NE-140 Regional Project Meeting: Mountainbrook Inn, October 25-27, 1989, Gatlinburg, TN

- Dennis Fulbright-Chairman
- Lou Shain-Secretary

Objective 1: Hv, dsRNA, Dissemination of Pathogen

Peter Bedker-Rutgers University

He started looking at American chestnut in 1988, and began by sampling swollen superficial cankers in New Jersey, and he found some of the cankers contained dsRNA. He mentioned that *Endothia parasitica* was very easily isolated from a large tree with swollen superficial cankers.

He chose four sites in New Jersey and worked with 30 isolates from each site and tested them for virulence in apple and found that 45 of 120 isolates had significantly reduced growth, compared to Ep 155. He found isolates from superficial cankers that were more virulent than Ep 155, and conversely, he found less pathogenic isolates from normal looking cankers (he was not certain if the trees were pure American chestnuts).

He extracted from the 45 isolates that had reduced virulence and only two contained dsRNA. He is in the process of single sporing to try and get a handle on the biology of the isolates.

He found two additional dsRNA-containing isolates (they came from three trees at two sites in Monmouth County) and labelled one of the isolates NB88-58. He compared these four dsRNA-containing isolates and they had a single dsRNA band in excess of 13 kb-similar in size to the largest band in Ep 713. He did spot hybridization on nylon and probed with randomly primed cDNA from NB88-58, and under highly stringent conditions the cDNA from NB88-58 only hybridized with RNA from NB88-58.

Brad Hillman made cDNA clones of NB88-58, constructed restriction maps and is in the process of sequencing clones. He has 4 of 13 kb of the genome sequenced, and it includes both ends. He is comparing with Ep 713, and right now there are regions of great similarity, as well as regions that are not very similar. At the nucleotide level, there is 50-60% similarity between NB88-58 and Ep 713, suggesting a common progenitor.

Colin McKeen-Ontario

He formed a chestnut council in Canada.

The area in southern Ontario is the northern range of the American chestnut, and they have quite a few blight-free trees (27" dbh is the largest with many in the 15-20" range).

The Arnd tree has been infected for at least 10-15 years. It has very rough bark and yields Hv isolates. Isolates from this tree have oppressed growth-it grows only one-fifth the rate of a virulent isolate (in apple).

He finds many basal cankers that involve more than half the diameter, but they seem to produce callus as well.

He uses a combination of mud-compresses and Hv inoculations to control canker expansion.

He found many isolates with a white growth on the edge of the cultures, and he wondered if it was a contaminant-Sandy Anagnostakis offered to help.

He is growing chestnut seedlings in a box on the south side of his house-these will be transplanted next spring.

Scott Enebak-West Virginia University

He wants to characterize dsRNA from WV isolates, and he is using isolates with single dsRNA bands that are 10 kb.

He looked at cultural morphology and found that morphology is quite variable and many of the isolates are highly lobey.

He chose 89 isolates that contained dsRNA and determined the virulence of 50 of the 89 in dormant excised stem pieces and golden delicious apples, and found little correlation between the stem pieces and the apples. He found a couple of isolates that are as low in virulence as GH2, and several that are move virulent than Ep 155. From these 50, he chose two with low virulence, two with moderate virulence and two with high virulence for inoculations in a pathogenicity study in living trees.

What effect does the dsRNA have on the fungal host and what is the dissmemination potential of the above six isolates? He is in the process of single sporing these six isolates to obtain both dsRNA-containing and dsRNA-free conidia, from each isolate, for paired inoculates in living trees.

Do cankers start out containing dsRNA or is it acquired later, and is dsRNA related to developmental age? He chose 11 stromal and 11 pre-stromal cankers and obtained two isolates from each canker and extracted for dsRNA and found: 70% (16/23) of stromal cankers contained dsRNA and only 5% (1/19) of pre-stromal cankers contained dsRNA.

Neal Van Alfen-Utah State University

1. Characterization of VIR1/VIR2 (stands for Virulence)
   a. Overlapping coding sequence
   b. Different 5' ends-they are in the process of mapping the 3' ends
   c. Genes have been deleted (400 bp region that has been inserted in a selected marker)
   d. They should have been named Spo1/Spo2, since they don't affect virulence, but sporulation
   e. Transcription run-off studies-isolating nuclei and looking for transcripts in vitro. Does the dsRNA affect trasnscription? They have worked out the system to answer this question and they are ready to start.
2. Cryparin-lechtin

They have 50 amino acids sequenced. They have developed an antibody which is good for histochemistry, but not very good enough to pull out a probe.

3. Laccase-polyphenyl oxidase produced by Virulent strains but downregulated in the presence of dsRNA
   a. one isozyme close to purity
   b. extracellular
   c. differential expression

Polymerase Studies:

1. Nature of products of RNA polymerase:
   a. full-strength ssRNA, plus sense
   b. some evidence for negative sense products
   c. full-length ssRNA is released
2. Nature of RNA polymerase
   a. replication of intermediate formed
   b. strand displacement synthesis
3. Purification of RNA polymerase
   a. Complex of RNA-protein can be isolated with polymerase activity
   b. Partially purified active complex has two proteins

Population Genetics Studies:

1. Mitochondrial DNA is polymorphic
   a. Random within mitochondrial genome (40% of the random clones detect RFLP's)
   b. All RFLP's are length polymorphisms
   c. Isolated populations are not polymorphic (interaction with other populations is necessary for polymorphisms)
   d. RFLP types are not a function of geography
2. Nuclear DNA does not appear to be polymorphic

They looked at RFLP's from 10 trees at the National Colonial Farm and found 4 different RFLP types.

Dennis Fulbright-Michigan State University

Don Nuss at the Roche Institute has sequenced all of Ep 713, and has found 4 open reading frames.

Fulbright has been at the Roche Institute for a 6 month sabbatical and he decided to clone the 9 kb segment of GH2 for his sabbatical work.

He has some isolates that lose certain typical GH2 bands, and some of the isolates with missing bands are less pathogenic than GH2 and some are more pathogenic.

-He isolated the top band of GH2 on acrylamide and electroeluted.

-Used reverse transcriptase and turned into cDNA.

-Radioactive labelled to look at the restriction sites.

-Out of 9 kb, he has 7 kb cloned into DNA; checked with Ep 713 and found no direct sequences.

-Wants to eventually go back to the isolates that have lost bands and see how similar they are.

In an orchard where GH2 was placed in 1982 (Sally Garrod's plot), they found an isolate in 1986 with a band that is heavier than the top band in GH2. The isolate is very debilitated and has a unique morphology. The isolate was found in a natural canker near the soil line on a tree that was

not inoculated with GH2, but has a number of cankers on it that have the GH2 banding pattern.

Chris Durbahn-Michigan State University

She is cloning dsRNA from RC1 (Ross Common)-has 2.8 and 1.3 kb bands. It is very different from other hv isolates in that the largest band is less than 3 kb (most isolates have a heavy band that is 8 kb or greater). She wants to determine if there are any open reading frames.

She isolated the two bands, electroeluted, added poly T's and used a vector to cut at PST1 site and got about 400 clones. The RC1 dsRNA probe was lighting up everything with both radioactive and nonradioactive systems, so she is attempting to work out the problems.

Nini Mahonti-Michigan State University

She is working on mitochondrial plasmids, and using CL 25 (Crystal Lake). She has found that CL 25 has no multisegmented dsRNA, although it can be used as a biocontrol agent because it is transmissible. They have extracted for dsRNA many many times and have never found it.

The factor in CL 25 is:

-transmitted in 20% of the conidia

-maternally inherited in sexual crosses

-cytoplasmically transferred via hyphal anastomois

In mitochondrial DNA isolations:

-treated with DNase

-broke open the mitochondria

-found a plasmid in the mitochondria-actually they found two plasmids, 6 and 10 kb

-the plasmids have no homology with the mitochondrial DNA

-they tested strains from Europe, Michigan, West Virginia and Maryland, both virulent and hv, and have found the plasmid in every strain.

-the plasmids are very conserved-they hypothesize that they are circular, and have no direct affect on hypovirulence.

Sandra Anagnostakis-Connecticut Ag. Exp. Station

She was sent five isolates of *Endothia parasitica* from Beijing, China. She converted the isolates with both Italian and French hv and sent the isolates back to China with the understanding that they are to work with the isolates only in the laboratory.

She still gets letters from nurserymen around the country stating that Conn. Ag. Exp. Sta. (CAES) sent them hv slurries in years past, and now they want more. She has little idea what they were sent-she has to go back over the files of Dick Jaynes and Jack Elliston to see if she can find out what these people were sent. She stressed the importance of keeping track of where hv is going for future reference. They are now reporting hv in New Jersey, and CAES sent hv slurries to certain areas in NJ in the past and what we are now seeing may simply be dissemination of those hv strains.

Mark Double-West Virginia Univesity

In conjunction with Gary Griffin at Virginia Tech, 12 plots were established in the spring of 1988 in a 5-year old clearcut in Pocahontas Co., WV to look at dissemination of hv. Six plots were cleared of all competing vegetation and only chestnut was left standing. Six plots were not cleared. Hypovirulent strains were introduced in three of the six cleared plots, and three of the noncleared plot. The remaining six plots (three cleared and three noncleared) had no hv introduction.

The purpose of these plots is to test Dr. Griffin's hypothesis that in areas where competing vegetation is removed, chestnut will continue to sprout, which will support a population of *E. parasitica*, and subsequently, hv will be supported by the high level of the host fungal population.

Four hv strains were used in the hv introduction plots, and those four strains collectively were able to convert 60% of the virulent isolates in our culture collection, *in vitro*. The idea behind using a number of hv isolates with broad conversion capacities is to try and cover as many v-c types in the wild as possible and give hv a distinct advantage. Many dissemination studies have been conducted in the past in WV, but the hv isolates diployed had narrow conversion capacities.

In the fall of 1988 and the spring of 1989, new infections were sampled and isolate morphology examined. Three percent (2/63) cankers from cleared/hv introduction plots yielded hv isolates in the fall 1988 sample, compared to 14.6% (7/48)

and 21% (5/16) from the cleared/hv and non-cleared/hv introduction plots, respectively. No hv isolates have been recovered in the plots without hv introduction.

In addition, canker morphology was evaluated at the onset of the experiment; only 7 cankers were noted as apparently superficial. These observations will continue annually along with mearurement of basal area of chestnut and competing vegetation.

William MacDonald-West Virginia University

Dissemination tests established in July 1982 were designed to measure the spread of V and European hv in a partial canopy setting. Dissemination was evaluated by sampling new cankers 2 times/year and examining the morphology of the isolates recovered. Hv strain dissemination continues to predominate (75%) of new infections on trees where hv inoculum was established in 1982. In contrast, cankers on adjacent trees that received no hv inoculum have largely been classed as V (92%). To date, disease progress has been comparable among all treatments; there does not appear to be significantly greater survival of hv inoculated trees.

Gary Griffin-Virginia Tech

In his test areas he has managed plots(competition continually removed) and control plots(no management of competing hardwoods) and he reported on the data after 10 years, in terms of the number of superficial cankers.

% Apparent Superficial Cankers/Sprout Cluster

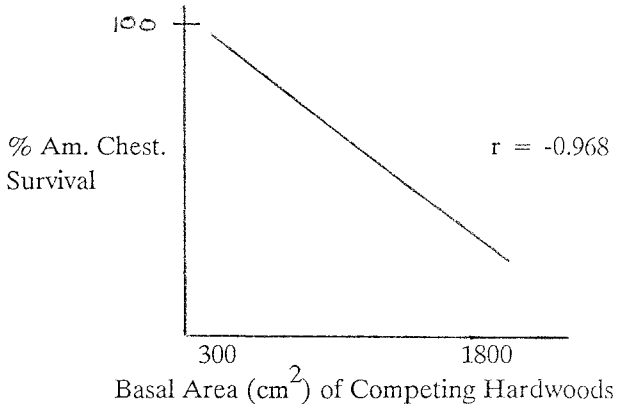
Treatment	Location		
	Parsons	Marlinton	Sinking Creek
Managed	89	19	17
Control	0	0	0

Survival of Am. Chestnut Sprout Clusters (%)

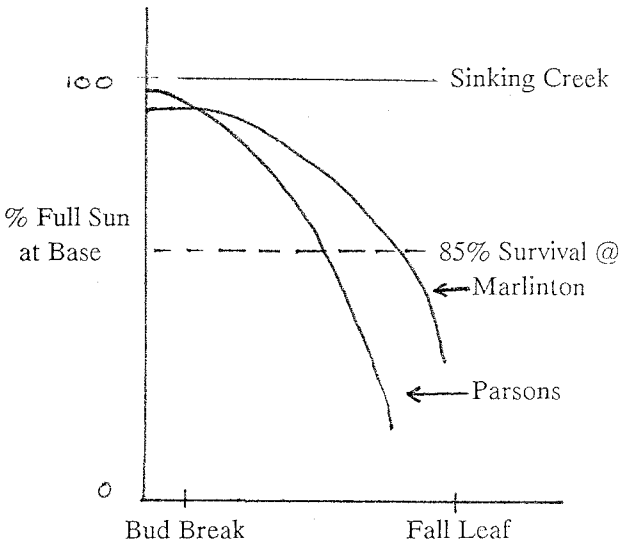
Treatment	Location		
	Parsons	Marlinton	Sinking Creek
Managed	70	85	89
Control	0*	50	54

*Not a single living stem

To determine hardwood competition he measured the basal area and plotted against the survival of American chestnut and the following graph resulted:



He also feels that light and water are important in competition of hardwoods and Am. chestnut, and he measured the % full sun at the base of the trees at various times during the year for the three sites: Parsons, Marlinton and Sinking Creek (all are 14-year old forest clearcuts).



He also reported that deer browse is severe in clearcuts.

He also reported on mites, and stated that 30% of the isolates that were isolated from mites were low in virulence. Two of three isolates examined were positive for dsRNA. The one isolate that was negative for dsRNA was inoculated into stems, and the resampled at 5 places on the resulting canker-some of the reisolates had dsRNA and some did not.

Dr. M. A. Khan, a visiting scientist from India spent a year in the lab of Dr. Griffin examining dsRNA from 60 apparently superficial cankers. He also conducted pathogenicity tests with these isolates and it looks like 20 of the 60 have reduced virulence.

Objective 2: Growth & Physiology of Pathogen; Responses of Castanea

Sandy Anagnostakis-Connecticut Ag. Exp. Station

She reported on the 100 Am. chestnut seedlings that were planted at Rocky Hill (4 blocks, 5 rows in each block and the seedlings planted 5 feet apart). Two of the blocks were covered with shading (to cover 37% of the sunlight) and two blocks were unshaded. She plans to inoculated the seedlings in two years (with Ep 155 and Ep 748-hv) and look at the rate of canker growth.

Lou Shain-University of Kentucky

He stated that there are large differences in susceptibility between American and Chinese chestnuts and the hypothesis is that there is some compound(s) that is induced in the infection process. In order to try and test this hypothesis, they took bark strips of chestnut with the periderm removed and challenged with both v and hv. They also gassed some of the strips with ethylene (done over increasing periods of time) and after challenging, the stips were dried, ground and examined for putative, phytoelexin compound. They found a compound, but the results were not repeatable.

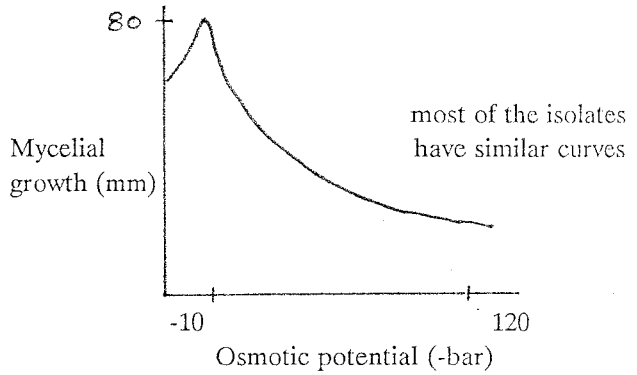
He also looked at proteins from chestnuts challenged in a similar fashion as above. He found two bands similar in weight to kitinase and gluconase. He then did amino blots:

Protein ---nitrocellulose---antibody added

Found that both species (American and Chinese chestnut) are capable of producing kitinase and gluconase. He is now trying to discern if one of the species produces more quantity of kitinase or gluconase.

Shasjian Gao-University of Kentucky

He is interested in water stress. He noted that canker expansion differs at different times during the year and maybe water has an important role in the disease process. He tested the osmotic potential of six *E. parasitica* isolates.



When he added NaCl or KCl, he noted that the hv isolates show a different sensitivity.

He also tested conidia germination and found that conidia were less sensitive to osmotic potential. He stated that conidia can germinate at, as low as -60 bars, but the germination process takes much longer. When he added NaCl, some of the hv conidia failed to germinate at -60 bars.

He also examined water effects on canker expansion-he treated excised stems with Polyethyleneglycol at 0, 30, 40, 50 and 60 grams PEG/100 ml of water. He dipped the lower end of the stems into the appropriate PEG/water mixture and sealed the upper ends of the stems with wax. He then inoculated the stems with Ep 155 and found that canker expansion was greater in the PEG treated stems, and the largest canker expansion was with 40 grams PEG/100 ml water.

PEG	Canker Size (mm2)
0	small canker
30	750
40	1075
50	900
60	640

He reported using non-dormnat stem pieces with good results.

Graciele Foulas-Virginia Tech

She reported on her hypothesis that *E. parasitica* utilizes tannins as a carbon source. She is looking for an enzyme that is produced by *E. parasitica* that breaks down tannin into gallic acid and glucose. She reported on her methods. She looked first at different extraction methods and found the following:

Method	Protein Concentration (ug/ml)
Turrax homogenization	20
Grinding with sand	40
Sonication	80
Braun homoginization	170

She chose sonication.

She then measured the activity of the enzyme by measuring the amount of gallic acid formed and found the maximum activity was at day 6 for a standard v strain (WK).

She found the optimum pH for the enzyme production was 5.5.

Now that she has a base line method for assaying the enzyme, she will continue with her work on tannin utilization by the fungus.

Bill Powell-College of Env. Forestry-SUNY

He has two major interests, fungal transformation and electrophoretic karyotyping.

1. Fungal transformation and vector construction studies yielded the following data:

Vector	Transformant/microgram
pHA2	1.5×10^6
pHRC12	1.3×10^5
PFT1	3.7×10^3

He used the following isolates and found that they all transformed at about the same rate: Ep 155, Ep 713, Ep 287, 501-3, Ep 67, Ep 29, PA1, and PA2. He stated that the rate of transformation for *C. parasitica* was far greater than for other fungi, such as *Fusarium* sp.

2. Electrophoretic karyotype using (CHEF) Contoured Clamped Electric Field. He uses pulse-field electrophoresis to separate DNA. The larger the DNA the longer it takes to get oriented in the field and begin moving. For chromosomal DNA, he used continuous switching times of 60 minutes for 14 days. He found a distinct chromosomal pattern of 7 bands that range from 3 megabases to 13 megabases. He indicated that *C. parasitica* has at least 7 chromosomes, and there may be more. He compared the *C. parasitica* isolates with *C. cubensis* and found that *C. cubensis* was distinctly different-none of the bands matched.

Fred Hebard-American Chestnut Foundation, Farm Superintendant

He reported on some histology work. Both Chinese and American chestnut form lignified zones and they respond equally to the fungus, but, the fungus responds differently in the trees. The fungus forms mycelial fans much more rapidly in American chenstnut and the fans move through the lignified tissue.

The mean dry weight of a v strain (Ep 155) cultured for 14, 22, and 29 days on filtered bark broth of Chinese and American chestnut

Culture Age	Bark Broth	
	American	Chinese
14 days	121	195
22	113	111
29	98	117

Chinese broth supported at least as much growth as the American broth.

He raised the questions: How does the fungus kill the bark? and Is Chinese chestnut different from American chestnut? A convenient assay for a toxin is via ethylene evolution by the host since the stimulus of ethylene production is at a distance close to the canker center.

Dick Rhode-Administrative Advisor-U. of Massachusettes.

NE-140 has just finished the 2nd year of a 5-year project. He explained that money comes from the USDA to Experiment Stations and a 25% formula is used, in that 25% of those funds are made available for research with other states. He clarified that joining a regional project does

not automatically qualify a researcher for money, it just allows their Exp. Station Director to fund travel to regional project meetings, etc.

Congress passed a continuous resolution to fund the budget until Oct. 25. He said that the deficit reduction sequestered 5.3% of the budget. It looks however, that the Hatch Fund money will be the same as last years-actually it has been at the same as it has been for the past 8 years. He mentioned at 0.47% reduction in the budget will be used for the war on drugs.

He talked a little about the National Research Concil's report: **Investing in Research**. If Congress puts money into research, it will pay off. The report recommends $500 million for agriculture in the 1991 budget. The report states that the United States is losing its competitiveness in the world, and this money would be for competitive grants, for both individuals and interdisciplinary groups.

Merritt Nelson-USDA/CSRS

He reiterated that NE-140 is a model for what regional projects are supoosed to be, and he stressed that interdisciplinary approaches are the way of the future.

He talked a little about funding, in that the approach for funding is to work through the system, but he cautioned that it is a very cumbersome approach, but not to despair.

Sandy Anagnostakis-CAES

She is still working on oxalic acid production by *E. parasitica.* She is attempting to find a liquid system that is suitable for radioactive labelling. She is using 11 strains of *E. parasitica*. One of the strains is almost avirulent (methionine requiring), and another strain is lower in virulence that normal (cream and temperature-sensitive). She is also using 9 standard strains (3 normal; 3 with Italian hypovirulence; 3 with french hypovirulence). She is using autoclaved cellulose sponges , covered with PUDO cellophane, onto which she is inoculating the fungus. Her results are that the avirulent strain does not produce much oxalic acid, and the flat mutants that she is using produce a fair amount of oxalic acid.

In her isozyme experiments, she is looking at peroxidase enzymes. She is using only agarose, and she has seen peroxidase differences in different strains.

Dennis Fulbright-reporting for Andre Vannini

Vannini, a visiting scientist from Italy, worked in Fulbright's lab for a year, studying oxalic acid. He brought several isolates from Italy, all homologous and found that the hv strains didn't produce much oxalic acid, in comparison with v strains. He took single spores from isolate TR55/a (from Italy) and compared the amount of oxalic acid produced by several American hv strains.

Strain	Oxalic Acid mg/gm dry wt. mycelium)
Cl1-16 (v)	20.6
GH2	8.5
R1	7.7
E-6	7.7
M18 (dsRNA free)	9.5
M14 (dsRNA containing)	16.0
M7 (dsRNA containing)	3.0
TR57/a	3.0

He found that polyphenoloxidase is elevated in chestnut trees inoculated with hv. Laccase is an inhibitor of polyphenoloxidase and Vannini found that with hv, laccase is shut down and polyphenoloxidase is allowed to increase.

Philip Gordon-New York Botanical Garden

In the last 19 months, he has logged 32,000 miles in Connecticut alone looking at chestnut trees, and he has discovered a large ingression of foreign germ plasm. He discerns American chestnut by means of the exclusion principle-you know what's not American, and you proceed by excluding species.

Chestnut is hard not to find. He is finding seedlings coming up, growing into trees, flowering, and some of the trees are 24" dbh. He calculated (from a 2 square mile area) that there are 2,000 fruiting American chestnuts in Connecticut.

The state of Connecticut is now committed to restoring the American chestnut, and he proposed a scenario for the restoration of the American chestnut: raise seedlings in the state nursery and plant them in state forests, in addition

to selling seedlings directly to the public (being sure to inform the public that these trees will probably succumb to the blight). Foresters will report all flowering chestnut trees on state and private land. By working with the Nature Conservancy in terms of land use, seedlings can be grown everywhere.

Jerry Payne-USDA, Byron, GA

He reported on ten years of experimentation on the oriental gall wasp, *Dryocosmus kuriphilus*. The wasp was found in Georgia in 1974, and subsequently spread to Alabama, averaging 15 miles per year. The wasp seems to move much better to the north than it does to the south.

Chestnut is the most important nut tree crop in the world-it has the most acreage worldwide. The U.S. imports 20 million pounds of chestnut each year. The gall wasp is the most important pest worldwide and there is no control.

The insect is 3 mm long, shiny black, and has 14 segments in the antennae. Only females have been found in North American, so all you need is one insect in an orchard. The gall wasp attacks all *Castanea* species. It overwinters in the galls, and comes out of the galls in May-June (95% come out the last week in May to the first week in June). The adults lay eggs in the axillary buds by inserting the ovipositor between the bud scales. In 40 days they hatch to instar larvae. There have been many treatments to the buds, but they have found no treatment that will kill the insect but not kill the bud. In every instance, when the insect is killed, so is the bud.

With gall wasp infestation, instead of getting normal bud break, you get a gall formed which shunts nutrients to the gall. The cherry red color of a bud (on a Chinese chestnut) is usually a good indication of a gall. Leaves from a gall are very small, and it not uncommon for every single bud on a tree to be infested.

Gall wasps will attack leaves and catkins as well.

Payne reported that they have attempted two methods of control:

1. They tried to cause the trees to break bud extremely early-in December, and maybe the wasps would come out and then freeze, but he found that 32 days later, galls had formed.

2. He decided to wait until bud break and then defoliate the trees. He reported that this is good control, but there is only a 2-day window for the control to work.

He reported that the percent loss has increased dramatically:

Year	Pounds of chestnut/acre	% Loss
1975	2729	0
1976	1454	47
1978	917	66
1979	729	73
1980	168	94

He is trying to use the gall wasp parasite (it has a very long ovipositor that it iserts into the gall, killing the wasp). The problem is that in Gerogia the gall wasp parasties often come out in the winter-they find no galls, and fly off looking for other hosts.

He also reported on the use of systemmic insecticides-he used levels high enough to kill the limbs, but the gall wasp still survived.

He cautioned that we might be bringing back the chestnut, but we had better consider the possibility of attack by the gall wasp, and right now, there is no control.

Objective 3: Breeding Technologies; Tissue Culture Work

Scott Schlarbaum-University of Tennessee

He stated that he has had terrible luck with successful grafts. He has coated the entire root stock and scion with wax with the hope that it would increase his success rate. He grafted 106 American chestnuts and had 14 survive; grafted 126 sweetheart chestnuts and 29 survived; grafted 99 Chinese and hybrids and 39 survived.

Tom Hall-Tennessee Tech

He decided to put together a plantation in terms of a horticulture crop. They collected nuts for food quality and are looking at 7 sites in Tennessee to plant the trees: 4 sites owned by the University of Tennessee, one site at Tennessee Tech, one site at Tennessee State, and one site at a nursery research station. He is looking for superior nut producers, both in terms of quantity and quality, and he is interested in material for breeding program as well.

Gary Grffin-Virginia Tech

He reported for Jay Stipes on *E. parasitica* on live oaks, and they are currently using nitrogen to increase the soil fertility.

He also reported that John Elkins went down to a large tree (36" dbh) in Wilkes County, NC and collected som scion material for grafting, but he lost some of his grafts to Hurricane Hugo. Elkins does intend to do some inoculations at a later date.

Dennis Fulbright for the National Colonial Farm

In a pathogenicity study several years ago, eight trees were inoculated and seven of them have died. The eighth tree is still living and producing callus.

Uniform clumps were selected for a pathogenicity study started in 1989. Each clump had 3 stems, and this pathogenicity study was duplicated at Stronghold, Inc, and in Tucker County, WV (the stems here, however were single stems and not clumps). The isolates chosed were: Ep 155, a isolate from the National Colonial Farm (NCF 5), an isolate from southwestern PA (Dunning), a isolate from the Sugarloaf planting, and and isolate from Pocahontas County, WV. After 4 months, there are no isolate differences, and it appears that the irradiation may be the difference in response of the trees.

Margaret Smither-Michigan State University

She is working on anther culture. She takes anthers, bleaches them and then places the anthers in a medium with hormones to obtain callus. To date, she has had a failure to induce embryogenesis. She has tried 3 techniques: root or graft shoot cuttings; micropropagation from shoot tips; anther culture. Her best shoots come from the shoot tip method. To date, she has had no roots from any of the three methods.

Sandy Anagnostakis-CAES

She reported that a presumably extince insect, *Cyanthadon* sp. (clear-winged chestnut borer) was found using experimental pheromones. It is now found by the hundreds in CT and it is also causing damage in CT.

She also reported on her crosses. She put 470 bags on female flowers in CT to get American pollen, for crosses with:

C. mollissima-got 86 nuts
C. dentata x *C. crenata* hybrids-got 31 nuts
C. mollissima x *C. seguia* hybrids-got 5 nuts
C. crenata-got 36 nuts
Clapper hybrid-got 250 nuts
C. dentata x *C. mollissima* hybrids-got 86 nuts

She stated that she is grafting using a complex hybrid made by Dick Jaynes and she will continue grafting onto those dwarf trees.

She has been examining old chestnut records, and the records indicate:

-in 1799 a large number of European chestnuts were planted in NJ, and Delaware. Mr. DuPont gave European chestnuts to his friends in NY City, Delaware and NJ.

-in 1876, Parsons in Flushing, NY imported trees from Japan

-in 1882, William Perry imported 1000 grafted trees into NY City

-in 1886, Luther Burbank imported 10,000 nuts into California

-in 1893, Luther Burbank, in his mail-order catalogues sold hybrids such as the Japan Giant

-in 1898, Luther Burbank was selling many hybrids at the Albion chestnut nursery in NJ

-in 1903, Sargeant sent the first documented *C. mollissima* to Boston, MA

Fred Hebard-American Chestnut Research Farm

Based on inoculations on five-year old seedlings in the greenhouse, American chestnuts had much larger cankers than Chinese, Nanking or F1 hybrids. Ethylene tests done also correctly rank the plants but there was a large variability (he suggested there needs to be 7-10 replicates).

Concerning the ACF farm, he planted in 1989:

-350 trees (some exotic chestnut planted)
-19 first backcrosses from Minnesota
-KY and IA first backcrosses
-Chinese demonstration plots
-test plots of American and Chinese

Fred harvested the following number of nuts from various places in the eastern US:

American x Clapper-250 nuts

Open pollinated Clapper-1000 nuts

Chinese x (C x A)-776 nuts

American x (C x A)-25 nuts

Americans near the Meadowview farm-300 nuts

Chinese grafting stock-273 nuts

Putative (C x A) x (C x A)-200 nuts

Business Meeting:

New Secretary nomination-Gary Griffin

Next year's meeting location-possibly Catoctin Moutain State Park, MD. The time for next year's meeting was Friday afternoon through Sunday noon, to accomodate those individuals with teaching responsibilities. It is tentatively scheduled for October 26-28, 1990.

Fred Hebard raised a question concerning the NE-140 cooperative pathogenicity study, that compared virulent isolates from Michigan, Connecticut, West Virginia, North Carolina, and Virginia. The 8 x 8 latin square was conducted at CT, WV, VA and NC, but the data was never published. Martha Roane agreed to put the data together, and it was suggested that all parties get their data to Martha by Dec. 31, 1989.

Concerning the 1991 International Symposium,(to be held at the Lakeview Resort and Country Club) Gary Griffin was elected to chair a subcommittee to oversee the details. Sources of funding were discussed, but it was agreed that a formal proposal is needed first. Bill MacDonald stated that from past experience, the cost of this meeting will be approximately $40,000. Sources of funding were discussed, and those organizations that may possibly contribute are:

-CSRS

-Tennessee Valley Authority

-Forest Service

-Norther Nut Growers Association (Phil Rutter, President of the American Chestnut Foundation, is also President of NNGA, and Jerry Payne is on the Board of Directors)

-Park Service

The major expenses will be airfare, published proceedings and the cost of Lakeview Resort

There was some discussion concerning the meeting format. It was proposed that the meeting start on a Saturday around 10:00 am with a banquet/social Sat. night. Saturday would be a day for general reports, planned lay talks, and spots for television, radio and newspapapers. The scientific meetings would start Sunday around 10:00 am. The NE-140 meeting would be encompassed in the Symposim, but the business meeting could be held at the end, Tuesday @ 5:00 pm.

Sandy Anagnostakis agreed to be responsible for the format and Dennis Fulbright agreed to write the proposal with the final document out by April 01, 1990.

Many names were discussed as to whom to invite as keynote speakers. Some of the names mentioned were:

From Turkey: Delan and Soylu

From Switzerland: Heineger

From New Zealand: Steve Choo

From Italy: Bisiach, Gobbi, Turchetti and Vannini

From Japan: Kobiashi

From Greece: Xenopoulos

From France: Jean Grente

From China: Liang and Lu

From Yugoslavia: Halambek

From Australia: Allson and Old

From Spain: Vietez