

The Connecticut Agriculture Experiment Station

Sandra Anagnostakis. Her hybrid grafting studies using C. sanguini & C. mollissima continues. These trees fruit early (less than one year). She has grafted some American and European chestnut but stated that it is difficult to test resistance of these trees, and she is using a weak auxotroph to test for resistance.

-Ceratocystis microspora has been found on some of the Scientist Cliffs hybrids.

-She is looking at JR, which is a single nuclear gene mutation that exhibits a "flat" phenotype, but does not necessarily contain dsRNA. She is using Ep 155 and Ep 67 with French and Italian hypovirulence agents and single sporing those isolates and looking for the "flat" phenotype.

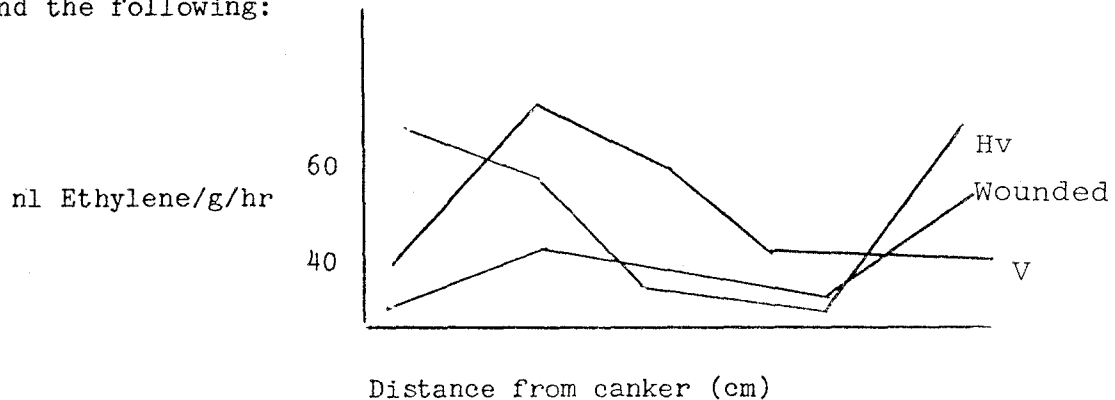
-She reported on the clearcut study set up in 1978 by Dick Jaynes and Nancy DePalma in which they cleared 4 circular areas (25 m each) leaving the chestnut and girdling other species. They sprayed with a Hv slurry mix once a year for six years. In 1982 they cut an additional 25 m on one-half of the 4 circles. She reported Hv is everywhere. She is trying to quantify the Hv spread, by using the two circles with the best chestnut. So far 50 vc types have been found in 200 sampled cankers. She indicated that there are more cankers/stem, with diverse morphology.

Jack Elliston. He reported on the natural spread of Hv agents in the plot of American chestnut trees at the Experiment Station farm.

-He also reported on his continuing study concerning the interaction of possible donor fungi that may contribute dsRNA to E. parasitica. He challenged Ep 523 at 1, 2, 3, and 4 days after the Ep 523 colony had begun to grow. He used four Penicillium spp. and one Trichoderma sp. and found that one of the Penicillium sp. disrupted the growth of Ep 523.

University of Kentucky

Fred Hebard. He is continuing his study on ethylene production in chestnut stems. He measures ethylene by taking bark plugs, inserting them into vacutainer tubes, lets them sit for 24 hr and then pulls out 1 ml for GC analysis. He has found that ethylene production increases with injury. He measured ethylene production at distances from a canker, and found that ethylene peaks at about 2 cm from a canker and then drops off. He also looked at virulent, hv and wounded trees in association with ethylene prod., and found the following:



Hebard (cont.)

-He also inoculated American chestnut and scarlet oak with V and Hv and found that American chestnut exhibits a quadratic effect indicating a repression of stimulation and a linear effect in scarlet oak indicating no repression of the stimulation of ethylene.

-He found early stimulation of ethylene in American chestnut, while little stimulation of ethylene in Chinese chestnut or scarlet oak when mycelial plugs of V or Hv were added.

Kevin Scibilia. He has been looking at the effect of a pre-symptomatic spray. From his 1986 field data he found an aggressive Hv strain converted significantly more cankers than a debilitated Hv strain of the same genotype. He found that a pre-symptomatic spray would protect a stem for a maximum of 10 weeks. He used Ep 780 conidia to try and determine how many conidia it takes to convert a canker, the data are as follows:

Ep 780 conidia/sq cm	Cankers Converted/ Total		
	Unsealed	Sealed	
40,000 (13.5 mil/ml)	24/26	9/10	Sealed=covered with a latex caulk
4,000	14/26	4/10	
500	5/26	3/10	
40	6/26	0/10	
0	0/26	0/10	

-He also looked at the effect of a spreader-sticker in aiding canker conversion. His data is as follows:

Conversion of V cankers inoculated 10 wk after painting with Ep 780 conidia determined at 2 and 5 wk

	<u>2 weeks</u>	<u>5 weeks</u>
In water	7/75	29/74
In Nufilm 1/1600	5/59	10/58
In Nufilm 1/600	3/75	17/71
Sterile water	0/45	0/44

-He presented data on the conversion efficiency of conidia from aggressive and debilitated Hv strains which were painted on chestnut sprouts inoculated with corresponding V strains:

Hv strain	Relative aggress.	Cankers converted/total
758	+++	20/23
63	++	23/24
779	++	24/24
901	+	2/12
1105	+	14/20
717	-	24/24

-With mixtures of Hv strains, he found:

Mixtures of Hv strains	Cankers converted/total
HI(2) aggressive	24/24
HT(2) debilitated	17/22
HI(2) & HT(2)	21/23
HI(2) and HI(1)	24/24

Scibilia (cont.)

-He also reported on conversion of Ep 155 and Ep 523 with Hv 780 (A) and Ep 721 (B):

Treatment	Percentage of Cankers Converted		
	A	B	Total
Sterile water	0	-	0
Tank mix of A&B	100	85	92
2 hr mix of A&B	70	70	70
2 wk mix of A&B with A first	100	16	60
2 wk mix of A&B with B first	15	85	50
A alone	95	5	50
B alone	5	75	40

Lou Shain. He discussed his interest in dissemination and the dynamics of canker conversion. He initiated cankers with a methionine requiring auxotroph allowed, the cankers to grow and then inserted a Hv plug (same genotype) at the base of the canker. He was looking to see if the cankers were converted, would Hv conidia be produced. The cankers were two mo old when they were challenged, and after interaction, cirrhi were induced to look at the spores. He showed overlays of the cankers and they showed that in some cases the periphery of the cankers were converted after 3 wk, and then with time there was conversion into the center of the canker. No Hv cirrhus was found in cankers interacting for 9 weeks. He did find some Hv cirrhi in cankers allowed to interact for 32 weeks, although only a few. There is still one set of trees to be examined 52 weeks after challenging.

-He initiated cankers with the same V isolate as above (Ep 289) and attempted conversion with mycelial plugs and conidial sprays. He found that those treated with plugs were readily converted, while those treated with the conidial spray were not.

Michigan State University

Patty McManus. She discussed her research dealing with water relations and how the fungus effects water movement. She found that the fungus decreases conduction in the stem and the fungus can be isolated from the xylem. She reported on hydraulic conductance on stems with and without cankers:

	Hydraulic conductance per unit length			
	Portion of Stem			
	Entire	Distal	Middle	Proximal
Normal Canker	2	219	1	144
Abnormal Canker	8	13	10	-
Healthy Stem	595	601	1043	1038

-She used a saffranin dye in the xylem and showed that in healthy stems most of the conductance is in the current years xylem, while in infected stems the dye was greatly reduced and there was no dye in the main ring axis.

-She found tyloses clogging the xylem elements and wanted to know if the fungus was also present, so she chipped away at the rings and plated out the chips, and presented the following data concerning percent fungal recovery:

McManus (cont.)

		Region or Growth Ring				
	Bark	1	2	3	4	5
Normal Canker	100	88	88	75	50	38
Abnormal Canker	88	86	100	86	--	--
Healthy Stem	0	0	0	0	0	0

-She showed slides of wood pieces which were incubated in a fluorescent stain and hyphae were evident in the vessels.

Dennis Fulbright. He stated that to date no West Virginia dsRNA has been found to be homologous with GH 2. They have found a DNA plasmid from two Crystal Lake strains in the mitochondrial fraction. The plasmid also has been found in a V strain from the same site.

-He updated the results from Sally Garrod's research: 20 trees at the Crystal Lake site were used to study movement of three marked strains, namely CL 116 (V), CL 116 with PCNB resistance, and CL 116 PCNB resistant with the GH 2 background. Plates containing the various isolates were tied onto the respective trees and movement was evidenced by isolate morphology and presence of dsRNA. The experiment was started in 1982 and the plates were replaced three times a year. In 1983 4 trees had Hv cankers (2 on inoc stems and 2 on noninoculated stems). In 1984 the Hv strain had moved to 2 additional trees (1 inoc and 1 noninoculated), and in 1985 had moved throughout the plot. They have recovered one isolate that contains a dsRNA band that is heavier than the heavy band in GH 2.

-They combined the dsRNA from CL 116 (GH 2 background that has 3 bands) and CL 116 (RC 1 background that has 2 bands) and produced an isolate (E) that has 5 bands. Upon single sporing the "E" isolate, some single spores had all 5 bands, some had the GH 2 parental pattern (3 bands), some had the RC 1 parental background (2 bands), while some of the single spores had 0 bands. One of the isolates (E ss6) had only 2 bands (RC 1 parental). It was subsequently single spored, and the following banding patterns were found: all 5 bands; no bands; 3 bands; and 2 bands. He stressed that most of the RC 1-like isolates yielded 0 bands-it was only the one isolate (E ss6) that gave the above pattern.

-He ran an agarose gel with GH 2; E; E ss6; and CL 116 and probed with dsRNA from GH 2 and found homology with all of the isolates. He postulated that there might have been some DNA contamination in the dsRNA. It still hybridized with the L and M bands. He used Hind III and lost lower band homology. If the probe is clean and the blot is correct, then there may be homology with the dsRNA and DNA.

-In conjunction with E ss6, he went back to the RC site and got another isolate, TDH. He found that this isolate had three bands (not corresponding to the GH 2 pattern) and the heavy dsRNA band is larger than the original heavy band. Upon single sporing of TDH, he found some single spores have only the top band, some have the top and bottom, while some have only the bottom band.

Roche Institute

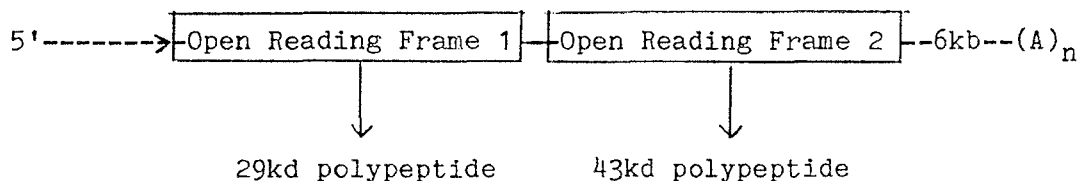
Don Nuss. He reiterated the structural analysis of the GH 2 dsRNA bands:

large band	9.0 kb
middle band	3.5 kb
small band	0.9 kb

Nuss (cont.)

The large band and the middle band are homologous, while neither of these bands is homologous with the small band. They plan on regenerating a full clone of the small strand—they will concentrate on the small strand to answer questions about homology.

He reported that Ep 713 contains a poly A region which is not homologous with GH 2 dsRNA. His group has generated a cDNA library of the Ep 713 dsRNAs. They have characterized and sequenced cDNA clones that correspond to the 2.8 kb non-homopolymer terminal portion of Ep 713 dsRNAs. They used strand-specific probes to show that single-stranded RNA corresponding to poly A-containing, but not polyU-containing strands, were present in the cytoplasm of the hyphae, indicating poly A-containing strand is the coding strand. Sequence analysis of independent cDNA clones indicated 5'-terminal portion of Ep 713 coding strand consists of a 497 nt non-coding leader followed by a 957 nt ORF, a 108 nt intergenic region, followed by a second ORF (at least 1,231 nt). A cDNA clone encompassing most of the 2.8 kb sequence was subcloned into transcription vector pGEM and translation of synthetic transcripts containing ORF 1 yielded a 29 kd polypeptide, while ORF 2 yielded a 43 kd polypeptide.



University of Minnesota

Dave French. He reported that in conjunction with the vegetative propagation in Horticulture, Paul Reed left for Nebraska and Mary Hosier has left for a private company. Their major thrust is still breeding.

Charles Burnham. He showed Arthur Graves' rating scheme:

	Chestnut (2n=24)	
Chinese Chinkapin (<u>C. seguinii</u>)	II,III,IV	
American Chinkapin (<u>C. pumila</u>)	III	
American Chestnut (<u>C. dentata</u>)	V, susceptible	
Chinese Chestnut (<u>C. mollissima</u>)	I, resistant	
Japanese Chestnut (<u>C. crenata</u>)	II	
F ₁ hybrid CXA	II,III	
F ₁ hybrid JXA	II,III	

Clapper's study:

			Cankers		
	None	Very small	Small	Large	Very Large
C.A. X C	51	52	19	11	7
		3	:	1	

Advantages of Backcrosses:

1. Only a few kind of crosses are necessary
2. Numbers needed can be estimated
3. Each resistant tree used for the next backcross is test-crossed with American.
4. Open pollination in seed orchards will produce the large numbers needed for F₂ and the final increase.

Burnham (cont.)

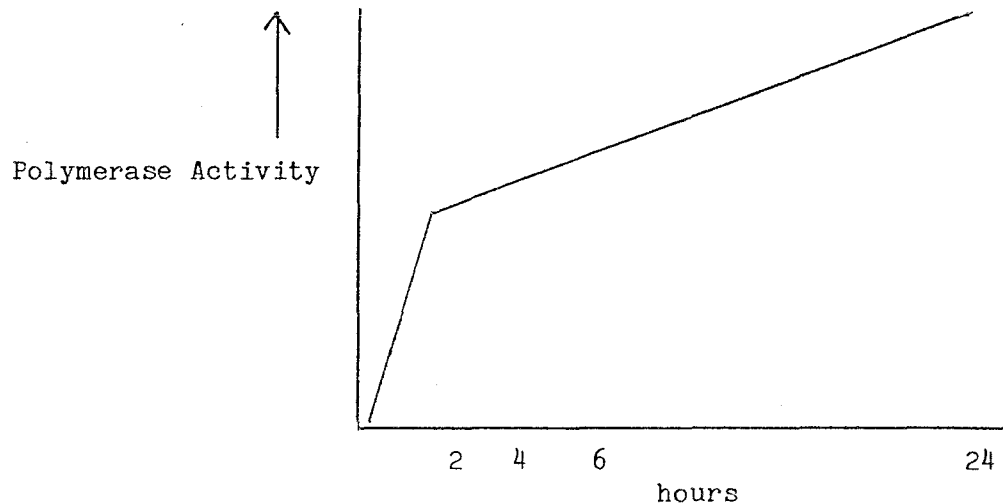
	Recurrent Parent in %
F ₁	50%
First backcross progeny	75%
Second backcross progeny	87.5%
Third backcross progeny	93.7%

University of Tennessee

Scott Schlarbaum. He is writing up a proposal for chestnut breeding, and is using Charles Burnham's approach. The proposal is out for funding now. He raised the question as to what is the best material to use for resistance, Chinese, Japanese, and where is it obtained? Gary Griffin suggested the Nanking cultivar as a good choice of Chinese material. He also stated that Japanese material is much more variable than Chinese. Scott stated that he hoped to get some material from mainland China.

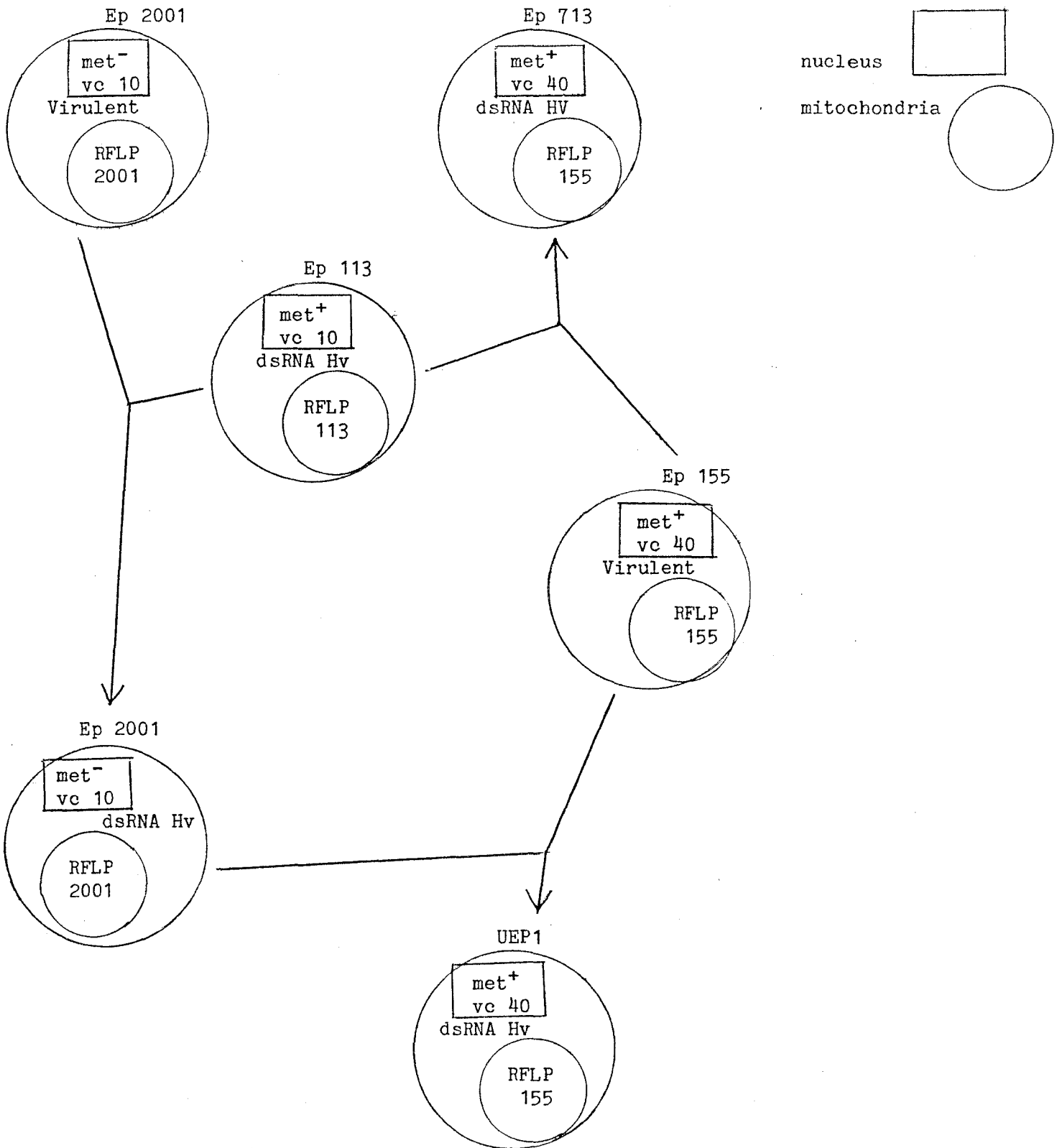
Utah State University

Dane Hansen. She reported that they can isolate vesicles from Ep 113 and polymerase activity is associated with the vesicles.



-They have isolated vesicles through a sucrose gradient and assayed along the gradient-the assay peaks where there are vesicles. dsRNA is associated with the vesicles and high polymerase activity. The polymerase activity is dependent on Mg-the optimal concentration is 5 millimolar.

Neal VanAlfen. He raised the question-what is the cytoplasmic Hv agent doing to the fungus, and what effect is it having? Does it affect the virulence of the fungus? He is working on a molecular model with strains that differ only in the presence or absence of the virus-like particles:



-They made a genomic library, isolated poly A RNA and made cDNA and added it in a 10-fold excess of the poly A from Ep 155. They used this as their probe, and found there are uniquely expressed messengers that are only found in the virulent strain. They looked at protein expression of two isogenic strains (UEP 1 and Ep 155/2) using 2-dimensional gels stained with comassie blue. They found some proteins expressed in the V and not the Hv. Some

Van Alfen (cont.)

proteins are partially or totally shut down, suggesting that the virus-like particles are very specifically regulating the fungus.

-He reported on the detection of a 24kd protein from Ep 155 which peaks at 10 days. He asked what does this protein do, is it a lectin? It was found to agglutinate red blood cells which is one property of lectins. Histologically, where does this protein accumulate? To answer this question, they sectioned cultures and embedded them in plastic, and found that this polypeptide accumulates along the surface of the hyphae. It is not expressed in the hyphae below the agar, nor the hyphal mat-it is only expressed in the aerial hyphae. Perhaps this is one of many polypeptides that is found in V and not Hv which may affect cell agglutination.

-They have introduced hygromycin resistance. They have made protoplasts of the fungus and introduced DNA from whatever source and grow out the protoplasts to screen for resistance. To date, two Aspergillus spp. have been the best promoters.

Wexford Soil and Water Conservation District, Michigan

Rick Denell. He stated that more than 100,000 seedlings of American chestnut have been raised and sold over the last 10 years. Last year (1986) they collected 10,000 nuts and were hoping to collect 20,000 this year. Their hopes have been surpassed as they have collected 80,000 nuts to date. They collected 200 pounds from the Magnificent Seven grove alone.

-They have initiated taking 50-100 nuts from each grove to study germination. They are also taking \$1.00 from the cost of each seedling to be put into an account to purchase land for a nursery. He also said there are plans to begin a northwest Michigan chestnut foundation-not to impinge on the American chestnut foundation, but there is a great deal of interest in that part of Michigan.

Ontario, Canada

Colin McKeen. Granny Smith apples are still being used to test his isolates for their degree of virulence. He stated that in Ontario there has been little planting beyond the natural range, and consequently, most of the blight-free trees are isolated and not producing nuts.

-The largest American chestnut tree in Ontario is 65-66 cm and about 18 m tall, with some cankers that have yielded dsRNA.

-In 1985 he had looked at 5 sites with chestnut, 9 isolates of Ep, 3 of which yielded dsRNA. By 1987 he has looked at 9 sites, 15 isolates, 4 of which contain dsRNA (dsRNA analysis has been performed at Michigan State University). His slowest growing isolate, from the ARN tree does not contain dsRNA. He has recovered dsRNA from isolates obtained at Moravian Town and Delhi-they have a white morphology. He commented that the fungus does not seem to be spreading very far.

-One of the trees which he examined, from Delhi, did produce viable nuts last year and he has germinated 8 seedlings from this tree.

Virginia Tech

Gary Griffin. He reported for Al Dietz who was unable to attend. The chestnuts at the National Colonial Farm were supplied by Dietz after irradiation with gamma rays from Cobalt-60. He passed out a reprint by Dietz from the 1978 chestnut symposium which gives additional information regarding the irradiation.

Griffin (cont.)

-Concerning his continued work on clones of large surviving American chestnut trees, he has established two plantations which will be tested in a few years for blight resistance. He has collected scion wood from the Clapper tree in Connecticut and has grafted onto trees at the Virginia Tech Horticulture farm in Blacksburg, where the Nanking and Maling trees are located.

-Since good grafting trees are hard to come by, John Elkins has attempted stooling, and he has had about 10% success.

-He discussed his clearcut research which was set up 4 years ago-all non-chestnut is removed every other year. He is finding apparently superficial cankers and he has gone to a rating system to avoid destructive sampling. He is marking the cankers to see if they remain stable and he has found that some have become unstable after one year.

National Colonial Farm

Donald Strasburg. He wanted the technical committee to assist the farm staff with some of their questions regarding the chestnut orchard. A statement was made that the committee would come up with some guidelines within the next year to address some of the questions that Strasburg raised. It was decided that they should continue to mow the existing orchards, and a third orchard should be started.

-He stated that 17 bushels of nuts had been collected this year, and they are continuing to donate them to the Maryland Department of Natural Resources.

American Chestnut Foundation

Phil Rutter. He showed slides from 9 founder trees at West Salem, WI-the trees were planted about 1880. There are 50 acres, 10 of which are pure chestnut with 5,000 individual stems and 900 canopy stems.

West Virginia University

Sue Whalen. She reported on some of the work of Ed Bosnoyan who looked at the homology between dsRNA and genomic DNA. He found that dsRNA from Ep 811 (a Hv isolate) hybridized to genomic DNA of Ep 811 and Ep 421, an isogenic strain without dsRNA. He also looked at sequence homology between a number of dsRNA isolates, from Italy, Michigan, West Virginia and Virginia. Under high stringency, Italian dsRNAs were homologous. A Michigan isolate and a Virginia isolate showed homology with one another, but not with the Italian isolates.

-She also reported finding RNA-dependent RNA polymerase activity and reverse transcriptase in two V and two Hv strains.

Adam Michna. Stomata and perithecial production and vegetative compatibility (v-c) types are being evaluated at three Michigan and three West Virginia sites. Cankers examined from the dsRNA containing sites in Michigan (County Line and Frankfort) have fewer v-c types and perithecia than cankers from the dsRNA-free site (Kellogg Forest). Overall, cankers examined from WV exhibit greater numbers of perithecia and represent more v-c types than cankers from Michigan.

Bill MacDonald. Stomata and perithecia production were examined from winter cankers obtained from three Michigan sites and two WV sites. Overall, the WV cankers exhibited more stomata (17 vs 11 stomata/sq. cm.) and perithecia (68 vs 30 perithecia/sq cm) than cankers from Michigan.

West Virginia (cont.)

Mike Likins. He is studying the same set of cankers as Michna but looking at the occurrence of dsRNA from two points in each canker. dsRNA has been associated with all cankers examined, to date, from County Line and Frankfort, and it has not been detected from any cankers from the Kellogg Forest. From cankers in WV, dsRNA has been recovered from slightly more than one-half of all cankers so far examined.

Mark Double. He reported on data from long-term dissemination studies involving trees in clearcut and canopy settings. After five yrs, Hv isolates were found to spread more commonly on inoculated stems in the canopy (83%) vs inoculated stems in the clearcut (31%). Hv strains were found to be disseminated among plots in the clearcut, while Hv spread in the canopy was mostly limited to the plot where the inoculum was introduced.

Steve Jakobi. He reported on 2 years worth of data from his dissemination plots in which he has made artificial punch and branch wounds, made a four different times over a one year period. Most cankers arising from the artificial wounds have been associated with the punch wounds (55%), and wounds made in the summer months are much more likely to become infected than wounds made in the winter. Dissemination of Hv strains has been poor, to date (only 15 of 495 cankers).

Jeff Sillick. He is looking at a number of parameters, including chestnut stem surface area, bark colonized by Endothia and occurrence of dsRNA from different age clearcut and canopy sites in West Virginia, Virginia and Maryland. The greatest number of infections occur between 5 and 50 cm on the main stem, although basal infections account for the greatest area of bark infected. To date, 59% of the cankers examined contain dsRNA.

Dale Hindal. He reported on the work of Rick Bennett, who examined healthy and infected chestnut bark for calcium oxalate and oxalic acid. He used isogenic V and Hv strains to initiate cankers and found oxalate content was not different in V and Hv cankers. He observed calcium oxalate via SEM in both healthy bark and fungal hyphae, and found the greatest density of crystals in the older region of the hyphae.

BUSINESS MEETING

1. Deadline for annual reports to Dr. MacDonald is December 15, 1987.
2. Next year's meeting will be held in Connecticut and hosted by Drs. Elliston and Anagnostakis.
3. The 1989 meeting will be held in Tennessee with Dr. Scott Schlarbaum as host.