NE-140 Technical Committee Meeting
Biological Improvement of Chestnut (*Castanea* sp.)
Toftrees Resort, State College, PA
19-21 October 1990

Attendance:

Project leaders or representative from participating stations or agencies:

Connecticut: Dr. S.L. Anagnostakis
Kentucky: Dr. L. Shain
Michigan: Dr. D.W. Fulbright
New Jersey: Dr. P.J. Bedker
Roche Inst.: Dr. D. Nuss
Tennessee: Dr. S. Schlarbaum (absent)
USDA-CSRS: Dr. J. Barnes (absent)
USDA-SEA: Dr. J. A. Payne (absent)
Virginia: Dr. G. J. Griffin
West Virginia: Dr. W.L. MacDonald

Administrative advisor: Dr. R. A. Rhode, Univ. Mass. (absent)

Other participants:

Connecticut: Dr. P. Gordon
Maryland: Dr. E. Seligmann, Jr., Dr. Al Webb
Michigan: Ms. C. Durbahn, Ms. Nibedita Mahanti, Mr. D. Huber
New Jersey: Dr. B. Hillman, Mr. Matt Brown, Dr. Ronny Shapiro, Dr. Gill Choi
New York: Dr. M. Milgroom, Dr. W. Powell, Mr. Stan Wirsig
Pennsylvania: Dr. D. Davis, Dr. B. Nash, Ms. Mary Torsello, Ms. Marian Lander Ms. Coralie Bloom, Dr. Glen Stanosz, Mr. Glenn ?
Vermont: Mr. J. Herrington (Exec. Dir., ACF)
Virginia: Mrs. L. Griffin, Dr. F. Hebard, Dr. M.K. Roane
West Virginia: Mr. M. Double, Mr. S. Enebak, Dr. Val Ulrich
Ontario: Dr. and Mrs. Colin McKeen, Dr. G. Boland, Mr. Martin Dunn

The meeting was called to order at 1:00 pm October 19, 1990 by Chairman Shain. Bruce Nash, who served as local host and arrangements Chairman made several announcements. The group was welcomed by Assistant Dean Robert Todd from The Penn State University. The meeting was organized by Regional Project objectives.

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

Sandra Anagnostakis, Connecticut Ag. Exp. Station

In a blight resistance test, 2 standard virulent (v) isolates were used, Ep 155 and Ep 389. American chestnuts in an area where hv has never been introduced were inoculated, along with a group of *Castanea.mollissima* (from northeast China as documented by USDA records), two specimens of *C.crenata*, one *C.henryi*, some F1's and a backcross (B1). Only green bark tissue was used and the stems ranged in size from 3-5". The bark was peeled away for final measurements at 119 days and horizontal

expansion was measured to determine resistance. Resistance rankings are as follows: Japanese (most resistant) followed by B1, Chinese, *C. henryi* and American. She now plans to concentrate on Japanese.

Peroxidase tests have been started using the methods of Frank Santamour. Peroxidase has been linked to rapid suberization of tissue and potentially may be involved in wound healing. Santamour's method includes bark scrapings that are ground up and run on starch gels, but Sandra uses 2 dormant buds, run on agarose or acrylamide and stained for peroxidase. A new peroxidase isozyme type with a faster-moving band than Santamour's was found in three *C. dentata* clones. *C. crenata* and *C. henryi* also produce a single band, but they are different from C. dentata's type. *C. mollissima* produces two bands. She hopes to use isozyme markers to look at progeny. The results are summarized as follows:

C. dentata	AA
C. crenata, C. henryi	BB
C. mollissima	AA, AB, BB

Martha Roane, Virginia Tech

The 1985 NE-140 pathogenicity study, devised by Jack Elliston and tested in CT, WV and VA has been analyzed. The data from the three sites were analyzed statistically in each of three categories: canker area, number of stromata/canker, and number of stromata with perithecia, and ranked via the three sites. The final group ranking was as follows: Ep 155 (most pathogenic), Ep 523, SOSM (Duke),WK(VPI), CR (VPI), 5-9-1B (WV), CL1-16BLBO (MI), and CL1-16 (MI) (least pathogenic). She has volunteered to write the manuscript for publication and will submit it to the *Canadian Journal of Botany*, since they have no page charges.

Gary Griffin, Virginia Tech (reporting for Graciela Farias)

With respect to tannins the fungus grows more rapidly on American chestnut, as compared with Chinese, but there was no significant difference in growth at the end of the experiment (using winter bark). She has found that tannins are higher in American than Chinese, but the tannin level drops just before tannase peaks, so other enzymes may by involved.

Her other area of work is tannase purification, but this work is just beginning. Her problem is that a great quantity of protein is necessary. She plans on looking at the degree of specificity of tannase.

Mary Torsello, Penn State University

A survey of *C. parasitica* on scarlet oak (60-80 years old) in 8 stands during the summer of 1990 was conducted. Transect lines were established in each stand and basal and bole cankers were recorded. A flashlight was used in the sampling process to look in the bark cracks for stromata. Bark samples were taken whether or not the fungus was visible. In terms of *C. parasitica* on scarlet oak, her preliminary results are as follows, based on 821 trees: basal cankers 6.9%, bole cankers 6.3%, both basal and bole cankers 1.7%. In comparison to Nash's data in North Carolina, taken in the early 1980's, there are more bole cankers in PA than in NC. Of those trees exhibiting symptoms, 69% had visible signs of the fungus (stromata visible 58.5%, stromata/perithecia visible, 8.1% and mycelial fans visible 2.4%).

Fred Hebard, American Chestnut Foundation Research Farm

He showed the mathematical model he developed to show that blight incidence increases after clearcutting.

He showed data relating to occurrence of old trees with hv-type cankers. Cankers were described as healing or non-healing and he presented data as to the number of provisional hv cankers.

Colin McKeen, Ontario, Canada

He stated that the Canadian chestnut council is moving slowly but confidently. The council met May 9 of this year and William MacDonald was the guest speaker. He noted that MacDonald gave two very interesting presentations. On May 12, McKeen was interviewed on CBC radio, and this interview has brought a good response and public awareness.

He stated that he has used the term "comeback" or recovery over the past several years and he has received some criticism. He uses the term based on the following information given by Professor Fox on Ontario in the late 1940's.

> *Chestnut blight entered Canada at Niagara Falls in 1920. In 1946 a survey was conducted in Ontario, and Prof. Fox states "...in 1939 there were still a host of chestnut trees in Ontario, some infected but still bearing heavily. By 1946 no trees are still alive and bearing-the vast majority are blasted before hey reach 6 feet".*

Ontario is on the northwest fringe of the natural range. There are still 60 or more blight-free American chestnuts from 15-70 cm dbh. He conducted 2 surveys this year for epidemiological information. For hv testing he is using the Arner isolate (a slowly growing isolate) by inoculating it into cankers. He inoculated it at 23 sites during May/June, but it's too early to talk about conversion. He has now identified 4 or 5 isolates that he feels are better than the Arner isolate.

Lou Shain, University of Kentucky (reporting for Mr. Shaojian Gao)

Because previous studies suggest a role for bark turgor in the development of canker diseases, studies were initiated to determine the effects of water potential in this disease relationship. Various osmotica were used: NaCl, KCl, sucrose and salt mixture, with six isolates of the fungus: Ep 155, 915, 780, 421, 717 and 758. Mycelial growth and conidial germination were monitored on osmotically adjusted corn meal agar. When osmotica was KCl the fungus could withstand -9MPa (megapasquals or bars). Growth was extended using sucrose (-12MPa). There was evidence of sodium toxicity at -6MPa. Conidial germination could withstand more osmotic potential than mycelium.

To address what is happening with the host, excised chestnut stems were preconditioned at various relative humidities: 100%, 90%, 70% and stems soaked in water. The stems were inoculated with Ep 155 via the cork borer method, seven days after preconditioning. Largest cankers were produced on the most water-stressed stems (70%), while the stems soaked in water produced the smallest cankers. As bark moisture decreased, canker expansion increased. Conidial infection of chisel wounds was not favored by low bark moisture-all treatments were similar in terms of canker area.

Effects of water stress on host bark/water status changes were measured *in vivo*. Monthly samples were taken and the lowest month was February (-2 MPa) while July was the highest (-0.8 MPa). The pathogen can handle the low osmotic potential (down to -6 MPa)-it's the tree that can't withstand the low osmotic potential. Thus, bark water stress seems to have less effects on the pathogen than on host resistance.

Amino acid and soluble carbohydrate accumulation was examined in terms of effects on water stress. Although proline and alanine increased in bark with increasing time there was no correlation between these amino acids and water stress.

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 Hv 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 Hv inoculation. Material is being processed for additional studies on antifungal compounds which may be produced in response to challenge.

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 or 8%). Plots will continued to be monitored.

Gary Griffin, Virginia Tech

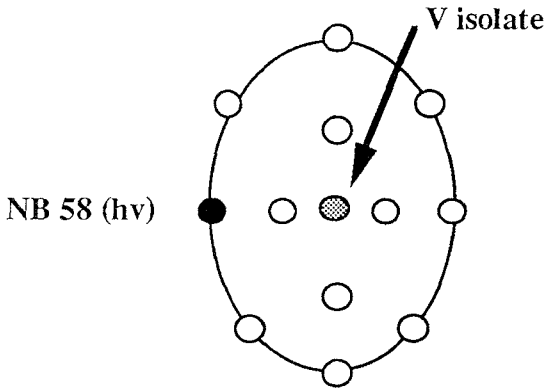
In conjunction with the data reported by Mark Double, Griffin is examining the same plots for apparent superficial cankers. In the cleared/hv inoculated plots 13 cankers are apparent superficial; 5 in the cleared/no hv plots; 1 in the non-cleared/no hv plot and no apparent superficial cankers in the non-cleared/no hv plots. He has measured the degree of canopy competition in the non-cleared plots using 6.1 meter radius subplots and the basal area of competing hardwoods ranged from 230 cm^2 to 516 cm^2. To give some perspective to the data, basal data less than 500 cm^2 will result in almost 100% chestnut survival, 50% survival for basal areas of 1200 cm^2, and greater than 2000 cm^2 will result in no chestnut survival. Competition, even in the non-cleared plots, at this point, is relatively low.

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

Peter Bedker, Rutgers University

Virulence/aggressiveness of isolates of *C. parasitica* is being assessed in apple inoculations, dormant stems, growth in culture and field inoculations. He screened 243 isolates from 7 sites on Granny Smith apples. Each apple is inoculated in 3 places-two unknowns and Ep 155, and the mean difference of growth is used as a growth index. From the 243, he chose 18 isolates that covered the range of virulence-fifteen were less virulent than 155 and 3 were more virulent (in the original test). These 18 isolates were inoculated into apples again and the data was different-essentially 2 groups of hypovirulent isolates. These 18 isolates, and Ep 155 were inoculated into dormant stems and again 2 hv groups emerged. In 1990, young stems in Clarksburg, NJ were inoculated using the 18 isolates and Ep 155-each stem was inoculated with all 19 isolates in a randomized block design. The isolates broke out into two main groups-those averaging 0.16 mm growth/day, and a group averaging 0.5 to - 0.7 mm growth/day. The isolates were then inoculated on PDA, and radial growth was measured. Two hv groups were distinguished. All the data was put together to determine what is the best predictor of isolate virulence (based on field testing) and the single best predictor of growth is radial growth on PDA. There were 4 isolates that were quite debilitated and the clustered at the far end of the liner regression-if those isolates are removed from the data, the results may indicated a better predictor.

In Hv recovery studies, virulent isolates were used to initiate cankers in the field, and the NJ Hv isolate (NB 58) was used as a challenge isolate. Conversion of the thallus was examined at 4, 8, 12, 16, 24, 32 and 64 days. A cDNA probe (1.2 kb) that resides near the 3' terminus of the dsRNA was used as a probe against isolates from bark plugs taken in the sampling scheme, shown as follows:



Based on the above scheme, conversion progressed through the V thallus, so by day 64 all but two sample spots were converted.

Lou Shain, University of Kentucky

Investigations continue to assess the movement of cytoplasmic hv in chestnut blight cankers (similar to that of Bedker's discussed above). Cankers were initiated with Ep 289, a methionine-requiring (met^-) V isolate. This genetic marker permitted the distinction of cohabitation and conversion. Hv isolates (of same and different vegetative-compatibility groups, and non-methionine requiring or met^+) were introduced at 1 or 2 points and cultures from the sample areas showed that the Hv agent moved through mycelium around the periphery of the cankers within 3 weeks when the v-c of the V and Hv inocula were the same. Conversion of mycelium in the canker interior proceeded more slowly. Conversion of mycelium also was delayed but occurred when V and Hv inocula differed in v-c and v-c network. Based on sampling, the Hv agent is moving through the mycelium more rapidly than V mycelium is moving through chestnut bark Cirrhi are induced *in vitro* and then sampled. With few exceptions, cirrhi continue to yield V, met^- cultures up to 26 months after challenge, even though underlying bark yields Hv, met^- cultures. This lack of movement of the Hv factors may substantially limit Hv spread. Cankers also were sprayed with conidia and the pattern of conversion is much more complex.

Michael Milgroom, Cornell University

Using Parsons,WV v-c data, collected from 1978-82, spatial patterns were used to show the distribution of v-c types within plots. There were 12-27 v-c groups per plot and spatial patterns were used to try and establish whether sexual or asexual canker initiation is most common. The data was analyzed three ways: all cankers present, new cankers relative to cankers present, and data sets 1 and 2 but with multiple occurrences of same v-c groups eliminated. He found that aggregations of v-c groups occurred in all plots, the aggregations increase over time and aggregations due to multiple occurrences of same v-c groups appear on the same trees. Patterns suggest clonal reproduction on same tree (nearly half of all new cankers).

An alternative to typing cankers to assess movement, he has developed molecular markers to study sexual and asexual reproduction (RFLP's). He sees polymorphisms-there are two probes and each is unlinked and segregates as single genes. He also has isolated some repeated probes-he has looked at 38 isolates and found 37 patterns, so it is

almost a fingerprint. The advantages of RFLP's over v-c typing are: genetics easily understood, allele frequencies can be calculated, rare alleles can be identified and a large number of independent markers can be identified. The disadvantage is cost of capital expenses and supplies. He hopes to use RFLP's to determine how far conidia are moving and what % is attributed to asexual vs sexual.

Scott Enebak, West Virginia University

Many dsRNA isolates from Appalachia were recovered by two former students, Mike Likins and Jeff Sillick. He examined 90 of their isolates morphologically, and selected 50 for testing in apples. From these data, he selected 2 isolates with high virulence, 2 intermediate and 2 with low virulence for inoculation in a field test. The isolates show significant differences in lesion size among isolates with similar dsRNA banding patterns.

Studies have been started which compare the effect of dsRNA on the pathogenicity of selected isolates. Parent strains with dsRNA and progeny, with and without dsRNA (this relationship is defined as a "family") were inoculated in a field test in the summer of 1990. Of the six families examined, after two months growth, there is no significant increase in lesion size within a family when comparing dsRNA-containing and dsRNA-free isolates.

Complementary DNA libraries (cDNA) of three distinct dsRNAs were constructed to examine the interrelationships of dsRNA. Isolate SR-2 contains 1 band (12kb), isolate D^2 contains 2 bands (1.5 and 12kb) and isolate C-18 contains 11 bands (ranging in size from 1 to 5 kb). Two plasmids from the libraries of D^2 and C-18 and five plasmids from SR-2 were ^{32}P labeled and used to probe dsRNA preparations of European and North American origin. With the D^2 and C-18 isolates, the recombinant plasmids used as a probe hybridized only to its own template dsRNA-no other dsRNAs. While SR-2 did not hybridize to the other dsRNAs tested (D^2 and C-18), recombinant plasmids did hybridize to the single banded dsRNAs from other isolates of Appalachian origin, suggesting that the single-banded dsRNAs common to central Appalachia are common.

Don Nuss, Roche Institute

Their goal is to completely analyze all the bands in one isolate and they have chosen Ep 713. The strategies for determining molecular basis of transmissible hypovirulence are as follows:

1. Identify gross structural properties by direct analysis.
2. Orient and characterize cDNA clones of dsRNA; preform sequence analysis and translational mapping to determine genetic organization and gene expression strategies.
3. Introduce into strains of *C. parasitica*, cDNA copies of the dsRNA by DNA-mediated transformation or synthetic transcripts by transfection.

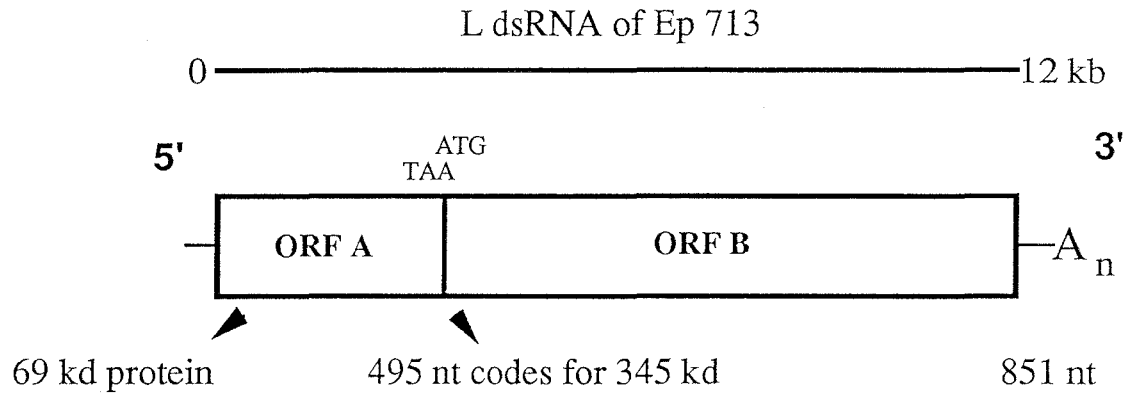
Ronny Shapiro, Roche Institute

He discussed strategy 2, as listed by Don Nuss. Their focus is on Ep 713 with a banding pattern as diagramed below.

L dsRNA (13 kb)

M dsRNA (9 & 11 kb)

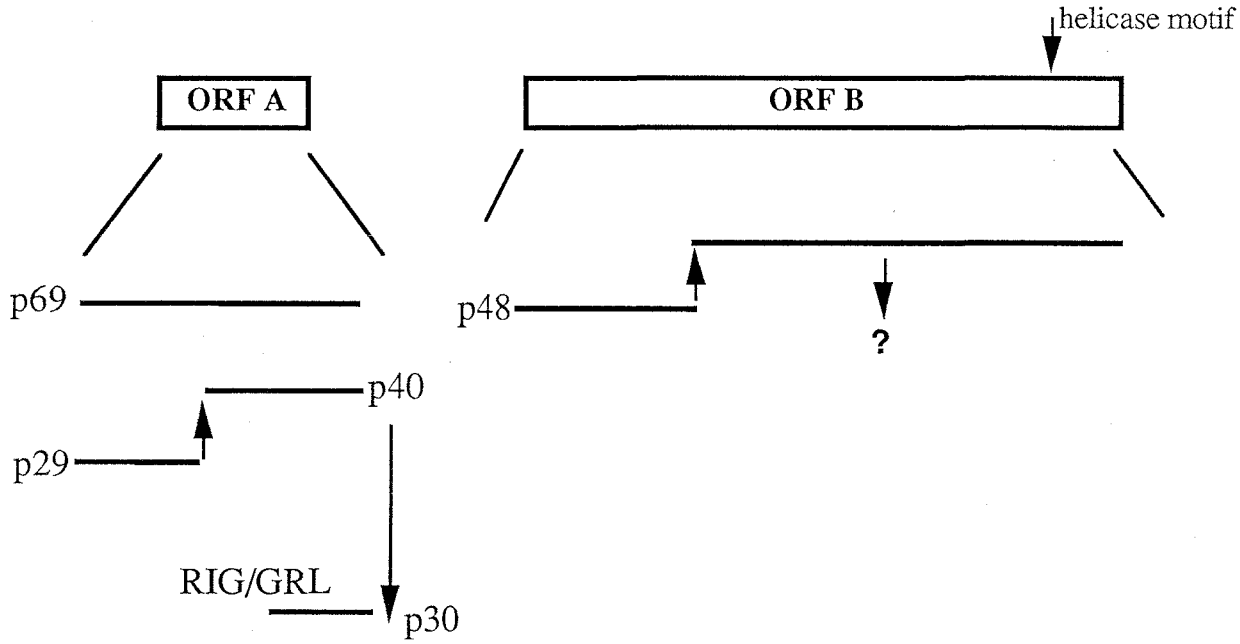
S dsRNA (1.5 and 3.6 kb)



12,700 base pairs

The two open reading frames produce protein of 69 and 345 kilodaltons.

These data were confirmed by the autocatalytic co-translational method as shown *in vivo* below:



He believes that we are dealing with a virus. Some bands are internal deletions and they are not always deleted. Sometimes they appear and sometimes they disappear.

Gil Chou, Roche Institute

He discussed strategy 3, as stated by Don Nuss. He is cloning *C. parasitica* genes and wants to use DNA complementary to dsRNA to understand what dsRNA does to the fungus. He wants the fungus to accept foreign DNA so they can begin to understand the basic functions. The following genes are being worked on: endothiapepsin, gluconase-3-phosphate dehydrogenase, laccase, cellulase and ubiquitin. Two of the characterized genes are:

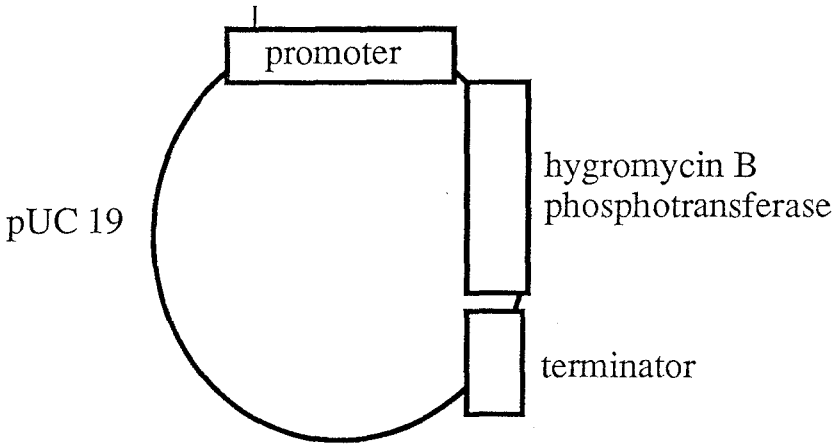
epn-1	endothiapepsin	419 aa

gdp	gluconase-3-phosphate dehydrogenase	337 aa

From sequencing information, canonical signals for transcription regulation are:

	5' Upstream	
	CAAT box	TATA box
epn-1	CAAT	TATA
gpd-1	CAAT	--

The *C. parasitica* transformation vector is shown below:



	Promoter	Terminator
pEPNHY2	epn-1 585 bp	epn-1 802 bp
pEPNHY3	epn-1 2100 bp	epn-1 802 bp
pCPGHY1	gpd-1 1700 bp	gpd-1 600 bp

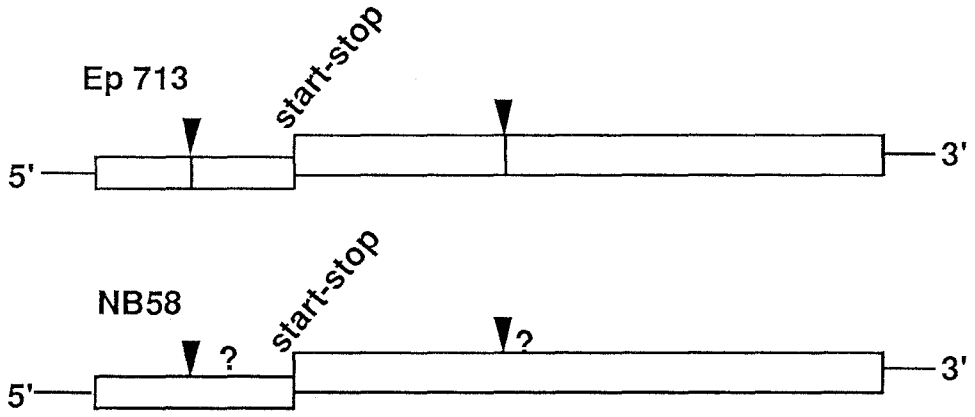
Brad Hillman, Rutgers University

He is working with the one-banded New Jersey isolate NB58-88. He has made a cDNA library to dsRNA and he's made overlapping clones. He has mapped 100 cDNA clones by sequence analysis. The structure of NB58-88 is similar in size to Ep 713. Some of the NB58-88 cDNA clones hybridized to Ep 713 and Ep 747.

Matrix comparison at the nucleotide level indicates the 5' terminal regions of Ep 713 and NB55-88 are 60% similar. However, once the first open reading frame is reached, there is no similarity-past the ORF, similarity picks up again (see diagram on following page). As far as the 3' terminus, there is greater alignment at the 3' terminus than anywhere. The 3' termini themselves are different but the region is similar.

He is examining Ep 747, a "flat" mutant. cDNA libraries have been made and all Ep 747 clones hybridized with Ep 713. Using clones to both termini and internal Ep 713 clones, there is not an integration of viral genome into fungal genome to explain flat

mutants, no matter how long the film is exposed. After examining 2 kb of Ep 747, it is 80-85% similar to Ep 713.



He has also examined Scott Enebak's C-18 isolate. It is an orange isolate but it's debilitated. C-18 has 11 dsRNA bands with no large band in the 12 kb region, as is common with most other Appalachian isolates. The 11 bands are equimolar in that all or none go into conidia. He took cDNA library to see if a major band with deletions could be found. He has not found a band that shares sequence similarity with another band. Five of the 11 bands have been examined to date, and all are unique. Speculation at this point is this may be a reovirus. Virologically, this is a distinctly different type of agent than Ep 713.

Bill Powell-SUNY ar Syracuse

He is separating chromosomes using electrophoretic karyotyping (CHEF). Protoplasts are lysed to separate chromosomes. He gets a pattern of 6 DNA bands, possibly 7. The chromosome patterns has been compared among *C. parasitica* isolates and they are all constant.

Chromosome Number	Avg. Size (megabases)
1	5.4
2	6.7
3	5.2
4	4.8
5	3.8
6	3.2

Estimated genome size: 32.1

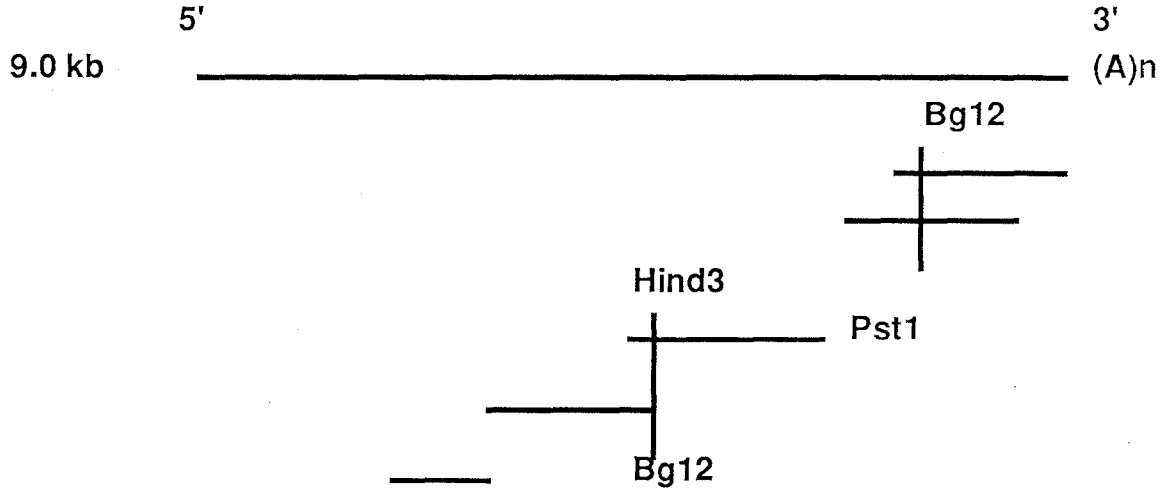
The bands can be blotted, and he has found that the #3 band contains ribosomal DNA. The vir gene which is expressed in V and not Hv, hybridizes with #3 band. He has looked at the following isolates:

US (Ep 42, Ep 3, Ep 155 2 from PA); Italy (Ep 67, Ep 501); France (Ep 113) and the banding pattern is all the same, so he feels that this is an indication that ascospores are the main source of propagation in the fungus

Concerning vegetative-compatibility typing, McCormicks red food coloring (18-24 drops/500 ml) accumulates in the barrage zone, making v-c typing easier to read.

Dennis Fulbright, Michigan State University

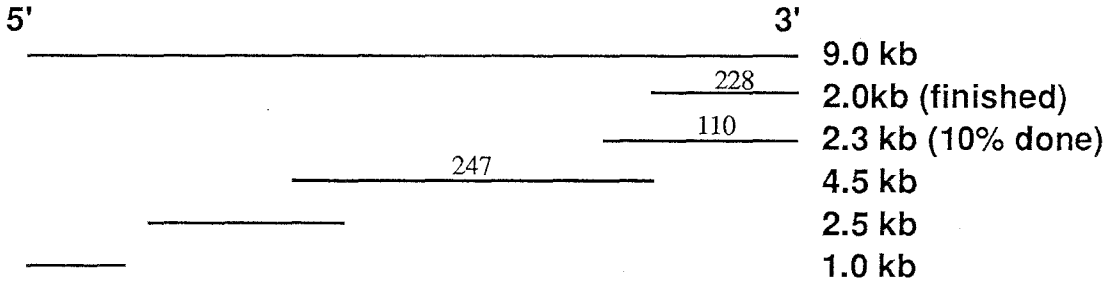
The biology of GH2 is hv, but 50% of the conidia that are "parental" looking and can kill seedlings. Ten percent of those isolates are extremely debilitating and have 100% of their conidia that contain dsRNA. Below is a diagram of a portion of the 9.0 kb band and the enzymes which have been used in the characterization.



Chris Durbahn, Michigan State University

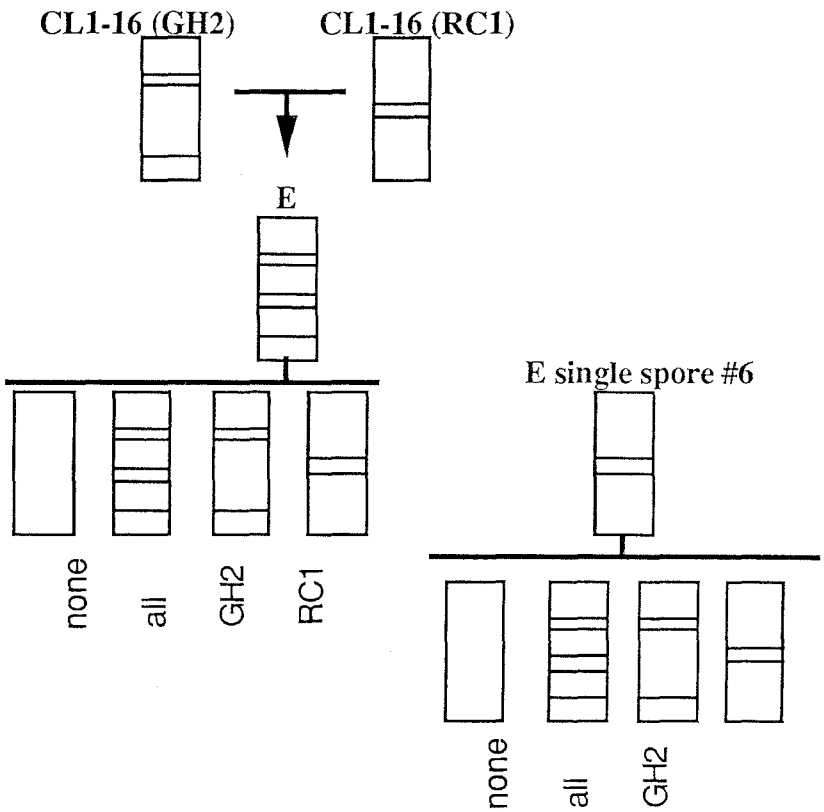
She is working on two projects: complete sequencing of the GH2 top band and putting two dsRNA types into one strain.

Concerning the sequencing of the GH2 top band, she is using the following scheme:



She has found no ORF-she has a lot of start sites, but they terminate quickly (~500 bases).

She is attempting to put 2 dsRNA types into one strain. The diagram on the following page explains her findings. She has used CL1-16 (with a GH2 backgound-3 bands) and mixed with CL1-16 (with a RC-1 background-2 bands). The single spore of this mixed isolate yielded a isolate, E, with all five bands. When E was single spored, four patterns emerged: no dsRNA bands, all five dsRNA bands, GH2 pattern or RC1 pattern. One of the RC1-like isolates, that was unique in that it was very slow growing, was single-spored, and it yielded four patterns, similar to those just described.



In an attempt to answer the possibility that dsRNA had inserted into the DNA, she probed the DNA of the fungus with cDNA 228 (see previous page) of GH2. The clone recognized the RC1 type dsRNA, but GH2 and RC1 dsRNAs are not homologous. She has digested Ess6 total DNA with many restriction enzymes and Bgl II and Hind III give only one band.

Nibedita Mahanti, Michigan State University

She is looking at CL 25, which is hypovirulent but contains no dsRNA.. It is transmissible, has reduced virulence and is successful in biocontrol. The hv factor is carried in 20% of conidia, is maternally inherited in sexual crosses and is cytoplasmically transferred through hyphal anastomosis, but it is not associated with dsRNA. She has decided to look at the mitochondria. She has cloned a 25 kb marker, and through experiments using chloramphenicol resistance and nuclear pigmentation, she thinks mitochondria transfer during hyphal ananstomosis, since mitochondria from one strain have been detected in the cytoplasm of another strain after pairing the cultures on cellophane.

She is also looking at cytochrome oxidase and she has found some cyto. oxidase activity in CL 25, but it's defective.

Dave Huber, Michigan State University

He is just starting his Ph.D. program and he will be looking at v-c groups and hopes to find a gene that is involved in anastomosis and clone.

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

Dennis Fulbright, Michigan State University

Many trees around Ohio have non-lethal cankers. These trees are all M1s from Al Dietz. Ep 155 was inoculated and it is growing well in some trees.

The trees in Gaylesville, WI are the source of the trees at the National Colonial Farm at Accokeek, MD.

He chose 20 isolates from National Colonial Farm and the Bob Evans farm in OH and inoculated them into apple and they're all relatively aggressive, however, they do not grow well in trees.

He is continuing his nut grafting and tissue culture (in 9 months the tissue cultures have gone from buds to shoots).

Bill MacDonald, West Virginia University

Five isolates of *C. parasitica* were inoculated on replicate trees growing in West Virginia (non-irradiated) and two locations in Maryland (irradiated), Stronghold and National Colonial Farm at Accokeek, 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.

Gary Griffin, Virginia Tech

He and John Elkins at Concord College (Athens, WV) had a good year grafting. Crosses made 8 years ago were inoculated with the Virginia V strain, WK. The 1 year results will be available in May, 1991.

He went to Lessane State Forest in VA this past year with Al Dietz to inoculate Al's trees. He paid tribute to Dietz, who died in August, 1990.

The airport planting at Virginia Tech was discussed. The site was established in 1974 and a blight epidemic developed and the larger stems in the spout clusters died. There is one tree that hadn't died, and some resistance was possible. It was inoculated with a standard V strain, and it died. Now the sprouts are nearly as large as the original trees and they have many superficial cankers.

A visiting scientist from Kashmir, India, Dr. M.A. Kahn spent a year in Dr. Griffin's lab. He worked with 53 *C. parasitica* isolates that were inoculated in a forest clearcut, along with their standard V isolate, WK. After four and one-half months length, width and degree of superficiality were determined. Based on the data he identified four clusters:

Classification	# of Isolates (out of 53)
Virulent	9 (including WK)
Intermediate V	18
Intermediate Hv	20
Hypovirulent	6

Some of the intermediate Hv isolates still cause some degree of necrosis. The cankers rated as Hv were completely superficial.

Lucille Griffin, Gary's wife has been assaying these isolates via dsRNA extraction. She has done all but 4 of the isolates, and she is replicating the extraction four times. She has found dsRNA positive isolates in all four classifications listed in the above table. Mostly a single band is found (of a size intermediate between the L and M band of Ep 713). Replicate extractions have been almost identical. They are in the process of diluting Ep 713 and GH2 to get sensitivities the same as unknowns on the gel during electrophoresis.

Sandra Anagnostakis, Conn. Ag. Exp. Station

The crosses she made last year were planted this year at the Exp. Station and at a nearby farm. She feels they'll see cankers within 4 years at which time she can evaluate. She is hoping to get information on how easy it is to evaluate resistance in the face of Hv.

She also did many crosses for arboreta.

Nuts were collected from a dwarf hybrid (*C. seguini* x *C. seguini*). She now has trees that flower early. She mailed Scott Schlarbaum ~2,000 nuts from these dwarf trees.

She went to the Forest Products Lab at Madison, WI and went through the USDA import records, in an effort to locate surviving imported *Castanea* spp. trees She succeeded in finding some valuable trees in CT (trees planted in 1916 with good parentage records that can now be used in breeding work). She read a letter written to Wilson Pomenoe af the USDA in 1923, regarding tree 58602 (parent of Maling and Nanking varieties). The letter stated that collection of nuts (destined for the US) occurred all over China, not just in one location. That explains why there is such a great deal of variability now.

Peter Bedker, Rutgers University

John Kuzer at Rutgers is involved in a backcross breeding program. He has 100 nuts from his previous crosses. Plantings were established near campus and at a research farm (several hundred acres). Last spring 100 Chinese and <100 American chestnuts were planted.

Philip Gordon, Connecticut Forest and Park Association

His organization strives to enhance the living of CT residents through trees. Through cooperative agreements, he has two tasks:

1. He has taken a population count of all American chestnuts in CT and they are found in almost every woodlot, mostly as an understory shrub or small tree. He has 6 state foresters make monthly reports of Am. chestnut and Phil visits the sites and verifies. He is looking for fruiting populations so he can collect nuts to give to CT residents (via a nursery outplanting). He works with many organizations and his goal is to plant 2.5 million seedlings.
2. He have his thoughts on survival mechanisms. He has a transect 2.5 miles around his home in Old Lyme and he examines the chestnut within this transect on a weekly basis. On Higby Mt., burrs were falling-he noticed how the burrs fell, and made particular attention to a burr that bounced uphill. He proposed that trees can develop uphill from parent trees because the burrs act as a bouncing ball. He also noticed that withered burrs sometimes one good nut. He has planted some of the nuts from withered burrs and he has 100% germination. Some trees that are dying produce nuts as small as peas. These will germinate and produce miniature plants, that will eventually take off and form normal-size seedlings. He believes that sprouts represent whole plants through somatic embryogenesis. He feels that with the possibility of somatic mutation, sprouts are not clones from one root system, but represent separate entities.

Fred Hebard, American Chestnut Foundation Research Farm

He planted 300 seedlings last year (including 60 backcrossses from the Douglass F1). He made crosses in CT and has about 200 backcrosses from the Clapper chestnut. He will be testing at various times for resistance. He harvested 1300 nuts this year from controlled pollinations.

There are now a total of 800 trees at the farm. He is budding using the epicotyl budding graft.

Bill MacDonald, West Virginia University

He paid tribute to Jim Comp who died this past fall. Colin McKeen suggested that NE140, in some way use the name of some of these senior citizens to carry on their memory.

Business Meeting

Thanks were extended to Bruce Nash and Penn State.

Sandra Anagnostakis was nominated as secretary for 1991.

Possible 1991 meeting sites are Meadowview (Hungry Mother State Park) VA suggested by Fred Hebard and Rutgers (pine barrens) as suggested by Peter Bedker. The new chairman (Griffin) will speak with both parties and make a decision.

Several individuals voiced favor for the weekend meeting format. Next year's meeting will be scheduled the last weekend in October.

Jack Barnes sent a letter and he indicated some points for NE-140's consideration:

-1990 farm bill
-National research initiative
-National Academy of Science research report
-the new plant pathologist at CSRS is Gabriel (who replaced Fulkerson)
-NE140 is slated for renewal in 1992. A renewal statement is needed by summer 1991.

The technical committee officers should draft the framework of objectives.

Gary Griffin and Sandra Anagnostakis reported on the International Conference, slated for spring, 1992. The meeting was initially scheduled for fall, 1991, but funding timeframe was not suitable for a fall meeting. The federal budget isn't approved until late October/early November, and that is too late to hold a meeting-what if the funding doesn't come through, then all plans are made with no money, so the suggestion was sometime in the summer of 1992.

The following people have been invited to talk: Carl Leopold (environmental issues, ecology); Dick Jaynes (history); Jerry Payne(chestnut industry in the US); Bonuous (?), Italy (status of chestnut in Italy); Vietez, Spain (chestnut physiology). Fred Paillet has agreed to be the evaluator.

The tentative meeting will start on a Saturday. The public, ACF members, growers, and press will be invited, and Leopold, Jaynes, a hypovirulence speaker yet to be selected and Payne will speak on Saturday.

The scientific session will run from Sunday through Wednesday. Each section has a main speaker whose expenses are 1/2 paid, and a resource person who is knowledgeable in an area, but not specifically on chestnut. Their expenses will be 1/2 paid and they will be expected to attend all scientific sessions and, in a written report, describe 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	Not organized	Not organized
Molecular Basis of Hv	Gobi(Italy)	Don Nuss (Roche)
Tree Breeding	Ellingboe (WI)	David Burke (Princeton)
Tree Ecology	Heineger (Switzerland)	?
Tree Propagation	Vietez (Spain)	Frank Santamour (USDA)