Michigan virus into the resident Wisconsin strain. In June 1994, new infections were treated that had arisen since June 1993. In addition, all cankers were sampled at multiple locations and the resulting isolates assayed for v or hv morphology. The results of those isolations are as follows:

Cankers Treated in	% Hv Isolates Recovered
1992	21%
1993	40%
1994*	7%

*sampled prior to treatment

We have confirmed by v-c testing that a single strain of *C. parasitica* still exists at the West Salem site. All isolates appear compatible and they also are readily converted in culture by the Wisc. 25-1/COLI 11-1 isolate. There is no explanation, at present, for the failure of the introduced isolate to convert the entire canker thallus.

Mark Double, West Virginia University

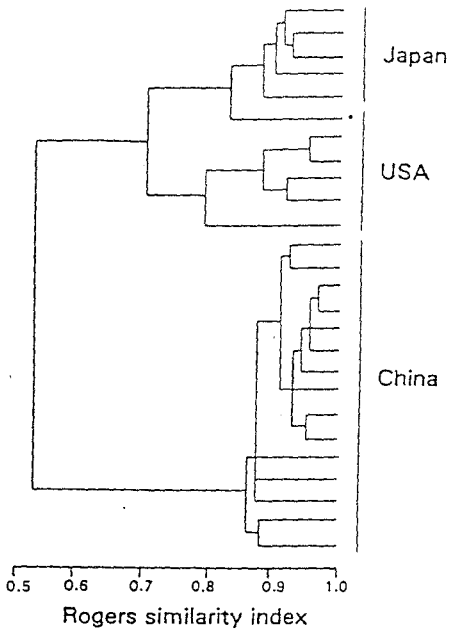
He reported on the cooperative experiment with Gary Griffin of Virginia Tech, in which cleared and non-cleared plots are compared to discern disease incidence and movement of hv isolates. In the spring of 1988, plots were established in a 5-year old clearcut with abundant chestnut regeneration. Of the 12 plots established, 6 were cleared of competing vegetation; the remaining 6 were not cleared. Four European hv isolates were inoculated in 3 cleared and 3 non-cleared plots by scratch-wounding 25% of the trees. New infections were sampled and isolate morphology examined twice a year, from 1988-94. The following conclusions can be made:

- The cut-over area is not 11 years old and tree mortality has reached ~95%.
- Mortality has been equal in all plots, regardless of the vegetation treatment.
- In excess of 1,300 cankers have been sampled over a 6-year period.
- Hv dissemination has been better on trees upon which hv inoculum was established
- In all settings, dissemination has been disappointingly low (~10%)
- Trees that contain hv inoculum have had fewer cankers.

As the experiment enters is 6th year, next generation trees >2.5 cm dhb will be added to the study.

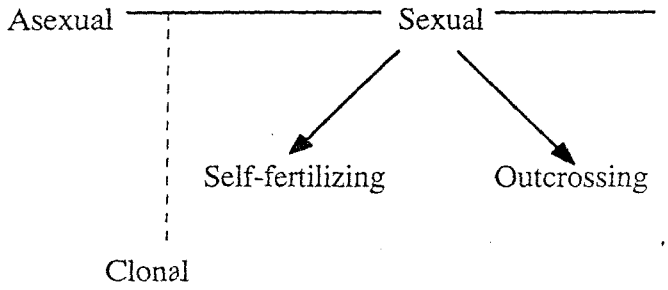
Michael Milgroom, Cornell University

1. Population subdivision in North America, Asia and Europe. He sample 15 populations of *C. parasitica* in eastern China, 5 in Japan, 9 in the eastern U.S. and one each in Switzerland and Italy. Sample sizes averages 20 per population, yielding a total of 755 isolates. The genotype at 8 RFLP loci were determined for each isolate, generating the following dendrogram:



The major findings from this study are *that C. parasitica* populations in the U.S. and Europe are more similar to those in Japan than China and that those in Japan and China are quite distinct. These results corroborate the claim that *C. parasitica* was most likely introduced into the U.S. from Japan on imports of *C. crenata*. The European isolates clustered more closely with the U.S. isolates, raising questions as to their origin. No correlation of RFLP to geographic locations in the U.S. Since chestnut blight has only been in North American for 100 years, there has not been enough time to establish diversity–it is still random.

2. Reproductive Biology of *C. parasitica.*

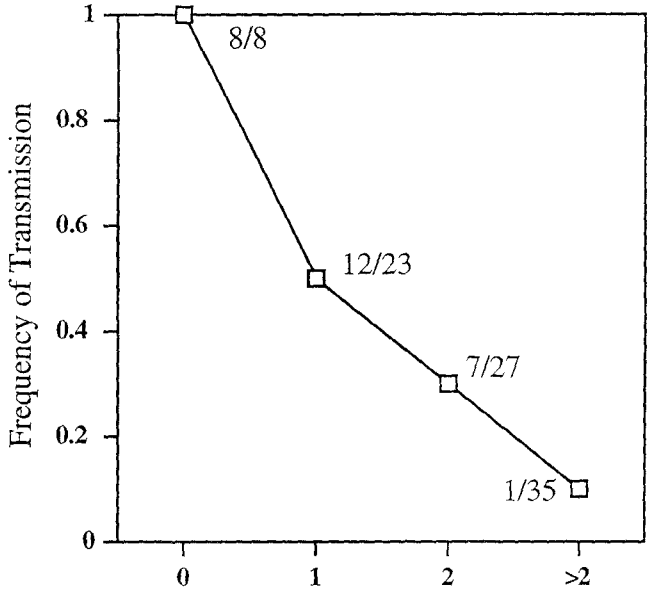


Inbreeding is a deviation from random mating on a continuum from random mating to selfing. Five populations of *C. parasitica* from the eastern U.S. and four European populations were looked at for segregation of various markers among progeny to estimate outcrossing rates.

Population	# of Perithecia	Outcrossed:Self	t
Depot Hill, NY	56	41:15	0.73
Danby, NY	25	17:8	0.68
Mt. Lake, VA	22	16:6	0.73
Parsons, WV-1	24	19:5	0.79
Parsons, WV-2	32	26:6	0.81
Lumino, Switzerland	30	29:1	0.97
Gnosco, Switzerland	27	24:3	0.89
Bergamo, Italy	26	22	0.85
Valdossola, Italy	26	20	0.77

He had not been successful in getting *C. parasitica* to cross when single-spore isolates are kept in culture axenically.

3. Transmission of hv within and among vegetative-compatibility groups (Yir-Chung Liu). The frequency of hv transmission is negatively correlated to the number of *vic* genes different between donor and recipient. The time it takes for transmission to occur is not correlated to the number of *vic* genes different.



If transmission occurs, it usually occurs within the first 1-2 days, regardless of the number of *vic* genes different.

4. Interactive particle-system model. A spatially explicit model was developed to model the transmission of hv viruses in order to determine how hv isolates should be released in natural settings. The model assumes that each individual site is a function of the states of each neighboring site, thereby making it an interactive particle model. Each site can be vacant (no tree), or occupied by an uninfected tree, tree with virulent canker, or tree with hv canker. Using the model, they found:

- V-c diversity effects transmission of hv.
- Spatially random deployment of hv works best in most cases of low v-c group diversity.
- Genetically engineered hv strains are generally more effective than cytoplasmic strains because 100% of the conidia are virus-transmitted.
- For 2 v-c groups difference, the random approach of dissemination works best.
- For more than 4 v-c groups difference, it does not matter, but random dissemination is the worst.
- Engineered strains will spread faster that cytoplamsic forms, even with more than 4 v-c groups difference.

Terry Tattar, University of Massachusetts

They tried to find hypovirulence in Massachusetts, but they have failed to detect any hv isolates. A *Trichoderma* sp. was isolated from a 35 cm tree in Sunderland, MA; this particular isolate is antagonistic to *C. parasitica*, as shown on PDA plates co-inoculated with the two fungi. The effectiveness of *Trichoderma* was tested in the laboratory, using bark and xylem tissue from

freshly peeled bark of American chestnut. Hyphal growth of *C. parasitica* was stopped within 2 days after application of the *Trichoderma* spores and when pretreated with *Trichoderma*, no *C. parasitica* growth was observed.

The *Trichoderma* isolate was used in the field on 5-10 cm trees in the Cadwell Experimental Forest in Pelham, MA. A *Trichoderma* spore suspension was applied to actively growing blight cankers and the cankers were then wrapped with Saran wrap. This was done in the fall of 1993 and samples were taken in November 1993, May and August, 1994. In approximately 50% of the *Trichoderma*–treated cankers, canker expansion had been retarded and *C. parasitica* could not be isolated. He believes there is potential for use with seedlings.

Mark Mount, University of Massachusetts

They plan to look at other microorganisms for biocontrol potential. Currently, they are looking at *Streptomyces tendae*, an Actinomycete that produces nikkomycin, a competitive analog of chitin synthesis; it prevents normal chitin synthesis in some fungi. Using a yeast extract/malt extract media (pH 6.8), it appears that nikkomycin has dramatic effects on cell wall formation of *C. parasitica*. They would like to insert the nikkomycin gene into *Trichoderma*.

Jimmy Maddox, Tennessee Valley Authority

TVA is a land and tree management group, headquartered in Norris, TN. He is out of the Muscle Shoals, AL office where he is involved with fertilizer development technology. Several years ago, their new director was interested in biocontrol with a focus on river water quality. Maddox's primary activity is with allelopathy with aquatic plants. Corollary activities include entomology and plant pathology.

Fred Hebard shipped *C. dentata* nuts to Maddox and they have been planted in pots and in conetainers. His data is as follows, 84 days after planting:

VAM Treatment (pots)	Height (cm)	Stem Diameter (mm)
GES 329	91.0	7.62
GET TVA	90.6	6.71
Control	100	8.95
VAM X_{Δ}	-9.2%	-20%

VAM Treatment (conetainers)	Height (cm)	Stem Diameter (mm)	Leaf Area (cm^2)
Acid Tolerant	26.7	4.0	627
GES 329	28.7	4.0	657
GES 31L	29.8	4.1	610
GET TVA	28.2	3.6	564*
VAM X_{Δ}	+16%	+15%	+23%

* Leaf scorch

One isolate burnt the leaves, similar to chlorine toxicity. This particular isolate enhances chlorine uptake; when muracid was discontinued, the problem was resolved.

Suggestions:

1. In terms of working with hypovirulence, the TVA might be able to work our massive dissemination techniques (i.e. helicopters). Maddox is looking at a 5-year project with applied technologies. The TVA is looking at chestnut at a reforestation species, especially at Cooper Basin, TN; they currently are screening legumes in that area.

2. Biomass. If chestnut could be stimulated sufficiently to get good growth, pole size material could be used and chipped for fuel. Under power lines, the TVA encourages agricultural activity. Could a chestnut nursery be established under a power line? The offer is open to interested individuals.

OBJECTIVE 3. Continue efforts toward developing blight-resistant chestnuts utilizing both tissue culture and traditional breeding methods.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

1. European Chestnut. The origin of *C. sativa* was probably the Caucasus Mountains between Russia and Georgia. She received 80 nuts from the Cavca Biosphere Reserve and she sent one-half of the nuts to Scott Schlarbaum. Of the nuts planted in Connecticut, 13 seedlings were outplanted. The trees are obviously distinct from *C. dentata*. Resistance to *C. parasitica* will be tested in 5 years.

2. Hybrid's Resistance. Canker expansion rate, caused by Ep 155, is generally 1 mm/day in Connecticut. Five *C. dentata* and 19 hybrids were inoculated with Ep 155 and canker expansion was measured. The data is as follows:

Tree	Canker Expansion (mm/day)
Castanea dentata	1.0–1.4
American x Japanese	0.8
American x Chinese x American	0.4

3. *C. dentata* Resistance. F. Hebard and Sandra have been comparing expansion rates of blight cankers on *C. dentata* sprouts in Connecticut and Virginia for three years. Three strains (Ep 155, Ep 389 and Weekly) were grown on PDA in CT and a set of cultures mailed to Hebard. Inoculations were done in the field in both states on the same day, using a randomized block design with 12 trees inoculated at each site. Canker expansion rates seem to be similar at both locations, indicating that there is little blight resistance in the two provenances of trees.

4. Breeding Progress. In 1993, her crosses resulted in 96 seedling, a 10% loss, attributed to drought. All of these crosses were for gall wasp resistance. She continues to get requests for seed from all over the world.

Scott Schlarbaum, University of Tennessee

1. Nut Plantations/Cultivar Test. He reported on his established nut plantations/cultivar test for disease resistance and nut productivity and evaluation for gall wasp susceptibility. The test will include 400 trees and 20 cultivars planted in Tennessee at: the Ames plantation; University of Tennessee; Tennessee State University; Tennessee Technical University; and, the Tennessee Division of Forestry. The Tennessee State and Ames sites are one-half planted; the remaining sites will be planted within the next two years. The plantings will be an incomplete block design with 5 trees/block. All sites will be managed as orchards.

2. Baiting Trials. He has been conducting baiting trials with turkeys in an area near Knoxville. He received bulk chestnuts from Greg Miller in Ohio that were sized into three classes. The chestnuts were put out with acorns, walnuts, milkduds, etc. for a preference test in 1993. The turkeys ate the chestnuts as well as anything else, but they seemed to have a preference for smaller chestnuts. In 1994, only chestnuts were used and the preference seemed to be chinquapins, followed by American chestnuts, followed by Chinese chestnuts.

3. Asiatic and Hybrid Climatic Chestnut Test. Evaluations were made of chestnut plantations that were established over the last six decades in eastern North America by J.D. Diller and R.B. Clapper. The plots range from Connecticut to Alabama to Missouri. He and Sandra Anagnostakis have measured all plots with one exception, the plot in Alabama. Generally, there is

1-3% survival in all stands. The remaining trees are 60–80 feet tall; some are still standing but are dead. His conclusion is that Chinese chestnut is a short-lived tree in the eastern U.S.

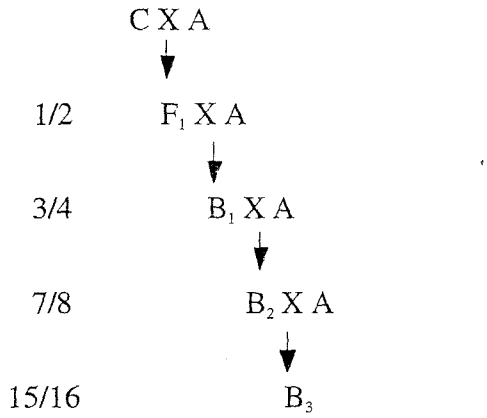
4. TVA Planting. Jerry Payne felt that gall wasp has been at the TVA planting near Chattanooga for about two years. The planting has been quasi-managed as an orchard since it was established in 1946-47. Schlarbaum has collected 12 families of chestnuts and he is looking at various characteristics.

5. Gall Wasp. Flowers and leaf buds are infested by gall wasp. It produces a toxin that can kill a tree, but generally reduces a trees' vigor. James Hill Craddock, a plant pathologist, most recently at the Institute of Tree Culture at the University of Torino in Italy, will be working with Schlarbaum, specifically with rooting and cuttings.

Fred Hebard, The American Chestnut Foundation, Meadowview Research Farm

He reported on his techniques used at the Meadowview Research Farm. He plows and fertilizes and then lays strips of plastic into which a hole is punched for the trees. He places a aluminum cylinder around the trees, piles soil around the cylinders to prevent them from blowing away and places Styrofoam cups over the cylinders. He has found that the cups positively effect plant emergence; he can get 85% emergence using the cups.

His breeding diagram is as follows:



He is working toward several lines of blight resistance, rather than a single line. Currently, he has 15 lines for 2 sources of resistance (B_2). The objective is to make third backcrosses and distribute B_3F_3 nuts.

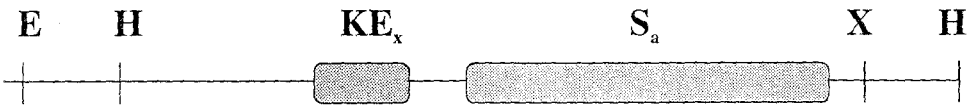
The B_3 have not been tested yet, but current evidence leads to 2 genes responsible for resistance.

Shaojian Gao, Roche Institute of Molecular Biology

He is looking at antifungal hydrolases in terms of host resistance. A polygalacturonase (PG) enzyme was purified from Ep 155 via several steps (ultrafiltration, cation exchange and gel filtration on Sephadex G-75) yielding a protein with a single band, 42 kDa. As little as 15 ng of the protein can be detected. The purified enzyme induced browning of the inner bark of chestnut. The enzyme also hydrolyzed isolated cell wall materials from both American and Chinese chestnut bark, *in vitro*. PG activity was detected in infected chestnut bark by a "cup-plate" assay and by isoelectric focusing (IEF) and overlay-gel activity staining. Ep 713 induced small cankers on American chestnut and produced small amounts of PG *in vivo* compared to its isogenic virulent strain, Ep 155. Lower PG activity was detected in cankers on resistant Chinese chestnut as compared to those on susceptible American chestnut. Tannin isolated from the bark of American chestnut inhibited PG more than tannin isolated from Chinese chestnut bark. A proteinaceous extract from Chinese chestnut bark was about 15 times more inhibitory to the *C. parasitica* PG that a similar protein extract from American chestnut. This PG inhibitory protein (PGIP) was active

also against the PG of *C. lindemuthianum*, but not the PG from *Rhizopus* spp. The PGIP from Chinese chestnut bark, eluted on Sephadex, yields 2 banks and is a basic protein.

In his more recent work at Roche, he cloned the gene to see what this enzyme is doing. The following is the restriction map of a 2.1 kb genomic DNA fragment that contains the PG gene.



Lou Shain, University of Kentucky

PGIP might be a possible source of host resistance. To test this, a Chinese x American chestnut F_1 and 12 F_2s, provided by F. Hebard were assayed for PGIP activity against purified *C. parasitica* PG. The F_1 was intermediate in PGIP activity between American and Chinese chestnut (cv Nanking) as shown in the following table.

Polygalacturonase Inhibitory Protein (PGIP) in Bark of American and Chinese Chestnut.

Sample	Species	% PGIP Inhibition
Nanking	Chinese	100
12/1/89	Chinese	100
3/9/90	Chinese	92.6
6/22/90	American	100
Nanking x American	American	75.3
CCR	American	42.4
12/1/89	American	13.0
3/9/90	American	31.5
6/22/90	American	19.8

This very limited sample suggests that PGIP may be inherited quantitatively and therefore may be controlled by multiple genes. All genotypes designated as resistant to blight by an inoculation test were high in PGIP activity as seen in the following table.

Polygalacturonase Inhibitory Protein (PGIP) in Bark of some F_2 American x Chinese Chestnut.

Sample	Disease Rating	% PGIP Inhibition
12	Resistant	99.6
70	Resistant	98.4
907	Resistant	73.1
395	Susceptible	38.8
600	Susceptible	50.0
21	Susceptible	14.4
389	Susceptible	35.4
901	Susceptible	31.9
387	Susceptible	97.7
292	Susceptible	97.4
459	Susceptible	95.9
879	Susceptible	89.7

This may suggest that PGIP may be necessary but not sufficient for host resistance.

Scott Merkle, University of Georgia

His approach to resistance is from the side of the host plant. He believes that a combination of hv and a resistant host will be the best way to develop a timber-type tree. He is trying to develop a gene transfer system via somatic embryogenesis (*in vitro* regeneration system). Somatic cells go through maturation, and his laboratory has been working with various tree species, such as Yellow poplar, magnolia and oak. He has X-plants from immature embryos from Wisconsin, New York, Connecticut, Pennsylvania, North Carolina and Georgia.

His laboratory is working on two projects simultaneously and hoping to bring them both together:

- a regeneration protocol
- a gene transfer protocol

1. Regeneration protocol. Growth regulators are used to produce and proliferate early globular stages and then are removed to allow the developmental stages to occur. He is trying to find the optimum zygotic stage to use as the embryo and he has found that the early stages work best. Embryos go though various stages, such as early globular stage to heart stage to torpedo stage. There are up to 20 ovules in a chestnut fruit; usually one becomes dominant. On 2,4-D medium, an ovule will clone itself and form a mass. Shake culture is used to separate proembyogenic masses. Then, they size fractionate the masses onto filter paper to separate the embryos. They are still testing treatments by using several carbohydrates. They have found that fructose is favored over sucrose. Their best "seedling" came from the following treatment: a 10 week cold treatment followed by a 6-hour desiccation. They are still looking at various potting mixes.

. Gene transfer protocol. He needs cultures of globular cells that are synchronous. He uses a "gene gun" system, whereby he bombards plated material with gold microprojectiles that are coated with plasmid DNA, that encodes a selectable marker. The DNA is attached to a carrier that is forced into the cells via high-pressure helium. He assays for transient GUS expression 2 days following bombardment. He then transfers his bombarded material for selection and he looks for kanamycin resistant colonies. He uses the following substrates in his analysis:

GUS Assay	**Substrate**
Histochemical	X-Gluc
Fluorometric	MUG
Spectrophotometric	p-NPG

He has the gene transfer protocol worked out. He now need to select a gene that will be useful (antifungal properties). His problem is the regeneration of plants. He can occasionally get entire plants, but that is rare. He is continuing to work on the regeneration problem while searching for using a useful gene to insert.

Business Meeting

Terry Tattar was elected secretary for 1995. Chairman-elect for 1994 is Bradley Hillman; the 1995 meeting will be hosted in New Jersey.

John Anderson, Administrative Advisor. He believes that the NE-140 research is focused, it is noble and it is an important scientific problem. He believes that we aim high and the research borders on the exceptional. He senses the patience of some working in classical plant pathology. The project meets all qualifications with good cooperation. He had the following suggestions:

- limit lecture and debate to adhere more to the agenda
- arrange the program by objectives
- everyone should have an official copy of the project
- the "official" minutes need to be shorter, limited to 1–1 1/2 pages that includes
  - -agenda
  - -those in attendance
  - -date and time of next meeting
  - -officers
- the minutes need to be out within 30 days

- need to issue a progress report (3 pages)
  - include the facts essential to the objectives
  - plans for next year
  - list of publications (not included in the 3 pages)

Jack Barnes, CSREES. The Secretary of Agriculture, Mike Espy signed orders which begin the implementation of the reorganization of the Department of Agriculture. "Through this reorganization, we will save taxpayers $3.6 billion and improve service to our many diverse customers," said Espy. Reorganization of the USDA will eliminate 14 USDA agencies, close 1,100 field offices and reduce staff by up to 11,000. There are some programmatic restructuring problems in CSREES; how can you separate science programming and funding? The acting administrator of CSREES is Dr. Bill Carlson.

Hatch and McIntire–Stennis funds are at the same level as 1994. Most other funding has been reduced from 1994.

Fund	1994 (in million)	1995 (in million)
IPM	$3.04	$2.731
Pesticide Clearance	$6.345	$5.711
Water Quality	$1.474	$1.327
Pesticide Impact Assess.	$1.474	$1.327

In competitive grants for 1995:

Plant Systems	$37 M
Animal Systems	$23.1 M

The meeting was adjourned at 12:00 pm on Tuesday, November 1, 1994.