NE-140 Technical Committee Meeting
Biological Improvement of Chestnut (*Castanea* sp.)
Hungry Mother State Park, Marion, VA
18-20 October, 1991

Attendance:

Connecticut:	Sandra Anagnostakis, Philip Gordon
Idaho:	David McCarroll
Kentucky:	Lou Shain, Shaojian Gao
Massachusetts:	Terry Tattar, Mark Mount, Jong-Kyu Lee, Richard Rohde
Michigan:	Dennis Fulbright
Minnesota:	Philip Rutter
New Jersey:	Don Nuss, Matt Brown, Gil Choi, Bao Chen, Mark Craven, Bradley Hillman, Samuel Kim, James Polashock, Ping Wang
New York:	Michael Milgroom, William Powell, Yir-Chung Liu, Rabia Rizwana, Ningchih Chang, Janna Beckerman, Marcia Federman, Patricia Rocha, Stan and Arlene Wirsig
Pennsylvania:	David Davis, Mary Torsello, Jennifer Mix
Tennessee:	Scott Schlarbaum
Texas:	Neal VanAlfen
USDA-CSRS:	Clifford Gabriel
Virginia:	Gary and Lucille Griffin, Fred Hebard
West Virginia:	William MacDonald, Mark Double, Scott Enebak, Val Ulrich, John Elkins
Ontario:	Colin and Beatrice McKeen

The meeting was called to order at 1:30 pm October 18, 1991 by Chairman Griffin. Fred Hebard, who served as local host and arrangements Chairman made several announcements. The data presentations are organized by Regional Project objectives and presented by station.

Richard Rohde, University of MA, Administrative Advisor

He stated that the project terminates September 30, 1992 at which time there needs to be a revised project. Once a project is revised, it must be approved by the committee of three. They meet in November, February and July. Once approved, it goes to the committee of nine. He urged the committee to revise the project by February. He hesitated to say that a one-year extension is possible. He has received written inquiries from Syracuse, Massachusetts, Rutgers, and Texas concerning formal participation in the committee. Dr. Rohde urged these stations to include their research in the project for 1992.

Clifford Gabriel, USDA/CSRS Representative

He stated that Jack Barnes is on a 15-month Presidential appointment. He gave budget figures for FY 1992. From conference committee reports, the breakdown in dollars as follows (in millions):

Hatch	$168.0
National Research Initiative	97.5
Plant Systems	40.0
National Resources/Environ.	18.0
Integrated Pest Management	4.457
McEntyre-Stennis	18.5

He reported on the following items:

- 1990 Farm Bill--in the national genetics resources program (subtitle C) there is a program that might support microbial culture collections.
- Pest Management Strategy Subcommittee is a subcommittee that is serving as an umbrella for numerous pest management groups--he hopes to push for increases in IPM Special Grants.
- EPA regulations--trying to get FIFRA to decrease scope of organisms that require EPA modification before field testing.
- Personnel:
  - -Charlie Hess has resigned as Assistant Secretary of Science and Education--a replacement has not been selected
  - -Arthur Kelman is the new chief scientist at CSRS for NRI
  - -O.W. Barnett and Ken Barker will be working part-time with CSRS the next few years
- National Research Initiative support for 1991
  - -2,722 proposals requested $637 million
  - -590 proposals were supported at $69 million for a 22% success rate:

-Plant Responses to the Environment	$ 5
-Forest/Rangeland/Crop Ecosystems	3
-Plant Genome/Plant Genetics	10.4
-Plant Pathology/Weeds	5.4
-Crosscut Areas	
-Plant Genome	10.5
-Forest Biology	6.4

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 Ag. Exp. Station

She reported that the CT. Ag. Exp. Station has a good collection of old chestnut photos, since the Station has been in existence since 1875.

A new study site has been established in a clear-cut in a CT state forest. Two plots (about 2,000 ft apart) were almost totally cleared during the winter of 1990-91. This site will be mapped next spring, cankers noted as they appear, and in one plot cankers will be sprayed with a mixture of strains, all flat and originally from these plots, and changed in the lab to flat H. *C. parasitica* was isolated from stumps within the site last summer and three isolates from each plot chosen for conversion to H and flat selection. Potential flat strains have been selected and are being tested for stability and their ability to convert to confirm H.

Rocky Hill is a small CT chestnut woodlot, with chestnut blight. Fifty trees >1" were first mapped and cankers treated as they appreared. H was introduced 1983, but no H has been introduced since 1989. Twenty-seven new trees reached one-inch in diameter during this year and were added to the map. The long hot, dry spell took a heavy toll on chestnut tree tops, but there was still good nut production. Thirty-seven new cankers were found this year, with 36 v-c groups. Only 2 of the 37 isolates were white.

Lou Shain, University of Kentucky

In an attempt to determine if multiple H agents may be more efficient in biocontol, cankers were established on American chestnut using Ep 289, a V methionine auxotrophic strain (v-c 71). Cankers were then challenged with H isolates, Ep 713, 780 (v-c 40) or a strain that contained H

agents from both Ep 713 and 780. Hypovirulence was transmitted through mycelium to encircle cankers challenged by Ep 713-780 within 3 weeks. In contrast, those cankers challenged with each H agent separately were not encircled with 44 weeks. The data are as follows:

Canker Circumference and Percent Recovery of Bark Isolates 3, 9 and 44 Weeks After Challenge with H

	Canker Area (cm)			% Recovery of H		
Challenging H Strain	3 wk	9 wk	44 wk	3 wk	9 wk	44 wk
Ep 713 [F]	62	126	407	3.9	32	0
Ep 780 [I]	69	128	340	0	0	35
Ep 713/780	60	66	159	91	100	100

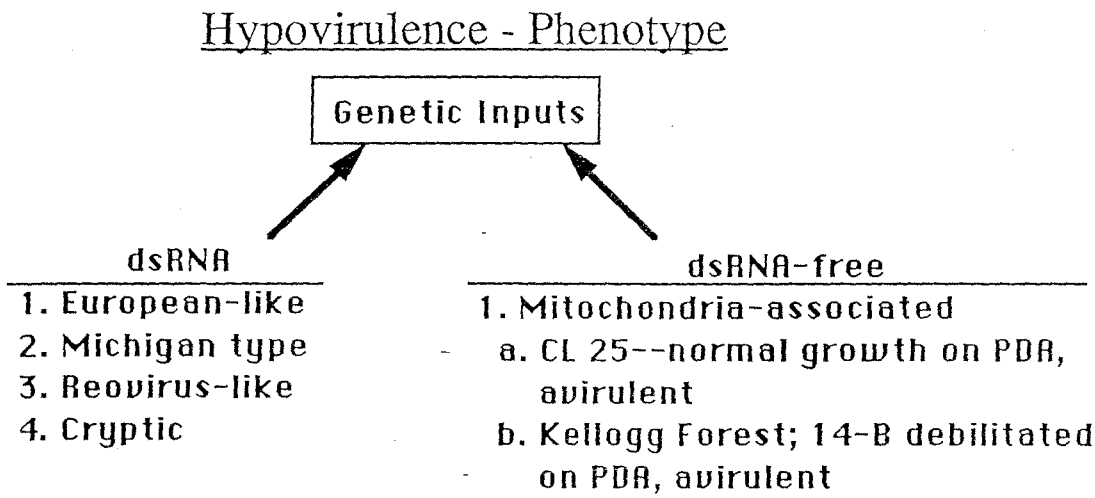
The production of H conidia was extremely low for all cankers. Even though the underlying bark has white mycelium, cirrhi still produce V conidia, as seen in the following table:

Number of V and H Conidia Detected in Cirrhi of Cankers 3, 9 and 44 Weeks After Challenge with H

Challenging H Strain	3 Weeks		9 Weeks		44 Weeks		Total	
	V	H	V	H	V	H	V	H
Ep 713	53	0	55	0	210	0	318	0
Ep 780	54	0	27	0	81	1	132	3
Ep 713/780	54	0	71	0	255	1	350	1

Dr. Shain reported that their original Ep 713 isolate was lost. They attempted to retrieve Ep 713 by pairing the original parent isolates: Ep 155 and B2025. The "new" Ep 713 differs from the original in that the latter is a non-sporulator with a higher titer of dsRNA.

Dennis Fulbright, Michigan State University



CL 25 from Crystal Lake, MI has many characteristics of dsRNA-associated H but harbors no detectable amount of dsRNA. The H phenotype is carried in 20% of the conidia, maternally inherited in sexual crosses and can be transferred to other V strains via hyphal anastomosis. This suggests that there is a cytoplasmic "virulence inhibition factor" (VIF) other than dsRNA responsible for H in CL 25, as dsRNA is not maternally inherited.

Study of cytoplamsic agents in CL 25 revealed the presence of two low copy number plasmids in the mitrochondria. These plasmids are about 6 and 10 kb in size. To screen other strains of *C. parasitica* for the presence or absence of these plasmids, a portion of the 6 kb plasmid

was cloned and was used to screen other strains. All V and H strains of *C. parasitica* tested revealed the presence of this plasmid. These results suggest that the 6 kb plasmid is not directly involved in the expression of H in CL 25. Hybridization studies suggest that the plasmid has no homology with the mitochondrial genome, and can thus be defined as a true mitochondrial plasmid.

Since H in CL 25 is maternally inherited, a study on the possible involvement of mitochondria was initiated. Respiration studies of CL 25 and other dsRNA-free H strains revealed that the cyanide-insensitive or alternate oxidase pathway accounted for as much as 85% of the total respiration in these strains, compared to 8% in V and 16% in dsRNA-associated H strains. This study suggests that a respiratory defect, like "vegetative death" in other fungi, may be closely associated with dsRNA-free H.

Alternate Oxidase Pathway as Percentage of Total Respiration in Different Strains of *C. parasitica*

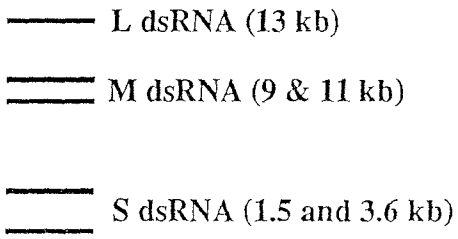
Strains	Phenotye	Presence of of dsRNA	Alt. Oxid. as % of Total Respiration	Description
Ep 155	V	-	9.0	From CT
CL1-16	V	-	10.5	SCI from Frankfort
14B-N1	V	-	17.5	SCI from Kellogg
4-C	V	-	8.0	From cured GHU4
CL25ss4	V	-	13.5	SCI from Cry. Lake
CL24ss18-9	H	-	71.5	SCI from Cry. Lake
14B-D6	H	-	84.5	SCI from Kellogg
14B-N6	H	-	71.0	SCI from Kellogg
4-C	H	-	82.0	Converted c CL25
GHU4	H	+	53.0	From Grand Haven
GH2	H	+	16.5	From Grand Haven
Cl1-16(GH2)	H	+	15.0	Conv. with GH2
R1	H	+	6.5	From Ross Common

Don Nuss, Roche Institute of Molecular Biology

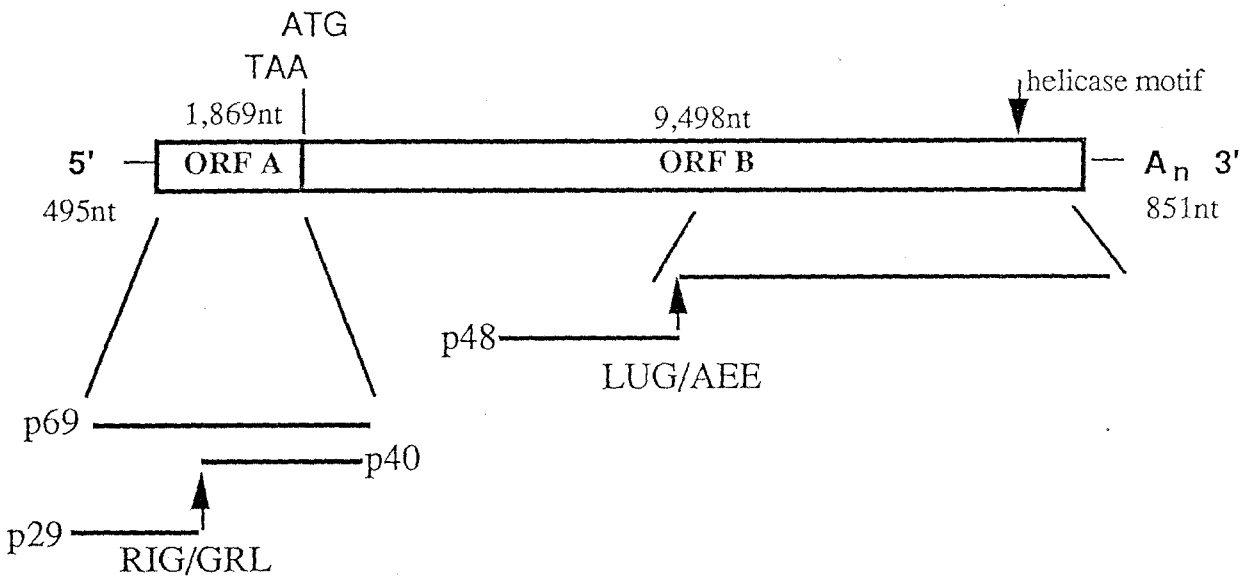
He posed four questions his laboratory is trying to answer:

1. Are hypovirulence-associated dsRNAs of viral origin?
2. Are dsRNA-encoded gene products responsible for traits exhibited by hypovirulent *C. parasitica* or are these traits the result of a reaction of the fungus to the physical presence of the replicating dsRNA?
3. Can hypovirulence be uncoupled from associated traits such as reduced levels of sporulation or pigmentation?
4. Is the expression of a specific subset of fungal genes modulated in hypovirulent strains or is hypovirulence the consequence of general metabolic alterations?

The strategy was to identify gross structural properties by direct analysis of dsRNAs and then orient and characterize cDNA clones.



The current view of the genetic organization and gene expression of the L-dsRNA in strain Ep 713 (shown above) was found to consist of 12,712 bp, excluding the poly(A):poly (U) homopolymer domain, as seen below:



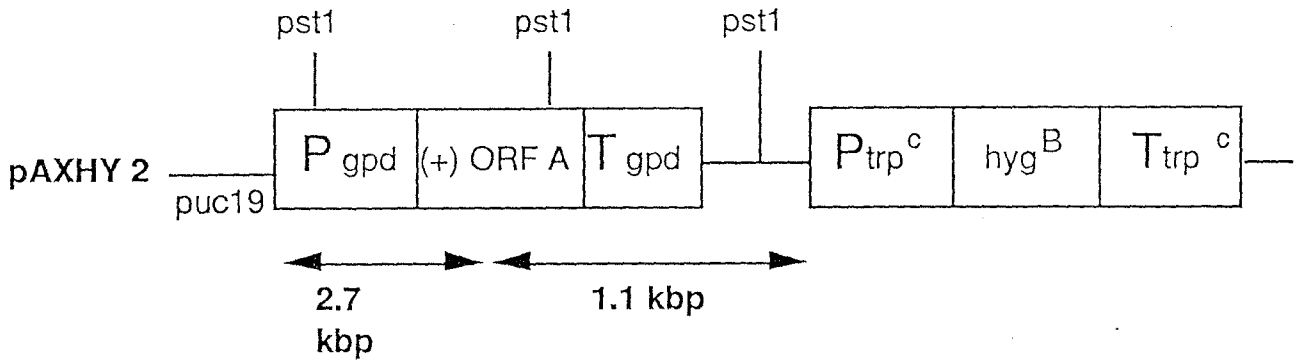
The strand which terminates with a 3'-poly(A) contains two large open reading frames designated ORF A and ORF B. ORF A is preceded by a 495nt non-coding leader sequence which contains six potential initiation codons each of which is followed closely by one or two termination codons. This leader sequence reduces the level of *in vitro* expression of downstream coding regions. ORF A encodes two polypeptides, p29 and p40 which are released from a polyprotein p69. Cleavage occurs during translation between GLY-248 and GLY-249 and is mediated by p29, i.e. cleavage is autocatalytic. The ORF A/ORF B junction consists of the sequence 5'-UAAUG-3'. Translational mapping studies indicate that the UAA portion of the pentanucleotide serves as the termination codon of ORF A, while the AUG portion is in the 5'-proximal initiation codon in ORF B. Presumably, p29 and p40 or both polypeptides are involved in catalyzing additional steps in processing of the predicted ORF B-encoded polyprotein. A helicase motif was identified within the carboxyl-terminal domain of ORF B and may represent a functional domain of an encoded polymerase. Studies are now in progress to identify processed forms of the ORF B-encoded polyprotein in an effort to obtain a complete view of the expression strategy for this portion of L-dsRNA of Ep 713.

Computer assisted analysis revealed that similarities among the predicted amino acid sequences of L-dsRNA and member of the single-stranded RNA potyvirus group are not limited to the p29, p48 and HC-Pro proteases. Additional domains of similarity included a putative RNA-dependent RNA polymerase, a RNA helicase and a cysteine-rich region of unknown function similar to the N-terminal portion of the potyvirus helper component protein. Phylogenetic trees derived from the alignment of the L-dsRNA polymerase domain with all known viral RNA-dependent polymerases strongly suggests that this hypovirulence-associated dsRNA and potyvirus genomes share a common ancestry.

The name "HAV" (hypovirulence-associated virus) has been submitted to the International Committee on Viral Taxonomy, thereby forcing them to consider this as a true virus, rather than a dsRNA, and rule on a proper name.

Gil Choi, Roche Institute of Molecular Biology

He made transformation vectors, as indicated below:

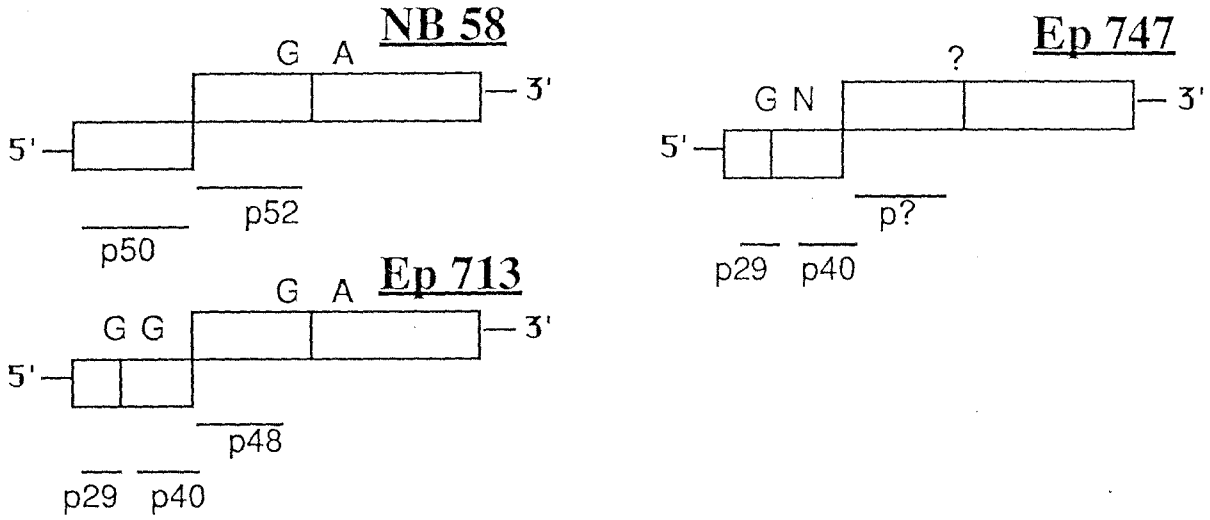


He reported that transformation of strain Ep 155 with a cDNA copy of ORF A, under the control of the *C. parasitica* glyceraldehyde-3-phosphate dehydrogenase promoter, conferred traits similar to those exhibited by strain Ep 713. In contrast, transformation with the identical vector that contained ORF A in the reverse orientation resulted in no phenotypic alterations. Transformants containing OFR A in the sense orientation [(+) ORF A transformants] were reduced in pigmentation to the level exhibited by strain Ep 713, but, unlike the Ep 713 colonies, did develop a very low level of pigmentation very late in the culture period. These transformants also produced approximately 40-fold less conidia than the untransformed Ep 155 strain. The transformants also were considerably reduced in laccase production as indicated by the degree of the color reaction on Bavendamm's medium. The observation that a specific L-dsRNA coding domain, in the absence of replicating hypovirulence associated virus RNA, is sufficient to confer certain traits that are normally exhibited by the corresponding untransformed hypovirulent strain established two important points. First, these results provide the first direct evidence for a cause and effect relationship between the viral dsRNA present in a H *C. parasitica* strain and specific traits associated with that strain. Second, they demonstrate that these phenotypic traits are not the result of some general reaction of the fungus to replicating viral RNA, but are caused by the action of a specific viral coding domain.

Matt Brown, Rutgers University

He found that NB58 is related to Ep 713 by hybridization. He has been working with two proteases from Ep 713. Clones of NB58 were mapped and compared to Ep 713 and Ep 747. From the 5' end of ORF A, Ep 713 and Ep 747 compare 85% at the nucleotide level, while NB58 and Ep 713 compared at the 50% level.

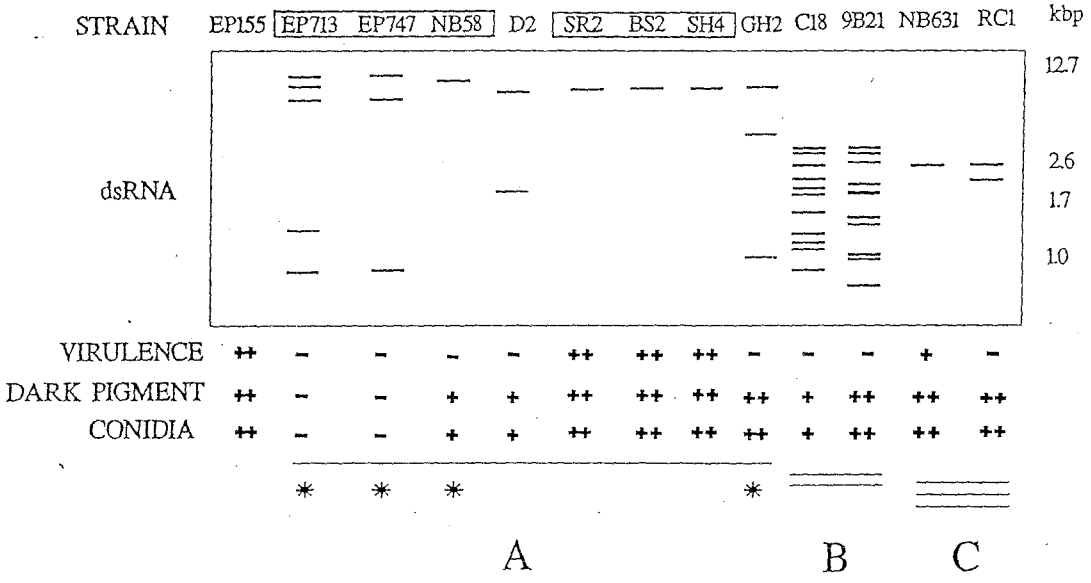
He has found no protease activity in the ORF A region for NB58. There is good sequence similarity in the ORF B region between NB58 and Ep 713. The representation of NB 58, Ep 713 and Ep 747 are shown on the following page.



Brad Hillman, Rutgers University

He discussed dsRNA from different hypovirulence-associated viruses form Europe and North America, and presented the following table:

DOUBLE-STRANDED RNAs FROM DIFFERENT HYPOVIRULENCE-ASSOCIATED VIRUSES FROM EUROPE AND NORTH AMERICA



Isolates that are boxed share sequence similarity. Isolate origins are as follows: Ep 155, CT (V); Ep 713, France; Ep 747, Italy; D2, southwestern PA; SR2, BS2, southeastern WV; SH4, northwestern VA; GH, Grand Haven, MI; C18 and 9B21, southwestern WV; NB58 and NB631, New Jersey.

He assumes that the smaller bands are not necessary--the information is organized on the large dsRNA. Isolates C18 and 9B21, both from Brush Mountain in WV are virologically entirely different than the others. They are packaged in virus-like structures; all bands migrate in an all or none fashion; and all bands are of equal titer, suggesting these two isolates contain a virus in the Reoviridae family. Visuses in this family frequently have insects as one of their hosts (and therefore, as vectors).

Yir-Chang Liu, Cornell University

She is interested in why some H isolates convert and other do not. She hypothesized that there are different populations of the fungus coupled with genetically diverse populations. She paired H and V isolates in various vc groups and showed in the following table that the higher the difference in loci the more difficult conversion becomes as shown in the following:

vc	# Loci Different	Number of Conversions	Number of Days For Conversion
5+39	1	2/5	3-4
10+40	1	5/5	1-3
10+17	1	5/5	1-3
5+8	2	1/5	1-3
5+17	4	0/5	
8+17	5	0/5	
5+10	7		
5+5	0	5/5	1-3
10+10	0	5/5	1-3
17+17	0	5/5	1-3

Virulent:

Strain	vcg
Ep 67	10
Ep 42	5
Ep 78	17
Ep 155	40
Ep 36	8
Ep 392	39

Hypovirulent:

Strain	vcg
Ep 43	5
Ep 113	10
Ep 78-H	17

DNA fingerprinting was performed on isolates from Michigan and West Virginia to see if isolates within the same v-c groups display the same fingerprint.

Site	v-c Group	Fingerprint Similarity
County Line, MI	same v-c group	similar except for 2 isolates
Frankfort, MI	same v-c group	similar except for 1 isolate
Pocahontas County, WV	different v-c groups	very dissimilar

Clonal reproduction may be dominant in Michigan, based on the above data.

Neal Van Alfen, Texas A&M University

The three projects in his laboratory are:

1. How does the virus replicate and move?
2. How do cytoplasmic genes move in the population of the fungus?
3. How does the virus regulate fungal genes?

In an attempt to answer the second question concerning mechanisms, mitochondrial DNA is being looked at because it is very heterogeneous. Few polymorphisms occur in nuclear DNA because its quite homogeneous. Since there is more heterogeneity in *C. parasitica* mitrochondria than in any other fungal system, the mechanisms that are causing some entity to move from one strain to another are being studied.

The question of how the virus regulates fungal genes is being addressed by isolating genes down-regulated by the virus, determining the function of these regulated genes, and identifying the genes which regulate these cloned genes. He speculates that the virus disrupts specific pathways within the fungus. Four genes have been cloned that are down regulated: early sporulation genes (Spo1 and Spo2), Laccase, and a cell-surface structural protein has been isolated (cryparin). The characteristics of two of these genes are as follows:

Spo2	Cryparin
down-regulated by dsRNA	hydrophobic, cell-surface protein
by gene regulation, an early sporulation gene	lectin-like properties
some homology with Spo1	similar to cerato-ulmin
transcript of 650 bp	tissue specific
four short ORFs in transcript (do not know whether it expressed as a protein or not)	N-terminal sequence is similar to structural proteins
	down-regulated by dsRNA

Laccase A is also being worked with in his laboratory. Laccase A is an extracellular glycoprotein with a molecular mass of 77 kDa.

His summary of research is as follows:
-specific fungal genes are down-regulated by dsRNA
-dsRNA-effected genes are coordinately regulated
-there are multiple groups of dsRNA-regulated genes
-a portion of viral symptoms are mimicked by gene deletion

Scott Enebak, West Virginia University

He reviewed findings by two former graduate students, Mike Likins and Jeff Sillick. They surveyed *C. parasitica* isolates from WV, MD and VA and found that 30-35% of native isolates contain dsRNA. Scott's objectives for his doctoral work were to study the morphology, dissemintation potential, virulence and genetic relatedness of native dsRNAs. He initially chose 50 isolates, inoculated them into excised stem pieces, and chose three isolates with reduced virulence. These isolates were from Savage River, MD, Stillhouse Road, Marlinton, WV and Hankey Mt., VA. He single-spored these isolates, plated them out for morphology and extracted dsRNA from 40 single spores/isolate. More than 85% contained dsRNA, indicating the native dsRNA is transmissible. He then looked at the dissemination potential of several other isolates as, follows:

Isolate	Percent Transmissible dsRNA
Waldrop	100%
Savage River 2	94%
Hankey Mt. 3	87%
Boy Scout Camp	10%
Stillhouse Rd. 4	80%
Nathanial Mt. 2	90%

To study related virulence, he chose isolates with dsRNA, and single-spored isolates without dsRNA and put them into stems. He found no difference in virulence for any of the isolates with

or without dsRNA. He then used a 3kb clone from SR2 to probe some of the other isolates and found that the WV isolates share sequence similarity.

Isolate C-18 was collected from a V canker in WV. It is composed of 11 electrophoretically distinct dsRNAs, ranging in size from 1 to 5 kb, which segregate in an all-or-none fashion in single conidial isolates. The virus alters cultural morphology, reduces virulence when compared to dsRNA-free progeny and can be transmitted into other isolates of *C. parasitica* via hyphal anastomosis. Using cDNA cloned from a library representing the viral genome as hybridization probes, 7 of the 11 segments have been identified as being unique with respect to the hybridization properties in northern blots. The clones also do not hybridize to the Reovirus, wound-tumor virus, or to another 11-segmented dsRNA from WV (9-B-2-1). An icosahedral particle, approximately 60 nm in diameter has been purified from the mycelium using phosphate buffer extraction techniques. Double-stranded RNA extraction of phosphate buffer material yields 11 segments of dsRNA. He concludes that this virus is comparable to other viruses in the family, Reoviridae.

Mark Double, West Virginia University

In the spring of 1988, plots were established in a 5-year-old clearcut with abundant chestnut regeneration in Pocahontas County, WV. Of the 12 plots established, 6 were cleared of competing vegetation; the remaining 6 were not cleared. Four European Hv isolates with broad conversion capacities were inoculated in 3 cleared and 3 non-cleared plots by scratch-wounding 25% of the trees in each plot. New infections were sampled and isolate morphology examined during fall, 1988; spring and fall, 1989; and spring, 1990. Cankers yielding isolates with Hv morphology from Hv inoculated plots have remained somewhat constant, but disappointingly low (average of 10%). Almost 55 % of the H isolates have been recovered from trees that received H inoculum. Cankers are resampled on an annual basis to discern any change in isolate morphology. Resamples after 1 year indicated that 87% of the cankers yielded isolates morphologically similar to the initial samples. Similar results were obtained for resamples collected after two years, although a greater proportion of isolates, that were initially V, yielded H isolates after two years.

Colin McKeen, Ontario, Canada

He stated that Dr. Greg Bolyard, from the University of Guelph, is on sabbatical at the Vancouver Viral Laboratory. Martin Dunn, Dr Bolyard's student has been comparing Ontario cultures of *C. parasitica* with West Virginia cultures. He has found that in Ontario, dsRNA is similar to that in West Virginia (single band; lack of virrulence control, etc).

Colin is continuing to inoculate V cankers in Ontario with the Arner isolate. This isolate has been examined by Fulbright at Michigan State University and no dsRNA has been detected. The Arner tree is now 30" dbh with many healing cankers.

Gary Griffin, Virginia Tech

Trees in norther VA (Fairfax County) have cankers that are superficial at the vascular cambium. Ten more isolates were taken from these trees in 1991 and two, possible three isolates segregrate as H in pathogenicity trials. They have the 12 kb band.

Four year ago, a visiting scientist from India, Dr. M. Khan worked with 60 isolates taken from "normal" cankers. Four of these 60 isolates, presumed to be V, had one large dsRNA band, but they were V in a cluster analysis. These isolates were inoculated into trees in a clearcut and the cankers were 80-100% necrotic to the vascular cambium.

OBJECTIVE 2: To study the growth and physiology of the pathogen and responses of Castanea spp. and related genera to infection.

Sandra Anagnostakis, Connecticut Ag. Exp. Station

She reported that her fungal genetics work is proceeding, albeit, slowly. Selfing in *C. parasitica* was examined by inoculating a cream, temperature sensitive strain into American chestnut stems. One group of stems was harvested after 2 months and all 16 perithecia examined were outcrossed. The other group of 21-month old cankers were harvested in April 1991 and the ascospores were examined. From this group, 44 perithecia were selfed, 23 were partially outcrossed and 10 outcrossed. This may be an explanation of what is happening in plots with little v-c diversity.

Michael Milgroom, Cornell University

He raised the question, can H be successfully deployed in North America? His approaches to answer this question are via mathematical modeling and molecular genetic markers. The Cornell group is looking at two main mechanisms, reproductive biology of the fungus and dispersal of fungal propagules.

Concerning reproductive biology, they are looking at conidial vs ascospore reproduction and the frequency of self-fertilization. All cankers in a 25 x 25 meter plot at Mt. Lake, VA were sampled, yielding a total of 39 cankers. Ascospores were collected and examined using DNA fingerprinting and single-copy RFLPs. Using the DNA fingerprinting, 33 different patters were found in the 39 samples; 6 genotypes were found twice. In terms of gametic disequilibrium, 89% of the fragmented pairs were random. With respect to outcrossing, they found that 14 of 20 perithecia with segregating fragments corresponds to 70% outcrossing.

The summary of conidial/ascospore reproduction and self-fertilization are:

1. Most reproduction appears to be sexual -- only 6/39 are putative clones.
2. Except for 6 clones, the null hypothesis of random mating could not be rejected.
3. Outcross rate is 70%, leaving 30% self-fertilization.

He also is interested in a comparison of the genetic diversity of *C. parasitica* in China versus that in North America . To test the hypothesis that the diversity is greater in China, he compared 16 Chinese isolates with 17 American isolates. The data is as follows:

Characteristic	China	No. America
# Polymorphic Probes	8	2
Average # banding patterns/probe	2.5	16
Average gene diversity	0.22	0.08
# Multilocus haplotypes	12	10*

* = 7 from a single 3-locus probe

There were 7 to 12 fragments/genome in American isolates and only 2 to 4 in Chinese isolates, indicating many fewer fingerprint patterns for Chinese isolates. Two Chinese isolates were quite similar to two North American isolates.

William Powell, Syracuse University

He discussed the chestnut program at Syracuse and introduced his graduate students: Rabia Rizwana (UV mutations of vic genes) and Ningchih Chang (chromosome mapping) who are working on biological control, and Janna Beckerman who is involved with the identification of possible resistant genes in *C. parasitica.*

Rabia Rizwana, Syracuse University

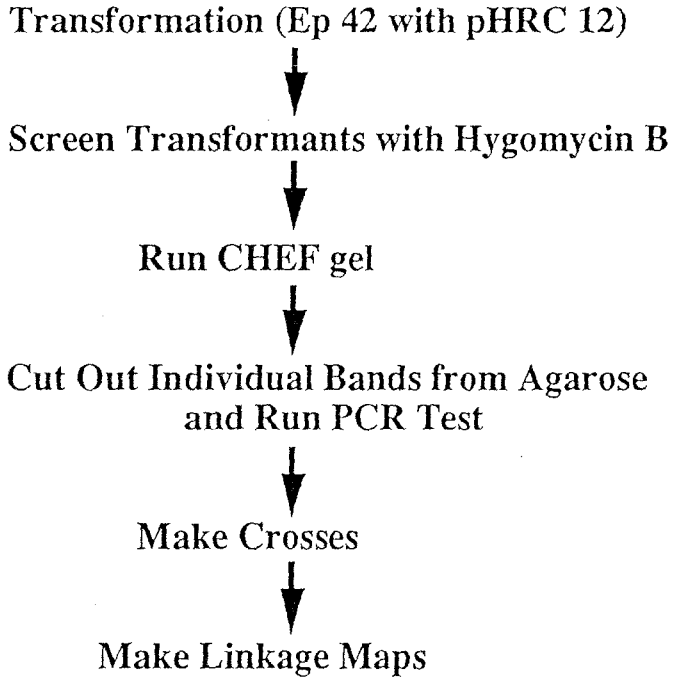
Her long-term aim is to knock out v-c gene(s) to allow for universal compatibility. She is screening by selecting complimentary auxotrophs and forming heterokaryons. She is working with the following isolates, developed by UV mutations:

v-c Group	Characteristic
5	met - ATCC
8	arg - ATCC
16	arg - leu -
39	arg - leu -

Four heterokaryons were formed and resolved. Two of the isolates switched from vc 39 to vc 5 (gene vic1 [vic = vegetative incompatible]), two isolates switched from vc 8 to vc 39 (vic2), and two switched from vc 39 to vc 5. To make sure this data didn't represent a parasexual event, one strain was UV-treated and the strains were plated. Three mutant isolates were recovered from vc 8; one changed form v-c 8 to v-c 39 and two isolates switched from v-c 8 to a new v-c group. Three mutants were recovered from vc 39, and are, as yet, untyped. She feels the genes responsible for v-c have been successfully altered by UV radiation.

Ningchih Chang, Syracuse University

Her goal was to produce a set of transformants with Hygromycin B resistant gene marked chromosomes. Her protocol is as follows:



She plans to:
1. Use the transformants in crosses to form linkage maps.
2. Line CHEF data with genetic cross data.
3. Provide starting points for chromosome walking to adjacent genes.
4. Clone linked-genes by making a library of individual chromosomes.

Jong-Kyu Lee, University of Massachusetts

He isolated 102 strains from cankers in the Amherst area, and found 54 v-c types. These were best converted by CT H strains #752 and #43 (Italian and French H). Growth rates of converts and normal strains were compared on PDA and PDA + ground chestnut bark. Growth was best on PDA + ground *C. dentata* bark.

He compared the strains in living stems, and on the inner surface of peeled bark and on peeled stems. The *in vitro* test allowed good separation of V and H strains.

He has tried various additives to media to distinguish V and H strains, and Bavendamm's method was best.

Lou Shain, University of Kentucky

Studies on the basis of resistance and susceptibility of Chinese and American chestnut, to chestnut blight are continuing. Chinese and American excised stems segments were challenged in the dormant and growing season with V and isogenic H strains. Stem segments were processed with increasing time after challenge by stripping off the bark, grinding it up and analyzing protein profiles. Glucanase and chitinase, enzymes implicated in the lysis of fungal cell walls, were detected in both American and Chinese stems, following V or H inoculation. In addition to SDS PAGE and immuno blotting, as reported last year, detection of chitinase also was confirmed by electrophoresis of native protein and assaying enzyme activity directly on overlay gels containing glycol chitin and calcofluor white. Resolution of glucanase from native bark protein on gels has not yet been as successful. Attempts are being made to bioassay native proteins of both chestnut species on *C. parasitica* before and after challenge.

Dr. Zvi Solel, Bet Dagan, Israel, spent an 8-month sabbatical in Dr. Shain's lab. His initial objective was to develop a suspension cell system for chestnut. Such a system would be of great value for studies in host-pathogen interactions. Numerous protocols were tried, but none satisfactorily disrupted chestnut callus into suspension cells. Calli of American and Chinese chestnut were then challenged with the pathogen. Generally calli from resistant material, particularly cultivar Nanking, supported less fungal growth and were less discolored than calli from American chestnut. Challenged and non-challenged calli were extracted in organic solvents decreasing in polarity. Extracts were bioassayed directly on TLC plates. Conidia of *Cladosporium cucumerinum*, a common test organism for antifungal activity, or *C. parasitica* were used in these bioassays. Results did not identify specific antifungal compounds that could be correlated with resistance. This preliminary study, however, demonstrates the importance of using the target organism rather than a test organism for such bioassays. Zones of inhibition and stimulation differed markedly between the two organisms tested.

Mary Torsello, Penn State University

Four of the most severely *C. parasitica*-infected oak stands, located during the incidence survey of 1990, are being used to study the radial growth of infected versus non-infected scarlet oaks, using dendrochronology, to determine the influence *C. parasitica* has on growth. The four stands are located in Fayette, Centre, Fulton and Franklin Counties. Fifteen infected, co-dominant scarlet oaks were paired with the closest healthy S. oak, giving a total of 30 trees/stand. Two increment cores were removed from each tree at dbh, air dried, glued into wooden mounts and sanded to enhance ring boundaries. The core analysis is currently underway and the rings are being measured on a Bannister Incremental Measuring Machine interfaced with the microcomputer program TRIMS.

Gary Griffin (reporting for Graciela Farias),Virginia Tech

Her work in finding tannase produced by *C. parasitica* is progressing. The activity of tannase (tannin acylhydrolase) was determined by estimating the amount of gallic acid produced during hydrolysis of methylgallate. When the fungus was grown on allepo-tannic acid, mycelial extract had higher activity than culture filtrate and highest activity occured 6 days after inoculation.

The pH optimum of the tannase was 5.5 and maximum activity was observed at 28 C. Growth of the fungus was higher on American chestnut total aqueous extracts than on Chinese chestnut total extracts during the first 3 days, with no significant differences in dry weight after 3 days. The tannase was purified with mono-Q columns and superose and purified to homogeneity by ammonium sulfate fractionation, anion-exchange and gel flitraton chromotography. Graciela estimates the weight of the tannase to be 22,400. She plans to do kinetic studies.

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

Sandra Anagnostakis, Connecticut Ag. Exp. Station

In a continuation of tree chemistry, tannins were extracted from green bark of two each Chinese, Japanese and American chestnut trees, and compared with commercial tannins using a radial diffusion assay that precipitates protein. No differences were found. When a V strain of *C. parasitica* was grown on buffered media containing these extracts, growth was enhanced, but no inhibition was seen and no differences in growth rate were found.

With regard to the peroxidase isozyme, she reported that data will be published in *HORT SCIENCE*. She is looking at isozymes by isoelectric focusing and the bands are clearly distinguishable. She will look at peroxidase levels in infected and non-infected bark.

Reporting on tree breeding, she reported that 200 hybrid nuts were produced this summer using Rocky Hill chestnuts as female parents.

Janna Beckerman, Syracuse University

Her goal is to genetically engineer resistance in American chestnut, but she must find a suitable gene to clone. A 45 kd protein was formed when bark strips were challenged with conidia, and it was induced in both American and Chinese chestnut. The protein was extracted on filter paper disks and used to challenge conidia. There was more conidia control (suppression of growth) with the protein from the unchallenged bark. A protein from another organism was found that is quite effective against conidia at 10^7 conidia/ml. This protein, effective at pH down to 3.5, will be transformed into American chestnut.

Scott Schlarbaum, University of Tennessee

He reported that since the mid-1980's, he has been grafting American and Chinese chestnut. All of his hardwood stock, however, was lost in the winter of 1989 due to a hard freeze and he has had to start his grafting over again.

He obtained dwarf chestnut stock from S. Anagnostakis which he has used in his grafting program. The TN Park Service will take all the grafts he has produced over the last two years. He is contracting a private nurseryman to do much of the grafting work.

He has applied to CSRS in conjunction with Tennessee State University for construction of a large greenhouse in TN.

He plans to travel to Japan to obtain scion from 12 gall wasp-resistant cultivars.

Philip Gordon, Connecticut Forest and Park Association

He reported on two separate areas of work:

1) CT service foresters report their findings of American chestnut. He has cooperative research with the CT Bureau of Forestry in which fruiting populations of American chestnut are being established. In 1989 there were an estimated 6,000 fruiting American chestnuts and the estimate in 1991 is 50,000, probably because more people were looking for them.

2) His second area deals with the CT state nursery. The nursery will accept nuts and distribute them, and it is hoped that 10,000 seedlings will be distributed next year with that figure pushed into the millions in a few years.

He talked about the survival mechanisms of American chestnut. He is always looking for oddities in the forest, and he found a tree with 1 living branch that contained many small burrs. He collected the burrs and found chestnuts the size of peas. He planted these nuts and got seedlings from all 24 nuts. These seedlings were quite small through half the season and then grew normally.

He hopes to do some work at Yale University with regard to somatic embryogenesis. He stated that root collar sprouts come up through somatic embryogenesis and each sprout has its own root system. It is possible to obtain resistance through somatic embryogenesis.

Fred Hebard, American Chestnut Foundation, Meadowview Research Farm

He discussed V isolates that were chosen from a normal distribution and selected throughout a wide array of pathogenicities. He believesthat canker length is a better index for comparison, than canker area.

He showed scanning electron micrographs to illustrate differences in glands from American, European, Japanese, and Chinese chestnut. American glands are 4-celled (hot-cross bun); Japanese glands are multi-celled with two distinct halves (Italian bread loaf); Chinese glands are confined to the mid-rib and are capitate columns; European glands are clavate or baseball bat-shaped, often laying on the surface.

He then discussed the morphological characteristics of American and Chinese chestnut.

Characteristic	American	Chinese
Stem color	red	green
Stipules	fall off	remain attached
Lenticles	small	large

He discussed evidence that simple hair in interveined regions of Chinese chestnut leaves are controlled by single dominant gene, in crosses with American chestnut. He speculated that red stem color is probably an anthocyanin, so there must be a gene that controls the presence of condensed tannins and red stem color.

Bill MacDonald, West Virginia University

Five isolates of *C. parasitica* were inoculated on replicate trees growing in West Virginia (non-irradiated trees) and two locations in Maryland (irradiated trees) to evaluate host resistance. Isolates performed comparably on the irradiated trees at the two Maryland sites. The greatest growth occurred at the MD sites during the 1989 season followed by the 1990 season. Winter canker development was almost absent at these two sites during 1989-90. On trees in West Virginia, the greatest growth occurred during the 1990 season, followed by the 1989 season. In contrast to the Maryland sites, measurable canker expansion occurred during the 1989-90 winter period.

During the final evaluation period (May 1991), cuts were made into the canker margin (along the x and y canker axis) to evaluate the extent of inner bark necrosis. These measurements revealed that, for the trees at the two Maryland sites, about 50% of the necrotic outer bark was underlain by healthy phloem tissues. This was in contrast to cankers on West Virginia trees where extent of external bark necrosis corresponded closely to the death of the inner bark.

John Elkins, Concord College

Selected trees that showed some resistance were taken to a natural setting for evaluation. Chestnut saplings, in the forest, were cut at the base and scion wood was added from the selected trees showing resistance. The graft union was covered with growth tubes to insure good establishment. The trees used are Gault and Floyd crosses.

One tree in Beckley, WV that was very good at accepting grafts has died, however, John has been stooling this tree and the new trees will be taken to a natural setting.

He discussed that after his grafted trees get up to pollination size, the pollen is rubbed off, run through a 10 mesh screen, then through a 40 mesh screen and then desiccated over calcium chloride and stored in the freezer. Two-year old pollen is still able to pollinate, whereas he found that 9-year old pollen was not able to pollinate. His technique in pollination studies is to remove the male catkins and add pollen bags (brown paper lunch sacks) over the female flowers. John stated that he does not remove the leaves from around the female, while Fred Hebard does remove the leaves. After 10 days have elapsed, the bags are removed, the female flower is misted with water, and then coated with pollen, using a brush. The bag is then placed back on the flower to prevent outside pollination.

John conducted chestnut grafting clinics in NC, WV and VA during 1991. Members of the public were instructed in grafting techniques in forest clearcut or plantation sites using American or hybrid chestnut scions.

Gary Griffin, Virginia Tech

Trees from 1983 crosses were grafted and the most promising of these trees were cross pollinated and the nuts outplanted. These trees were then challenged with inoculation with *C. parasitica*. This data will be presented next year.

Discussion of the 1992 International Chestnut Conference

The conference is scheduled for July 11-15, 1992 at the Sheraton-Lakeview Resort and Conference Center in Morgantown, WV. The international chestnut growers meeting will meet Wednesday through Friday, preceding the conference.

The resource people will listen to all talks given in their session and will write a report describing areas of strengths and weaknesses. The following speakers have tentatively agreed to speak:

Topic:	Main Speaker:	Resource Person:
Fungal Ecology	Hambeck (Yugoslavia)	John Leslie (Kansas)
Fungal Physiology	Vannini (Italy)	Bob Scheffer (Michigan State)
Taxonomy	Undecided	Undecided
Molecular Basis of Hv	Gobi (Italy)	Undecided
Tree Breeding	Ellingboe (WI)	David Burke (Princeton)
Tree Ecology	Heineger (Switzerland)	Fred Paillet (Colorado)
Tree Propagation	Vietez (Spain)	Frank Santamour (USDA)

The monetary picture as of October, 1, 1991 is as follows:

CSRS	$7,000
Forest Service	$7,500
WVU Experiment Station	500

There are two outstanding sources of funding: Park Service and the State and Private Forest Service Unit in Morgantown.

A question was raised as to whether the CSRS funds could be used for foreign travel. Dr. Gabrial state that travel from CSRS was zeroed out with the feeling that units should pick up travel expenses. The money can be spent on publications. Dr. MacDonald estimated $7,500 would be needed to publish a proceedings if it's done through West Virginia University.

Initially the Saturday keynote speakers were to be paid expenses in full; the main speakers were to be paid one-half expenses and the resource people were to be paid expenses in full plus an honorarium. Chairman Griffin raised the question that with the lack of firm funding, and the question as to whether monies already appropriated can be spent on travel, should the meeting be restructured or cancelled all together?

The comments from the floor were as follows:

-MacDonald	A policy needs to be set for honoraria
-Fulbright	Recind the money to the resource people; invite them anyway but do not agree to pay their way.
-Van Alfen	Questioned if private individuals could fund a portion of the conference since we are holding a general session.
-MacDonald	According to Bob Wallace, possibly 400 growers will attend the preceding growers meeting and many of them may stay for the conference.
-Hebard	He questioned the different honoraria for domestic versus foreign speakers.
-Van Alfen	He felt a set amount should be set for honoraria. He added that the executive committee should figure out the total cost to put on the meeting, the total amount appropriated from various funding agencies and then determine the difference. That figure should be divided by the number of people expected to determine the registration fee.

Business Meeting

Thanks were extended to Fred Hebard for his work at local arrangements coordinator. Michael Milgroom was tentatively elected as secretary for 1992 with Sandra Anagnostakis assuming the position as chairperson.

A synopsis of the executive council's discussion of the International Meeting was given by Chairman Griffin:

- the honorarium system will be $1,000/speaker (and $500 to Al Elingboe)
- Drs. Jaynes (CT) and Bounous (Italy) were dropped from the conference.
- Carl Leopold's talk was moved to Friday evening and Jerry Payne to Saturday morning.
- The molecular biology session was moved to Saturday to accommodate the virologists who have a national meeting beginning Sunday in NY.
- A budget of $25,000 was adopted.
- March 01, 1992 was given as the final date for submission of papers.

Discussion centered around the loss of Dick Jaynes from the program. It was suggested to keep Dr. Jaynes, but delay his invitation until the funding situation is clarified. A vote was taken to accept the meeting as outlined--the vote passed with one dissention.

Possible objective for a new NE-140 project might include:

-gene and gene products of dsRNA and the fungus
- ecology of the tree and dissemination of H
-breeding for resistance

It was suggested that each Experiment Station submit the following in their annual report:

-objectives
-research for the past 10 years
-rationale
-methods
-publications
-references
-chestnut research publications

Dennis Fulbright and Michael Milgroom agreed to put the new project report together and stations should submit their annual reports to Dr. Fulbright by **November 15, 1992**. Dr. Rhode suggested that individuals who contribute through each Experiment Station should be included in the report.

The annual report is due March 15, 1992.

Dr. Shain reminded everyone of the International Forest Pathology meeting which will be held in Montreal in August, 1993.