NE-1015 Technical Committee Meeting
Biological Improvement, Habitat Restoration, and
Horticultural Development of Chestnut by Management of Populations, Pathogens and Pests
National Conservation Training Center, Shepherdstown, WV
October 26-29, 2006

Attendance:

Connecticut:	Sandra Anagnostakis, (Connecticut Agricultural Experiment Station)
Kentucky:	Lynne Rieske-Kinney (University of Kentucky)
Maryland:	Donald Nuss, Gil Choi, Fuyou Deng, Quihong Sun, Xuemin Zhang, Diane Shi (University of Maryland Biotechnology Institute); Robert Strasser (Hood College)
Michigan:	Andrew Jarosz, Dennis Fulbright (Michigan State University)
Mississippi:	Tom Kubisiak (USDA-FS)
New Jersey:	Bradley Hillman, Sara Baxer, Joanne Crouch (Rutgers University)
New Mexico:	Angus Dawe—chair-elect (New Mexico State University)
New York:	William Powell (SUNY-ESF), Alice Churchill, Keiichi Sudo, Michael Milgroom, Cristina McGuire, Marin Brewer (Cornell University), Steven Jakobi (Alfred State University)
North Carolina:	Paul Sisco (TACF)
South Carolina:	Laura Georgi, Haying Liang (Clemson University)
Tennessee:	Hill Craddock (UT Chattanooga)
Vermont:	Michael Vayda (University of Vermont)—Administrative Advisor
Virginia:	Fred Hebard, Bob Paris, William White (TACF, Meadowview)
Washington:	Nicholas Wheeler (Molecular Tree Breeding Services, Centralia)
West Virginia:	William MacDonald—chair, Mark Double (West Virginia University)

The meeting was called to order by Chairman MacDonald at 8:15 am on October 27, 2006 at the National Conservation Training Center (NCTC) in Shepherdstown, WV. MacDonald introduced Jim Willis who welcomed the group to NCTC, a 540-acre facility. NCTC, opened in 1997, was constructed as a training center for the Department of Fish and Wildlife, an organization with 8,000 employees. The facility was intentionally built too large for the purpose of expanding educational opportunities. The facility also serves other Federal agencies, USGS, Park Service, USDA, etc. but fifty-percent of current use is for educational groups. The facility was built with low maintenance in mind (e.g. stainless steel roofs). There was a concerted effort to make the facility blend in with the WV landscape and a lot of local stone was used in construction of the buildings. Willis encouraged educational groups to take advantage of the facility. Chairman MacDonald then asked attendees to introduce themselves.

<u>OBJECTIVE 1</u>. To improve chestnut trees for reestablishment in forest ecosystems, and chestnut cultivars for nut production by selection, breeding, and marketing, and determine the cultural criteria of all chestnuts for successful production in nurseries, orchards, and/or natural settings.

Fred Hebard—The American Chestnut Foundation, Meadowview

Hebard indicated that the TACF is trying to transfer blight resistance from Chinese to American chestnut. The two species are crossed resulting in trees that are 50% Chinese and 50% American. Each backcross generation reduces the Chinese genes by a factor of one-half. The hypothesis was that by the third backcross, trees would be $1/15^{th}$ Chinese and $15/16^{th}$ American. The $15/16^{th}$ chestnuts hopefully will resemble American chestnut in form and behave like American chestnut in the forest. At each step in the backcross process, resistance to chestnut blight is retained. This is done by inoculating trees with the blight fungus and selecting for resistance. At the $15/16^{th}$ level, trees will be intercrossed to get trees that are homozygous for blight resistance and then release trees into the forest. Blight resistant trees are selected at each backcross. He presented the following summary of the backcross method.

C X A	Proportion Chinese	Proportion Resistant
F1 X A	1/2	All
B1 X A	1/4	1/4
B2 X A	1/8	1/4
B3 X B3	1/16	1/4
B3F2 X B3F2	1/16	1/16
B3F3	1/16	All

Currently, they are in the process of producing the 3^{rd} backcross F2 generation and starting to produce the 3^{rd} backcross F3 generation. The number of trees at Meadowview in 2006 is listed in the table below.

	Number of		
Type of Tree	Nuts or Trees	Sources of Resistance	American Backgrounds
American	2162		235
Chinese	692	51	
F1	523	22	90
B1	425	15	33
B2	1559	10	91
B3	3818	9	77
B4	9	1	1
B1-F2	769	4	8
B2-F2	341	5	9
B3-F2	12376	2	29
B3-F3	121	1	2

F2	710	6	6
F3	6	1	1
Other	2502		
Total	26013		

The research farms in Meadowview cover 133 acres. The expectation is that VA trees may not grow well in ME and vice versa so there is a vigorous chapter breeding program, conducted by volunteers, underway by the TACF for local adaptation. These state programs are coordinated by three individuals (Paul Sisco southern region, Sara Fitzsimmons, mid-Atlantic and Leila Pinchot, New England). Most of the states have large numbers of backcross trees as seen in the following table.

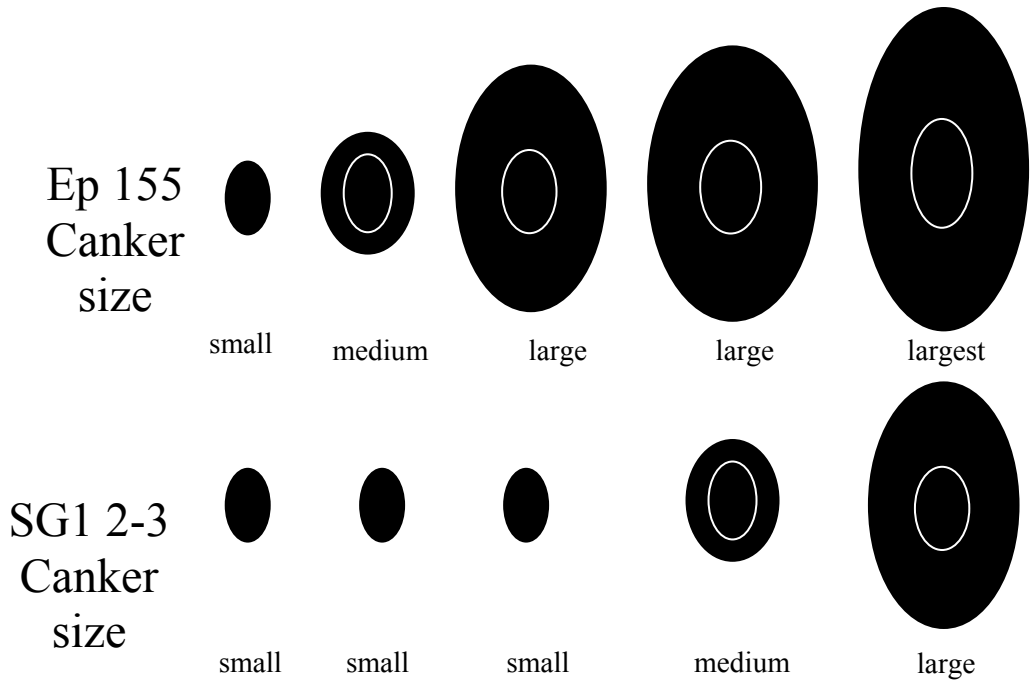
	Number of		
State	**Nuts or Trees**	**Sources of Resistance**	**American Backgrounds**
Maine	1962	2	39
Vermont	110	1	2
Massachusetts	4502	2	46
Pennsylvania	4837	2	38
Maryland	689	1	11
Indiana	3931	1	19
Kentucky	802	2	7
Carolinas	1064	2	14
Tennessee	1287	1	10
Alabama	267	1	8
Total	19451		

The harvest for 2006 is as follows:

Pollination Type	**Cross**	**Number of Nuts**
Controlled Pollinations	American x American	225
	B1	483
	B2	652
	B3	83
	B4	23
	F1	245
	Chinese x Chinese	1920
	Crosses between European, Japanese & Chinese	409

	Large American x Chinese or Japanese	72
	Large American x Small American	401
	Large American x Large American	739
Open Pollinations	B1-F3	2657
	B2-F2	182
	B2-F3	6601
	B3-F2	17168
	B3-F3	97
Total		31957

For blight resistance ratings, Hebard uses the cork borer/agar plug method when trees are 2-4 years old. He found that younger trees tend to die as a result of the inoculation and that is why he generally inoculates trees when they are older. Hebard uses two virulent strains of the fungus, Ep155 and SG1 2-3. There is some variability with the hybrid trees but there is little variation in testing American chestnut. Hebard uses the following rating scheme for cankers inoculated with Ep155 (more aggressive) and SG 1 2-3 (less aggressive).



When these two virulent isolates are inoculated into a highly resistant trees like 'Nanking' and Mahogany" they produce very small cankers. In a slightly less resistant tree, there will be some growth beyond the initial lesion. In resistance class #3, Ep155 produces a large canker while SG1 2-3 produces a small canker. As trees decrease in

resistance. Ep155 still look the same while SG1 2-3 cankers get larger. Hebard stated that he used to measure the length and width of each canker to evaluate resistance, but there are simply too many cankers to evaluate and the subjective rating scale works quite well.

Hebard showed data on the evaluation of BC3 for resistance; he expects 1/16th or 1/64th of these trees to show high levels of resistance (blight resistance class 1 or 2) as seen in the following table.

Code of Mother Tree	Code of Resistant Grandparent	# Progeny Tested	1	2	3	4	5
CH271	CL285	120	2	6	29	50	33
CH199	CL112	35	0	6	14	10	5
CH34	CL198	84	0	7	11	27	39
CH726	CL130	91	0	3	17	40	31
CH283	CL98	247	5	28	82	69	63
CH520	CL287	145	1	7	30	42	65

Above data are based on the first season of canker expansion and there has been more canker expansion in these trees than expected. Hebard stated that he may not recover high levels of resistance from BC3F2s at this point. Part of the reason he isn't sure is that the above test contained no controls. The trees were tightly spaced (1' x 7' spacing) and there is no standard of comparison. He will try to rectify this. He is going to do a staged inoculation beginning with SG1 2-3 and thin out the most susceptible tress and inoculate with Ep155 after a few more years. This will hopefully give a better representation of their resistance.

The American Chestnut Foundation had a scientific review this summer conducted by Laura Finns (University of Idaho), Ron Phillips (University of Minnesota), Bob McIntosh (Australian wheat breeder) and Glen Stanosz (University of Wisconsin).

Hebard showed pictures of a stand of B1F2, 'Clapper', 'Graves' trees that were hit by gall wasp. Since gall wasp is a clonally reproducing insect, it may have adapted to this stand. In some instances, gall wasp is controlled by parasitic wasps. Hebard indicated that he could spray for gall wasp but an insecticide that would kill gall wasp also would kill the beneficial wasps.

Bob Paris—The American Chestnut Foundation

Paris talked about looking at resistance in Chinese chestnut to try and determine the number of genes responsible for resistance and see if there are any other sources of resistance for the breeding program that they are not currently utilizing. The three cultivars that serve as a source of resistance for the breeding program are 'Mahogany', 'Meiling' and 'Nanking'. Paris wanted to take a look at other Chinese trees to try and determine if there was any segregation for the resistance genes. They had available 18 different Chinese chestnut trees at the Meadowview farm and he used those in conjunction with the three aforementioned cultivars. More than1500 chestnuts were collected from the crosses of the 18 Chinese with each of the three cultivars. While the main focus was on Chinese chestnut, other "exotic" chestnuts were used, 409 chestnuts were harvested from the following crosses: Japanese x Chinese; Japanese x European and Chinese x European to see if there is any value in other species of chestnut, not

necessarily to serve as a single source of resistance but as a modifier or helper—things that can be added in and taken as a whole to increase resistance.

When offspring of Chinese crosses are challenged with the blight fungus, very small cankers are produced and it can be difficult to assess resistance. Paris will look at different 'races' of the fungus to see if he can find something a little 'hotter' than what they are using currently. Another way is to examine extended progeny and look segregation in a more susceptible state.

Questions were raised about the single-gene theory along with the possibility that resistance may be the lack of a gene. Hebard indicated that the lack of a gene can be looked at in the backcross method. There could be a mixed system. Is resistance partially dominant? It must be to explain the variability in resistance. Paris stated that he hopes to increase the number of sources of resistance.

Hill Craddock—University of Tennessee-Chattanooga

A dairy farmer walked into Craddock's office a few years ago and asked about growing chestnuts on his 300-acre farm to diversify his farm economy. The man had done his homework and simply asked 'what is the best chestnut to grow?' A chestnut cultivar trial using 20 cultivars was established using Amy, Eaton, Paragon, Gideon, Lindstrom 67, Lindstrom 99, Byron 3-3, Revival, Nanking, Smith, Colossal, Qing, Peach, Meiling, Bouche de Betizac, Willamette, Mossbarger, Shing, Sleeping Giant and *C. henryi*. Over the past four years, they have planted 60 different cultivars, all grafted clones. The textbooks state that chestnuts will begin to produce nuts after five years; in this trial after only three years, they harvested 500 pounds of chestnut, more than they knew what to do with. Next year, they will have to start marketing the chestnuts. Sisco commented that Fulbright, three years ago, suggested that marketing precede planting.

The experimental design was 20 main varieties (20 trees of each of 20 cultivars for 400 trees) planted in four 5-tree plots (only four replications per cultivar). Statistical power was sacrificed to look at five trees in a row. It is intuitively obvious what the cultivar differences are. Eventually, they will be thinned to two/plot because they are too close together. Some cultivars are really good.

This year he has no graduate students. He presented a map of breeding orchards with pedigree information. His breeding work is in conjunction with the TN chapter of TACF. His work is with 'citizen science' working with enthusiastic amateurs. Everyone has their own chestnut story. He feels like he needs a documentary film maker to follow him around because the stories are as exciting as the biology. His work takes place in a spectacular part of the world, the Cherokee National Forest about 5,000 feet. The ecology is dynamic. He mentioned hemlock wooly adelgid as a devastating problem in the Smokey Mountains. He also showed southern pine beetle which releases chestnuts.

The following table shows yields from his trials. Last year was a bust because of bad weather, but 2006 was a banner year mostly because of his students. They collected more than 3000 seeds this year but more than a 1000 were from one tree.

Year	Number of nuts
1998	241
1999	213
2000	462

2001	292
2002	614
2003	1518
2004	4658
2005	659
2006	3136

Over the years, he averages 1 nut/bag. This year, one tree averaged 5 nuts/bag, a spectacular year. Zero nuts in no-pollen control bags.

Most importantly, he is taking pollen from Fred Hebard which has been screened for blight resistance and American morphology and backcrossing into the TN population. Apparently it is working. Fred said that full resistance may not be recovered from BC3F2. Craddock showed pictures of BC2F2 (from Hebard seed) and they are beautiful (10 years old and 40' tall) and the cankers are almost invisible. They are better than the Chinese controls. The nuts for the *Phytophthora* screening are being sent to South Carolina. *Phytophthora* is a big problem in the southeast.

F1	72
TACF Backcross hybrids	2226
American x American	278
Phytophthora screening	218

Grafting is a fun, exciting hobby. All of his cultivar material is propagated by grafting. He has also been grafting native germplasm into the breeding orchards. The Park Service allows them to take material out of the park but they are not allowed to take any foreign material into the park. He has collected in the Great Smokey Mountains and grafted that material. He showed photos of his cloned material.

In response to a NE-140 meeting several years ago, Rob Doudrick suggested a larger test for resistance. When screening is conducted, it is one individual and there is no opportunity to replicate that and we are not learning much about the host/pathogen interaction from a single individual. But, we can clone those trees. He showed slides of TACF BC2F2 individuals that were screened for blight resistance. He has 8 or 10 clones in the guard rows around his cultivar plots. He called his statistical model, 'casualization' as they were planted in the order they came off the truck. These trees are ready now for an experiment and he made them available to the committee for a host/pathogen interaction study.

While in Turin, Italy doing his doctoral work, Craddock sent some chestnut material to the US. Some of that material is now in commercial production. He can now purchase 'Primato', a precocious Japanese/European hybrid, that he introduced. This is personally gratifying to think that he has contributed to chestnut growing in North America.

This year he was working with Mahn-Jo Kim of the Korean Forest Research Institute. Kim spent a year at John Carlson's lab at Penn State. Kim sent Craddock 10 commercial varieties from Korea. Craddock is interested in these for: (1) commercial cutivars and (2) possible sources of blight resistance and (3) possible sources of *Phytophthora* resistance and (4) possible sources of gall wasp resistance.

Chestnut cultivars graft-propagated in March 2006 at the USDA-APHIS-PPQ-PGQP facility in Beltsville, MD

Cultivar	Origin	Species	Note
Arima	Japan	*C. crenata*	
Daebo	Korea	*C. mollissima* x *C. crenata*	KFRI patented variety
Ginyose	Japan	*C. crenata*	Very large nut
Ibuki	Japan	*C. crenata*	
Ishizuchi	Japan	*C. crenata*	
Okkwan	Korea	*C. crenata*	Released by KFRI in 1965
Riheigur	Japan	*C. mollissima* x *C. crenata*	
Sandae	Korea	C. crenata	Korean native cultivar
Tanzawa	Japan	*C. crenata*	
Tsukuba	Japan	*C. crenata*	

Only 'Sandae' is a Korean native cultivar. The others are either Japanese or hybrids. He is particular interested in 'Daebo', a *C. mollissima* x *C. crenata* cross that was made to improve nut quality because the Japanese chestnut are not considered high quality because of sugar content. The inner skin adheres to the pellicle in Japanese chestnut. He was able to do this work at Beltsville at the Plant Quarantine Center.

He mentioned *C. henryi* germplasm that he obtained from Greg Miller and it by far the fastest growing germplasm that he has. It outgrows everything that he has in the orchard. They produce one nut per burr. He showed slides of variegated leaves of *C. henryi* and he doesn't know the cause of the variegation. As a nursery manager, he should destroy it, but as a scientist, he wants to keep it. Hillman suggested grafting. If it is not transmissible, then it is a transposon, not a virus.

Craddock showed slides of one-year growth of 'Colossal' and 'Shing' cultivars and they are growing very well. They are very productive.

Sisco asked Craddock to tell his White House story. The Forest Service called Craddock for a replacement chestnut tree at the White House. Apparently, the White House grounds crew over-watered the chestnut tree supplied by Fred Hebard and they were worried that it might be noticed, so Craddock supplied a tree.

Paul Sisco—The American Chestnut Foundation-Asheville

Interregional Cooperation. He is the TACF regional science coordinator for the south and he is responsible for KY, TN, GA, NC, SC and AL. He has a program with Ozark chinkapin in AR. VA has just become a chapter. The southern Appalachians are set to start at the New River. One thing he has encouraged is inter-regional cooperation because all chapters have the same problems but they have different strengths. There was a southern regional science meeting at Bendabout Farms in February. Fred Hebard, Bob Paris and Kim Steiner attended. At the meeting Craddock talked about his BC2F2 trees. Some of the BC2F2 trees are growing well and some have died of *Phytophthora*. In August, the southern Presidents gathered at the TN river gorge.

Breeding for resistance to *Phytophthora cinnamomi.* Sisco suggested that some of the *Phytophthora* researchers might be brought into the NE-1015 meeting because it is a major disease issue with chestnut. The symptoms are the tree begins to die and wilts within two weeks. A nice-size seedling dies within a few weeks. A black stain develops on the roots. He showed a map of the distribution of ink disease and *P. cinnamomi* on American chestnut, Allegheny chinkapin, and Ozark chinkapin from a 1945 publication by Crandall, Gravatt, and Ryan.

The areas of the Ozarks, northern Alabama and Mississippi and western Tennessee along with the Appalachian mountains were affected by *P. cinnamomi* some 60 years ago. Joe James, a physician, found *Phytophthora* on his farm, and he went to Clemson and elicited the help of Steve Jeffers and his lab. They purchased six large horse troughs, filled them with sterile soil and added stings as row markers and planted different families of chestnuts and chinkapins along with controls. Markers indicated where one family starts. These were replicated in the six times in the six tubs and they got very consistent results last winter. After the seedlings had grown up for about a month, they were inoculated with rice grains that had been impregnated with *P. cinnamomi.* After 21 days, plants start to die. The tubs are continually flooded with water and the water that comes out of the tubs is tested also. The *P. cinnamomi* used in the experiment was an isolate from Joe's farm so there was not introduced isolate used. In December, the trees were dug up and rated on a 0-3 scale (0=no visible lesions; 1=lesions on fibrous roots; 2=lesions on tap root; 3=dead). American chestnut is uniformly susceptible; the trees were ravaged and look like match sticks. Chinese chestnuts had very healthy root systems after growing in the same conditions. Both Japanese and Chinese have resistance. Sisco's preliminary conclusions were:

- Resistance is governed by a single gene from Chinese chestnut.
- The resistance gene is not completely dominant. F1's have high survival but their roots have lesions.
- About half of the BC3 and BC4 families coming from Meadowview contain the resistance gene
- More families are being screened this summer and will be screened next summer from seed provided by cooperators in the Pennsylvania Chapter.

There was a question as to the susceptibility of chestnut to *P. ramorum* and the work at Fort Detrick indicates that American and Japanese chestnut are both susceptible. The thought was that *P. cinnamomi* was introduced from the Indies, Sumatra perhaps, (for cinnamon) possibly into the ports of North Carolina. There were widespread reports of tree mortality in the Piedmont, dating from 1823. Crandall thought it was from cork oak imported from Portugal because *P. cinnamomi* had been reported prior to 1923 in Portugal.

Chestnut vs chinkapin. There is evidence of integration between chestnut and chinkapin. Allegheny chinkapin has 1 nut/burr and it has hairy leaves. It can grow 20-30' tall whereas Ozark chinkapin is more tall tree-like in its form. Fenny Dane at Auburn is doing DNA work on the chloroplast and she has found the Ozark and Allegheny chinkapins are different. In looking at all *Castanea*, it appears that Japanese chestnut is the outlier. American chestnut is closely related to European and more related to Chinese than Japanese chestnut.

Project on Chestnut Genetics. This is part of a four-year (9-1/2006 to 8-31-2010) project funded by NSF. The total award was $2.7 M. The goal is to map the *Fagaceae* family**,** or beech family, characterized by alternate leaves with pinnate venation, flowers in the form of catkins, and fruit in the form of nuts, one to seven in a scale or spiny husk that may or may not enclose the nut. Chestnut, oak and beech all have 12 chromosome pairs. The species to be studied are:

Principal: *Castanea mollissima* (Chinese chestnut)
Secondary: *Castanea dentata* (American chestnut)

Tertiary: *Quercus rubra* (Northern red oak)
Quercus alba (White oak)
Fagus grandifolia (American beech)

About 70% of the markers in oak work in chestnut. One of the questions to be answered is: Are there resistance markers in oak since it can contract chestnut blight? The people involved are: Ron Sederoff and Chris Smith (NC State University), Bill Powell (SUNY-ESF), Tom Kubisiak (USDA-FS), Sandra Anaganostakis (Conn. Ag. Exp. Station), Sara Fitzsimmons, Fred Hebard, Paul Sisco and Leila Pincho (TACF) and Nicholas Wheeler (Molecular Tree Breeding Services, Centralia, WA). The international collaborator is Antoine Kremer of France, winner of the Wallenberg Prize for his studies on oak and European chestnut.

Some of the tools that hope to be developed are:

(1) More molecular markers
   a. Single Nucleotide Polymorphisms (SNPs) are less variable -- useful for species-specific markers
   b. Simple Sequence Repeats (SSRs) have many possible alleles. These markers are useful for many purposes, including identifying parents and following specific alleles

(2) A more accurate genetic map. One use of a more accurate map is to help identify the location of blight resistance genes and any correlated (linked) traits.

Benefits to TACF's breeding program of an expanded set of molecular markers:

1. Genes for Blight Resistance. What genes in Chinese chestnut are conferring resistance, how many genes are there, and where are they located on the Chinese chromosomes? Why do we want to know how many genes? The answer will affect how many seedlings we need to plant out to recover at least one tree with all the resistance genes. In the following table, numbers in parentheses are the number of trees needed at the 90% confidence level.

Number of unlinked loci	BC family	F2 family
1	1:2 (4)	1:4 (8)
2	1:4 (8)	1:16 (36)
3	1:8 (18)	1:64 (147)
4	1:16 (36)	1:256 (589)

Do 'Clapper', 'Graves', 'Nanking', or other sources of blight resistance have different loci for resistance or different alleles for resistance at identical loci? The answer will affect how many sources of resistance we need to incorporate into our program. If they have different loci, we may want to "pyramid" all these loci into a single source of resistance or put mixtures of different sources of resistance into one location. Too many unlinked loci make the numbers unmanageable.

Number of unlinked loci	BC family	F2 family
1	1:2 (4)	1:4 (8)

2	1:4 (8)	1:16 (36)
3	1:8 (18)	1:64 (147)
4	1:16 (36)	1:256 (589)
5	1:32 (73)	1:1024 (2356)

The ultimate goal is to clone the DNA sequence for at least one of the blight resistance genes. How might we clone a resistance gene? Map-based cloning can be used. An ordered set of Bacterial Artificial Chromosomes (BAC) equals a physical map of the chromosome. The goal is to have markers on both sides of the resistance locus and a continuous set of BACs between them. A BAC library has been developed from the Chinese chestnut cultivar 'Mahogany' by Laura Georgi at Clemson University. How might we narrow down the region in which a resistance gene is located? Association mapping can be used with 'Clapper' and 'Graves' BC3F2 families at Meadowview and 'Graves' BC3 families at Penn State University. The need for advanced lines is where Hebard's trees will be very valuable. Association mapping can help answer what Chinese segments are common to most or all of our resistant selections after several generations of backcrossing. How could we then prove that a certain DNA sequence is a resistance gene? If a gene is found, it will have to be cloned with the help of Scott Merkle (University of Georgia) and Chuck Maynard (SUNY-ESF). Shoots are multiplied with cytokinin and then rooted with auxins. The plantlets are grown, inoculated with virulent *C. parasitica* and evaluated for resistance. If the putative loci for blight resistance were all cloned, they could be inserted as a single linked block (a "cassette") using the tools of genetic engineering. Or, if all the genes are alike, one could be inserted with a strong "promoter" regulatory sequence. Benefits to TACF's breeding program are an expanded set of molecular markers.

2. A calculation of the percentage Chinese remaining in our elite hybrid could be determined. It can then be discerned as how much Chinese chestnut remains in our seed orchard parents?

3. Discover contaminants and determine the pollen parent of the open-pollinated BC3F2 families in the 'Clapper' and 'Graves' seed orchards. Are any of the pollen parents contributing more resistance than others?

4. How genetically diverse are our seed orchard parents compared to American chestnut in the wild? How much genetic diversity have we recovered both at Meadowview and in our State Chapter breeding programs? Genetic markers do not code for the genes for adaptation, such as timing of bud burst in the spring. We need to measure adaptive traits on a whole tree basis, although we may find that some markers are linked to the genes controlling these traits.

Finally, the recommendation from the Reviewers at the Recent Audit of TACF Science was that "We recommend that there be a close collaboration of the TACF staff with the NSF grant participants in order to encourage research that will advance the breeding program."

Sandra Anagnostakis—Connecticut Agricultural Experiment Station

List of mature trees at CT (pure species). She has data on where these trees came from. If researchers need germplasm, she can provide scion wood for grafting. The list of mature trees is:

Castanea dentata (232 total)
Lockwood Farm:
- Michigan Soil and Water Conservation District, 1981, 83 trees
- Roxbury, 1988, 2 trees
- Watertown, NY 1989, 4 trees
- Norwich, CT, 1984, 1 tree
- Rocky Hill, CT, 1985, 43 trees
- Michigan and Wisconsin (from Thor, TN), 1976, 71 trees

The Chestnut Plantation
- Asheville, NC, 1933, 1 tree
- Thomaston, PA, 1933, 1 tree
- Roxbury, CT, #4 open pollinated, 1959, 1 tree
- Rosbury, CT, #1 open pollinated, 1959, 1 tree

Windsor
- Crosses of MI and WI trees at Lockwood Farm, 1989, 24 trees

Castanea pumila **(**2 total)
Lockwood Farm
- Empire Chestnut Company, Carrollton, OH, 2000, 1 tree

The Chestnut Plantation
- Empire Chestnut Company, Carrollton, OH, 2000, 1 tree

Castanea ozarkensis (65 total)
Lockwood Farm
- Crosses of plantation trees, 2002, 3 trees
- Ozark plateau, LeFlore County, OK, 2003, 57 trees

The Chestnut Plantation
- Russellville, AR, 1936, 5 trees

Castanea alnifolia (3 total)
Lockwood Farm
- Lafayette County, FL, 1985, 2 trees

The Chestnut Plantation
- Unknown source, 1936, 1 tree

Castanea sativa (7 total)
Lockwood Farm
- Bursa, Turkey from Arif Soylu, 1990, 5 trees
- The Black Forest, Germany, 1984, 1 tree
- Cavcas Biosphere Reserve, Russia, 1983, 1 tree

Castanea sequinii (4 total)
Lockwood Farm
- Cross of two trees 1998, 1 tree

The Chestnut Plantation
- USDA #70317 seed called “Mo lut tsz” 1930, 3 trees

Castanea henryii (6 total)
Lockwood Farm
- Nanjing Botanical Garden, from Liu Liu, 1991, 4 trees

The Chestnut Plantation
- Lu-Shan Botanical Garden, from R.C. Ching., 1935, 1 tree
- USDA #104058, Anhwei Provence, 1935, 1 tree

Castanea crenata (17 total)
Lockwood Farm
- 'Japanese Giant', Rochester, NY, 1990, 2 trees

The Chestnut Plantation
- Higashiyama, 2001, 4 trees
- Sakurayama, 2001, 2 trees
- USDA #78626, Oguriyama, 1929, 1 tree
- Korea from Col. E. Thompson, 1993, 1 tree
- 'Japanese Giant' from H.N. Folk, 1930, 3 trees
- USDA #104014 from Temple Forest, Koyasan, 1935, 1 tree
- USDA #104016, Numakunai Erinsho, 1935, 1 tree

Private Land
- 'Parsons' Japanese, 1876, 2 trees

Castanea mollissima (60 total)
Lockwood Farm
- USDA #7275, 1939 1 tree
- USDA # 7284, 1939, 1 tree
- USDA # 7273, 1939, 1 tree
- USDA #36666 from Beijing, 1939, 1 tree
- USDA #108552, K'ueii Lee, 1962, 1 tree
- Nanjing, from Liu Liu, 1992, 1 tree

The Chestnut Plantation
- USDA #70315, NE China, 1935, 2 trees
- Lu-Shan Botanical Garden, 1935, 1 tree
- Wen Chia Shih, 1972, 5 trees
- USDA #78744, Tiger Paw, 1930, 1 tree
- USDA #36666 open pollinated, 1943, 1 tree
- J.B. Gable, Stewartstown, PA 1938, 2 trees
- 'Mahogany' selfed, 1960, 1 tree
- FP 530 from Tientsin, 1935, 1 tree
- USDA # 104061, Mau, from Peter Liu, 1935, 2 trees
- USDA #104063 from Peter Liu, 1935, 4 trees

Private Lands
- USDA #58602 from J.H. Reisner, 1926, 33 trees
- USDA #36666 from F.N. Meyer, 1913
- USDA #39721 from S.S. Knabenshue, 1914

Controlled crosses. Anagnostakis mentioned briefly some of the crosses she has conducted.
- Crosses for molecular mapping. Leila Pinchot did the actual work in the bucket truck, putting 250 bags on each of two trees. There was good yield on one tree. This will be repeated.
- Crosses for gall wasp resistance studies.
- Backcross breeding program for *C. ozarkensis* for resistance to chestnut blight and *Phytophthor*a. Anagnostakis is conducting a joint project with the University of Missouri because graft incompatibility in chestnut is a huge problem. Anagnostakis is doing controlled crosses and cooperators at the University of Missouri will be grafting scions of the parents onto the offspring looking for evidence of genetic control of graft incompatibility.
- Crosses for orchard trees to develop cultivars that will do well in Connecticut.
- Crosses for timber trees.

Japanese chestnut and planting designs. Anagnostakis agreed 20 years ago that Hebard would use Chinese chestnut for his sources of resistance while Anagnostakis would use Japanese chestnut. If they had different genes for resistance, the genes could be combined. She showed photos of advance breeding lines that she has been selecting. In addition to selecting for blight resistance, she is also selecting trees that leaf out late, because in the cold climate of CT, it would be nice if they didn't get frozen as soon as they made leaves. Anagnostakis is looking at how to plant trees in the forest. She plants trees in recent clearcuts. She showed photos of a site with granite soils and lots of clay. She planted advanced F2 trees and there has been a fair amount of mortality; she will replant next year. There is a lot of American chestnut in the area around the clearcut. She samples all the cankers in the area, converts them with either Italian or French hypovirulent strains and she uses those strains to inoculate the natural cankers. She wants the American population of trees to remain alive so that they will cross with the hybrids. Some of her oldest plantings are 8 years old. Her hybrid trees are BC3 with Japanese as the resistant parent with northern American chestnuts as the other parent. She also has Chinese BC2. She is now making crosses between those various hybrids. The Chinese hybrids that she has are all male sterile and if they are planted with Japanese BC3 that are all male fertile the offspring of the Chinese BC2 will have the Japanese as the male parent.

Hypovirulence at Lockwood Farm. Anagnostakis showed pictures of American chestnut trees (from MI and WI) 23 years after the last treatment with hypovirulent strains. Treatments were conducted for 4 years using a cocktail of five hypovirulent strains. Most of the trees now exceed 25' in height. Half of the trees keep dying back and resprouting. About 15% of the original stems are still alive and never died back, whereas 33% of the trees died once, resprouted and they are now doing quite well, despite being heavily cankered. Hypovirulence is keeping the sprouts alive. There is clearly a difference in the American population in its response to hypovirulent strains. This phenomenon may be more than just vc types. Tree genetics, competition and time also may be factors. Some trees seem predisposed to die and treatment with hypovirulent strains will not be effective. Anagnostakis indicated that it may be a minimum of 20 years for sprouts to begin to survive in conjunction with hypovirulent treatments.

Michael Gold, Michele and Ken Hunt—University of Missouri (written report)

Chinese market analyses. In 2004, a nation-wide survey of chestnut producers in the U.S. was conducted. Results showed that the U.S. chestnut industry is in its infancy. The majority of chestnut producers have been in business less than 10 years and are just beginning to produce commercially. Volume of production is low (less than 1.5 million pounds). U.S. chestnut producers are mainly part-time or hobbyists with small, manually harvested operations. The majority of respondents sell fresh chestnuts. Demand exceeds supply and prices often exceed $3.50.lb. Barriers to success in the chestnut business include the lack of information for producers, retailers and consumers, 5- to10-year time lag to get a return on investment, and shortage of available chestnut nursery stock of commercial cultivars. There are also concerns related to pest and disease control and market uncertainties. Lengthy quarantines for cultivars form other countries and lack of chemicals registered for use with chestnuts can also be considered barriers to success. Chestnut growers' associations, universities, state and federal

agencies must join their efforts to fund and support chestnut research and industry development.

Graft union failures of Chinese chestnut trees. In May 2005, 20 each of Qing and Auburn Cropper seedlings were chip-budded with bud tissue removed from the same seedling. Graft unions formed on all of the trees, but very few of the chip buds broke and produced new growth. It is unknown if the lack of new growth was due to the use of small chip buds, environmental conditions, or some other unidentified problem. This experiment was repeated on August 22, 2006. A second study was conducted to identify the optimum time for chip-budding. Auburn Super was chip budded onto seedlings each of Qing and Auburn Cropper on July 21, Aug. 15, Sept. 1 and Sept. 19. Twenty trees of rootstock were budded at each date. In Spring 2007, grafting success will be evaluated.

Delayed union failure of Chinese chestnut trees. By summer 2006, about 33% of the Qing trees grafted in 2002 on seedling rootstocks of Miller 72-138 or Miller 72-66 have died. Failed trees tested negative for TsRSV, CLRV and ArMV viruses and also negative for phytoplasma using generic detection primers in Dr. Jerry Uyemoto's lab. In 2006, Dr. Robert Martin also had negative results using double-stranded RNA extraction methods.

Dwarfing rootstocks for Chinese chestnut cultivars. In March 2005, trees of the following combinations were grafted to evaluate the dwarfing potential of Little Giant as a rootstock and as an interstem:

Eaton/Cropper	Easton/Little Giant/Cropper
Auburn Super/Cropper	Auburn Super/Little Giant/Cropper
Easton/Little Giant	Auburn Super/Little Giant

In April 2006, 12 replications of each tree combination were field planted. During the first growing season, all tree combinations except Auburn Super/Little Giant and Eaton/Little Giant produce primary burs. From a grower's perspective, the lack of bur development during tree establishment would be a very positive attribute. Trunk cross-sectional area of the scion at 5 cm above the graft union, winter injury ratings of rootstock and interstem tissue, tree height and spread, annual yield and yield efficiency data will be recorded annually for 10 years. In March 2006, chestnuts obtained from Connecticut were sown to produce rootstocks. These chestnuts include: Lockwood, King Arthur, Little Giant and Hope. These seedlings will be used as rootstocks and grafted with two scion cultivars in April 2007. Trees will be field planted and evaluated as described above.

Reproductive biology of Chinese chestnut and thinning of secondary burs. Since 2005, a study has been conducted to characterize early chestnut fruiting (from primary and secondary burs). 'Willamette' and 'Peach' trees planted in 2002 are being utilized for this study. The objectives of this study are to determine the fate (i.e. return bloom, bur set, and nut production) of shoots that fruited the previous year. Relationships between vegetative growth and fruiting will also be determined. In 2005, all fruiting shoots per tree were tagged. Twenty replications of 'Peach' and 17 replications of 'Willamette' each cultivar were used in this study. Measurements included number of primary and secondary flowers and burs produced, total primary and secondary nut weight and number, yield per tree, shoot length to primary and secondary burs, total shoot growth, leaf number, and dry weight per fruiting shoot, and increase in trunk circumference. All measurements were repeated in 2006. In 2005 and 2006, the

percentage of secondary burs on chestnut trees in the repository at the Horticulture and Agroforestry research station was rated. In 2006, the following cultivars in the repository produced secondary burs on 51-75% of the main scaffold branches: 'Crane', 'Orrin', 'Armstrong', 'Douglas #1', 'Maraval' and 'Belle Epine'. Moreover, this heavy production of secondary burs was apparent on these cultivars much earlier (Aug. 3) than m any other cultivars that produced secondary burs later (by Sept. 3). Some trees, such as 'Auburn' 'Homestead', 'Miller 723-76', 'Simpson', 'Carr' and 'Miller 72-105' did not produce any secondary burs this season. Since 2004, there has been only one growing season (2004) in which none of the chestnut trees produced secondary burs. The lack of secondary flowering may have been due to unusually cool temperatures and above-average rainfall during June, July and August or the trees were in an 'off' year of alternate bearing. Weather records indicated that 2004 was the coolest summer on record since 1950 in Missouri. In 2006, a study was conducted to: (1) determine the effect of secondary bur removal on primary nut weight and size; and (2) ascertain the effect of thinning on subsequent vegetative and reproductive growth the following growing season. In late August, limbs of 'Willamette' and 'Peach' trees with small secondary burs were identified and primary burs were enclosed in loose netting to prevent loss of nuts. The following four treatments were imposed on chestnut trees: (1) removal of secondary burs; (2) secondary burs left intact on limbs; (3) tagging terminal limbs with secondary catkins (but no secondary burs; and (4) tagging terminals without selected and tagged as treatments. All fruiting limbs on each trees were included in the study to maximize the number of replications per tree. In 2007, bur set and nut yield will be evaluated. In another experiment, chemical thinning of secondary burs was also investigated using applications of NAA, Accel and Sevin alone or in various combinations applied on Aug. 2, 2006. Preliminary results show that there was a high rate of drop of secondary burs in many of the treatments by Aug. 30, perhaps due to high temperatures in the month. However, two of the hormone treatments increased the rate of drop by two weeks after application. Data will be collected and treatments will be repeated next season. As these studies progress, the search continues for the 'ideal' cultivar that produces an optimal yield of large, primary nuts each year with little or no secondary flowering.

Nitrogen fertilization of Chinese chestnut. In 2004, a study was initiated to determine the optimum timing and formulation of nitrogen applications. The objectives of this study were to: (1) compare growth and fruit yield of 'Qing' chestnut trees fertilized at various times with equivalent amounts of nitrogen; (2) determine the nutrient concentrations of leaves of 'Qing' chestnut trees fertilized at various times with equivalent amounts of nitrogen; and, (3) evaluate the SPAD-502 chlorophyll meter as a tool to estimate foliar nitrogen content. In March 2002, 48 kg N/ha was applied in the form of slow release fertilizer (Pro-Grow 33-3-6, Scotts-Sierra horticultural products, Marysville, OH) to the chestnut trees. In March 2003, 77.3 kg in the form of NH_4NO_3 on June 9. An analysis of 'Qing' foliage in 2003 showed a slight nitrogen deficiency. In 2004, the nitrogen rate was increased to 150 kg N/ha. In the current study, the following treatments were applied in 2005 and 2006: (1) 150 kg N/ha in the form of slow-release fertilizer (Pro-Grow 33-3-6) applied on March 1; (2) 75 kg N/ha in the form of NH_4NO_3 applied on April 1 and on June 15; and (3) 75 kg N/ha applied on April 1, 30 kg N/ha applied on June 15 and 45 kg N/ha applied on Oct. 10, all in the form of NH_4NO_3. Six

single tree replicates of each treatment were arranged in a randomized complete block design. The following protocol was followed in 2005 and 2006. Trunk cross-sectional area (TCA) was measured at 30 cm from the soil surface on Nov. 1 to determine the increase in growth due to treatment. In early Mar. 2006, five terminal shoots per tree were tagged and the length of these shoots was recorded on Nov. 1. On May 15, June 15, July 15, Aug. 15 and Sept. 15, measurements were obtained with a SPAD-502 meter on four leaves from the middle portion of the current season's growth on each tree and were collected for foliar analysis of percent nitrogen by the Leco method. Burs were harvested to determine fruit weight per tree. Data analysis is in progress.

John Carlson—Pennsylvania State University (written report)

New full-sib families for genetic linkage mapping studies. (Carlson lab; supported by TACF). For the past four years, Alex Reinke has assisted us to make pollinations with *Castanea mollissima* var Mahogany pollen (from Sandy Anagnostakis' Mahogany trees at CAES) onto burs on the Alec-R American chestnut tree in the Moshannon State Forest in central PA. Unfortunately the pollinations in 2006 were the last ones possible on Alex-R as the tree has finally succumbed to the blight. In total we obtained app. 135 seed which Sara Fitzsimmons of the PA-TACF chapter and honors students in the School of Forest Resources assisted us in planting in a demonstration orchard at the university's Stone Valley Research Forest. Hopefully construction of a linkage map with the F1 trees can commence in 2007.

In June of 2006, Sara Fitzsimmons and volunteers from the PA and New Jersey chapters of TACF planted 645 Chinese chestnut nuts for us at the Stokes State Forest in New Jersey. The nuts were collected in 2005 by PA, Connecticut and New Jersey volunteers from three pedigreed Chinese chestnut trees. The 215 or so trees in each family are being grown at very close spacing, one foot between trees and five to seven feet between rows, for a period of approximately 3-4 years. At the end of 3 years, the trees will be tested for resistance by direct inoculation with the chestnut blight fungus. Measurements of the resulting cankers will be taken and will subsequently will be tested for genetic correlation with DNA marker data for each tree. We also hope to identify enough full-sibs among the 3 families by DNA fingerprinting for the construction of genetic linkage maps for Chinese chestnut.

Site evaluation and planting method study of American chestnut (Kim Steiner and Tim Phelps, School of Forest Resources at Penn State) Tim Phelps continued examinations of suitability of a range of native forest sites, and of planting methods, for restoration of American chestnut that were initiated in May, 2005. Field planting of large containerized stock for this study was completed in Spring 2006. Additional containerized stock had been field planted along with direct seed treatments in Spring 2005. This is our second large scale field study to evaluate site conditions for American chestnut restoration. Previous attempts in three central PA State Forests suggested that competing ground level competition and deer browsing were primary culprits to low success. For this new round of trials seven sites within a 5 mile radius were established within Penn State's Stone Valley Experimental Forest in an effort to limit variations of climatic conditions, mostly precipitation. The sites varied among other factors by competing vegetative composition and density, overstory removal method, topography, and edaphic characteristics, but all included deer protection by woven wire exclusions.

The trials consisted of five planting regimes including two direct seed and three containerized seedling methods factored with chemical control, or no control, of competing vegetation. Height measurements will be recorded yearly for six years and periodically thereafter until most trees succumb to the blight.

PA-TACF Activities (Sara Fitzsimmons, Regional Tree Breeding Program Coordinator, TACF) All of the PA-TACF projects at Penn State University reported last year continued on pace in 2006. These projects include 1) roguing of 2000 trees inoculated in the BC3F2 Seed Orchard in the Penn State University Arboretum, 2) planting of additional American x Chinese F1 trees (reported above by Carlson) at the university's Stone Valley Demonstration Orchard, and 3) breeding of 4th generation material by the PA Chapter in support of The American Chestnut Foundation's goals of that is regionally adapted, blight resistant and American in all other characters. The Chapter has 33 locations throughout Pennsylvania where American x Chinese F1 crosses have been planted along with progeny collected from local American chestnuts (open-pollinated seed lots). At each of these orchards, app. 60 Chinese trees have been used to create the F1 progeny, increasing the range of sources of resistance for the program. To date, the Chapter has located and identified over 600 American trees or groups of trees of which just over 200 have been used to collect open-pollinated progeny. The PA Chapter will begin breeding of 5 th generation, BC3F2 material in the near future, after its selections start to flower.

Dennis Fulbright—Michigan State University

Fulbright stated that the chestnut growers in Michigan were organized into a cooperative. Because chestnuts are being harvested and commercially released, a beer brewer was attracted. Recently, a chestnut beer has been produced (the first beer brewed from chestnut in America in decades). The beer is produced under the label 'jollypumpkin.com' and chestnut chips are being used; these are thinly sliced dehydrated chestnuts. Another brewer in Michigan, the Great Baraboo Brewing Company also is making chestnut beer.

There are 37 chestnut growers in Michigan and one in the state of Washington that are part of the CGI, chestnut growers incorporated. They grow primarily Chinese chestnuts and the European x Japanese hybrid, 'Colossal'. All the new orchards in Michigan are primarily 'Colossal', although the majority of harvested nuts are Chinese chestnuts. Since chestnuts were being harvested, Fulbright saw the need for a commercial chestnut peeler, so a USDA-World Development grant was written to obtain a peeler. The peeler is housed in a MSU facility dedicated to chestnuts. An individual interested in chestnuts, Ernie Rogers, went to the Jackson County Extension Agent and asked if anyone was working on chestnuts at Michigan State University. The Extension agent identified Fulbright and as a result of that contact (12 years later), Michigan now has a 140-acre farm and a $3.6M endowment dedicated to chestnut.

Products made by the CGI are fresh chestnuts, peeled frozen chestnuts, chestnut chips and chestnut flour. Thirty pounds of Michigan chestnuts were donated by the CGI board of directors to the Maryland Chapter of TACF for the November 10 gala dinner at ThorpeWood. Chestnut chips rehydrated in 10 minutes. CGI products include:

- Fresh chestnuts
- Peeled frozen

- Chips
- Breading
- Flour
- Chestnuts in maple syrup
- Chestnut puree in maple syrup
- Chestnut salsa
- Chestnut beer
- Dehydrated chestnut soup

<u>OBJECTIVE 2</u>. To evaluate and integrate multiple approaches for the biological control of the chestnut blight fungus and other pathogens and pests that threaten chestnut, by investigating host/pathogen/parasite relationships from the molecular to the ecological level.

John Carlson--Pennsylvania State University (written report)

Molecular Genetics Studies (John Carlson, Haiying Liang, Kelly Deitrick, Scott DiLoreto, School of Forest Resources and Huck Institutes for the Life Sciences)
Genomic tool development for the Fagaceae (Funded by the National Science Foundation)
After many years of grant proposal submissions, a major grant of $2,739,325 over four years was recently by the National Science Foundation's Plant Genome Research Program to develop genomics resources for five important species in the Fagaceae family - American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea mollissima*), northern red oak (*Quercus rubra*), white oak (*Quercus alba)*, and American beech (*Fagus grandifoli a*). The effort to obtain the funding for this project was facilitated by workshops and conference calls set up by Susan McCord and Bob Kellison of the Institute of Forest Biotechnology in Raleigh, North Carolina. Ron Sederoff, Distinguished University Professor of Forestry and Co-Director of the Forest Biotechnology Group is the lead investigator for the project. Co-principal investigators include Sandy Anagnostakis at the Connecticut Agricultural Research Station, John Carlson at Penn State University, Bill Powell at SUNY's College of Environmental Science and Forestry in Syracuse NY, Paul Sisco of TACF, and Jeff Tompkins of the Clemson University Genomics Institute. Other collaborators involved in the project include Laura Georgi at Clemson University, Fred Hebard of TACF, Jennifer Koch at the US Forest Services lab in Delaware Ohio, Tom Kubisiak at the US Forest Services Southern Institute for Forest Genetics, and Dahlia Nielsen and Chris Smith at NC State University.

For each of the species, DNA libraries will be constructed, expressed genes will be discovered by high throughput DNA sequencing, and DNA sequence databases will be established. All of these resources will be made available immediately to the scientific community, to enable more in-depth research on these important species. However the primary focus of the extended project will be on chestnut, including fine scale mapping of the chestnut genome and the comparison of chestnut genes and maps to oaks, beeches and other forest trees. A major objective is an integrated genetic and physical map of chestnut that would become the basis for discovery of the blight resistance genes in Chinese chestnut, and eventually sequencing of the whole genome. Detailed molecular

information for chestnuts and their close relatives will be available for the first time at the whole genome level which we hope will help accelerate the breeding programs for chestnut for all possible applications, including urban and rural forestry, food crops, timber, high quality wood products and ecological restoration.
Work on the project began in the Schatz Center for Tree Molecular Genetics, which John Carlson directs, immediately after notification of the award this summer. Samples of all of the tissues of American chestnut, Chinese chestnut, northern red oak, and white oak trees were collected. Oak trees were sampled from the Penn State campus collection, while chestnut trees were sampled with Sandy Anagnostakis' assistance from the CAES collection. American beech tissues were collected by Jennifer Koch from the US Forest Service's beech breeding program in Delaware. Furthermore, Fred Hebard provided stem samples from the Meadowview Farm from American and Chinese chestnut trees before and after inoculation with the blight fungus.

The Carlson and Powell labs have prepared RNA from all of these samples to date, except beech. The Powell lab at Syracuse will use the RNA preps to prepare nine cDNA libraries over the next year - (1) Pooled genotypes whole plant tissue for Chinese chestnut, (2) Pooled genotypes infected stem tissue for Chinese chestnut, (3) Pooled genotypes uninfected stem tissue for Chinese chestnut, (4) Pooled genotypes whole plant tissue for American chestnut, (5) Pooled genotypes infected stem tissue for American chestnut, (6) Pooled genotypes uninfected stem tissue for American chestnut, (7) Selected parental genotypes whole plant tissue for Northern red oak (*Quercus rubra*), (8) Pooled genotypes whole plant tissue for white oak (*Quercus alba*), and (9) Pooled genotypes whole plant tissue for American beech (*Fagus grandifolia*).
The Carlson lab completed sequencing of the gene transcripts from American chestnut cankers previously, and has initiated sequencing of the gene transcripts from Chinese chestnut cankers. Over the nest two years, sequence data will be generated for all of the cDNA libraries above, by Penn State and in the Clemson University Genomics Institute (CUGI). Jeff Tomkins at has already started a Fagaceae Family Genes Database at CUGI that will house all of the sequence data from this project as well as from the EU Fagaceae genome project and others. please checking at http://www.genome.clemson.edu/projects/fagaceae/ for updates on sequencing progress in these projects.

Variation in Genome Content among Resistant Backcross Progeny (Funded by TACF. A former student in the Carlson lab developed a dot blot protocol to screen for the amount of American versus Chinese chestnut genome in individual trees, as reported previously. In a test run, we validated the protocol by testing individuals in the three backcross generations of TACF. A graduate student, Kelly Deitrick, is now conducting our first practical application of the dot blot technique by screening large numbers of individual trees for their genome content in the third backcross generation of TACF's breeding program. Results if this project will be reported next year.

Assembly of BAC Clone Contigs for the Two Major Blight-Resistance Loci in Castanea mollissima (With Albert Abbott and Laura Georgi at Clemson U; funded by TACF). We have made great progress on our goal of preparing a contiguous set of BAC clones that span the two major blight resistance QTLs from *Castanea mollissima*. Scott DiLoreto in the Carlson lab saturate the areas of the C. *mollissima* genome around the Cbr-1 and Cbr-2 loci with many additional DNA markers using a bulk segregant analysis

(BSA) approach. Kelly Deitrick is now mapping the position of these new DNA markers to ensure that they are locate at the resistance loci. The Clemson University Genomics Institute accomplished our second objective of increasing the depth of the BAC libraries to ensure that there are enough clones in the libraries to completely blanket the Cbr-1 and Cbr-2 loci. Laura Georgi at Clemson has had great success in isolating clones from the BAC libraries by hybridization to the DNA markers from the QTL and from BSA. She is now sequencing the ends of these clones to help order their positions at the QTL. The end sequences have already revealed interesting gene sequences and we look forward to full sequencing of the BACs in the future.

Tom Kubisiak (USDA-FS, Southern Institute of Forest Science, Saucier, MS)

Chloroplast/nuclear markers in *Castanea* (as a whole). The impetus of this work was driven by TACF and their need to identify trees in nature to ensure that trees are truly American chestnut. There was a lot of European, Chinese and Japanese chestnut planted all across the Appalachian region. As researchers are looking at large surviving trees in the forest, are they truly American chestnut or hybrids? We don't want to take steps backward in the program. When we say a tree is 15/16th American, we want to make sure that it is truly 15/16th and not less because a hybrid was used mistakenly for a pure American parent. He first started focusing on chloroplast DNA markers; those are obviously not the best because they are maternally inherited but it was a good place to start. With Fenny Dane as a collaborator at Auburn University, they sequenced three different regions of the chloroplast genome and looked at polymorphism mutations within these regions. They wanted to identify species based on these regions. They did find some regions that appear to be species specific and are hence useful for saying that at least maternal lineage of a tree is either American type, Chinese, etc. With that data, they also did a phylogenetic analysis. *Castanea* has been divided into sections based on cupule (a cup-shaped involucre, occurring especially in the oak, beech, chestnut and hazelnut, formed by fused bracts) characteristics. The three sections are: (1) Section *Eucastanon* comprises five species: *C. mollissim*a, *C. seguinii* from China, *C. crenata* from Japan, *C. dentata* from North America, and *C. sativa* from Europe, all with the characteristics of three nuts per cupule; (2) Section *Balanocastanon*, has a widely disputed taxonomy, with taxa exclusively found in the southeastern United States. It contains just one species, *C. pumila* Mill., with two varieties: var. *pumila* and var. *ozarkensis*, characterized by one nut per cupule; and, (3) Section *Hypocastanon* consists of just one species, *C. henryi* (Skan) Rehder & Wilson, which is found in a restricted area in southeast China and is sometimes called the Chinese chinkapin because it is characterized by a single nut per cupule. Kubisiak stated that the decision to base the phylogeny of the number of nuts per burr is actually not very good for *Castanea*. Based on chloroplast markers, it turns out that chestnuts are more closely related to their geography. *C. henryii* (Chinese chinkapin) is more closely related to the Chinese chestnuts than it is to our American chinkapins. The nut and burr/cupule characteristic is not a very good way to distinguish among or to hypothesize the phylogenetic ancestry of *Castanea*. The paper of this work has been published (Lang, Dane and Kubisiak. 2006. Phylogeny of *Castanea* (*Fagaceae*) based on chloroplast trnT-L-F sequence data. Tree Genetics and Genomes 2: 132-139). As a side note, they were able to find markers that appear to be specific for particular species in the chloroplast genome but this is not

sufficient to distinguish a hybrid. Chloroplast markers, in and of themselves, are not useful for determining if a tree is a hybrid. They shifted their focus and their goal was to find nuclear DNA markers that may be predictive of ancestry. They used some of Bill Powell's ESTs that he submitted to GeneBank. He started with 80 sequences that he crunched through the sequencer. He developed 55 primer pairs and he sent those to Fenny Dane and she ran those primer pairs against a battery of American chestnuts. Her idea was to do a preliminary screen and look at those that had introns. They wanted to find those ESTs where the primer pairs were spanning an intron. The assumption was that if they were going to find variation more than likely mutations are going to accumulate at an intron which are not coding for anything. Eleven of the fifty-five spanned introns. Dane eventually focused in five ESTs and did in-depth sequencing across all species (following table).

	Number of single nucleotide polymorphisms (SNPs) or indels				
Species	**Region 17 600 bp**	**Region 32 600 bp**	**Region 40 750 bp**	**Region 38 600 bp**	**Region C126 400 bp**
C. dentata	1 SNP		2 indels but not completely specific	Indel with overlaps (not species specific)	
C. pumila	1 SNP				
C. sativa			1 SNP, 3 bp intel		
C. mollissima	1 SNP			Indel with overlap (not species specific)	8 SNPs
C. sequinii	2 SNPs				
C. henryi					
Asian specific	4 SNPs	10 SNPs	Large indel		
C. crenata	1 SNP				

They need to be able to screen these polymorphisms across a very large germplasm of all of the *Castanea*. The goal is to find those polymorphisms that are truly species specific. They would like a limited number of makers and they need to be highly predictable. Kubisiak feels that better markers than the ones they currently have will come out of the *Fagaceae* project.

Pathogen study. Pathogen work is being conducted in conjunction with colleagues at Cornell University. A genetic linkage map of the fungus has been constructed and they did find markers linked to some genes. One marker was found that segregates with the vic2 gene. Bulk segregate analysis provides a lot of information without a lot of work. They have found some markers within a reasonable distance of two vic genes. They are trying to identify cosmids to pull out clones that are positive for markers for vic2.

Development of more markers. There are 6-7 chromosomes in *Cryphonectria*. Cyril Dutek is a collaborator on this project. To date, 115 primer pairs have been

developed; 53 are polymorphic. However, these did not coalesce the genetic map very well. They cloned RAPD markers and found an area 3 cM from a mating type locus. There is a very highly polymorphic region around the mating type locus. Some of the markers in this region have been cloned.

Don Nuss—University of Maryland Biotechnology Institute, Shady Grove

Sequencing *Cryphonectria parasitica* genome. The strategy involved: (1) organizing a *C. parasitica* genome constortium; (2) identifying and organizing a *C. parasitica* community which consisted of assembling contact information research summaries; and (3) collecting support letters. A proposal was submitted in March to JGI-DOE, (Joint Genome Institute, Department of Energy) entitled 'Genome sequencing in the chestnut fungus, *Cryphonectria parasitica'*. It was approved in 2006. Don Nuss, Alice Churchill (Cornell University) and Michael Milgroom (Cornell University) prepared a proposal and sent out a draft. Other participants on the grant proposal include: John Carlson (Pennsylvania State University), Baoshen Chen (Guangxi University, China), Angus Dawe (New Mexico State University), Bradley Hillman (Rutgers University), Dae-Hyuk Kim (Chonbuk National University, South Korea), Thomas Kubisiak (USDA-FS), Myron Smith (Carleton University, Canada), Neal Van Alfen (University of California, Davis) and Michael Winigfield (University of Pretoria, South Africa). Letters of support were obtained from a number of people around the world. For 2007, the community sequencing program JGI identified 13 small eukaryotic genomes for sequencing, only four of which were fungi (*Pleurotus ostreatus, Heterbasidion annosum, Cryphonectria parasitica* and two *Neurospora* species). JGI takes DNA and RNA and does an 8-10X shotgun sequence of the expected 40 mb genome of *Cryphonectia*. They then assemble the genome sequence. They also will generate another 20,000 ESTs for annotation purposes. (The consortium already has about 10,000 ESTs). They will then do an automated and directed annotation. They also will provide a web-accessible database with tools for mining and comparative genome studies for the community and they hopefully will support a Jamboree (where the community gets together at Walnut Creek, CA, to go through the annotation in detail). They will maintain a user-friendly database. Xuemin Zhang made the DNA that was sent to JGI. The DNA of Ep155 went through several quality control screens. Different size fragments of the total genome were made from the DNA by either shearing or the use of restriction enzymes. They usually make a number of different libraries, depending on the size so that they can have different lengths of sequences with different overlaps. This is all automated and JGI runs through thousands of clones each day. Then they take all the sequence information and assemble it. The computer puts the overlaps together to form a contig. They will do about 400 megabases worth of sequence to get 10X coverage of the 40 mb genome. This is a lot of shotgun sequencing and computer assembly. Then they do annotation and go through certain algorithms and identify open reading frames, introns, etc. The central dogma is go from DNA to RNA and you have introns which usually have landmarks so you know what sequences so the computer can sort this all out. In addition, the ESTs are generated by sequencing either one or both ends of mRNA. For the EST library, Nuss has sent a combination of Ep155 grown in rich liquid and on cellophane (no virus is involved). The transcripts of mRNA that have polyA tail from which cDNA are made and these are sequenced either at the 5' or 3' end and that is

the EST. These ESTs represent the open reading frames. All of this information is used to prepare a draft of the genome that is assembled with some indication where the ORFs are. Nuss is hopeful that by this time next year, there will be a draft of the genome.

The above is the role of JGI. What are we going to do? We need to have a coordinated effort to annotate the genome sequence in depth and produce a joint publication. He is hopeful for a lot of visibility out of this publication. He would hope that we can identify research topics that can lead to multi-investigator proposals. There are a number of examples in the *Neurospora* communities where they have formed groups; this is very powerful. Granting agencies want to see more and more of mulit-investigator, multi-institutional proposals and this grant lends itself to that.

Transcription factor. This study was conducted principally by Fuyou Deng with help from Todd Allen who spearheaded the microarray effort in Nuss' lab. A transcription factor, CpST12 (a homologue of Ste12, a transcription factor of yeast), is down-regulated by hypovirus infection and required for virulence and female fertility of the chestnut blight fungus. It also regulates the expression of a subset of hypovirus-responsive host genes. Ste12 is a transcription factor in *Saccharomyces* that is activated in response to stimulation by a mating pheromone through a G-protein coupled receptor that leads to induction of various genes required for mating. A homologue of this transcription factor has been reported to play a role in sexual development, reproduction and virulence in a number of filamentous fungi. In *C. parasitica,* hypovirus infection and disruption of G-protein signaling both result in virulence attenuation and female sterility. They found Ste12 through the microarray studies. A number of years ago, members of the lab put together an EST library and they used that to make a spotted array. They spotted 3,864 ESTs, (spotted twice) onto a glass slide and used that to look at the effect of hypovirus infection on the expression of a number of *Cryphonectria* genes. Those genes represent about 2,200 unique genes of the fungus. They expect 10,000 to 12,000 genes. RNA was isolated from uninfected Ep155 or that infected with CHV1-Ep713 and labeled by making a cDNA copy in a way that allows a tag with either a green or red fluorescent dye. They were mixed together and then hybridized to spots of the ESTs on the glass slides. If there is no transcript present, there is no signal. If there are equal amounts of red and green before and after infection, it is yellow. If the amount of transcript goes up as a result of virus infection, it is red. If it goes down, the spot is green. This is all done 4-6 times for statistics and it is not quantitative. In general, they run around 80-90% accurate. In the case of just infection with CHV1-Ep713, about 14% of the genes were altered in a significant way, either up or down (more than 2-fold) as a result of infection. Gil Choi showed a number of years ago that hypoviruses interfere with the G-protein signaling pathway, which is one of the major pathways that all cells monitor the environment and then respond to the environment. They were able to show that a good portion of the genes that are responsive to virus infection are also regulated through G-protein signaling by looking at the effect of knocking out an alpha-subunit or a beta-subunit. A number of genes can be identified (45 genes) that were regulated, either up-regulated or down-regulated, in the same direction as a result of disruption of one of the subunits the G-protein signaling pathway or virus infection. An EST corresponding the Ste12 homologue was found to be down-regulated as a result of infection by different viruses as a result of the mitochondrial hypovirulence phenotype as a result of disruption of a number of the G-alpha subunits, and this was validated by real-time PCR. That

suggested that this transcription factor may be playing a role in some of these events of gene regulation. Deng cloned this gene, part of which is shown below. It is the alignment of Ste12 homologue homeodomains. These are other viral Ste12 homologues (*Neurospora crassa* and *Magnporthe grisea*).

This shows a high level of 98% identity for all of these different Ste12 homologues indicating a high level of confidence that the EST corresponds to Ste12 homologue of *Cryphonectria*. Deng then did a knock-out. Below is the full-length gene, 3 introns, about 2500 bp ORF with about 700 amino acids. Deng introduced the hygromycin resistance gene and then knocked it out. The transcript corresponding to the gene is not present in the knockouts and reduced in the virus-containing strain.

The phenotype looks very similar to Ep155 when grown on PDA. There was a 4-5 fold increase in conidia made, otherwise there was no effect on growth. This Ste12 homologue is not required for growth under these conditions or for the production of asexual spores. It is reduced significantly in virulence as shown in the following table which lists the virulence and sporulation of cpst12 deletion mutants.

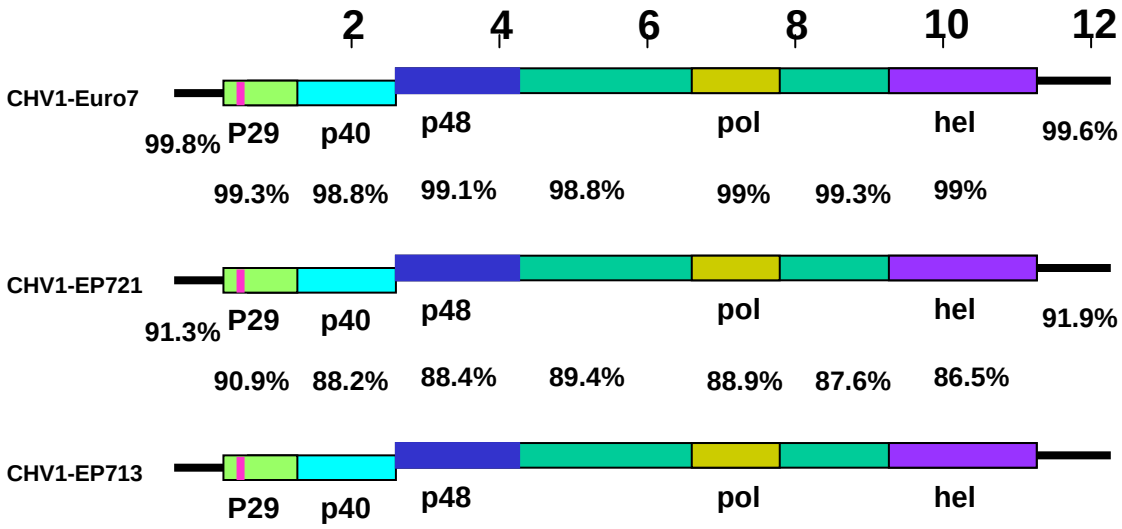
Strain	Characteristic	Canker area (mm^2)		Nur
EP155	Wild type	1856.2	A*	7.43
Δcpst-E1	cpst12 knockout mutant	374.8	B	2.67
Δcpst-E7	cpst12 knockout mutant	629.1	B	3.05
EP713	Hypovirulent	113	B	0
		SE=169.2		SE=

There is a significant reduction in canker size. Complimentation with the cDNA of cpst12 and virulence is restored to the Ep155 level along with conidiation. The knockouts are female infertile.

From the microarray, the results from the effect of disruption of cpst12 are about 152 fungal genes that were changed in their expression levels. Forty-seven of those overlapped with genes that were responsive to hypovirus infection. In many cases they altered in the same direction. Summary points of Deng's work:

- *cpst12* is a Ste12 homologue.
- *cpst12* is down-regulated by hypovirus infection.
- CpST12 is required for:
  - female fertility
  - virulence
  - regulated expression of a subset of hypovirus-responsive host genes
- Provides link between hypovirus-mediated down-regulation of a specific transcription factor, reduced virulence, female infertility and the regulated expression of a subset of hypovirus-reponsive host genes.

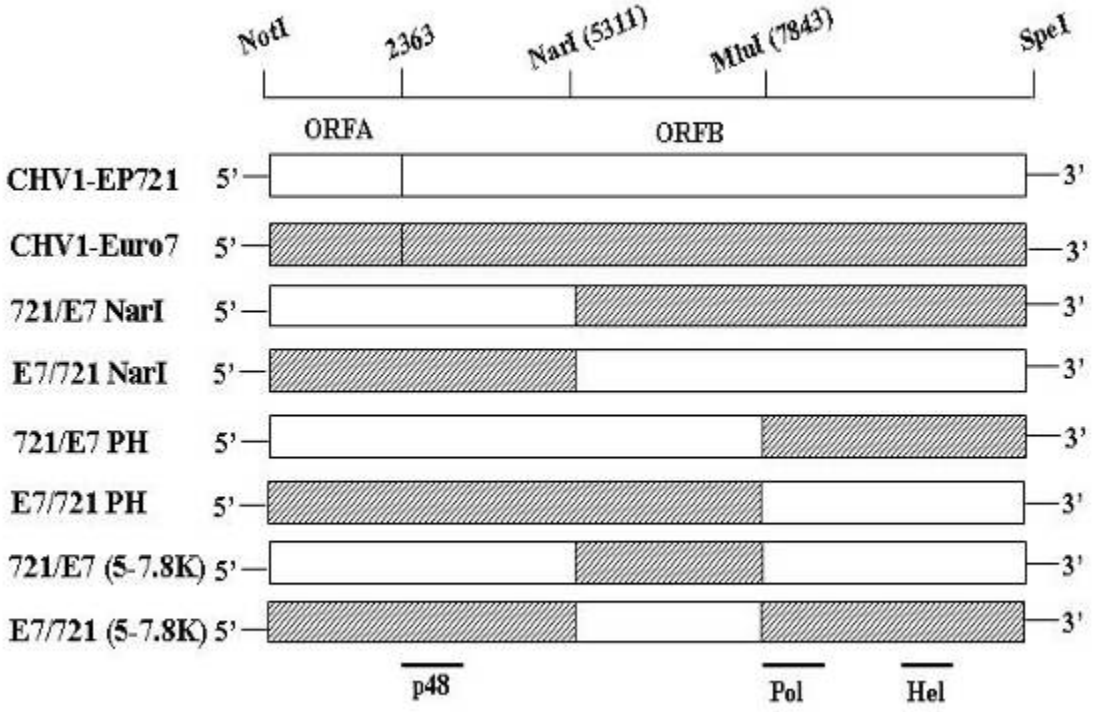
The genome sequence, full-length infectious cDNA clone, and mapping of viral dsRNA accumulation determinant of hypovirus CHV1-EP721 (the third hypovirus); this is a collaborative study with Baoshen Chen, former lab member. Ep721 looks much like Euro7.



Identity at the nucleotide level

The nucleotide identity is shown above for Euro7, Ep713 and Ep721 (all CHV1 hypoviruses). There is a high level of identity between Euro7 and Ep721 (more so than with Ep713). There are only 46 amino acid differences between Euro7 and Ep721. At the RNA level, Ep721 has a low level of RNA but phenotypically, it cannot be

distinguished from Euro7. Because they are so similar, chimeras can be made to look at the combinations of ORFs as seen below (the hatched areas represent Euro7).



NarI means the 5' end up the NarI site and PH indicates the polymerase helicase domain. The 5-7.8KB is the region between the NarI and MluI site (the area of focus). Chen is mapping the low level of RNA accumulation. The transmission of virus in conidia is severely reduced in Ep721. He has identified a correlation between the accumulation of viral RNA and its transmissibility through conidia which has relevance to what happens in the field.

If the N-terminal part up to the NarI site comes from Euro7, transmission to conidia is high; if it comes from Ep721 it is low. The determinant for the low level of RNA accumulation lies in the 5-7.8 kb site. There are only 7 amino acid differences in that region (just past p48 and just before the helicas region) between Euro7 and Ep721. Summary points are:

- CHV1-EP721 is the third hypovirus for which an infectious clone has been developed.
- CHV1-EP721 causes a phenotype very similar to CHV1-Euro7, however, CHV1-EP721 dsRNA accumulates to a very low level.
- The determinant for the low level of CHV1-EP721 dsRNA accumulation mapped to the 2.5 kb region in ORF B between p48 and the polymerase region. Total of 7 aa differences between CHV1-EP721 and CHV1-Euro7 in this region.
- Correlation between low hypovirus dsRNA accumulation and low hypovirus transmission rates through conidia.

Angus Dawe—New Mexico State University

Web site. Dawe has developed a web site (chestnut.nmsu.edu) for posting information related to chestnut and NE-1015. It can be used as a listserv and for email. It currently has no content

BDM-1, an essential component of G-protein signaling in *Cryphonectria parasitica*. Heterotrimeric G-proteins in Eukaryotic cells:

- Highly conserved
- Transduce variety of sensory signals
- Composed of three subunits Gα,β,γ that couple receptors with intracellular effectors
- Essential for virulence and other phenotypes in *C. parasitica*

Deletion mutants of Gα and Gβ subunits have drastic phenotypes.

Trait	Ep155	Δcpg-1	Δcpg-2	Δcpgb-1
Growth (wet wt)	100%	20%	90%	150%
Pigmentation	(+)	(-)	(+)	(-)
Conidiation	4×10^9	0	1×10^9	0
Virulence (canker area)	10	0	9	2

In the presence of hypovirus, the accumulation of these proteins is affected. Signaling proteins sit at the top of cascades that then amplify a signal.

How is G-protein signaling affected by the presence of the hypovirus? Dawe stated that the story of pathogenesis begins with three G-proteins, Gα, Gβ and Gγ. These are involved in downstream events where signaling takes place. The end result is nuclear gene regulation and phenotypic changes. Nuss' lab has identified Gα, Gβ and Gγ and phenotypic changes have been shown using certain deletion mutants. G-protein signaling is important for virulence. Modification of G-protein signaling pathways is an important component of the hypovirus phenotype. A relationship between Gα protein-signaling pathways and virulence in *C. parasitica* has been suggested by the deletion of the genes encoding two Gα subunits (*cpg*-1 and *cpg*-2). Absence of *cpg*-1 results in an avirulent strain that is also defective in asexual sporulation and pigment production, while a deficiency in *cpg*-2 leads to only minor phenotypic changes.

A gene involved in G-protein subunit function and subunit accumulation, *bdm-1*, (beta disruption mimic) can be deleted and the deletion of *bdm-1* produces a phenotype identical to Δcpgb-1. *BDM-1* is required for or facilitates G function. Moreover, disruption of either *bdm-1* or *cpgb-1* resulted in a significant, post-transcriptional reduction in the accumulation of CPG-1, a key G subunit required for a range of vital physiological processes. BDM is a negative regulator of Gβγ signaling, similar to mammalian phosducin; 23% of 291 amino acids are identical to mammalian phosducin (a regulator of G-protein signaling). In mammalian systems, there are both phosducins and phosducin-like proteins, whereas eukaryotes contain only phosducin-like proteins.

Dawe posed the question, "Is there microsynteny between Sordariomycetes?" There is conserved genomic arrangement of a G-protein β-subunit and a regulator of Gβ signaling. Dawe is attempting to expand available sequence information by: (1) using a cosmid library from Alice Churchill; (2) identifying and sequencing those genes containing *bdm-1* and/or *cpgb-1* using traditional methods; and, (3) possible enhancement

using the 454 sequencer denoted by John Carlson. He plans to look for synteny with *N. crassa*.

In microarray data, there is a high degree of overlap between profiles from Δbdm-1 and Δcpgb-1. If Δbdm-1/Δcpgb-1 data is compared to results with Ep713-infected mycelium, there are 32 genes that overlap. Dawe noted that the exact nature is not as important as the trend. As the protein sublevel, Gβ subunit is altered and *cpgb-1* is undetectable in the absence of *bdm-1*. Conversely, *bdm-1* accumulation also is affected by the absence of *cpgb-1*. Both *bcm-1* and *cpgb-1* appear compromised by the hypovirus.

Is BDM-1 the key to modulating G-protein signaling? Dawe has one graduate students and two undergraduate students working on methods to understand this interaction. He is putting a tag on *bdm-1* protein (8 amino acids at N-terminus) to help answer the following questions.

1) Do *bdm-1* and *cpgb-1* really interact?"
2) Assuming so, which bits of each are important?
3) What regulates the interaction?
4) How does it relate the phenotype and hypovirulence?

Commercially available beads were use to pull out BDM1 and BDM2. Is BDM1 a phosphoprotein? They took total protein lysates and followed BDM1. Dephosphorylated proteins are shifted in migration compared to untreated lysates. BDM1 is a phosphoprotein, but at this point in time, it is not known which kinase does the phosphorylation. In phosducin-like proteins in mammalian cells, the phosphorylation of that protein is very important in that is allows Gγ and Gβ to come together as seen in the following cartoon. BDM-1 can be a target for CK2 (casein kinase) phosphorylation and Dawe's lab now has an assay for BDM-1 phosphorylation using purified FLAG-tagged BDM-1 and lysates from various sources. There are a number of predictive CK2 phosphorylation sites in BDM1. Five sites are being looked at. Maybe one or all are being targeted. Mutants are made by changing serines to alanines so they cannot be phosphorylated. They can also be changed to aspartic acid which actually mimics phosphorylation. This will help answer the question as to which residues are important.
CK II:

- Implicated in the phosphorylation of over 300 different cellular targets
- Includes AP-1 transcription factor (altered in microarray studies in presence of hypovirus)
- One CK2 EST clone (regulatory subunit)
- Not on the "changed" list from microarray studies
- May reflect altered activity/targeting at protein level by hypovirus with potentially huge effects post-translationally
    - Proteomics (this is where the genome project will be very helpful).

Where is BDM1 located? They are using a GFP tag (green fluorescent protein) to detect BDM1 in the fungus. Preliminary microscopy shows strong localization of BDM1.
Ongoing projects include:

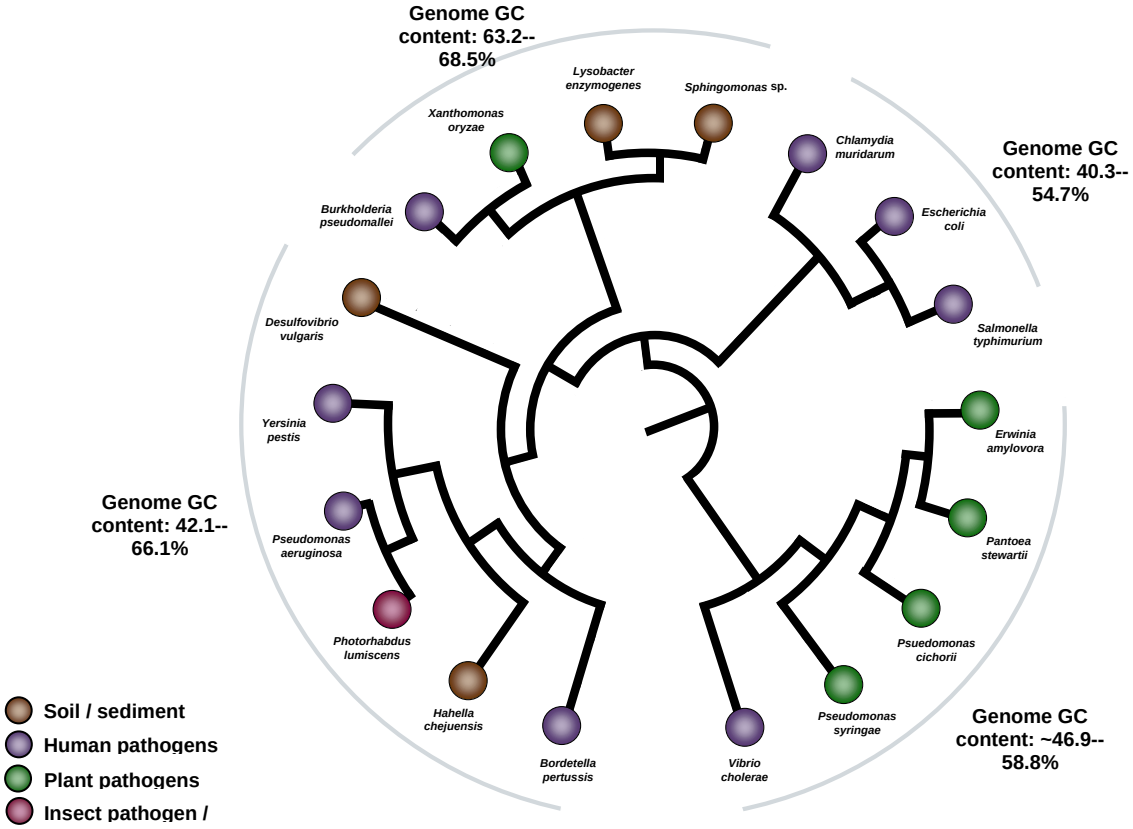
- Transformation of individual (10 total) phos-site mutants in *bdm-1* deletion and WT (EP155) strains
- All mutants are FLAG tagged permitting their use in the *in vitro* phos/dephos assay

- Preliminary observations:
  - Ala mutants do appear to complement deletion
  - But…in EP155 seem to induce very rapid and intense pigmentation
  - Not unlike a construct that over-expresses CPGB-1
- Use the *in vitro* phos/dephos assay to determine if there is a difference in CK2-mediated phosphorylation in the presence of hypovirus
- Validate GFP reporter, use mutants to ask if phosphorylation is required for localization
- Use FLAG-tagged BDM-1 in conjunction with myc-tagged Gβ and V5-tagged Gγ to establish *in vivo* interaction
- Use the phos-site mutants to determine if phosphorylation at those specific sites:
  - is affected by hypovirus infection
  - help determine cellular localization
  - affect interactions with or of Gβ / Gγ

Bradley Hillman—Rutgers University

***Cryphonectria* pathogens and extrachromosomal elements.** Hillman's lab focused on extrachromosomal elements in general, including transposons. Joann Crouch is working on this topic currently, and hopefully, she will have a report next year.

Lysobacter enzymogenes is a Gram negative, soil-inhabiting bacterium that has a Type III secretion system, employing an injectorsome (this project was instituted by a Rutgers colleague, Don Kobayashi, a microbiologist). Hillman is using *L. enzymogenes* to study fungal/pathogen interactions. The closest relatives of this bacterium are: *Sphingomonas, Xanthomonas, Burkholdaria. Lysobacter enzymogenes* produces lytic enzymes and antibiotics prolifically. It enters and likely multiplies in lower eukaryotes (a number of which there are complete sequenced genomes): true fungi, stramenophiles (*Phytophthora*), nematodes *C. elegans*), lower plants (*Physcomitrella*). The bacterium is being sequenced (Kobayashi, Hillman, Lawton, Crouch, Sullivan; TIGR). Not only does *Lysobacter enzymogenes* kill lower eukaryotes, but it multiplies in the cells. Kobayashi has a lot of pictures of *L. enzymogenes* "busting its way out" of fungal cells. It is clearly an internal pathogen. This fungal/pathogen interaction intrigued Hillman. Kobayashi wrote a sequencing project for the Lysobacter genome that was funded by USDA/NRI. Below is Crouch's phyogenetic tree of bacteria with TypeIII secretion systems, based on GC content:



Lysobacter is a highly GC bacterium so it is difficult to work with. An experiment was conducted using *Colletorichum cereale, Magnaporthe oryzae and C. parasitica* whereby droplets of *L. enzymogenes* were dropped onto the surface of each fungus. Large lytic zones were produced on *M. oryzae* and *C. cereale*, but phenotypically *C. parasitica* is not affected, presumably because of its hydrophobin content.

Reovirus Rather than a single piece of genomic RNA that is infectious, Reoviruses are true dsRNA viruses that have a package and within a single package, all of the dsRNA pieces are there. The individual pieces are not infectious; the package is necessary. For the package to be transcriptionally active, you have to strip away outer proteins to get to the transcriptionally active core that contain all the dsRNA that then make their ssRNA messages that then encode the various proteins. Current Reovirus projects are:

- Expression of 11 segments from baculovirus vectors in insect cells (Suzuki, Supyani)
- Completion of MyRV-2 sequence (Festa, Stout)
- Double infection of MyRV-1 and MyRV-2 (Stout)
- Microarrays of MyRV-1- and MyRV-2-infected *C. parasitica* (Deng, Nuss)

Reovirus properties are:

- 10-12 dsRNA segments
- Found in mammals, invertebrates, plants, fungi
- Terminal sequences conserved, but slightly different for each segment
- Particle important for replication, capping, mRNA production

- Very important for early RNA studies
- Discovery of capping (Aaron Shatkin)
- Studies on translation initiation (Marilyn Kozak)

Hillman showed slides of two mycreoviruses, MyRV-1/9B21 and MyRV-2/C18; the isolates containing these viruses were isolated less than 20 miles apart in southeastern WV. The two viruses have very different effects on the fungal host.

Reovirus-infected isolates can be used as females to produce dsRNA-containing ascospores; this is different from other *C. parasitica* viruses with the exception of mitochondrial viruses. These are very rare viruses; as a consequence, they may not be vectored. MyRV-2/C18 is lost fairly readily; it is harder to keep the virus than it is to get rid of it. They are transmissible by anastomosis, but MyRV-2/C18 does not transmit readily. In the figure below, a comparison of MyRV-1/9B21 and MyRV-2/C18 of the differential expression of genes is given. Eighty-five genes are affected in the same direction in both viruses. A good number of the genes are outside of the Venn diagram so they are affected differently in an infection with the C18 virus as opposed to the 9B21 virus. They are clearly worth looking at, not just because they are simply reoviruses, but they are two individual reoviruses that have very different effects on the fungus. He does not have picture of the overlap between the reovirus infected and hypovirus infected isolates.

Expression of 11 segments of reovirus MyRV-1/9B21:

- Protein expression of complete cDNA clones of 11 segments individually from baculovirus vector in insect cells
- Suzuki identified segment 3 as the segment that is the capping enzyme
- Guanylylation assay shows that segment 3 is viral guanylyltransferase

Mycoreovirus-1/Cp-9B21 segments structure and function are listed below.

Segment number	Size (bp)	Protein	Protein size aa (Mr x 10^3)	Structure / Function
1	4127	VP1	1354 (151.8)	RdRp; sequence similarity to coltivirus segment 1
2	3846	VP2	1238 (138.5)	Possible methyl transferase, similarity to coltivirus segment 2
3	3258	VP3	1065 (120.8)	Guanylyltransferas
4	2269	VP4	720 (79.8)	Similarity to coltivirus segment 4; myristoylated; major capsid?
5	2023	VP5	647 (72.8)	Similarity to coltivirus segment 5?
6	2056	VP6	649 (73.4)	Similarity to MYRV-3/RnW370 segment 6 and coltivirus segment 10
7	1536	VP7	481 (54.1	Proline rich, leucine zipper
8	1539	VP8	469 (51.2	
9	1072	VP9	297 (32.9)	Sequence similarity to MYRV-1/RnW370 segment 11
10	975	VP10	247 (27.8	
11	732	VP11	101 (11.5)	

Laura Georgi—Clemson University

What is a chestnut BAC library and why do we want one? A BAC library is an ordered collection of random fragments of chestnut genomic DNA propagated in *E. coli*. The average insert size is about 90,000 bp or about 1/10,000th of the coverage of the total genome size. She has almost 110,000 such fragments. The DNA in this case is from *Castanea mollissima* cv 'Mahogany'. A BAC library is used to assist in the identification, cloning and manipulation of genes such as those conferring resistance to chestnut blight and for map-based cloning. She showed areas of QTLs, quantitative trait locus (RFLP markers) for CD175 (on linkage group B) and CD145 (on linkage group F). CD175 is about 3 cM from a QTL for blight resistance on linkage group B. Robert Bernatsky's sequence for this marker resembles a gene for phosphoglucan, water dikinase (involved in starch metabolism) in *Arabadopsis thaliana*. It is the right size and the internal EcoR5 site which is also consistent with the original marker.

John Carlson submitted a grant from TACF for continued funding for physical mapping to double the size of the BAC library. Carlson's group sequenced a collection of plasmids, including for RFLP probes on the original interspecific map. They obtained the same sequence for CD145 and CD146. Using HinDIII digest, Georgi pulled out 6 BAC clones form CD175 and two for CD145. The probe from which Georgi selected the BAC on CD145 matches with 137D4/R (they are within 63 kb of each other) on *Populus* chromosome VIII. The hybridization probes that she used to pull the BACs out of the library are called overgo probes, fairly large oligonucleotides that overlap in bases. She discovered a restriction enzyme site in the 'Mahogany' that was not present in *C. dentata*.

Michael Milgroom—Cornell University

Milgoom is currently involved in four projects concerning the population biology and genetics of *C. parasitica*: (1) linkage mapping with Tom Kubisiak; (2) MAT heterokaryons and parasexualtiy with Cristina McGuire Kiril Sotirovski, Mihajlo Risteski and Marin Brewer; (3) cloning *vic* genes with Cristina McGuire, Tom Kubisiak, Alice Churchill and Myron Smith; and, (4) genetics markers with Tom Kubisiak and Cyril Dutech (France).

MAT heterokaryons and parasexuality. Much of the work he presented was part of Cristina McGuire's Ph.D. work. Marin Brewer has also been involved in this project. Kirl Sotirovski and Mihajlo Risteski are collaborators from Macedonia who have been working in Milgroom's lab. They stumbled on heterokaryons while looking at what they thought was selfing in *C. parasitica*. When they do a PCR assay for mating type, they can find some isolates that contain both mating type idiomorphs in the same individuals. If they take single conidial isolates that they think are heterokaryons, they segregate into one mating type or the other. This is a classic case of a heterokaryon. They never find a single conidial isolate with both mating types. This work was done using hyphal tip cultures to preclude mixed cultures. This is a strange finding for an Ascomycete fungus. Heterokaryons are not typical in Ascomycetes unless they are caused by a mutation. In the case of *C. parasitica*, there is no mutation involved because MAT-1 and MAT-2 alleles are very different. They are so different that they are not called alleles but idiomorphs. Their hypothesis is that this is a parasexual recombination event. One of the ways to at parasexuality was to look at heterokaryons in the *C. parasitica* population in West Salem, WI. At the time of sampling, West Salem was a clonal population. They wanted to detect recombination when there was no sexual reproduction. They found heterokaryons in West Salem. They began using codominant markers (SCAR) and they found an isolate that had both MAT-1 and MAT-2, as seen for WS-3, haplotype V in the table below.

vc type	Fingerprint haplotype	Mating type	SCAR loci						
			327-1075	499-900	IO7-650	N14-1200	RO4-775	IO1-1990	IO1-800
WS-1	A	2	187	125	270	422	96	103	151
WS-2	G	1	169	115	280	422	87	94	167
	J	2	169	115	280/270	422	87	94	167/151
	T	2	169	115	280	422	87	94/103	167
WS-3	L, V, Y, Z	2	169	125	293	401	96	94	167
	V	1/2	169/187	125	293/270	401/422	96	94	167
	α	1	187	125	293	422	96	94	167
WS-4	β	1	187	115	280	401	87	94	167

It looks like for the codominate markers, they found multiple alleles which is a good indications that these could be heterokaryons. They looked at single conidial isolates

from haplotype V and they could get MAT-2 single conidial isolates, MAT-1 single conidial isolates. This was considered to be heterokaryosis and recombination. In another vc type, MAT-2, haplotype J, there were two isolates in MAT-2, and two alleles were found at several loci. The summary of the West Salem work to date is as follows:

- Clonal population (at the time of sampling)
- Heterokaryons
  - *MAT*
  - SCAR markers
- Recombination of *MAT* and SCAR alleles from heterokaryons
- No evidence for sex or mutation in *MAT*

The only explanation is that there is some type of parasexuality going on in this population. Milgroom stated that while this type of phenomenon occurs readily in the laboratory in fungi, it is assumed that it does not occur in nature because vegetative incompatibility prevents the formation of heterokaryons. In this population at West Salem, the conclusion was that they were getting heterokaryon formation between different vc types.

They are attempting to extend this story to another clonal population of *C. parasitica* in Macedonia and Greece. Between 1996-2000, about 800 *C. parasitica* isolates were sampled. About 94% of the isolates were the same vc type (EU-12) and 97% of all the isolates they collected were MAT-1. Is this a clonal population? If they are clonal, are there heterokaryons? If there are heterokaryons, is there evidence for recombination? They were repeating the same questions they posed for the West Salem population. They found the following from the 1996-2000 samples.

	vc	***N***	***MAT***	**SCAR**
Greece	12	53	1	A
Macedonia	12	100	1	A
	12	1	2	A
	1	6	2	B
	2	20	2	C
	10	1	1	D
	22	4	1	E

They are using 11 different loci. In Greece, they collected over 200 isolates; genotyping was conducted on 53 isolates. All the isolates are in EU-12, and all are MAT-1 and all are in genotype A. In Macedonia, 101 isolates were genotyped SCAR A and 100 were MAT-1, while one isolate was MAT-2. The table lists other vc types that were found (EU-1, EU-2, EU-10 and EU-22). Each one of these different vc types had a different SCAR genotype. This strongly suggests that this is a clonal population. Two independent markers both correlate. Sexual recombination can be ruled out of this population. The dominate clone in this population is also the dominate clone in Sicily. The Greece/Macedonia population was resampled in 2005 as seen in the following table.

vc type	***N***	**SCAR**
12	149	A
2	18	C
22	4	E

Again, the majority of the isolates were EU-12, SCAR A. It was mentioned that EU-2 is always SCAR C while EU-22 is always SCAR E.

Mating type data in EU-12 clone in four populations in Macedonia is shown in the following table.

Population	1/2	1	2
Frangovo	1	63	5
Vratnica	0	67	30
Glogi	0	67	0
Osoj	0	70	1
Total	1	267	36

This data shows a lot more MAT-2. In the earlier sample (1996-2000), 97% of all isolates were MAT-1; this has shifted some in the samples taken in 2005. Only one isolate was identified as a MAT heterokaryon. There has been a shift over time, especially in Vratinic, where about a third of the population is MAT-2. No perithecia have been detected. While the term 'selfing' is not entirely accurate (Milgroom does not know what term should be applied since there is no apomixis), there is no segregation of markers but there is segregation of mating type. A heterokaryon can go through meiosis and form perithecia (within the same vc type). When they studied natural populations and said that 30% of the population selfed, that means that they found no segregation of any markers. Later, they figured out that mating type could segregate. Selfing, by definition, is no marker segregation. They are now going back and looking at heterokaryons with more markers. Milgroom speculated that a heterokaryon forms between unlike nuclei (a maternal type and an invading type or male). If there is any incompatibility in the vc type, the male chromosome is lost with the exception of the mating type gene because there is an incompatible reaction. When a heterokaryon forms, it can undergo some sort of parasexual change where the chromosome of the male is lost except for the mating type gene. During sexual recombination, vc is turned off so heterokaryons can be formed during the mating process. Mating type does not affect vc and therefore plasmogymy is not affected. Nested PCR was conducted to detect the other mating type idiomorph. What they found was quite a few that are MAT-1 but if they do nested PCR, they can find the MAT-2 idiomorph; the same is true for MAT-2. There is a really highly skewed ratio of nuclei that are heterokaryons. This can be less than 1 in a 1000 nuclei.

Population	1/2	1	1(2)	2	2(1)
Frangovo	1	57	6	5	0
Vratnica	0	58	9	16	14
Glogi	0	62	5	0	0
Osoj	0	55	15	1	0
Total	1	232	35	22	14

MAT-2 and heterokaryons increased markedly in <10 yr in this *C. parasitica* population. Additional markers (SSRs) in progress to look for heterokaryons and hyphal tip and single-conidial isolates will be continued. The big unanswered question is, 'how do these heterokaryons form?'

Positional cloning of *vic* genes. Vegetative incompatibility is a self-nonself recognition system that triggers cell death and prevents heterokaryon formation. It also inhibits virus transmission (but not completely). The genetics of this system have been identified through classical genetics. It is a multilocus system and 6 *vic* genes have been identified; each has a different effect on virus transmission. More *vic* loci that are present in *C. parasitica* have not yet been identified. If any allele is different at one or more loci, they are incompatible. Or, to be compatible, two isolates have to have all identical alleles at all the vic loci. Why clone vic genes? First, to understand the mechanisms of vegetative incompatibility and maybe, with the goal of trying to enhance virus transmission. Maybe there is a way to turn off vegetative incompatibility. Second, he is interested in the evolution of *vic* genes because he is interested in maintaining viruses as a selective force. In *Neurospora*, vic genes are under selection but it is not known why.

Linkage group XII has a marker that is 4.5 cM from vic1 and a marker that cosegregates with vic2 on linkage group VIII. The idea is to take the linked markers, use these to screen a cosmid library (made by Churchill) for the linked markers and then use the positive cosmids and transform them into strains of *C. parasitica*. If you transform into the same *vic* allele that it already has, it should be a normal transformation. If you transform in a different *vic* allele, it would be an incompatible reaction and give very few or no transformants. If you transform in a cosmid that doesn't have a *vic* allele, it would be a normal transformation. This is exactly what was done in *Neurospora* for cloning het genes (heterokaryon incompatibility). Using the marker that cosegregates with *vic*2, they have been able to find a cosmid where there is a differential reaction. One or two transformants in the cosmids with different alleles are obtained rather than hundreds with the control. They are still at the very early stages of this research but they are excited because they think they finally have a cosmid that contains a *vic* gene. They need to begin chromosome walking for vic1 and do some additional mapping. They are also excited about the *Cryphonectria* genome sequence project to assist with their work. They also want to do additional mapping to find markers for other *vic* genes.

Lynn Rieske-Kinney—University of Kentucky

***Dryocosmus kuriphilus* (gall wasp).** This is a tiny wasp and the adults are active only briefly during the summer. They are cyptic during most of the life cycle (9 months) and only during bud break are they active. Females lay eggs within the bud and they sit there throughout the summer, fall and winter. Only when the plant begins to grow do the eggs hatch and the resulting gall occurs. Early galls are very difficult to detect. It is not immediately evident on a tree which buds are galled and which are not. Gall wasp is thought to have originated in southern China, and it appeared in Japan in 1941. It has been managed through resistant varieties of chestnut but a more virulent strain of the wasp seems to have evolved and resistant varieties are no longer effective in that part of the world. Gall wasp appeared in the US in 1974 (in SE Georgia on Chinese chestnut) and in Europe in 2002. A private grower in Georgia brought infected plant material into the U.S. and in 1975, gall wasp appeared in another located near Byron, GA about 18 km from the original site. By 1976, it expanded its geographic and host range (Japanese and

European chestnut). From 1974-1979, gall wasp expanded about 15 miles per year. By 1983, it continued to spread, getting as far west as Auburn, AL and as far south as Tipton, GA. By 1992, it appeared on American chestnut. It showed up in Chattahoochee National Forest in 1993 and by 1995, it had spread 635 km in 20 years. Gall wasp reached the TACF farm in Meadowview, VA by 2001 and there were satellite infestations in several Ohio locations between 2001-2005. By 2006, it appeared on the MD/PA border but it is not known if this was a natural dispersal or if there was a commercial chestnut grower in the area.

Dryocosmus kuriphilus has spread for 32 years in the US. Expansion has been to the north and northeast. Recreation and tourism may be an explanation for its spread, as there is suitable host material along the Appalachian Trail. Also, weather patterns are in the prevailing winds. There has been little southward movement (this may be due to a lack of host material). Plant movement is certainly the primary culprit in gall wasp movement. Risks associated with movement of material are extremely high. Inspections are only marginally effective since the insect is cryptic throughout most of its life cycle. Plant breeders can be educated but the truth is that if breeders want to move plant material, they will. Phytosanitary certification is not much of an option. There are only four states that have any kind of quarantine against moving *Castanea*. We have to learn methods of management. In her lab, they are looking at biocontrol agents, parasitoid wasps. *Torymus sinensis* has fairly effective suppression of D*. kuriphilus* in Japan and Korea. It was released in and around Byron, GA by Jerry Payne from 1978-1981. Release of this parasitoid was done in an era when regulations were very different than they are now. At this point, it is not known exactly what species of *Torymus* was imported. In her lab, they are evaluating: parasite recruitment of *Torymus* and other parasitoids; parasite dispersal; and, species identification of parasitoids they encounter. There are three infestations of *D. kuriphilus* in Virginia (2001), Kentucky (2003) and Ohio (2002) that they are examining. The Virginia and Kentucky sites are considered natural while the Ohio site is considered to be a satellite infestation. *Torymus sinensis*, the introduced wasp, occurs in Virginia and Ohio but does not occur naturally in Kentucky suggesting that the dispersal of *Torymus* lags behind the *Dryocosmus* expansion, as shown in the following table.

***Torymus sinesnis* emergence from collected galls**

Site (year infestation began)	Previous year's galls	Current year's gall
VA (2001, natural)	0.55 ab	0
KY (2003, natural)	0 b	0
OH (2002, satellite)	0.71 a	0

Given the fact that no *T. sinensis* was found in current year's gall, they are questioning if this is truly *T. sinensis*. In addition to *Torymus*, they found five native species of parasitioids that are associated with *Dryocosumus* galls. The most abundant of the five species is *Ormyrus labotus.* It is found fairly commonly in *Dryocosmus* infested areas. Its distribution is seen in the following table.

Distribution of *Orymus labotus,* another parasitoid wasp.

Site (year infestation began)	Previous year's galls	Current year's gall

VA (2001, natural)	0.14 a	0.12 b
KY (2003, natural)	0 a	0.41 a
OH (2002, satellite)	0.02 a	0.02 b

Orymus seems to travel with the *Dryocosmus* gall wasp, unlike the *T. sinensis*. A old cartoon, developed 30 years ago, from Jerry Payne indicated that removing galls and burning them is a good control measure for *Dryocosmus*. Rieske-Kenny stated that her data indicates that removing old galls is not a wise move. This does not reduce gall wasp distribution; in fact, removal of galls could reduce parasitoid populations.

ThorpeWood

The meeting moved to ThorpeWood, a property of Thorpe Foundation, an environmental education center located on a 116-acre nature preserve nestled in a secluded, heavily forested stream valley 1,500 feet high on Maryland's Catoctin Mountains. This venue was suggested by Paul Sisco and Robert Strasser. Sam Casselman, ThorpeWood director gave a brief introduction to members of NE-1015. The property was purchased originally by Merle Thorpe, an attorney who set up a trust with five trustees who were allowed to develop the property and define its use. It currently is used for youth education and community focus. There is a chestnut orchard on the property, due in part to the efforts of Robert Strasser. The buildings on the property are eco-friendly. The toilets are composting, the building was constructed with recycled lumber and rain run-off is used for aquatic plants. All soapy wastewater goes to a drainfield and the electric is from a wind-farm. ThorpeWood is located about 4 miles from Camp David.

Dennis Fulbright—Michigan State University

Irradiated chestnuts. There are irradiated trees at Stronghold, Inc., Sugarloaf Farm and the National Colonial Farm (NCF) in Accokee, MD. Many of the cankers at these two locations are non-typical—swollen, superficial cankers. The trees at these locations were part of an irradiation project in the 1950s conducted by Al Dietz who irradiated nuts with cobalt. The nuts were irradiated to a 50% kill rate and the survivors were outplanted at Sugarloaf and NCF. The best trees have an extended life, living long enough to harbor 10-12 years but they eventually die. Fulbright had a long-term project in 1989 with the following goals:

- Rule out environment and hypovirulence
- Determine level of resistance found in germplasm
- Describe the type of resistance found
- Determine if the highest level of resistance found is useful for a breeding program
- Determine if the tree(s) with highest level of resistance can be planted as a grafted tree for the landscape.

Trees were inoculated at both locations with virulent strains, including Ep155. After development of large cankers in first year, all cankers ceased expanding, but not on the control trees in WV. Ep155 cankers on the irradiated trees produced large cankers the first year and then significant callus developed the following spring. The last remaining stem from the 1989 inoculation died in 2006. Fulbright began collecting nuts from trees with callusing cankers in 1989. More than 2,000 nuts from various sites were planted in

nurseries in Accokeek, MD and in East Lansing, MI (MSU). Until 2004, more than 1,010 trees were still alive out of 1,248 that were transplanted to the research farm in Jackson, MI in 1993. Trees were large enough to inoculate with Ep155 in 2001. The results of the basal inoculations were very fast-growing cankers on 99% of the trees. The trees were removed in 2004 and sprouts came up from the stumps. The 1% of the trees that didn't die had very interesting cankers. The genetics of these trees were not sufficient as the cankers kept creeping along. Trees that had expanding cankers also were removed. The majority of the trees in the 1% category had massive amounts of callus were produced, but only on one side of the canker. The fungus would grow on the side with little or no callus and kill the tree. He never knew what trees were going to be successful, so many of the trees were untouched for several years. He showed pictures of Chinese chestnut inoculuated with Ep155—there was significant damage, but none of the trees died. Some of the irradiated trees produced different types of cankers—some cankers had significant callus where others had very little if any callus (target cankers). Natural cankers are now beginning to kill the tops of these trees. Small diameter stems are very susceptible to being killed quickly. There are only a few trees surviving from the 1,010 original trees. He is attempting to graft these trees using bud grafts. The trees are being grafted in an effort to study cankers away from the site to make sure canker reaction is not environmental. He hopes to put out a lot of these trees so he can study the effects of hypovirulence.

In a population of trees, different levels of tolerance may be found and different tree responses can be manifested during the infection process, as observed in canker morphology,. Fubright is not sure that irradiation is having much effect; he doubts that it is. He thinks there is a founder effect with a lot of trees from certain places that have been exposed to Ep155 and these trees are the result.

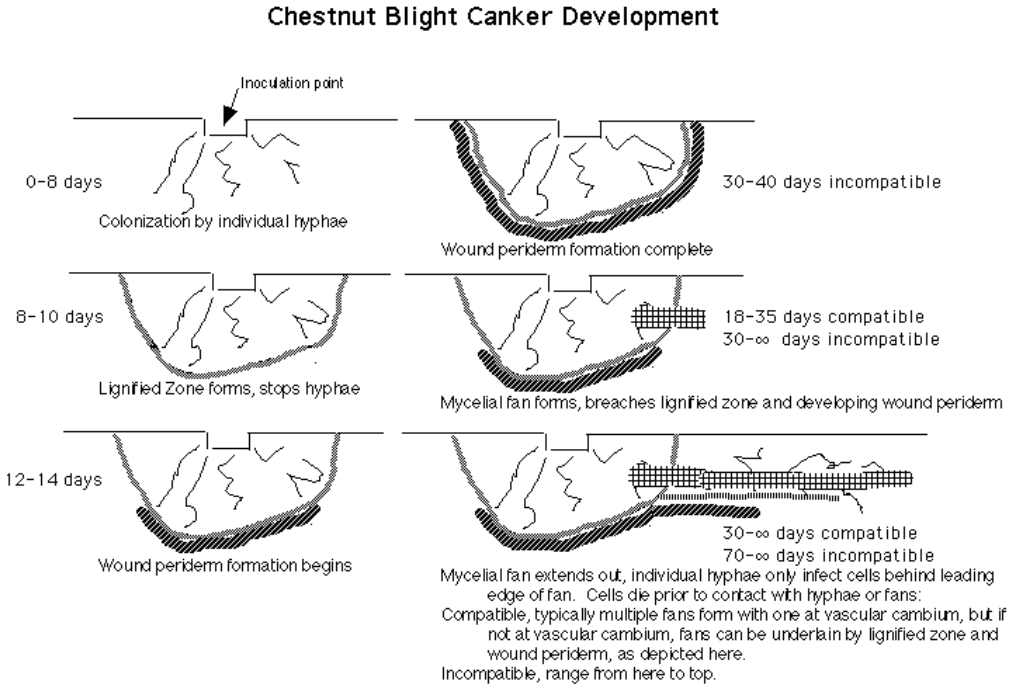
Crystal Lake site. This stand of about 2,000 trees in northern MI (Benzie County) was blight-free until 1976. The stand was inoculated with a variety of hv isolates, (Grand Haven 2, GHU4, etc) beginning in 1981. This was the first site that Fulbright began examining hv strains and genetically marked strains. By 1986-87, the trees were dying. They could not keep the trees alive with hypovirulent isolates. Callus would form on the cankers, but the trees died. He began to wonder if hypovirulence could ever work—were they using the right strains? Trees died and suckered for years. In 2006, the trees are recovering. There are abundant healing cankers. In some Michigan stands of chestnut that are exhibiting recovery, there are some trees that never recover—they continue to produce sucker sprouts. In Crystal Lake, there are both recovering and non-recovering trees. Some trees have big, ugly cankers, but that is what the County Line site looked like in 1980. At that time, Fulbright thought that County Line was one of the worst sites in MI, compared to sites like Grand Haven. But now, County Line is a fantastic grove of living trees. Fulbright stated that in his career, Crystal Lake is the only site that he walked into that was declining rapidly and has now gone into recovery as a result of hypovirulent introduction. In some trees, the crown is mostly dead, but one branch has continued to grow and the canopy is now replaced with foliage. He pointed out that there are more failures that successes, and recovery is definitely clumped. There are areas with no recovery and areas with significant recovery. His summary is that time needs to be considered as a factor in the successful treatment of trees with hypovirulence, as does the genetics of the trees involved in the recovery.

This led into a discussion of the West Salem, WI American chestnut stand. The 'word on the street' is that hypovirulence at West Salem is failing. To non-pathologists, West Salem looks much like stands in 1912 with dead and dying trees. There are pathogens in the forest other than chestnut blight. West Salem trees are also succumbing to *Armillaria* spp. and other pathogens. There are living stems at West Salem due to the persistent treatment of cankers of many years. There are swelling cankers on non-treated trees. With a trained eye, there are trees with swollen cankers in their crowns and the trees are still living. Fulbright finished with the statement, 'Let's do our job as plant pathologists and use this opportunity to try and fully explain what is happening in the stand before we declare or allow others to declare anything'.

Bill Powell—SUNY-ESF

The American chestnut research and restoration project. Powell presented work that has been done in conjunction with Chuck Maynard (SUNY-ESF) and Scott Merkle (University of Georgia) along with many graduate students and post-docs. This year, there are now transgenic chestnuts in the field—the first transgenic chestnuts ever outplanted. On June 7, 2006, the 'Wirsig' variety was planted in honor of Stan and Arlene Wirsig. Getting these trees out into the field is proof of the transformation concept. Now that trees can be transformed, the following putative resistance genes are available:

- Oxalate oxidase - from wheat (Dr. Randy Allen)
- Endochitinase - from *Trichoderma* (Dr. Gary Harman)
- CNO - endochitinase + NIa proteinase + oxalate oxidase (Hongyu Gao & Haiying Liang, former ESF graduate students)
- ESF synthetic antimicrobial peptides (small cationic)
- Ac-AMP1.2 antimicrobial peptide - analog from *Amaranth*
-



r. Armand Seguin, Canada)

- Possibly resistance genes from Chinese chestnut (from the NSF project)

Oxalate oxidase gene comes from wheat and is a natural defense gene of grains. Oxalic acid, in the presence of oxalate oxidase, is converted to hydrogen peroxide and carbon dioxide. Oxalic acid is produced by *C. parasitica* in cankers. They have looked at some transgenic (callus) tissue, constitutively expressing oxalate oxidase in the presence of oxalic acid and they found that callus tissue grown in the presence of oxalic acid have a decrease of about 4% in the lignin content. In the transgenic line that constitutively express the oxalate oxidase, there is no decrease in lignin content. Hebard developed a model of canker development. Based on that model, in the resistance reaction, the lignified barrier walls off the fungus and using oxalate oxidase, the oxalic acid will be degraded and the lignified area will be enhanced.

Oxalate oxidase summary:

- pleiotrophic effects - more durable resistance?
  - detoxifies oxalic acid (oxalate)Π
  - H_2O_2 possibly will enhance lignin formation
  - H_2O_2 possibly will enhance the expression of the chestnut's own defense genes
  - H_2O_2 has some antimicrobial activity
- easy assays (color change assay)
- shown to enhance fungal resistance in hybrid poplar
- public acceptance

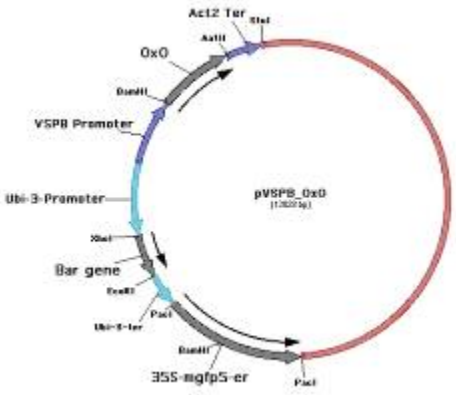
In addition to the number of genes that can be tested, there is a group of regulated promoters:

- *win*6.39 & *win*3.12 - poplar wound-inducible promoters (also some developmental expression) (Dr. Milton Gordon)
- ACS2, ACS9A, ACS10A - American chestnut vascular promoters (Dr. Bernadette Connors, graduate ESF)
- VspB - sucrose and wound-induced, auxin suppressed (vascular, soybean) (from Dr. Joe Nairn)
- EgCAD - Eucalyptus vascular & wound-inducible (from Dr. Armand Seguin); it has very good expression in roots
- *Cm*Gluc - *C. parasitica* & wound-induced promoter from Chinese chestnut (Khalil Howard, honors thesis project, in progress)

Over the years, these promoters have been put into a number of different plasmid constructs:

- pCEA1
- pCA1
- pCWEA1
- pOxO
- pGPOxO
- pWCPOT
- p35S-CPOT
- pSE39
- pVspB-OxO

- pMJMDEF



- pMJMEgCADdefensin

The construct of pVSPB-OxO is shown: The VSPB promoter drives the oxalate oxidase gene. It also has a Bar gene, a promoter for the herbicide 'Finale' and a GFP reporter gene, a very important gene for the transformation system.

Timeline for transformations:

- April 2004—the first transgenic American chestnut to make it to the field, LP28, was transformed by Linda McGuigan. This was selected for on 'Finale' selective media, using GFP.
- November 2004--after selection and bulking up,LP28 was ready for shoot regeneration (~ 7 months post transformation).
- March 2005—the first shoots were produced (~ 11 months post transformation)
- January 2006—the roooting of LP28 began
- March 2006—the first roots were seen (~23 months post transformation)

June 2006—the first transgenic American chestnut planted (~ 26 months post transformation)

Future plans:

- Fall 2007—transgenics with other vectors in pipeline
- Fall 2008—new constructs

Time for steps estimated from LP28: (each experiment ~2 years)

1) Propagate somatic embryos (continuous, bulking up 2 months)
    a) EI medium
2) Transformation (5 days)
3) Selection (~5 months)
4) Conformation of transformation & insert copy number (~2 months)
5) Regeneration to shoots (~4 months)
    a) EII medium
    b) EIII medium
    c) EIV medium
6) Propagation of shoots (4-12 months - note: lab closure & trouble shooting)
    a) Multiplication medium
7) Rooting (~2 months)
    a) Pre-rooting medium

    b) IBA dip and charcoal medium
    c) Pre-rooting medium
8) Acclimatization (~5 months)
    a) Chestnut soil mix, covered
    b) Opening cover for extended periods of time (qualitative)
    c) Increasing light exposure
9) Ready for field planting or resistance assays (~22 months post transformation)

The current bottleneck is acclimatization which is done by hand and is labor intensive. The smaller the shoot, the easier it is to root. The smaller the shoot, the more difficult it is to acclimatize. The choice is between small shoots with lots of roots or larger shoots which are easier to acclimatize. A programmable incubator is needed to increase output since the lids on the growth chamber are removed manually one hour a day, then two hours a day, etc. Out of 500 rooted plants, only 30 survived the acclimatization process. It is very difficult to transform plants and get them into tissue culture. Once a transgenic plant is obtained that has the required level of resistance, they will not be propagated via tissue culture; the transgenic plant will need to be outcrossed because:

- Less expense than tissue culture
- Larger numbers of propagules
- Increase genetic diversity by crossing to surviving trees
    - 1/2 progeny will contain new genes
- Need an easy selection for transgenics
    - Bar/Finale selection

They have had a theory that the herbicide Finale will work as a selection agent. An undergraduate did a research project using American chestnut and did a kill curve with Finale. The findings were that 2500 mg/L was sufficient to kill the non-transgenic plants.

On the 30 surviving trees, they will conduct some resistance assays. Small stems are inoculated with Ep155 and Ep42 using a small vertical cut with a scalpel and mycelial plug is inserted. The wound is wrapped with Parafilm for 5-7 days. Small cankers are produced on Chinese chestnut and the American chestnuts are killed. Powell recommends 3-mm diameter stems.

In their construct, their collaborators at Arbogen discovered a 700 bp deletion in the VspB promoter. When the construct was made originally (using about seven steps), it was tested thoroughly in *Arabidopsis* and they had nice vascular expression. In the transgenic American chestnuts, they have expression, but it seems to be very low. They have other trees in the pipeline that do not have the deletion.

Powell reported the results from inoculating one LP 28 transgenic stem and it had interesting results. In a comparative test with a non-transgenic stem using Ep42, the transgenic stem showed some wilting. It may not be resistant or it may be slower in reaction. He will conduct further tests on 10 stems.

Other constructs that are on the way include:

- 61 putative transgenic events with pGPOxO
    - Constitutively expressed OxO transgene, BAR, no GFP
- 14 putative transgenic events - co-transformations
    - pVspB-OxO

  - pSE39 - ESF12 antimicrobial peptide driven by the ACS2 vascular promoter, NTP2, GUS
  - pWCPOT - chitinase, NIa proteinase, & oxalate oxidase self-processing polypeptide driven by win6.3 promoter, NTP2
  - pMJMEgCADdefensin - spruce defensin driven by CAD promoter, NTP2, GUS
- New constructs being made:
  - OxO driven by *Cm* β-1,3-glucanase promoter, BAR
  - OxO driven by *Eg*CAD promoter, BAR
  - Reconstructing OxO driven by VspB promoter, BAR

SUNY-ESF also works with American elm. They began transforming elms several years after they began working with chestnut and they already have transgenic elms in the field. They are trying to get public awareness of transgenic trees and one of the first permits to plant transgenic trees on public property was in front of the SUNY-ESF library. Signs are posted at the trees explaining transgenics. Infrared detectors are in place to deter any damage. The permit is for 5 years.

Powell also is working on the Fagaceae project. Powell's lab is involved in the cDNA library construction. So far they have obtained samples from many people. From all of the samples, RNA was isolated, mass spec was conducted for concentration and an initial gel electrophoresis was run to make sure everything is intact before it was sent off to the report facility for analysis to make sure the quality is high.

Andrew Jarosz—Michigan State University

West Salem, WI inoculation study. He is looking at recovery from a population level by studying stands in Michigan and in West Salem. There may be some ecological differences in the level of susceptibility between the Michigan and West Salem stands. He offered a verbal argument as to why main stems have to die. When the chestnut blight pathogen gets into a susceptible host and the first canker is formed, the fungal strategy is to produce as many propagules, sexual and asexual, as possible. As long as there is a great deal of susceptible material in the population, there is no advantage for hypovirulence to be successful. He has studied the evolution of virulence of pathogens and from an ecological perspective, we need to get to a point where reduced virulence is favored. A situation needs to be created where it would pay to keep a tree alive compared to spreading to an uninfected tree. From Jarosz' perspective, that will happen when most of the susceptible material is gone—at which point, the current infected tree will be kept alive because there is not a lot of new material to become infected. In that case, reduced virulence is favored. It is during that stage when hypovirulence takes over as the trees recover because the advantage of hypovirulence is that it has horizontal and vertical transmission. There is no objective data to determine the benefit of hypovirulence on extending the life of large main stems. It is this hypothesis that drove this current study at West Salem with the objective of determining the benefit of hypovirulence in extending the health and life-span of the main stem of infected trees. The design includes:

- 118 trees scratch-inoculated with CHV1-Euro7
  - Initiated 2 cankers on each tree; one at the base and one at ~ 1.5m from ground.

- 46 control trees that were not inoculated

There are more hv-inoculated trees because Jarosz believes that population will be more variable. If there are some slightly less susceptible trees, they will survive longer. The control trees will pick up virulent cankers and die fairly quickly and he hopes to pick up the slight variation among trees in the population.

For assessing the trees, data includes:

- Diameter at breast height
- Crown health:
    - Size
    - Disease severity
- Epicormic sprouting (because most of the trees have infected crowns)
- Root collar sprouting (in an effort to determine if any of the root collar sprouts take over after the main stem dies)

In the future, the trees will be monitored annually until end of the property lease. The health and fate of inoculated versus control trees will be compared. We know from simply looking at the stand that many hypovirulent-treated trees are still living, despite a lost crown, while many of the non-treated trees are dead, but there is no hard data on survival., thus the reason for the test.

Alice Churchill—Cornell University

Secondary metabolism of *Cryphonectria parasitica*. Churchill is studying secondary metabolites of the chestnut blight fungus, which produces several known metabolites. The best characterized of these compounds is a family of orange and yellow aromatic polyketide pigments with diverse biological activities. The orange pigments produced by *C. parasitica* are the same color as the fungal fruiting structures present in cankers and are predicted to be responsible for their coloration. Pigments are just one example of the kinds of secondary metabolites produced by fungi. Why are fungal secondary metabolites important? Fungi are the sources of some very important secondary metabolites co-opted by the pharmaceutical industry to help combat human diseases. However, what purpose do they serve a plant pathogen? Specifically, why do we ca re that *C. parasitica* produces secondary metabolites?

- Convenient visual marker for following the fungus in cankers in the field
- Production of pigments is down-regulated by European Hypovirus infection
- How does a virus modulate fungal gene expression?
- Secondary metabolites play important roles in fungal biology
- Pathogenicity and virulence factors (e.g., toxins)
- Chemical self-defense (e.g., antibiotics, antioxidants, pro-oxidants (reactive oxygen species production)
- Fungal development (e.g., sporulation, appressorium formation)
- Signaling molecules (e.g., pheromones, modulators of signal transduction pathways)

What do secondary metabolites contribute toward the success of *C. parasitica* as a tree pathogen? How they serve the fungus as a chestnut pathogen or saprophyte is unknown.

We know relatively little about the roles of secondary metabolites in the biology of fungal plant pathogens in general. For each secondary metabolite pathway, most fungi

produce a family of compounds, each of which may vary to some degree in its biological activity. The only way to characterize definitively the roles of secondary metabolites in fungal biology is to identify one or more genes involved in the biosynthetic pathway and selectively disrupt those genes via targeted gene knockout (KO) methods. Then, one can compare the biological phenotype of the KO strains with those strains (wild type and controls) in which the pathway is intact and functional. Examples of phenotypes that might be altered in a secondary metabolite pathway KO strain include changes in virulence (e.g.,, due to loss of toxin production), pigmentation, development (e.g., ability to sporulate or mate), resistance to environmental stresses (e.g., heat, cold, dessication, reactive oxygen species, UV light), and nutritional requirements (e.g., iron acquisition).

The best-studied and most prominent secondary metabolites produced by *C. parasitica* are the aromatic polyketide pigments that color the sporulation structures and mycelium of the fungus orange. Production of these pigments is reduced in strains of the fungus infected with European Hypovirus. The first step in the biosynthesis of polyketides in fungi requires the activity of a large multifunctional, multidomain enzyme called a polyketide synthase (PKS). The Churchill lab has focused the majority of their efforts toward cloning secondary metabolite genes of the PKS class. Previously, her lab members used PCR to clone seven unique PKS fragments from *C. parasitica*. Each of these PKS gene fragments was hybridized to a genomic cosmid library of *C. parasitica* EP44; 60 cosmids were identified that hybridized to these PKS sequences. One cosmid was chosen for further analysis since it encoded a PKS gene whose expression was correlated with orange pigment production by *C. parasitica* cultures. A 37 kb genomic DNA insert was sequenced from this cosmid and 14 putative genes were identified in a presumptive secondary metabolite gene cluster. Most of the genes are highly similar to other fungal genes involved in secondary metabolite biosynthesis, regulation, or efflux. In addition to a PKS gene, the gene cluster contains genes similar to those that encode a protein folding enzyme, transcription factors, a transcription enhancer, monooxygenases, an oxidoreductase, a hydrolase, and part of a toxin transporter. These are the types of genes that are expected to be present in a secondary metabolite biosynthesis pathway.

C. parasitica PKS1 is most similar to other non-reducing PKSs having no characterized functions. The *PKS1* predicted protein is highly similar to several other uncharacterized fungal PKSs (E value of 0.0; greater than 60% identity). The Churchill lab disrupted *PKS1* in *C. parasitica* by *Agrobacterium tumefaciens*-mediated transformation and has begun characterization of the targeted gene knockout (KO) strains. There are no significant differences between the KO and ectopic control strains in colony growth rate on an agar medium or in Golden Delicious apple fruits, which are used as an alternate virulence assay. Pigmentation differences are evident on some media but not on others. Virulence and mating assays on dormant chestnut stems are in progress in collaboration with Sandra Anagnostakis (Connecticut Agricultural Experiment Station), as are efforts to identify potential differences in natural products chemistry, development, or nutritional requirements.

Future activities include:

- Confirmation of gene copy # of *PKS1*
- Utilization of differential gene expression in a variety of media to isolate sufficient quantities of *PKS1* natural product

- HPLC, electrospray MS, and NMR analyses to characterize *in vitro* chemical profiles and identify the *PKS1* natural product (in collaboration with USDA chemists Donna Gibson and Stuart Krasnoff, Ithaca, NY)
- Characterization of the *PKS1* KO phenotype on a variety of media and in chestnut stems
- Characterization of the *PKS1* KO phenotype macro- and microscopically to assess possible effects on development

In summary, there is a huge chemical potential in *C. parasitica:*

- 7 gene fragments have been cloned by degenerate PCR by the Churchill lab.
- 6 gene fragments have been cloned by EST analysis by the Nuss lab.
- There is some potential pathway overlap between the two sets of genes but most appear distinct.
- To date, as many as 13 PKS pathways and 1 nonribosomal peptide pathway are predicted.
- The genome sequence of *C. parasitica* (in progress by JGI-CSP) is predicted to reveal more polyketide, nonribosomal peptide, and terpene biosynthetic pathways in the form of gene clusters.
- Why are they there? What do they synthesize? What do they contribute to the fungus as a chestnut pathogen?? Do any contribute to the virulence of the fungus?
- Functional analyses in combination with analytical chemistry and novel biological assays will be necessary to determine differences between KO and WT strains.

Bill MacDonald—West Virginia University

Introduction of hypoviruses in West Salem, Wisconsin. Assessment of disease progress, spread of two hypoviruses and canker evaluation continues at the West Salem, WI site. Twelve permanent plots were established in 2001 in three areas of the stand designated as: 'Disease Center', 'Disease Front' and 'Beyond the Disease Front'. In 2001 and 2002, in the absence of hypovirus introduction, cankers within the plots were sampled and subjectively rated. Based on the lack of hypovirus spread, hypovirus treatment was reinstated in 2003 in all twelve plots. Two-thirds of all trees in each plot were treated with hypovirus CHV1-Euro 7 by either the traditional bark punch method or initiation of scratch wounds to create reservoirs of hypovirulent inoculum. One-third of the trees in each plot was left untreated to serve as trap trees to assess tree-to-tree spread. In 2004-06, all plots were sampled and treated. General findings for 2006 include:

- A total of 2235 cankers have been detected in the 12 plots since 1992; 635 cankers on living trees were sampled in May 2006.
- One-hundred, twenty-seven new cankers were discovered on trees within the twelve plots in 2006.
- The number of infected trees has remained relatively constant in the Disease Center plots (~92%) between 2001 and 2006. In contrast, the number of infected trees has risen sharply in the Disease Front plots (29% to 78%) and Beyond the Front plots (11% to 64%).
- Reservoirs of hypovirulent inoculum, provided by scratch-wounding areas of the bark, acted similarly to punch-treated cankers in terms of disseminating hypovirus to new infections. That is, non-treated cankers on trees that received hypovirulent

inoculum acquired hypovirus at twice the rate of cankers that developed on non-treated trees.

- Vegetative compatibility testing continues. WS-1 continues to be the dominant vc type in the stand; its recovery rate has remained steady for the past three years at 87%. WS-2 and WS-3 were found at rates of 1% and 8%, respectively.
- Mortality in the, Disease Center, Front and Beyond the Front is 42%, 30% and 22%, respectively.
- Hypovirus treatment appears to play a role in tree longevity. Sixty-six percent of the trees in the Disease Center that initially were treated with hypovirulent strains from 1992-1997 were alive in 2006. In contrast, 52% of the trees infected between 1998-2002, in the absence of hypovirus treatment, remain alive.

Mark Double—West Virginia University

The biological control potential of *Cryphonectria parasitica* strains containing an infectious cDNA copy of the hypovirus CHV1-Euro7. This work, begun by Bill Rittenour for his M.S. degree, is still in progress. Transmissible hypovirulence has not become established in most areas of North America as it has in Europe where it has been associated with biological control. Transgenic strains of *Cryphonectria parasitica* provide several mechanisms that may improve biological control. This study was designed to evaluate whether transgenic *C. parasitica* strains containing a cDNA transgene encoding the viral genome of CHV1-Euro7 show greater potential to biologically control blight than their cytoplasmically infected counterparts. Three treatments were employed that compared the following strains: transgenic hypovirulent (TG), cytoplasmic hypovirulent (CH), and virulent (V). Two types of cankers were initiated in each treatment to generate inoculum. To produce ascospore inoculum, naturally occurring and artificially established cankers were spermatized by painting cankers three times each summer with a conidial mixture that contained both mating types (MAT-1 and MAT-2) of the appropriate treatment strain (TG, CH, or V). To produce conidial inoculum, cankers were scratch-initiated (SI) on separate trees in June 2004, 2005 and 2006 by scratching the surface of the bark and painting the wounded area with a mycelial-agar slurry of the appropriate treatment strain (TG, CH, or V). Non-treated trees also were left to monitor natural canker development as well as hypovirus spread. In October, 2004 and 2005, tree condition and natural canker establishment were assessed for all trees. Most trees were asymptomatic after the first treatment season and the incidence of natural infection remained relatively low. By August 2006, there were 79 natural cankers in TG plots, 35 in CH plots, and 42 in V plots. Cankers also were sampled to determine the hypovirus infection status of the thallus. Although the purpose of the spermatization treatment was to produce ascospores, many treated cankers also acquired hypovirus from the treatment inoculum. Cankers occurring below SI cankers also acquired hypovirus. However, no transmission of introduced inoculum has been detected on trap trees in plot. Ascospore production was assessed by collecting bark discs in the fall of 2004 and 2005 and serially diluting ascospore contents from individual perithecia. Pigmentation and morphology were recorded for ascospores from all three plot treatments (TG, V, and CH). From the 2005 bark collection, 13,455 individual ascospore colonies were examined from 565 individual perithecia. Hypovirulent ascospore (HVA) isolates were collected from 75% of the spermatized cankers in TG plots. From those cankers, 68% of the perithecia yielded trangenic ascospores but at less than expected Mendelian ratios (OV:BV:OH:BH = 0.46:0.13:0.31:0.1). Pigmentation segregated as expected in V and CH plots conversion capabilities increased the biological control potential of transgenic strains. Continued monitoring canker development in 2007 will provide further information on the fate of the transgenic inoculum being produced and whether this approach results in improved biological control.

Michael Vayda—Administrative Advisor

Vayda indicated that he is pleased to be working with this group and he thanked everyone for a warm welcome. He hopes to assist the group as its administrative advisor. The current NE-1015 project runs from 1 October 2003 through 30 September 2008. Vayda commented that NE-1015 is a group of very passionate and dedicated people. He

has found the meeting to be stimulating with top quality research. The 2006 meeting is mid-way through the project (3 years). Vayda has to write a mid-point assessment that includes: accomplishments and progress of the team; interaction of the project team members; leverage of funding; and, the interdependency of team members. Vayda outlined important dates for a new proposal preparation (by technical committee), considering that it takes about 20 months for a project to be approved. Working backwards the steps to have a new project approved are:

- New project initiates 10/1/2008
- Project approved by CSREES 9/1/2008
- Proposal approved by NERA 7/1/2008
- Proposal reviewed by NERA 3/1/2008
- Finished proposal to NERA 2/15/2008
- Request to write multi-state project proposal 8/1/2007

Given the time constraints, an outline of the next project is due before the next annual meeting. Vayda posed the question, 'Does this group want to continue as a multi-state project?' NE-1015 is comprised of many individuals who are non-experiment station members, thus the leverage factor of this group is very high. Several project members do not have any experiment station appointments. A remark was made that the paperwork for joining the project is burdensome.

Technical Committee:

- Is more than collating station reports
- reviews progress towards objectives
- coordinates efforts of project members
- AES and non-AES contributions to project

If NE-1015 is to continue as a multi-state project, then

- NE-1015 members must demonstrate co-dependency & interaction
- identify participants and roles
- identify use of AES (seed) funding
- identify extramural (enabling) funding sources
- demonstrate outreach (extension?) efforts

A clear strategy for success must be demonstrated by defining the following:

- what is "success", when will the battle be won?
- how long until "success"?
- immediate term objectives
- how immediate term objectives will get us to "success"

The next steps for the technical committee include:

- Prepare List of Project Participants, including their affiliation, AES support, leverage, talents
- Identify co-PI grants generated
- Identify "economic impact"
- Appoint "Writing Committee" any volunteers?
- Set schedule for proposal writing

- Group input phase (how gather input, come to consensus?)
- Outline phase (long term, short term objectives, timeline)
- Grunt phase
- Polish/review phase

The project tends to broaden, and at some point efforts are diluted. The group needs to ask, 'what are the core objectives?' For an effective project, solid goals are needed so that all the pieces fit together. Vayda continued by saying that a clear vision must be articulated—a road map is required. There was some discussion if NE-1015 should remain as a northeast project or another division, or a national project. Vayda offered the potato germplasm project as an example of a national project where the funding was reduced to zero. Vayda recommended that the 1015 project remain a regional project and it should remain in the northeast.

Business Meeting

At the 2005 meeting, Angus Dawe was elected as Chair-elect. Dawe will chair the 2007 meeting, and agreed to host the meeting either in New Mexico or Virginia. Given that travel to New Mexico may limit participation, Dawe agreed to chair the meeting in Meadowview, VA. Sisco commented that holding the meeting near the Meadowview farm would be good for the NSF grant. For the chair-elect for 2007, Sandra Anagnostakis nominated Dennis Fulbright. The nomination was seconded by Hill Craddock. Fulbright was elected unanimously.

There was discussion about renewing the project. The following members agreed to act as a steering committee: Bill MacDonald, Sandra Anagnostakis, Dennis Fulbright, Fred Hebard, Hill Craddock, Paul Sisco, Don Nuss. Michael Gold was suggested as someone who might be willing to join the steering committee. MacDonald agreed to provide deadlines for the vision statement.

A tour of the Maryland Chapter of TACF chestnut orchard was conducted by Robert Strasser.

Vayda indicated that the 3-page summary of the 2006 meeting is due 60 days from the conclusion of the meeting.

Respectfully submitted,
Mark Double
November 2006

Milestone Accomplishments

2004 Milestones Accomplished:

- Nutritional analyses of nuts was conducted from orchard selections and cultivars of chestnut.(Anagnostakis)
- Hypovirulent strains of *C. parasitica* were developed and deployed for blight control on native chestnut trees at each of three clear-cut forest areas and one nursery are planted with hybrid chestnut trees (Anagnostakis)
- Site was selected for release of Euro 7 transgenic strains in West Virginia (MacDonald and Nuss)

2005 Milestones Accomplished:

- Market research analyses were completed and the findings reported (MO, MI). New selections of experimental lines from MO, CT, TN and MI are being established. (Gold)
- Orchard of advanced backcross chestnut for assessment of host resistance with hypovirulence was established in WV, albeit the planting failed due to raccoon predation of nuts (MacDonald and Hebard)
- Characterization of the role of hypovirus p29 in virus RNA accumulation in *C. parasitica* and virus transmission through conidia of the fungus (Nuss)
- Generation of polyclonal antibodies against 5 overlapping regions of hypovirus ORF B and construction of *a C. parasitica* database (Nuss)

2006 Milestones Accomplished:

- Publication of a *C. parasitica* EST database containing approximately 2500 ESTs (Nuss)
- Demonstration that hypovirus p29 suppresses RNA silencing in *C. parasitica* and in heterologous plant system, the first report of a mycovirus-encoded suppressor of RNA silencing (Nuss—not proposed)
- A proposal to sequence the *C. parasitica* genome was approved by the Department of Enegy Community Sequencing Program. A draft of the *C. parasitica* genome sequence should be available to the research community in 2007 (Nuss, Churchill, Milgroom).
- A proposal to develop more genetic markers and a better genetic map for the Fagaceae was approved by the National Science Foundation's Plant Genome Research Program. The results of the four-year project will be posted at http://www.genome.clemson.edu/projects/fagaceae/(Carlson, Sisco, Hebard, Anagnostakis)
- Chestnut market analyses completed and findings reported (Gold)
- New processed chestnut products introduced (Fulbright, Gold)
- New chestnut cultivars established in several cooperating locations (Fulbright, Gold)
- Role of canker age and vegetative compatibility on the perpetuation of hypoviruses determined, following their introduction into forest chestnut trees.

List of Publications 2005-2006

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