NE-1015 Technical Committee Meeting
Biological Improvement, Habitat Restoration, and
Horticultural Development of Chestnut by Management of Populations, Pathogens and Pests
White Eagle Conference Center, Hamilton, NY
October 14-16, 2005

Attendance:

California:	Debbie Wilk, Pam Kazmierczak (UC-Davis)
Connecticut:	Sandra Anagnostakis, (Connecticut Agricultural Experiment Station)
Maryland:	Donald Nuss, Lynn Geletka, Diane Shi (University of Maryland Biotechnology Institute, Center for Biosystems Research)
Massachusetts:	Timothy McKechnie (University of Massachusetts)
Michigan:	Andrew Jarosz, Carmen Medina-Mora (Michigan State University)
Missouri:	Michael Gold, Michele Warmund (University of Missouri)
New Jersey:	Bradley Hillman, Joanne Crouch (Rutgers University)
New Mexico:	Angus Dawe (New Mexico State University)
New York:	William Powell, Chuck Maynard, Linda Polin McGuigan (SUNY-ESF), Alice Churchill (Chair), Bruno Donzelli, Keiichi Sudo, Michael Milgroom (Cornell University), Steven Jakobi (Alfred State University)
North Carolina:	Paul Sisco (TACF), Ron Sederoff (NC State)
Pennsylvania:	John Carlson, Haiying Liang, Chien-Chih Chen, Kelly Deitrick (Pennsylvania State University), Sara Fitzsimmons (PA-TACF)
Tennessee:	Hill Craddock, Mark Alexander, Shannon Cagle, Lisa Worthen (UT Chattanooga)
Vermont:	Michael Vayda (University of Vermont)—Administrative Advisor
Virginia:	Fred Hebard, Bob Paris (TACF, Meadowview)
West Virginia:	William MacDonald (chair-elect), Mark Double, Paul Bogden, Shawn Kenaley, (West Virginia University); Laura Georgi (USDA-ARS AFRS, Kearneysville)
Canada:	Myron Smith, Fuad Tanha (Carleton University, Ottawa)

The meeting was called to order by Chairman Churchill at 8:30 am on October 14, 2005 at the White Eagle Conference Center in Hamilton, NY. Churchill welcomed the group and introduced the new administrative advisor for NE-1015, Michael Vayda, Associate Dean at the University of Vermont. Vayda, also professor of botany at UV, indicated that he was pleased to be chosen by NERA and he looks forward to working with the group.

<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.

John Carlson—Pennsylvania State University

Carlson gave a brief overview of the chestnut research being conducted at Penn State.

Siliviculture studies:

- **Site evaluation and planting method study of American chestnut**. Site conditions at seven forest sites within Penn State's Stone Valley Experimental Forest will be evaluated for site conditions for American chestnut restoration. Various planting methods, seed protections and vegetative competition also will be determined. Planting methods include direct seed via seed protector or one foot-tall tree shelter and containerized stock originating from 10, 40 and 173 cubic inch pots. Sites were laid out in a randomized complete block design with ten blocks of two replications per treatment. Existing vegetation was reset at all sites via mechanical equipment prior to planting. In addition, half of the blocks will receive a herbicide treatment (pre- and post-emergent) with applications as needed (likely up to three years). The first year consisted solely of post-emergent (RoundUp Pro 2%) applications. All sites were planted in recent harvest areas previously equipped with fencing where possible; fencing was erected where none existed previously. Height measurements will be taken annually after each growing season for the fist six years to determine best site establishment methods.
- **General evaluation of American/Chinese B2F2 seedling from TACF farm**. A B2F2 planting was established in 1996 as a progeny test. Approximately 1,000 seedlings were used in the study. Segregation for blight resistance is evident and some families showed marked blight resistance.
- **Proceedings**. The proceedings of the National Park Service chestnut conference held in Asheville, NC in May 2004 has been published. It can be viewed on-line at: http://chestnut.cas.psu.edu/nps.htm

Progeny Tests:

- **New F1 families for genetic mapping**. Crosses of a large American chestnut female parent tree ('Alex-R') were made with pollen from *Castanea mollissima* cv 'Mahogany'. Sandra Anagnostakis provided the 'Mahogany' pollen. There was good seed set in 2005 and the seed will be harvested soon.

Molecular genetics:

- **Variation in genome content among resistant backcross progeny**. A dot-blot procedure was developed to screen individual trees in the backcross generations to detect those with greater amounts of American chestnut genome after the selection for blight resistance, Results indicate a significant amount of variation in dot blot intensity among individuals in all three backcross generations that were tested. This indicates that this technique should be useful in identifying individuals with the greatest amount of American genome at each generation in the TACF breeding program (progeny can be chosen very early on the breeding program).

- **Morphological approach to species identity.** Matt Diskin is looking at the phenotypes of American versus Chinese chestnut. In looking at hybridization levels of BC3s, he found that there is variation in Chinese chestnut.
- **Assembly of physical maps for the two blight resistant loci in *C. mollissima*** (with Albert Abbott and Laura Georgi). The goal of this project is to prepare a contiguous set of BAC clones (bacterial artificial chromosomes) that span the resistance of *C. mollissima* QTLs (quantitative trait loci) for map-based cloning and sequencing of the blight resistance genes. The Cbr-1 and Cbr-2 loci are saturated with additional DNA markers. Bulk segregant analysis showed both resistant and susceptible markers.

Horticulture studies:

- **Accelerated growth production system (AGPS).** This work, conducted by Chien-Chi Chen, compared AGPS with direct seeding to compare seedling quality of American chestnut. Seedling quality has a crucial role in determining the success of forest plantations. Poor establishment and low survival are common problems with low quality seedlings. AGPS has been established in an attempt to shorten cultivation time and produce seedlings with desirable growth characteristics. In a study comparing AGPS and direct seeding (DS), plant height differences were significant (144.8 cm for AGPS and 49.5 cm for DS) as was seedling survival (86% for AGPS and 47% for DS. However, for American chestnut, transplanting containerized seedlings in the field has been observed to cause transplant shock and shoot or stem dieback. Leaf scorch is another factor that affects seedling performance in the field. Two studies have been developed for evaluation. The first study was conducted to look at mechanisms of leaf scorch in chestnut using the AGPS system and to determine protocols for reducing leaf scorch. Seedling were prepared and transplanted in the summer of 2003 at the PSU horticulture farm. Treatments included acclimation period (0, 30 and 90 days), shade (0%, 30%, 50% and 70%), water stress and antidesiccant. Preliminary data suggest that cuticle thickness and wax content were correlated with leaf scorch severities. American chestnut seedling produced by AGPS had less leaf scorch and better growth under 30-day acclimation with either 30% or 50% shade. A second study examined desiccation of seedlings and RPG (root growth potential). RPG is defined as the ability of a tree seedling to initiate and elongate roots when placed into an environment favorable for root growth. American and Chinese chestnut bare-root seedlings were purchased from a commercial source and placed in cold storage for 4, 10, 16 and 22 weeks under two storage temperatures (-1C and 2C). Seedlings from each treatment combination were potted into 1-gallon pots and grown in a greenhouse for 28 days at which time the potting material was removed and the roots were measured. Plant dehydration was greater in 2C after 4-weeks of cold storage. Chinese chestnut loses more water in cold storage than American chestnut. After 4-week cold storage, American chestnut requires longer time to break bud. Prolonged storage period may promote increased mold growth. Future plans include testing more storage temperatures, storage duration and storage methods to protect against dehydration. They also had nutrient disorder problems; they may look at nutrients further as the fertilizer may acidify the soil.

Sara Fitzsimmons—PA-TACF

In the PA-TACF regional breeding program, over 18,000 trees are planted and they have varied survival. Poor site selection and poor maintenance are the two factors that have the greatest effect on survival. On average, 35% of progeny are inoculated (using Ep 155 and SG1-2) at any given site. Of those progeny inoculated in the BC3 generation, 13% of the progeny are selected for further breeding. At Meadowview, about 1 of 8 are selected for further use.

Male Sterility. One of the major hypotheses currently being tested is the working of male sterility in controlled crosses with American, Chinese and Japanese chestnuts. Prior data suggest that American female to Japanese or Chinese male crosses yield 100% sterile progeny, whereas Chinese or Japanese female to American male crosses yield 100% fertile progeny. Preliminary data indicate that the above hypothesis is true although a major complication in defining the data is the contamination among seed lots. Sisco indicated that a mitochondrial trait may explain sterility.

Stone Valley F1s. In the field, chestnut growers have experienced germination rates from 20% to 80%, using a direct-seed method. In a greenhouse study of various F1 seed lots, Chinese x American germinated at a rate of 48%, compared to 63% for American x Chinese and 95% for open pollinated crosses. Once they germinate, however, survival is good.

PSU silviculture studies. In support of former plantings established in the late 1990s, a wide set of variables is being investigated that affect American chestnut in a forested areas. Site variables include: method of harvest, competing overstory and ground vegetation, slope, aspect and soil type. Planting variables include: direct seed with seed protection, direct seed with one-foot-tall tree shelters and containerized 0-0 and 1-0 stock. In terms of planting maintenance, half of the plantings will receive a herbicide treatment while the other half will not receive herbicides.

Fred Hebard—The American Chestnut Foundation, Meadowview

In the TACF breeding program, Hebard talked about the effective population size in terms of breeding backcross blight resistance into American chestnut. 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

Hebard decided that 20 lines would be enough as that would capture most alleles occurring at frequencies greater than 5%. He then presented the following table.

C X A1	C X A5	C X A9	C X A13
$F_1$1 X A2	$F_1$2 X A6	$F_1$3 X A10	$F_1$4 X A14

$B_1$1 X A3	$B_1$2 X A7	$B_1$3 X A11	$B_1$4 X A15
$B_2$1 X A4	$B_2$2 X A8	$B_2$3 X A12	$B_2$4 X A16
$B_3$1	$B_3$2	$B_3$3	$B_3$4

Hebard stated that they are using 20 American lines per source of resistance per locale. A persistent question has been, "What is the effective population size N_e of our breeding stock?" A famous rule in conservation genetics is the 50/500 rule which states that N_e of 50 is the minimum effective population size needed to avoid immediate collapse of a population from inbreeding depression while an N_e of 500 is needed for the slow loss of alleles by drift to be counterbalanced by addition of alleles from mutation. Hebard intercrossed BC3s to estimate inbreeding. Since chestnut does not self, several generations of crossing F2s must be conducted in an effort to calculate inbreeding coefficients. Hebard developed the following equation to ascertain an effective population size:

$$1 - \phi_{t = (1 - 1/2\ N_e)}{}^{t}$$

where N_e is a constant and ϕ equals inbreeding coefficient at generation t. Hebard presented the following table.

Effect of adding sets of 20 B3F3 progeny form our chapter breeding program on inbreeding and effective population size

# Chapters	Inbreeding Coefficient	Inbreeding Effective Population Size
1	0.02066	72
2	0.01166	128
3	0.00867	173
4	0.00721	208

Conclusions:

- TACFs breeding program will yield adequate effective populations sizes (although the projections have a number of assumptions that will not hold true, decreasing the projected N_e).
- There are more pressing worries such as whether the trees have adequate resistance to blight and whether they will have adequate forest competitiveness and whether the blight fungus will find a way to overcome resistance.

Lisa Worthen—University of Tennessee-Chattanooga

Worthen presented the Chattanooga chestnut tree project annual report.

Backcross breeding. Of the 5,437 seed that was collected in 2004, many were advanced backcross as shown in the following table.

Hybrid Type	# Nuts
F1	272
F2	290
BC1	134

BC3	1683
BC4	254
BC2F3	38
American X American	32
American Open Pollinated	252
Others	783
Total	5437

The harvest from Rutherford County, TN included 23 F1 and 254 BC4. Blount County, TN yielded 1202 nuts but many of the chestnuts at that site had gall wasp. Nut collection since 1998 is listed in the following table.

Year	1998	1999	2000	2001	2002	2003	2004
# Nuts	241	213	482	296	694	1918	4658

A single fourth backcross and two third backcross families were completed in 2004. If the harvest goes well, three additional backcross families will be completed in Tennessee in 2005. Mother trees include: McInturff Tree (Blount County), Gilliam Tree (Sumner County), Shriver Tree (Rutherford County), Cherohala Skyway, Ray Clements Tree and Clint Neel Tree. Two first backcross hybrid trees at Bendabout Farm were advanced to B3 in 2005 but the fruit set was low. They completed an intercross of two B2F2 families using pollen from selected trees at Jimmy Maddox's TVA planting at Muscle Shoals, AL.

In general, the nut harvest for 2005 is expected to be lower than 2004, due to a late frost and an August drought.

Hypovirulence and biocontrol. *Cryphonectria parasitica* has been isolated from mother trees and assessed for morphology. Cultures from the Schreiber tree are extremely slow growing. Also, *C. parasitica* isolates from a Macon County tree are abnormal. Some *C. parasitica* isolates from Walker County sectored and the Floyd County, GA isolates had two distinct zones of growth. Isolates from various trees were sent to Mark Double at West Virginia University who will examine the isolates for dsRNA.

Hill Craddock—University of Tennessee-Chattanooga

Chestnut cultivar trial at Chattanooga. Twenty cultivars were planted in four blocks. Cultivars include: *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*. The planting was initiated as a model for commercial production. Planting of this orchard is not complete; they had problems obtaining cultivars.

Problems associated with chestnut. Craddock highlighted several problems.

- ***Asian ambrosia beetle***. There is no effective control for this insect (*Xylosandrus crassiusculus*). The beetles bore into the trunk, leaving tell-tale spines of frass and sawdust. The beetles cultivate a fungus (ambrosia) upon which the larval brood feed. Infested trees usually quickly die. There might be a cultivar difference associated with this beetle as not all cultivars are attacked equally. Of the American X Chinese hybrid second backcross F2s, very few were attacked by beetles (Mossbarger) while 90% of some cultivars were killed.

- ***Frost damage***. There was a catastrophic frost on 24 April 2005 in Tennessee. Every shoot tip of *Castanea henryi* was affected. It will take more than a year for trees to grow out of this problem.
- ***Asiatic gall wasp***. In the summer of 2002, Asiatic gall wasp appeared in the Cuneo (Piemonte) region of Italy. The initial epidemic is catastrophic. Trees are dying and there are no normal shoots in many orchards; every bud has been transformed by gall wasp. Currently, researchers are looking for parasitoids of the wasp. The gall wasp problem in Georgia 20 years ago is no longer a problem, due to a wasp that is parasitic on *Dryocosmus kuriphilus*. Growers in Cuneo are using insect-proof netting over the 1-0 rootstock to protect them from gall wasp. Bud samples are taken weekly to inspect for gall wasp. Prior to grafting, buds are striped so there are no grafts for ovopositioning. The epidemic is spreading but it is restricted to the Cuneo region at present.

Sandra Anagnostakis—Connecticut Agricultural Experiment Station

Anagnostakis reported that the collection of chestnut species in Connecticut is probably one of the best in the world.

Tree growth. Anagnostakis showed pictures of trees from some of her CT plots that are planted with hybrid chestnuts. Hypovirulent strains are used on native trees in the plots in an effort to keep them alive so that they can flower and provide pollen to the hybrid trees to enhance localized resistance to chestnut blight. The hybrid trees, planted in green mesh tree shelters, have two American parents, Roxbury, CT and Watertown, NY. Hybrids in the forest plots include BC2 and BC3 trees crossed with either Japanese (J) or Chinese (C) chestnut; there are four families of trees (1=BC3(J) Roxbury, 2=BC3(J) Watertown, 3=BC2(C) Roxbury and 4=BC2(C) Watertown. In general, trees with Japanese background are taller than those crossed with Chinese chestnut and most of them have apical dominance. Trees in family 1 are the tallest of the four families. In a fourth planting site (Sessions Woods), there was a severe drought; no rainfall occurred between June and September. They make an attempt to visit each site once or twice a year to treat cankers with hypovirulent isolates, but the hay-scented fern was so lush that they have put of treatment until after the first frost.

In an orchard trial, 200 hybrids were thinned to leave only trees that exhibited some resistance to chestnut blight had good apical dominance and vigorous growth. Fifty trees remained after thinning; of those, nine exhibited late leaf emergence (a characteristic of CT American chestnut). Most of these trees are J X A and some of these trees will be selected for further testing.

Nut nutrients. Nuts from hand-pollinated crosses made in 2004 were collected in the fall and sent to a commercial lab for analyses (at $95/test). Protein levels ranged from 32 g/kg for Japanese chestnut and H1 X Hope to 102 g/kg for H1 X Lockwood. Total fat ranged from 5 g/kg for five crosses to 69 g/kg for American chestnut. Total carbohydrates ranged from 301 g/kg for H1 X Hope to 908 g/kg for Lockwood X Little Giant. Mean nut size (grams) ranged from 2.9 g for American chestnut to 29.8 g for Lockwood X Eaton. The laboratory report indicated that while all chestnuts had similar levels of sugar, the American nuts tasted sweeter.

The fatty acids measured included palmitic, steric, oleic, linoleic (omega 6) and linoleic (omega 3). High levels of oleic acid may be responsible for the sweeter taste. Anagnostakis reported that food science literature suggests that nuts with high oleic acid are more flavorful and nuts with more linoleic acid store better. American chestnut contained the greatest amount of oleic acid (37.3 g/kg) and linoleic acid (13.1 g/kg) than any other selection. In comparison, Japanese chestnut contains 0.2 g/kg of oleic acid and

1.1 g/kg of linoleic acid. These data suggest that pollinizers should be chosen carefully for their effect on a nut crop.

Michael Gold—University of Missouri

Competitive Market Analysis Chestnut Producers. Gold raised the question, "Why look at markets?" Widespread adoption of agroforestry in North America is lagging. Risk-averse producers are reluctant to establish agroforestry practices in the absence of readily available market information. Finally, market knowledge is a key ingredient in the success of profitable agroforestry enterprises that produce commercially valuable products.

Consumer research shows that it is necessary to redevelop the domestic market by reintroducing the chestnut as a food crop to a new generation of U.S. consumers. Studies in 2003 and 2004 were conducted to gauge consumer familiarity of with chestnuts and the key attributes that influence purchase decisions. Results show that consumers were unfamiliar with chestnuts. Most had never tasted a chestnut, but did have interest in exploring them as a new food. Quality and nutrition-diet-health were consistently listed as the most important attributes influencing purchase and consumption decisions for chestnuts

Consumer and market research led to the1st Annual Missouri Chestnut Roast in 2003. Almost 1,000 individuals attended. A consumer survey was conducted at the chestnut roast and the survey results were published in HortTechnology (2004). In summary, of the 232 individuals that completed the survey, 87% had no familiarity with chestnuts. In conjunction with pecans and black walnuts, again 86% of the respondents had no experience with the nuts in question. The second chestnut roast was held 2004. While 2,000 people were expected, 3,000 actually attended. A second survey was conducted in 2004 and it validated the 2003 findings. Consumer survey results indicated that most participants did not know that chestnuts need to be refrigerated or that chestnuts are very low in fat. The public (retailers also) know little about chestnuts. Education is needed to provide information about how to buy, store and prepare chestnuts along with information on nutrition and health benefits.

The objectives of National Producer Survey—2004/2005:

- To look at the U.S. chestnut industry from the producer's perspective considering the five forces that influence competition
- Understanding these forces, producers can find ways to react and maintain or develop competitive advantages to succeed in the industry.
- Study provides information to individuals looking to enter the marketplace, with chestnut production being either a potential alternative farm crop or an opportunity for people already in the orchard business to diversify into different markets.

National Producer Survey methods:

- A multi-step research methodology was used.
- First, U.S. chestnut producers were identified using secondary information (Internet, chestnut grower associations, and university colleagues).
- A database of producers (individuals and businesses) participating in the chestnut market was developed.

- Second, a questionnaire-based survey was designed to collect general information about the market participants and information specific to each of the five forces .
- Questionnaires were mailed to all individuals identified in step one.
- Using a snow ball approach, a question in each survey asked for names and contact information of other participants in the market.
- The newly identified individuals and businesses were added to the database and questionnaires were mailed to them.
- Out of 250 surveys mailed nationwide, 90 surveys were returned and analyzed (36% response rate).
- Industry is dominated by part-time producers (20% are full time farmers; 53% are part-time farmers; and, 27% are hobbyists .

National Producer Survey Results

- A young, undeveloped industry
- Vast majority of producers (96%) have been in the market less than 20 years and 64% less than 10 years
- Therefore, most orchards are new, just entering commercial production
- Commercial chestnut production begins sometime between 5 and 10 years after establishment, depending on location, management and other factors
- Survey Results - Chestnut Prices
    - Highest prices paid by restaurants, on-line customers, health and natural food stores, farmers markets, and on-farm
    - Lowest prices are offered by discount grocery stores, distributors, and wholesalers
    - Large range of prices received
        - Farmers markets: $0.75 to $6
        - On-farm: $1.50 to $6
        - Restaurants: $2.00 to $7

Full-time farming operations received better prices for chestnuts. Producers that grow chestnuts from cultivars, grow organic chestnuts and sell under a brand name obtained higher prices than those who sell generic seedling chestnuts grown conventionally. Organic food market retail sales growth has equaled 20 percent or more annually since 1990, from $1 billion in 1990 to $9 billion in 2002. Contrast this to growth rate of less than 5% for the overall US retail food market .

Potential income

- In the Midwest, Chinese chestnut trees begin bearing commercial quantities of nuts between ages 6 and 9.
- Planted at 30' x 30' spacing, and bearing 15-30+ pounds per tree, easily reach 1,000 pounds per acre - (20 pounds per tree).
- At the farm gate, Iowa growers receive $1.60 per pound for small-sized chestnuts
- Gross return@1,000 lb/ac = $1,600/acre.
- Demand for quality chestnuts currently exceeds supply!

Gold extended an invitation to attend the Missouri chestnut roast, an event that is open to the public in an effort to introduce the public to Missouri-grown chestnuts, pecans and black walnuts and Missouri wines and showcase the beauty of the Horticulture and Agroforestry Research Center and the Missouri River Hills. The event is Saturday, October 29, 2005 10 a.m. - 4 p.m.

Michele Warmund—University of Missouri

Union failure in newly grafted trees. In a 1994 paper by Huang, et al., graft failure was attributed to four phloem fiber bundles in the rootstock. After two seasons, an interruption of cambial continuity was observed in failed unions; this was probably caused by a proliferation of phloem fiber tissue. Warmund questioned how the phloem is being interrupted? To answer this, she did chip budding using *Qing* and *Cropper* to ascertain if the problem is anatomical or not. She plans to look at graft unions. She showed slides of the anatomical structure of *Qing* and *Cropper* internode 5.

Symptoms of graft failure include:

- Bulge at union
- Internal black line
- Profuse rootstock suckering
- Small scion leaves
- Heavy cropping

Warmund asked if this grafting problem might be viral induced.

- Two new chestnut viruses have been found in Italy and France, but not yet isolated.
- Sent sample to Jerry Uyemoto and they came back negative for nepovirus.
- Could it be leafhopper transmitted? They will test for ilarviruses in 2006.
- Is it graft transmissible?

Tissue was taken from graft unions to determine if:

- It might be *Fusarium* induced? PDA, and acidified PDA were used to plate out tissue and look for fungi. One non-*Fusarium* isolate was cultured but not yet identified.
- It might be bacteria induced? Tryptic soy agar was used to look for bacteria.

Virus indexing, using "clean" and "dirty" sources of scion wood, will be conducted in 2006.

***Little Giant* as a source of dwarfing for Chinese chestnut trees selected in Connecticut.** Since Chinese chestnut trees can grow to 40-60' at maturity, pest control, pruning and harvest are complicated by tall trees. The use of dwarfing rootstocks might be a way to have easier ground management, better climate acclimation and reduce production costs and increase profitability. Dwarfing rootstock affects tree size, anchorage, disease susceptibility, precocity, soil and climatic adaptability. *Little Giant,* originating from CT, was identified as a potential source of genetic dwarfing. Studies will be conducted to determine the graft compatibility with *Cropper, Eaton* and *Qing* cultivars. In May 2005, two scion cultivars (*Eaton* and *Qing*) were grafted, using whip and tongue grafts, onto *Cropper* or *Little Giant* seedling rootstock. *Little Giant* on its

own seedling roots was used as a control. Vegetative growth and productivity of trees grafted to *Little Giant* will be evaluated. In 2004, *Little Giant* budwood from CT was top-worked onto some border trees in existing plantings to produce interstem pieces of sufficient length to be grated onto *Cropper* rootstocks in 2005. The percent successful graft unions will be recorded after trees are produced.

Nitrogen fertilizer. Warmund is investigating the effects of formulation and scheduling of nitrogen on vegetative growth and productivity of Chinese chestnut. Current fertilizer application is recommended at 112kg N/ha. In other orchard crops, nitrogen is applied before July 1 to avoid late season growth and susceptibility to low temperature injury. Recent communications indicate chestnut tree damage in Iowa and Michigan by low temperatures occurring in late fall or winter. A study will be conducted to determine the optimum timing and formulation of nitrogen applications using *Qing* to compare growth and fruit yield and determine the nutrient concentrations in leaves that are fertilized at various times. A chlorophyll meter will be used to measure foliar nitrogen content. In addition, post-harvest application of nitrogen will be examined with respect to cold hardiness. In summary, proposed tests will be conducted to: (1) compare growth and yield of *Qing* trees fertilized at various times with equivalent amounts of nitrogen; (2) evaluate the efficacy of slow release fertilizer with that of NH_4NO_3 at equivalent rates of nitrogen; and, (3) determine the effect of post-harvest nitrogen application on the cold hardiness of trees and subsequent yield. Data collected will include: nutrient content of leaves, chlorophyll reading, trunk and shoot growth, low temperature injury, number of burrs and the nut number and weight.

Thinning secondary burrs of Chinese chestnut. Some chestnut cultivars produce secondary burs that do not have adequate time to mature. Thinning enhances fruit size, promotes return bloom, prevents biennial bearing, prevents limb breakage, balances vegetative and reproductive growth.

Professor Liu Kuiying at the Agricultural College of Zhanjiang Ocean University (China) has used SXC to thin catkins. Warmund plans to test SXC to see if it can reduce secondary burs. Warmund want to identify cultivars that produce secondary burs and determine the effect of secondary bur removal on primary nut weight and size. She hopes to ascertain the effect of thinning on subsequent vegetative and reproductive growth. Chemical compounds used for thinning efficacy will be evaluated.

Preliminary conclusion is that shoots that were thinned in 2003 had greater mean nut weight than those that were not thinned. Additionally, shoots that were thinned in 2003 set more primary burs in 2004than those that were not thinned.

GENOMICS SYMPOSIUM

Churchill indicated that as she organized this meeting, she realized that she knows little of chestnut genomics, so she wanted to bring together a focus of interest in genomic areas. Her goal is to educate the group in areas outside of individual expertise and help us think about the project in a collective fashion.

Paul Sisco—The American Chestnut Foundation-Asheville (Overview of chestnut genomics)

Uses of Genomics for TACF's Breeding Program. Sisco began work with the TACF in 1998 with a background in maize genetics. He focused his remarks on the

resources and challenges with respect to mapping chestnut. He highlighted: (1) the genetic map of chestnut; and, (2) loci associated with resistance. Sisco iterated that the holy grail for those advocating transformation in trees is to identify and clone the genes for blight resistance. Most of the work he reported on was initiated by Fred Hebard, who made genetic maps and crossed F1s to set up a mapping population in 1991. Leaf material was analyzed by Anagnostakis, Kubisiak and Maynard. These data were published in 1997; they found 12 linkage groups (12 chromosome pairs) with 3 loci associated with resistance. In setting out in this work, Sisco obtained two grants. One was to do RFLP with Ron Sederoff's lab at NC State University. The second was to compare the genetic map of European chestnut (and its 12 linkage groups) with American chestnut.

Objectives of the basic genetic map includes the following:

- To identify genetic loci in Chinese chestnut that are confer resistance (Bob Paris, a new hire at TACF will work on this problem).
- To determine the gene action of each locus (dominant, recessive, additive). If the genes are dominant or additive, they can be used in a backcross program.
- To identify the homozygotes for resistance in the seed production orchard.
- To aid in the recovery of as much of the American genome as possible. (The goal of TACF is to restore the American chestnut back into the eastern forest, not just a chestnut with some American traits).
- To monitor the level of genetic diversity in TACF's released seed.
- To aid in protecting TACF's material from unauthorized use. The TACF needs to be able to identify the material it releases to nurserymen.

The best-characterized mapping population is with the cultivar, 'Mahogany', a Chinese chestnut (from NE China) planted in 1929 at the Connecticut Agricultural Experiment Station (CAES). Its origin is USDA-PI #70315, a seed lot described as "hardy trees native in north-eastern China". Arthur Graves, who directed chestnut breeding efforts at CAES, crossed 'Mahogany' with several American chestnut trees. He also made a BC1 to American from one of the F1 hybrids. Fred Hebard used pollen from this BC1 tree (the 'Graves' tree) to initiate part of his backcrossing program.

Hebard crossed two F1 hybrids to create an F2 mapping population. He also backcrossed both F1 hybrids to a set of American chestnut trees in the Virginia mountains to create a BC1 mapping population. Some of these BC1 trees also have been used as parents in the backcrossing program at The American Chestnut Foundation's Meadowview Research Farms.

Time-line of genetic mapping experiments. 'Mahogany' BC1 genetic mapping population included Trees #4-52 and #4-31, F1 trees from CAES used as males and10 different southwest VA American chestnut trees as females. In 1990, the VA American chestnuts were pollinated and BC1 seed harvested. In 1991, BC1 seed was planted in "Graves" orchard at Meadowview. In June 1995, about 57 BC1 trees were inoculated with two strains of the chestnut blight fungus and the cankers were measured in August 1995. In 1997, 147 RAPD and 21 RFLP markers were used to identify 11 or 12 linkage groups. Three chestnut blight resistance genes localized to linkage groups B, E, and F. Sisco questioned whether linkage groups B and E are on same chromosome.

In order to answer the question as to whether B and E are on the same linkage group, the *in situ* hybridization approach was taken. FISH (Fluorescent In-Situ Hybridization) was used to study18S rRNA sites. While FISH gives good data, there are problems associated with this technique. First, large DNA segments are required for the fluorescent signal to be visible. The DNA clone used for hybridization must be large or the target must be large (a repeated sequence such as telomeres or ribosomal rDNA sequences). To address this problem, Laura Georgi at Clemson University is in the process of making a Bacterial Artificial Chromosome (BAC) library from Chinese chestnut. These will give us large pieces of DNA to work with. Second, FISH is technically difficult. Not many labs have the expertise. Labs that have the expertise are John Carlson at Penn State and Nurul Islam-Faridi at Texas A&M. Finally, FISH is very expensive.

Tom Kubisiak, using RAPD markers had good data. However, linkage group E could not be correlated with European chestnut linkage group #11 (it might be group B).

Synteny between the genetic maps of America/Chinese chestnut, European chestnut and two European oak species (Sisco's map from 2004 meeting).

Chinese/American Chestnut Linkage Group	European Chestnut Linkage Group	European Oak Linkage Group
A	1	2
B	11	7?
C	8	3
D	10	10
E	4	5
F	7	8
G	3	11
H	6	1
I	5	6?
J	12	12
K	2	4
L	9	9

Since the 1997 mapping paper by Kubisiak et al., (Phytopathology 87:751-759), over 300 additional genetic markers have been added to the 'Mahogany' F2 map. These include AFLPs, SSRs, 5srDNA, and a genomic probe for a cystatin gene. The SSRs and 5srDNA also allowed the correlation of the linkage groups identified in the 1997 paper with the linkage groups of European chestnut as published by Casasoli et al. in 2001 (Theor. Appl. Genet. 102:1190-1199.) The SSR data also revealed 18 contaminants among the 102 trees in the F2 mapping population. Reanalysis of the data with these 18 trees removed eliminated the resistance locus on LG "G" that was mentioned in the 1997 paper and added a resistance locus on LG "E" that was not mentioned.

There is still a question about whether Linkage Groups "B" and "E" represent one or two chromosomes. This is important, since resistance loci have been found on both Linkage Groups.

Pedigree of F2 Genetic Mapping Population (for table listed below)

- 4-52 F1 tree (CAES) x 4-31 F1 tree (CAES)

'Mahogany' Pedigrees of F1 trees:

- 'Mahogany' Chinese x Roxbury, CT, American tree #1 = 4-31 F1 tree (CAES)
- 'Mahogany' Chinese x Roxbury, CT, American tree #2 = 4-52 F1 tree (CAES)
- 'Mahogany' Chinese x FP551 American tree = F1 parent of 'Graves' tree

Pedigree of BC1 trees

- F1 parent of 'Graves' tree x Clinton Corner, NY, Amer = 'Graves' BC1 tree
- Several American chestnut trees x 4-31 = several 'Mahogany' BC1 trees
- Several American chestnut trees x 4-52 = several 'Mahogany' BC1 trees

Pedigree of BC2 trees

- Several American chestnut trees x 'Graves' = several 'Graves' BC2 trees
- Several American chestnut trees * x 'Mahogany' BC1 trees selected for blight resistance = several 'Mahogany' BC2 trees

Probability of non-correlation of loci with blight resistance

	Linkage Group	**4-31**	**4-52**	**3:1 Chin "1"**
F2 Map	B	0.08	0.01	<0.0001
93 Trees	E	0.04	0.002	0.005
	F	0.22	0.001	0.08
		30 Trees	*27 Trees*	*All 57 Trees*
BC Map	B	0.029	0.001	0.0001
57 Trees	E	0.008	0.007	0.0002
	F	0.003	0.014	<0.0001

Sisco stated that Chi square analyses indicate the deviation from 1:1. In LG B above in the F2 map, 0.08 is not a high correlation; it only represents 8% chance (0.001 is a good correlation). Sisco summarized that if you have 80 individuals and you get a 10:70 ratio when a 40:40 ratio is expected , then resistance is correlated with that locus. While the BC map is a complicated system (will not see recessives here), it is a much cleaner map. The bottom line, according to Sisco, is the backcross map is where we need to spend much of our efforts.

Ron Sederoff—North Carolina State University (NSF chestnut genomics proposal)

Sederfoff discussed mapping the chestnut genome with regard to a proposal to NSF Plant Genome Research Program (PGRP). Proposals for PGPR are due November 1, 2005. The targets are:

- basic research in plant genomics
- accelerate the acquisition and utilization of new knowledge;
- innovative approaches to elucidating fundamental biological processes in plants.

The focus is on plants of economic importance and plant processes of potential economic value.

Three kinds of activity will be supported by PGRP in FY2006:

(1) Genome-Enabled Plant Research (GEPR) awards to tackle major unanswered questions in plant biology on a genome-wide scale,
(2) Translational Research from Model Systems (TRMS) to transfer findings made using model systems to plants of economic importance; and,
(3) Tools and Resources for Plant Genome Research (TRPGR) awards to novel technologies to enable discovery in plant genomics.

NSF PGRP Manager, Anita Klein commented on the chestnut genome project:

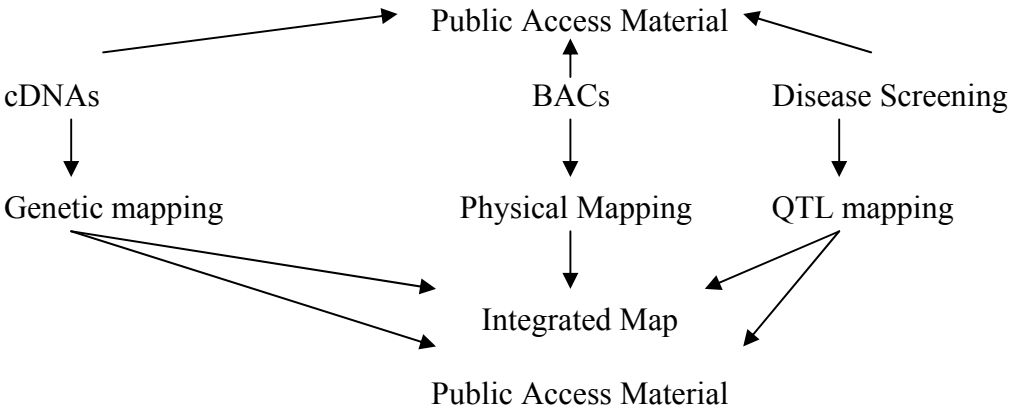
- You need to make a very compelling argument to justify the investment in chestnut.
- You span two of PGRP2006 target areas : Translation Research from Model Systems (TRMS), and Tools and Resources for Plant Genome Research (TRPGR).
- You need to decide on one. TRPGR seems most appropriate.
- Are you proposing Chestnut to be the model for the Fagaceae? Is anyone else doing genomics of the Fagaceae?
- Who will be the community of users for the Chestnut genomics tools?

How this proposal came about?. Sederoff discussed the role of the institute of forest biotechnology and the heritage tree program. Plans for 2004 were aborted by NSF, but there is a tentative 'go-ahead' for 2005.

Purpose of proposal:

- Develop the genetic and physical mapping resources needed to identify resistance genes by targeted sequencing.
- Develop chestnut as a model for the Fagaceae.
- Advance a genomic paradigm for rescue of trees endangered by pathogens and pests.

Outline of experimental plan is as follows:



Who will do what?

- Bill Powell: Project Librarian
- John Carlson: EST and BAC sequencing
- Laura Georgi/Bert Abbott: BAC mapping
- Paul Sisco: genetic mapping
- Tom Kubisiak: SNP marker development.

- Fred Hebard: chestnut breeding
- Ron Sederoff/Chris Smith: organize data/informatics.

Genetic problems to be addressed:
- QTLs: small populations overestimate effects
- Cloning genes by map based cloning can be extremely difficult if the functional genes have not been identified before.
- Current maps have fused linkage groups and segregation distortion.

Overview of the experimental plan
- Production of a high-resolution genetic map based on SNPs.
- Production of a high-resolution physical map based on BAC clones
- Associating QTLs for resistance with the integrated map.
- Fine scale genetic mapping of resistance QTL regions.
- Public access for all materials and information in this proposed work.

Specific objectives of the genetic mapping
- Construct libraries for ESTs of Chinese and American chestnut.
- Obtain 50,000 ESTs from each species and identify homologs and orthologs.
- Identify SNPs to distinguish Chinese and American genome segments.
- Create a high-resolution genetic map in Chinese chestnut.
- Establish the basis for SD in the AC x CC map.
- Resolve the basis of the missing linkage group in the AC x CC linkage map.
- Develop marker association genetic screening for BC3 and BC4 progeny for resistance.
- Physical mapping and integration
- Construct a physical map for *Mahogany*.
- Expand the available BAC library to 10X coverage.
- BAC EST hybridization screening,
- BAC fingerprinting and BAC end sequencing,
- Locate ESTs on genetic and physical maps to produce an integrated genetic and physical map.
- Identify BAC contigs in regions of the major QTLs for resistance.
- Fine-structure genetic mapping of the major resistance QTLs.

Target QTLs for host resistance--two have been identified (Kubisiak et al., 1997)

Genetic mapping. A high-resolution map will be made for Chinese chestnut using the cross of *Mahogany x Nanking* to obtain dense marker coverage and marker order in QTL regions using SNPs and SSRs. Association mapping will be done using CC species specific alleles in regions of QTLs to identify segments in AC BC3 and BC4 populations associated with resistance.

What will be the results?
- Model system for the Fagaceae
- Archive and database of ESTs

- Archive and database of BACs
- Database of SNPs
- Database with integrated clones, genes, markers and maps.
- Contigs defined for targeted sequencing.
- A platform to advance restoration of American chestnut.

Outreach, Training and Diversity:
- NCSU Diversity Coordinator: Thomas Easley.
- The Heritage Tree Program of Institute of Forest Biotechnology: Bob Kellison and Susan McCord.
- Technology Transfer through FORBIRC.
- Educational Outreach through a Kenan Fellowship: A Kenan Fellow in genomics, will be recruited for this project.
- Engages middle and high school teachers to create novel curricula in science.

Interactions with NSF sponsored program at St Augustine's College, Marvin Thompson: Director. There will be:
- Undergraduate lab course sessions in bioinformatics
- Project-specific undergraduate research training
- Participation in St. Augustine's Journal club
- University faculty exchange

What is really needed? There is no simple single path. We need to identify the known host resistance genes and look in Chinese chestnut for more genes, e.g. *Phytophthora* resistance. Also, hypovirulence suppression must be investigated along with engineering general resistance, e.g. chitinase. Once all the data is put together, the last step is the public/science interface.

Don Nuss—University of Maryland Biosystems Institute, Center for Biosystems Research (Chestnut blight fungus genomics projects)

Nuss indicated that there are several fungi that have their genomes mapped. The Broad Institute has several current projects, listed below.

Current Projects
- *Candida albicans* (strain WO-1)
- *Candida guilliermondii*
- *Candida lusitaniae*
- *Candida tropicalis*
- *Chaetomium globosum*
- *Coccidioides immitis*
- *Coprinus cinereus*
- *Cryptococcus neoformans*, Serotype A
- *Cryptococcus neoformans* variety *gattii* (Serotype B)
- *Histoplasma capsulatum*
- *Lodderomyces elongisporus*

- *Pneumocystis carinii* (human and marine)
- *Rhizopus oryzae*
- *Saccharomyces cerevisiae* (RM11-1a)
- *Stagonospora nodorum*
- *Uncinocarpus reesii*

Completed Draft Projects

- *Aspergillus nidulans*
- *Fusarium graminearum*
- *Ustilago maydis*

Current finishing projects on

- *Magnaporthe grisea*
- *Neurospora crassa*

Joint Genome Institute-Department of Energy

Finished or in progress	Pending
• *Trichoderma reesei*	• *Trichoderma virens*
• *Phytophthora ramorum*	• *Xanthoria parietina*
• *Phytophthora sojae*	• *Phycomyces blakesleeanus*
• *Phanerochaete chrysosporium*	• *Phytophthora capsici*
• *Mycosphaerella fijiensis*	

The Institute for Genomic Research (TIGR)

- *Aspergillus fumigatus* 32 Mb NIH funding
- *Cryptococcus fumigatus* 25 Mb NIH funding

What about *C. parasitica*? In 2001, there were only about 30 different *C. parasitica* genes in Genbank, so an EST project was initiated in Nuss' lab.

C. parasitica EST project included:

Minipreps	~5000
cDNA sequences returned	4448
Useful sequences	4217
Number of individual gene products represented in the library	~2200
Total base pairs sequenced	2,703,587
Undergraduate researchers supervised by Angus Dawe	2

About 20-25% of the coding potential of the *C. parasitica* genome is available at cogeme phytopathogen database, cogeme.ex.ac.uk (Nick Talbot's lab, Exeter University), NCBI/Genbank, accession numbers CB686454-CB690670.

The EST clones represent proteins from a broad variety of molecular functions that are involved in many biological processes. Through microarrays, they have been able to confirm that hypovirulence alters G-protein signaling.

There is a lot of microsynteny between viruses in *C. parasitica* and *Neurospora* and *Magnaporthe* (Sordariomycetes)? Nuss used these two organisms in context with conserved genomic arrangement of a G-protein ß-subunit and a regulator of G ß signaling.

Justification for *C. parasitica* genome sequencing project is plentiful:

- Phylogenetically, there is a lot of relatedness to *N. crassa* and *M. grisea*, although the later fungi have a completely different life style.
- It is the only fungal pathogen of trees to be characterized. The *C. parasitica/Castanea* system is the best characterized and it is the only host for which a robust mycovirus reverse genetics system is available.
- It is the only fungal host with experimental evidence for RNA silencing as antiviral defense mechanism.
- It has a robust DNA transformation system for genetic manipulation.
- There are well-developed fungal signaling pathways.
- Also, there are well-developed population genetics.
- Vegetative compatibility is important for hypovirus; it has practical application for biological control.

Resources brought to this project include:

- EST database representing 25% coverage of the *C. parasitica* coding potential (COGEME).
- Hope to have 2X coverage shotgun sequence (GS20 sequencer at Penn State) available soon.
- Linkage map based on RAPD (Kubisiak & Milgroom).
- Cosmid libraries are available at several labs.
- BAC library ?
- Advanced genome assemblies for related fungi *(N. crassa and M. grisea*).

The strategy is to organize a *C. parasitica* genome consortium. We need to identify and organize the *C. parasitica* community, by assembling contact information and research summaries. Support letters need to be collected and then two funding agencies can be targeted: JGI-DOE, NSF/USDA.

A *C. parasitica* discussion group met in Asilomar Conference Center (CA) in 2005. The group consisted of: Alice Churchill, Angus Dawe, Bradley Hillman, Pam Kazmierczak, Dae-Hyuk Kim, Donald Nuss and Debbie Wilk.

JGI-DOE/Microbial Genome Program. The White Paper deadline of July 14, 2005 was missed so a 5-page proposal will have to be submitted by December of 2005 to meet the July 2006 deadline. Items to include are:

- Relevance to DOE missions.
- Preliminary data on genome size, repeat content, suitability for shotgun sequencing, etc.
- Genome source.
- Post-sequencing follow-up will have to be developed.
- Availability of DNA transformation system.
- Biological novelty.
- Position is currently understood—we know where it stands in the16s RNA-based tree of life.
- Expected user community.
- Potential impact on scientific community at large.

- Explicit commitment to an acceptable data-release schedule.

Preparations are in place to work with JGI scientists to optimize yield from sequencing.

For the NSF/USDA Microbial Genome Sequencing Program, 20-30 awards are anticipated. The full proposal is due February 2006. The proposal looks that the justification of the organism selection, based on the basis of biological interest or agricultural importance, as well as community involvement, education and training, and societal impact. Teaching, training or outreach components must be incorporated into the proposal. In the aspect of outreach, they are in good shape, as the MD chapter of TACF has a high school module that travels to schools via an 18-wheeler and genomics aspects of this proposal could be incorporated into this teaching module.

Bradley Hillman—Rutgers University (Hypovirus genomes)

***Cryphonectria* virus genomics.** Viruses of fungi are generally simpler than plant viruses. The viruses of *C. parasitica* are all RNA. There are no known DNA viruses *of C. parasitica*. Viruses from 5 known families have been identified.

The only way fungal viruses spread horizontally is through anastomosis. Viruses can be transmitted between compatible, but not incompatible strains. Phenotypic changes follow virus transmission.

Five of eight described fungal virus families are in *C. parasitica*

Virus	**# in *C. parasitica*/Total #**	**Particle**	**Disease**
Narnaviridae (mitovirus)	1/~30	No	Yes
Hypoviridae (hypovirus)	4/4	No	Yes
Reoviridae (reovirus)	2/3	Yes	Yes
Partitiviridae (*Atkinsonella hypoxylon* virus)	`1/~30	Yes	No
Chrysoviridae (*Helminthosporium* virus)	1/4	Yes	Yes?

In terms of what the above viruses look like in *C. parasitica*, some have particles and some do not.

Hypoviruses:

- Contain lipid membrane with dsRNA inside membrane.
- No formal capsid (coat protein).
- The Hypoviridae was the first named family of viruses whose members lacked a coat protein.
- Structure and contents are poorly understood in terms of genome organization.
- CHV1 and CHV2 are relatively related,
- CHV3 and CHV4 are less related and poorly related to CHV1 and CHV2.

Where are *C. parasitica* hypoviruses found?

- CHV1 is all over Asia.
- CHV2 is found in one small part of China and in one spot in North America (NJ).

- CHV3 is in the United States.
- CHV4 is the most common hypovirus in North America.

CHV1 is currently the subject of cDNA microarray studies. CHV1 also has been found in Japan in *Cryphonectria nitschkii*. Two isolates of *C. nitschkii* are in different clades suggesting horizontal transfer. Hillman indicated that Milgroom confirmed that horizontal transfer between species can happen. Hillman questioned how common is interspecific transfer between *C. parasitica* and *C. nitschkii*.

CHV2 is rare. We do not know why it is among the most debilitating hypoviruses to the host.

CHV3 was identified in the late 1970s and it is prevalent in Michigan and Canada. CHV3 does not cross hybridize with CHV1 or CHV2. It has satellite RNAs associated with it but we do not know what contributions these satellites have.

CHV4 was isolated originally in West Virginia. This hypovirus is found throughout North America at a level of about 30%. It is the most common hypovirus in North America, but most fungal hosts are not symptomatic.

In comparative genomics organization, the hypovirus genome and potyvirus genome are compared to Endoraviruses. Hillman described areas of the hypovirus genome that might be of interest. He discussed the following:

- Area that might be associated with RNA silencing mechanism (a cysteine-rich region)
- HC-Pro homologue, a proteinase that affects symptoms (white phenotype in CHV1)
- Orf B proteinase, a paralogue of HC-Pro that might be essential to replication
- p40, a cis-acting protein affecting virus replication
- UDP-glucose, a sterol glysocyltansferase (the function of this gene is unknown—it is absent in CHV1 and CHV2. It may be an ancient acquisition from cellular genes)
- RNA-dependent RNA polymerase (usually have POL and HEL; there is good differentiation between CHV1 and CHV2 with CHV3 and CHV4. For the polymerase genes, CHV3/4 clade is more closely related to than to the CHV1/2 clade).

Mitoviruses properties:

- Simple mictochondrial virus and has been identified in only one tree in NJ
- Little apparent effect on fungal virulence and phenotype
- 2.7 kb dsRNA that encodes only RdRp
- Inherited in 100% of conidia, maternally inherited subset (~50%) of ascospores
- Passed by anastomosis; this may be associated with mitochondrial DNA recombination.
- Related to viruses that cause hypovirulence of Dutch elm disease fungus, *Ophiostoma ulmi* and *O. novo-ulmi*.
- Phylogenies of mitoviruses do not follow phylogenies of host fungi.

Reovirus properties:

- Found in animals, invertebrates, plants and now fungi
- 10-12 dsRNA independent segments
- Terminal sequence conserved, but slightly different for each segment
- Particle important for replication, capping, mRNA production
- Very important for early RNA studies in terms of the discovery of capping (Aaron Shatkin) and translation studies (Marilyn Kozak)

The *C. parasitica* reovirus has different phenotype from CHV1/2; it is more of a flat phenotype. Two *C. parasitica* reoviruses (MyRV-1/Cp-9B21 and MyRV-2/Cp-C18) were found 20 miles apart in West Virginia. These Reoviruses each have 11 dsRNA segments and the two viruses have different effects on the fungus phenotype. Both reduce virulence dramatically and they are inherited in 2-60% of conidia (not inherited in ascospores). Both Reoviruses have been sequenced completely and there is less than 50% amino acid identity.

There are many questions about *Cryphonectria* viruses:

- What are the details about fungus/virus interactions? There is good information on CHV1 but little else.
- We no little of the evolutionary biology of hypoviruses. Is there a missing link to be found in *Cryphonectria* between CHV1/2 and CHV3/4 lineage? More isolates from Asia need to be sequenced and we need more viruses from related fungi sequenced.
- What is the worldwide migration pattern of hypoviruses and *Cryphonectria* host?
- What is the fine scale ecology and evolution of hypoviruses? The West Salem site might be a good environment to study this question as the disease epidemic is relatively new.
- Why are hypoviruses so successful in *Cryphonectria* and mitoviruses so successful in *Ophiostoma*?
- What about close relatives of *Cryphonectria parasitica* viruses in other fungi? There is evidence for horizontal transfer between different *Cryphonectria* species—how common is this? How common is horizontal transfer to distantly related fungi?
- Mitoviruses are common in other fungi. Why do virus phylogenies not follow fungal phylogenies?

Finally, things we need:

- More sequence from dsRNAs that we already have
- Sequence from dsRNAs in strains we already have
- More dsRNA-containing strains, then sequence those dsRNAs
- *C. parasitica* genome sequence
- Sequences of *C. parasitica* isolates from different regions

John Carlson—The Pennsylvania State University (Overview of 454 sequencing technology)

Carlson spoke on the genomic sequence system. One possible route to get genomic data is by new technology—the Genomic Sequencer 20 system, from 454 Life Sciences (Roche Applied Science). It is a highly parallel system capable of sequencing 25 million bases in a four-hour period, about 100 times faster than the current state-of-the-art Sanger sequencing and capillary-based electrophoresis platform. The method

could potentially allow one individual to prepare and sequence an entire genome in a few days. The sequencer itself, equipped with a simple detection device and liquid delivery system, and housed in a casing roughly the size of a microwave oven, is actually relatively low-tech. The complexity of the system lies primarily in the sample preparation and in the microfabricated, massively parallel platform, which contains 1.6 million picolitresized reactors in a 6.4-cm^2 slide. Sample preparation starts with fragmentation of the genomic DNA, followed by the attachment of adaptor sequences to the ends of the DNA pieces. The adaptors allow the DNA fragments to bind to tiny beads. This is done under conditions that allow only one piece of DNA to bind to each bead. The beads are encased in droplets of oil that contain all of the reactants needed to amplify the DNA using a standard PCR tool. To perform the sequencing reaction, the DNA-template-carrying beads are loaded into the picolitre reactor wells; each well has space for just one bead. The technique uses a sequencing-by-synthesis-method in which DNA complementary to each template strand is synthesized. The nucleotide bases used for sequencing release a chemical group as the base forms a bond with the growing DNA chain, and this group drives a light-emitting reaction in the presence of specific enzymes and luciferin. Sequential washes of each of the four possible nucleotides are run over the plate, and a detector senses which of the wells emit light with each wash to determine the sequence of the growing strand. This new system shows great promise in several sequencing applications, including resequencing and *de novo* sequencing of smaller bacterial and viral genomes.

Technology differences between the conventional Sanger system and the 454 technology is listed below.

The Sanger technology employs:

- shotgun fragmentation of the genome
- cloning of the fragments into bacteria
- colony picking, microplate handling
- DNA purification from the clones
- sequencing by dideoxy chain termination
- electrophoresis
- whole genome mapping or assembly

The 454 technology employs:

- shotgun fragmentation of the genome
- fragments are clonally amplified on beads (800,000 beads per plate)
- DNA bead enrichment
- sequencing-by-synthesis on a pico titer plate device (about the size of the palm of a hand)
- whole genome mapping or assembly

The centerpiece of the Genome Sequencer 20 System contains both optics and fluidics subsystems, which are controlled by a computer subsystem. The fluidics subsystem ensures accurate reagent dispensing. It consists of a reagents cassette (which holds the reagent containers), a sipper manifold, pumps, valves, and debubblers. The fluidics subsystem flows the sequencing reagents across the wells of the pico titer plate device, and

moves the spent reagents from the pico titer plate device to the waste receptacle. The light resulting from the sequencing reaction travels through the back of the wells of the pico titer plate device and is captured by the CCD camera

Summary of steps include:

- Prepare DNA
- DNA is sheared to 500 bp fragments
- Adaptors are added to DNA and primer for sequence reaction.
- Attachment to beads.
- Anneal sstDNA to an excess of DNA captive beads
- Clonal amplification
- Break microreactors and enrich for DNA
- Raw images and peaks of light are collected and transformed into an overlapping set of sequences.
- Images are processed followed by signal processing
- Base call quality score

Carlson encouraged collaborators to look at the limitations and applications of the machine. He is hoping to publish several articles in the next year on current applications. Craddock used the following table:

Genome	**Base Pairs**
Hypovirus	12,700
C. parasitica	40,000,000
C. dentata	800,000,000

Since the 454 technology runs about 20,000,000 bp in a 4.5 hour timeframe, Craddock stated that it is incumbent for the group to take advantage of this system.

<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.

Laura Georgi—Clemson University and USDA-ARS Appalachian Fruit Station

She has made a BAC (bacterial artificial chromosome) library for *Castanea mollissima* cv 'Mahogany'. Using HinDIII partial digestion, she has about 55,000 clones and each insert is about 90 kb. Since the *C. mollissima* nuclear genome is estimated at 793 Mbp, her library coverage is about 6 times. The library and filter sets are available, at cost, from Clemson University (http://www.genome.clemson.edu). She will begin to construct physical maps around QTLs (quantitative trait loci) for blight resistance using markers generated by John Carlson's lab.

Bill MacDonald—West Virginia University

MacDonald reported on the work of graduate student, Bill Rittenour, who looked at the biological control potential of *Cryphonectria parasitica* strains containing an infectious cDNA copy of the hypovirus CHV1-Euro7. This work was conducted in cooperation with Don Nuss, University of Maryland Biosystems Institute, Center for Biosystems Research.

Transmissible hypovirulence has not become established in most areas of North America as it has in Europe where it has been associated with increased biological control of chestnut blight. 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: transgenic hypovirulent strains (TG), cytoplasmic hypovirulent strains (CH), and virulent strains (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 in June, July, and September 2004 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 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 formation. In October 2004, 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. There were 27 natural cankers in TG plots, 13 cankers in CH plots, and 20 cankers 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. Ascospore production was assessed by collecting bark discs in October 2004 and serially diluting ascospore contents from perithecia in the winter of 2004-2005. Pigmentation and morphology then were recorded for ascospores from all three plot treatments (TG, V, and CH). Hypovirulent ascospore (HVA) isolates were only collected from TG plots and at less than expected Mendelian ratios. Pigmentation segregated as expected in V and CH plots. The transgenic MAT-1 treatment strain effectively spermatized and hence produced HVAs on both initiated cankers and treated natural infections. The transgenic MAT-2 treatment strain did not produce morphologically distinct HVA isolates. To assess the increased conversion capability of HVAs, 18 HVA isolates were paired with 17 vegetative compatibility (v-c) types isolated from the study site; pairings then were examined for hypovirus transmission. Collectively, HVA isolates consistently (i.e. 4-5 conversions out of 5 replications) converted 12 of the v-c types; three additional v-c types were able to be converted at least once. When conidial inoculum produced by SI cankers was analyzed, the average percentage of hypovirulent conidia harvested was ~98% in TG plots, while no viable pycnidia (asexual fruiting bodies) were harvested from CH plots. Three of the 4 SI cankers analyzed from the V plots yielded all V conidia (i.e. 0% HV), but the remaining canker yielded 80% HV conidia. Overall, the production of HVAs with different conversion capabilities increased the biological control potential of transgenic strains. Monitoring canker development in subsequent years will provide further information on the fate of the transgenic inoculum being produced and whether this approach results in improved biological control.

Mark Double—West Virginia University

Double provided an update on the assessment of disease progress, spread of two hypoviruses and canker evaluation at the West Salem, WI site. Because of the ever-increasing task of sampling and assessment, twelve permanent plots were established in 2001 in three areas of the stand: '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 the Disease Front and Beyond the Front plots. Time constraints prohibited treatment of plots in the Disease Center. 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 and 2005, all plots, including the Disease Center, were sampled and then treated.

General findings for 2005 include:

- A total of 2004 cankers have been detected in the 12 plots; 628 were sampled in May 2005. Of the 1376 cankers not sampled, 509 were associated with dead trees and 867 were out of reach or merged with other cankers.
- One-hundred, seventy-six new cankers were discovered on trees within the twelve plots in 2005.
- Disease development (number of infected trees) has remained relatively constant in the Disease Center plots (~92%) between 2001 and 2005. 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 hv inoculum to new infections.
- Vegetative compatibility testing continues. WS-1 continues to be the dominant vc type in the stand; its recovery rate has remained steady at 87%. WS-2 and WS-3 were found at rates of 1% and 8%, respectively.
- Tree death in the, Disease Center, Front and Beyond the Front is 39%, 26% and 23%, respectively.
- Hypovirus treatment has seemed to play a role in tree longevity. Thirty-one percent of the trees in the Disease Center that initially were treated with hypovirulent strains from 1992-1997 are dead as of 2005. In contrast, 48% of the trees infected between 1998-2002, in the absence of hypovirus treatment, have died.

The state record tree, located in the West Salem stand, is an 80-foot tree with a 54-in diameter. The tree was uninfected until May 2003 when four cankers were detected on the root flares. All four cankers were of the WS-2 vc type. Sixteen additional cankers were found in September 2003, representing WS-1, WS-2 and WS-3 vc types. Four additional cankers were found in 2004. Cankers were treated in 2003 and 2004 with the appropriate hypovirus-containing isolate. The tree was not treated or sampled in 2005.

Shawn Kenaley—West Virginia University

Kenaley, a Ph.D. student, discussed his planned research with transgenic ascospores. To better understand the phenomenon of transmissible hypovirulence as a biological control strategy, the role ascospores and conidia serve in the epidemiology of the chestnut blight must be further refined. Present knowledge of the sexual and asexual stage of *C. parasitica* is based primarily on work conducted at the turn of the 20th century, following the discovery of *C. parasitica*. Previous studies have suggested that the roles of ascospores and conidia are strictly segregated; ascospores function as a source of primary inoculum and are responsible for the long distance spread and conidia serve primarily as spermatia and a secondary inoculum source. However, no field and/or laboratory studies have examined whether a level of redundancy exists between the function of these two spore stages. Transgenic strains possess two qualities that lend themselves to this study: hypovirus transmission to progeny (ascospores and conidia) and hygromycin resistance. These two traits serve as genetic/phenotypic markers, thereby allowing for the differentiation between treatment and wild-type inoculum, as well as, permitting treatments to be tracked overtime. The specific objectives of this study are to: (1) evaluate and compare the ability of ascospores and conidia of transgenic hypovirulent isolates to initiate infections; (2) determine whether transgenic hypovirulent ascospores, as well as conidia, can function as spermatia; and, (3) determine if transgenic hypovirulent ascospores and conidia are capable of transmitting hypoviruses to virulent cankers. This study, therefore, provides an opportunity to evaluate the potential role of transgenic inoculum, and determine the efficacy of transgenics as biological control agents.

Carmen Medina-Mora—Michigan State University

Medina-Mora reported on characterizing blight on chestnut trees and DNA fingerprinting of *Castanea* spp. using SSRs. The rationale of her research is that hypovirulence is thought to be the result of an interaction between a pathogen and hypovirus.

She posed several questions. Do trees differ in their degree of susceptibility? Can this play a role in terms of successful establishment of hypovirulence and biocontrol?

Anectdotal evidence—some trees never recover, even though they are in recovering populations in Michigan. Do these trees that never recover affect successful establishment of hypovirulence and biocontrol in a stand? Medina-Mora has noticed that there is variability in callus formation in an orchard on the MSU campus. She plans to utilize this population of trees to characterize chestnut blight. In the summer of 2005, she visually rated 266 trees in the MSU plot for callus (presence or absence). She randomly chose 50 trees from this population. She showed pictures of cankers with and without callus. All 50 trees were sampled (bark samples collected from 1-2 cankers/stem) and the trees were then cut at the base and allowed to sprout from the root collar. New sprouts were observed by the end of summer, 2005. *Cryphonectria parasitica* was isolated from cankers; she is now characterizing the isolates to assess whether they contain hypovirulent strains. When the sprouts are large enough (2006), she will inoculate with a virulent strain, a hypovirulent strain and standard strain Ep155. Rating of response to the first series of inoculations will be conducted in late 2006 and rated periodically

thereafter. She will determine if stems that showed callus before cutting show callus upon re-infection. Also, she will determine if the strain used for inoculation is responsible for callus induction.

In addition, she will develop SSRs (simple sequence repeats or micosatellites) as genetic markers of chestnut cultivars. She hopes the SSRs will be useful to: (1) distinguish cultivars, (2) validate clonal lineages; (3) help with paternity testing; and, (4) differentiate siblings and half-siblings. This study will utilize *Castanea* leaves. Using the DNeasy plant extraction kit (Quiagen), specific primers CsCAT2 and CsCAT41 (Marinoni et.al. 2003. Mol. Breeding 11:127-136) will be used. She will look at: 2 *Colossal* samples (C1, C2).
2 DNA extractions procedures and 2 PCR reactions. She hopes to address whether there are more suitable primers to use and if there are other tree populations that can be tested.

Chuck Maynard—SUNY-ESF

The American chestnut: transformation and whole-plant regeneration. The mission of this project is to conduct basic and applied research leading to the development of a blight-resistant American chestnut tree through genetic engineering and to reintroduce a population of these resistant trees back into forest ecosystems of New York and then the rest of the eastern US. The history behind this project began in 1988 with a grant from the American Chestnut Foundation. From early on, the project has had two parallel tracks: (1) gene transfer and plant regeneration, and (2) gene design, acquisition, and testing. The current chestnut project was started in 1997 through a New York State legislative act:
"authorizing the SUNY College of Environmental Science and Forestry to establish an American chestnut research and restoration program."

Transgenes and constructs used included:
Putative resistance genes encoding:

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

Regulated promotors:

- win6.39 & win3.12 - 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) (from Dr. Joe Nairn)
- CAD - Eucalyptus vascular (from Dr. Armand Seguin)

Vector constructs include:

- pCEA1
- pCA1: CaMV 35S - AcAMP1.2

- pCWEA1: win3.12 - ESF12/AcAMP1.2
- pOxO: CaMV 35S - oxalate oxidase (OA1 & OA2 transgenic events)
- pGPOxO
- pWCPOT
- p35S-CPOT
- pSE39
- pVspB-OxO
- pMJMDEF
- pMJMEgCADdefensin

Maynard showed slides of non-transformed and transformed OxO-stained *Arabidopsis* plants. The success story goes from shoots, to rooting to plantlet to the sandwich mix to the humid pot to fully acclimated plants to a field test.

In transformation studies, explants attempted or considered include: leaf discs; shoot meristems; germinating zygotic embryos; and, somatic embryos. Mayard then showed slides of zygotic embryos, ovule extraction, single ovules and ovule clusters.

Typical embryo establishment results:

- More than 90% of the ovules either die or become contaminated
- More than 90% of the surviving clean ovules form callus, but a small fraction of 1% do form somatic cell lines

Initial transformation protocol:

- Explant: 4- to 6-week-old somatic embryos
- Wounding treatment: 5 sec vortexing with sand
- Desiccation treatment: 0, 1, 2, or 3-days on moistened filter paper
- 1 week on Agro-kill media (E1 +200 carb)
- Then onto Selection media (E1-casein +200carb, + 6 PPT)
- Transferred every 2 weeks to fresh media

Multiple-Clone Study:
Desiccation Procedure protocol

- Add 200 ml sterile water to desiccation plates
- Transfer embryos to desiccation plates
- Incubate in the dark at room temp for 2 days
- Transfer embryos to Agro-kill media for 1 week
- Transfer embryos to selection media (E1 w/ Carb and PPT)

Agro-Transformation/Desiccation Procedure: Multiple-Clone Study American chestnut somatic embryos
6 clones:

- Pond 1-1
- 30015-2
- WB 348-5
- WB 275-27
- WB 296-10A-2

- WB 322-22A

Major accomplishments for 2004
- Optimized a transformation system
- Identified a set transformable and regeneratable parent clones
- Initiated large-scale transformation

Major accomplishments for 2005
- Isolated more than 200 transformation events
- Isolated 16 transgenic shoot cultures from 4 parent clones, including a 3-gene co-transformation event
- Rooted more than 25 transgenic shoots

Goals for 2006
- Root and acclimatize at least 10 plantlets from each of at least 5 transformation events
- Perform initial screening for blight resistance
- Establish a field trial with transgenic trees

Bradley Hillman—Rutgers University

Reoviruses. Ninety percent of the *C. parasitica* viruses found in nature are the hypovirus type, designated CHV. Reoviruses are very different from the CHV type. Two reoviruses of *C. parasitica,* C18 and 9B21, were obtained from West Virginia about ten years ago. In general, little is known of cell-to-cell movement of viruses in fungi; these reoviruses afford an opportunity to learn something about viral movement. Reovirus properties are as follows:
- Found in mammals, invertebrates, plants and now fungi
- Many human diseases are associated with reoviruses, as are viruses of cattle and insects
- 10-12 dsRNA segments.
- They are found across kingdoms.
- Terminal sequences are conserved.
- Particle is important for replication.
- There is no known nuclear component.

Reoviruses are fundamentally particle-associated viruses. There are double or triple structures containing the core, which is transcriptionally active. The covered core is not transcriptionally active, but it is infectious. Reoviruses contain exactly one segment of each of the 10-12 segments of dsRNA that constitute the viral genome, encapsidated in a single complex virus particle comprised of 6-8 proteins.

Myristoylation is a process of a short chain fatty acid addition to a protein. This is important in that this process leads to short-term association with proteins; cleavage of the fatty acid then allows for the release of a protein into the cytosol. *Rosellinia* reovirus will not be myristoylated because it has no G pattern. The mycoreovirus has a myristoylated protein in its outer capsid. One of the proteins on the outside of the capsid can have a myristoyl group. When reoviruses enter a cell, they enter in vesicles and the particles are striped to the core. The myristoylation site in reoviruses is similar to

mammalian systems. Hillman cloned the myristoylation gene to see if it is functional. One of the closest relatives to the myristoylation site is a form of an insect pox virus.

All eleven segments of C18 and 9B21 are found in one particle and are transmitted in an all-or-none fashion. Each core is transcriptionally active and all the enzymes necessary are in the particle itself. C18 virus is poorly adapted. The virus is lost easily; this could have been a horizontal transfer from another fungus in that canker.

C-18 and 9-B-2-1

- Were isolated in West Virginia about 20 miles apart.
- 11 segments segregate in an all-or-none fashion.
- dsRNA segments of 9B21 do not cross hybridize.
- Low cross-hybridization with homologous segments of C18.
- Different affects on virulence and phenotype.
- 9B21 virus causes more dramatic phenotype changes (greater debilitation).
- 9B21 virus substantially reduces *C. parasitica* virulence; it doesn't grow in apples.

Hillman tried to isolate these viruses while working in Nobohiro Suzuki's laboratory in Okayama, Japan. He succeeded in producing a good band in a sucrose gradient and they looked like reovirus particles; they were 80 nanometers, had 11 segments of dsRNA and proteins. He began working with 9B21 since it had a more stable phenotype. C18 in not a well-adjusted fungus; it loses its virus consistently. It has numerous sectors that are virus-free.

C18 and 9B21 were officially named Mycoreoviruses in the 8th ICTV report. Reoviridae is now subdivided into 12 general. Mycoreovirus has 3 species:

MyRV-1/Cp9B21
MyRV-2/CpC18
MyRV-3/RnW370 (for *Rosellinia necatrix,* a white rotter)

Mycoreovirus is most closely related to Coltivirus (a group of reoviruses that infect ticks).

Similarity of MyRV-1/Cp9B21 and MyRV-2/CpC18 and 9B21 is shown below:

Segment	% Similarity
S1	43%
S2	33%
S3	24%
S4	23%
S5	16%
S6	29%
S7	11%
S8	13%
S9	26%
S10	17%
S11	low

The genome size of the C18 virus is 25,050 bp while 9B21 is 23,426 bp.

The codon usage of C18 and 9B21 is dissimilar. Hypoviruses, particularly CHV1 and CHV4, are more similar to *C. parasitica* codon usage than are the reoviruses.

	CpC18	Cp9B21	*C. parasitica*
G+C	47%	42%	54%

Reoviruses—current and future research

- Complete the genome characteristics of CpC18
- Determine the particle protein—cyro EM in collaboration with José Castón
- Examine affects on fungal gene expression by northern blot and microarrays in collaboration with Don Nuss
- Examine CpC18 and Cp9B21 gene function and expression in collaboration with Nobuhiro Suzuki
- Infectivity in other fungi and other host types
- Expression of individual viral proteins in *C. parasitica*

Angus Dawe—New Mexico State University

Signal transduction. Dawe talked about signal transduction in *C. parasitica*. Hypovirus affects phenotype, virulence, radial growth rate and sporulation. This is all caused by a 12.7 kb cytoplasmically transmissible RNA element. This suggests a perturbation of cellular signal transduction pathways by 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 (see Dawe, A.L. et al. 2004. Microbiology 150:4033-4043). 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, posttranscriptional 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.

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 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.

Dawe has three graduate students and one undergraduate student 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.

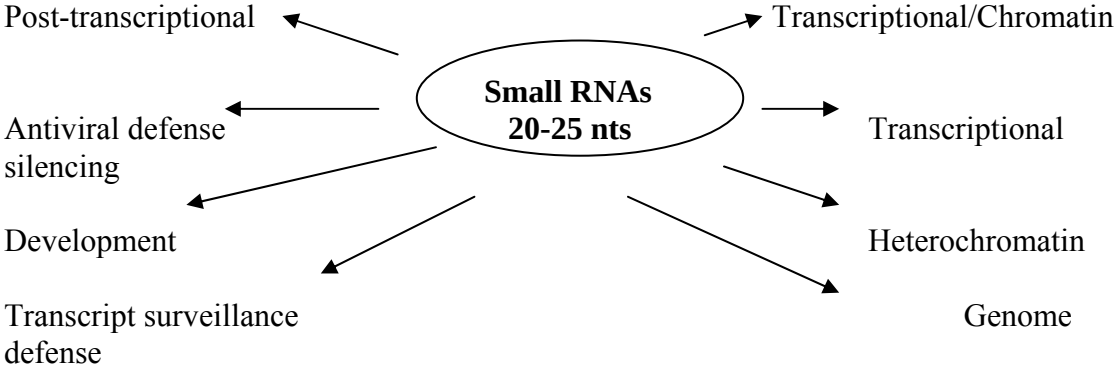
1) Do *bdm-1* and *cpgb-1* really interact?"
2) Assuming so, which bits of each are important?
3) What regulates the interaction?
4) How does it relate the phenotype and hypovirulence?

Fractals and growth. Dawe looked at colony morphology mathematically. This involves growing the fungus on PDA, looking at the shape of the colony, taking a digital image and allowing the computer to do all the work via box counting analyses. Digital squares are used to create boxes and the computer determines how may boxes it takes to cover a colony. The computer then generates a ratio of the number of boxes to the size of the colony. Ep155 is grown on PDA, reaches a fractal dimension of 1.85 over time (2.0 is a perfect circle). Conditions included 12:12 photoperiod (12-1500 lx) and temperatures ranging from 21-25C. The fractal dimension can be reduced by putting the fungus on cellophane. Growth on cellophane causes a reduction in the maximum fractal dimension. The colony size is filled less efficiently—maybe stress related. This is another quantitative measurement of phenotype. He reported that 713-infected Ep155 actually reaches the same fractal dimension as Ep155, but it takes longer.

Don Nuss—The University of Maryland Biotechnology Institute, Center for Biosystems Research

RNA silencing functions as an antiviral defense mechanism in fungi.. There is evidence that RNA-mediated sequence specific silencing of gene expression in plants (post-transcriptional gene silencing), animals (RNA interference) and, fungi (quelling). RNA silencing is regarded as an intrinsic defense mechanism of most eukaryotes against invasive nucleic acids such as viruses, transposable elements and transgenes. There is no experimental evidence that RNA silencing serves as an antiviral defense measure in fungi.

Nuss discussed siRNA and miRNAs.



Small interfering RNA (siRNA) is a powerful means to specifically knock-down a gene's message, and subsequently the protein level of the targeted gene. In this manner, cellular-based assays can be conducted in the absence (and presence) of the targeted gene's protein. The use of siRNA is replacing the methodologies of genetic knock-out technologies, dominant negatives and chemical inhibitors of protein activities. The advantages that siRNA provides over the alternative technologies are numerous—decreased time, decreased cost and increased specificity.

Nuss discussed p29, one of two polypeptides released from polyprotein p69 by an autoproteolytic event. A summary of p29 is as follows:

- Contributes to suppressed pigmentation and asexual sporulation by two apparently different mechanisms: direct interaction with host factors (works in absence of infection) and enhancing RNA accumulation.
- p40 and p29 Cys70 and 72 mutants do not function in trans.
- Activity domain mapped to Phe25 to Gln73.
- Enhances RNA accumulation and transmission through conidia in trans.
- Does p29 act as a suppressor of RNA silencing like related HC-Pro?

Hairpin RNA-mediated gene silencing exploits a cellular mechanism that recognizes dsRNA and subjects it and its corresponding mRNA to a sequence-specific degradation. When dsRNA is introduced into plant cells through hairpin RNA constructs, the result is a severe reduction of the target mRNA—the silencing effect being stably inherited over many generations.

Hairpin RNA-mediated gene silencing assay for *C. parasitica*:

- Silencing vector pGS
- Vector design based on plant gene silencing vectors developed by Waterhouse et al.
- Polylinker designed to facilitate cloning of inserts
- Benomyl-resistant β-tubulin as selectable marker

In relation to hairpin RNA-mediated silencing of EGFP transgene in *C. parasitica*, Nuss found a correlation between reduction in fluorescence, accumulation of gfp mRNA and gfp-specific siRNA.

Nuss posed the question, "Does hypovirus p29 act as a suppressor of RNA silencing in fungal host?" He conducted gene silencing in p29-expressing strains transformed with: (1) pGS - empty control; (2) pGS-2GFP; and, (3) pGS-2GFP.

Analysis of transformants included:

- 60 transformants of each strain for pGS-2GFP transformations
- 40 transformants of each strain for pGS control transformations
- Strains grown for 4 days on PDA
- GFP fluorescence analyzed with a fluorescence plate reader, excitation wavelength 488 nm, emission wavelength 507 nm, cutoff 515 nm.
- Background fluorescence in EP155 or P29T subtracted from transformant fluorescence values. Expressed as percentage of fluorescence in untransformed recipient strain.

Does hypovirus p29 act as a suppressor of RNA silencing in a heterologous plant system? Nuss attempted to answer this question in collaboration with Yiguo Hong, Warwick, UK.

- Hairpin RNA-triggered silencing was suppressed in *C. parasitica* expressing p29.
- Transformation of transgenic GFP-silenced strain with p29 resulted in an increased recovery of GFP-expressing transformants.
- Hypovirus p29 also suppressed both virus-induced and agroinfiltration-induced RNA silencing and systemic spread of silencing in GFP-expressing transgenic *Nicotiana benthamiana* line 16c.
- Circumstantial evidence that RNA silencing in fungi may serve as an antiviral defense mechanism.

Does disruption of the RNA silencing pathway in *C. parasitica* alter hypovirus-mediated symptoms and viral RNA accumulation? To accomplish this, Nuss infected a *C. parasitica* strain with a gene encoding the Dicer protein CPDc12. The result was severe symptoms and increased accumulation of viral RNA. This is the first experimental evidence for a role for RNA silencing as an antiviral defense mechanism in fungi.

Summary:

- Hairpin RNA-triggered gene silencing was suppressed in a *C. parasitica* strain expressing hypovirus CHV1-EP713 encoded protease p29.
- Transformation of transgenic GFP-silenced *C. parasitica* strain with p29 resulted in an increased recovery of GFP-expressing transformants.
- Hypovirus p29 also suppressed both virus-induced and agroinfiltration-induced RNA silencing and systemic spread of silencing in GFP-expressing transgenic *Nicotiana benthamiana* line 16c.
- Infection of *C. parasitica* strain in which the gene encoding the Dicer protein CPDcl2 was disrupted resulted in severe symptoms and increased accumulation of viral RNA.
- First experimental evidence for a role for RNA silencing as an antiviral defense mechanism in fungi.

Debbie Wilk—UC Davis

Wilk expounded on mycovirus CHV1 elements cofractionated with fungal host, *C. parasitica*, and trans-Golgi network membranes. She provided a bit of history:

- Dodds (1980)—found type 1 viral-like dsRNA with club-shaped particles in hypovirulent strains of *E. parasitica.*
- Newhouse (1983)—detailed that the general ultrastructure features of virulent and hypovirulent isolates were similar. Abundant membranous material was seen in the tip sections of the viral isolate (EP-4).
- Hansen (1985)—detailed that naked dsRNA is packed in fungal vesicles. Neutral sugars found in the vesicles are the same as those found in the cell wall.
- Newhouse (1989)— viral-like particles were found in other viral strains behind the hyphal tip in aggregates surrounded by rough endoplasmic reticulum, and a smooth cisterna or Golgi was found associated with these aggregates of VLPs (50-90nm correspond to apical vesicles). It was suggested that the virus is using the cytoplasmic machinery to package dsRNA and produce VLPs. This was consistent

with Hansen's findings that the VLPs contain carbohydrates of the kind found in the fungal cell wall in addition to dsRNA. In addition, the VLPs were located in the tip region, a location usually free of mycovirus particles in other fungi.

For membrane isolation and fractionation, fungal strains Ep67 and Ep 802 were grown in liquid cultures for 3 days and the mycelial pads were homogenized. The homogenates were centrifuged in three steps: (1) 5,000 x g 30 min; (2) 20,000 x g 30 min; and (3) 90,000 x g 90 min. She used heavy water-ficoll gradients for microsomal fractionation to look for vesicle accumulation. Using factions with and without magnesium, Wilk tried to determine where the ER migrates. Her summary of this work is as follows:

- Four CHV1 markers were found to accumulate in the microsomal fraction: dsRNA, Polymerase, Helicase and p29 protease.
- After the ficoll/heavy water fractionation, markers were found in fractions 6, 7 and 8.
- The dsRNA was CHV1-specific and same fractions in EP67 did not show presence of viral marker.
- Viral markers co-fractionate with peak in Kex2 activity and with AP-1, two TGN (trans golgi network) markers.
- ER marker KDEL and intermediate compartment marker migrated to upper fractions in the gradient. This showed a distinct localization between the ER and intermediate compartment and the viral vesicles and the TGN.

Conclusions:

- CHV1 replication complex co-localizes with membranes derived from the TGN of *C. parasitica.*
- CHV-p29 protease is membrane bound and localizes to the cytoplasmic face of the these vesicles.
- Membrane association determinants were shown to be within the C-terminal domain
- p29 C-terminal region is enough to direct p29 to the TGN vesicles in the absence of other viral elements.

Michael Milgroom—Cornell University

Milgoom is currently involved in two projects concerning the population biology and genetics of *C. parasitica*: (1) vegetative compatibility and linkage mapping with Tom Kubisiak and Joanne Davis and (2) MAT heterokaryosis and parasexualtiy with Cristina McGuire, Mark Double, William MacDonald and Jason Rauscher.

Linkage mapping. The objective of this work is to find markers that are linked to vegetative incompatibility (vic) genes. He used a mapping population from a cross between an Italian X Japanese isolate (to maximize diversity). Nearly 200 ascospore were obtained from this cross and vc typing was conducted on all ascospore isolates. Five vic genes segregated from this cross and 32 vc types were represented. Markers from primers included140 RAPDs (the problem with RAPDs is that they are very repetitive and therefore many chromosomes can be lit up). He also looked at 8 codominant SCAR markers and 4 Het-like ESTs (from Nuss and Dawe's library). What they found was a bit disconcerting—17 linkage groups but only 7 pairs of chromosomes (LOD=5). Markers linked to vic loci:

- vic2:1.1 cM and 5.6 cM (these two markers flank vic2)
- vic1:4.5 cM
- None closely linked to vic4, vic6 or vic7
- Het-like markers did not cosegregate with any vic genes

Another finding was that markers linked to MAT (37 markers linked—26 mapped at the same locus).

Summary:

- There are more linkage groups than chromosomes in *C. parasitica.* This may simply represent high levels of recombination.
- Markers are linked to 2 vic loci (Kubisiak estimates that they are within 80kb of these markers).
- Suppressed recombination near MAT locus is not a sex chromosome, but more likely an inversion

MAT heterokaryosis and parasexuality. Parasexuality characteristics include:

- Somatic recombination without meiosis
- First described in 1953
- Common in the laboratory
- Mechanism for asexual fungi to recombine

The objective is to understand the mating system of *C. parasitica*. Milgroom stated that Anagnostakis' theory (20 years ago) was that selfing in the fungus may occur because of heterokaryons (both mating types occur in the same thallus). Milgroom has found that MAT heterokaryons are common in nature; this allows selfing to occur. Parasexuality is a mechanism for asexual fungi to recombine; it is considered irrelevant in nature because of vegetative incompatibility. Milgroom showed an agarose gel of a sample of *C. parasitica* field isolates that had a single band indicating either MAT-1 or MAT-2. A few isolates had two bands indicating that they had both MAT-1 and MAT-2 genes. Milgroom believe that these isolates are heterokaryons. The MAT assay was conducted with hyphal tips. When MAT-1/2 isolates are single spored, the conidia are either MAT-1 or MAT-2. This is pretty conclusive evidence that these MAT-1/2 isolates are heterokaryons. Ascospores of these isolates have only one mating type. Heterokaryons do not persist through either conidia or ascospores. This indicates that heterokaryons must form *de novo* in nature.

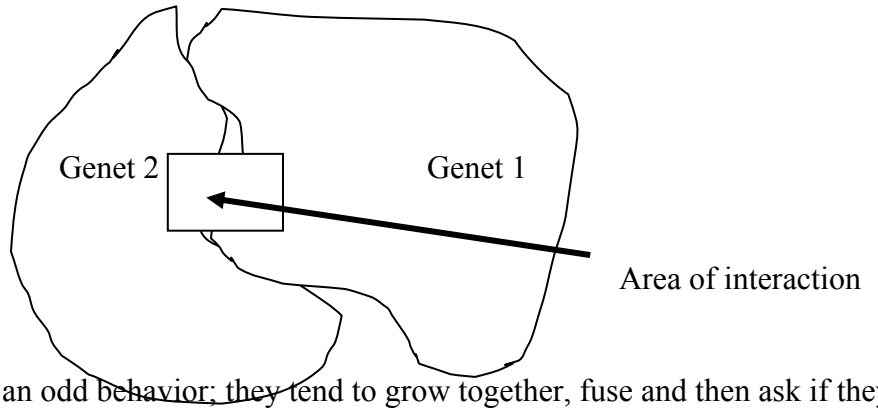
The current model of vegetative incompatibility is that it prevents heterokaryon formation (this is true for most loci). There is a correlation between the amount of interactive time of two incompatible hyphae and the frequency of virus transmission.

In order to show parasexuality, you have to show the presence of heterokaryons. More importantly, you have to reject the hypothesis that mutation or sexual recombination is causing heterokaryons. The West Salem chestnut population was a good locale to use to rule out sexual recombination as it was a clonal population of *C. parasitica*. He showed data from West Salem, collected between 1990 and 1999. Genotyping was conducted, looking at vc typing (N=1020), RFLP fingerprinting (N=225) and mating type (N=385). In 1990, the fungal population was clonal, all isolates were MAT-2 and there were no heterokaryons. By 1999, the population was mostly clonal (there was some limited recombination) and five MAT heterokaryons. How does the MAT heterokaryon form and where did the MAT-1 idiomorph come from

when most of the isolates are MAT-2? The short answer is that it had to have come from another vc type. They did find heterokaryons for mating type for the SCAR markers (in vc types 2 and 3). There is no evidence of sexual recombination or mutation in MAT; this suggests parasexuality. Milgroom believes that heterokaryons form between vc types, although this is contrary to most models.

Myron Smith—Carleton University, Ottawa, Canada)

One area of Smith's research is the interaction between hypovirulent factors and non-self recognition-associated programmed cell death in *C. parasitica*. Much of the work he reported focused on the incompatibility system in *C. parasitica* and some of the work was conducted by graduate student, Fuad Tanha, in conjunction with Don Nuss and Michael Milgroom. Smith asked why incompatibility systems are important. He used *Armillaria* as an example, citing a large area in Michigan colonized by this fungus. He showed a slide of two genets (genotypes) of *Armillaria* and indicated that it is a highly evolved system.



Fungi have an odd behavior; they tend to grow together, fuse and then ask if they are compatible. He did some background work with *Neurospora* to understand compatibility. He described two assays for compatibility: (1) a barrage assay; and (2) heterokaryon incompatibility.

There are six known vic loci in *C. parasitica* as shown in the following table.

Vic1	LG 18
Vic2	LG 8
Vic3	Unmapped
Vic4	LG 4
Vic6	LG 11
Vic7	LG 3

When a barrage formed, there is cell death and lysis of the cell. Smith discussed an experiment with EU-5 (hygromycin resistant isolate from Ep155-2) from Alice Churchill and an isogenic Ep155 that were transformed with benomyl resistance. These were paired with strains that differed at one vic locus at a time. Strains are grown on PDA with a cellophane overlay for 1-2 days until the hyphae touch. The cellophane is lifted off the PDA plate and placed onto a plate that contains both benomyl and hygromycin. If there are no differences at any of the vic types, there is nice heterokaryotic growth. If

there are differences at vic1, vic2, vic3, vic6 or vic7, there is a little bit of growth and then it stops. It appears that vic4 does not prevent heterokaryosis,, and this is surprising. This indicates that there is more than one incompatibility system at work in this fungus.

Smith showed slides of programmed cell death in an incompatible reaction between two *C. parasitica* strains; this included vacuolization. He discussed symmetric cell death in which hyphae from both strains die and asymmetric cell death in which the hyphae from only one strain dies. Smith cannot explain this phenomenon. Work from Sylvia Biella (published in 2002) was shown; she scored cell death/contact with percent virus transmission and there was a significant negative correlation—low cell death resulted in high virus transmission. Some strains have a high frequency of death per contact while other strains have a low frequency of death per contact; microscopy was used to look at contract between two hyphae. When contact was made between two strains, Biella scored whether there was cell death at one, both or neither strain. When one or both strains contain virus, there is often a change in cell death. This may simply be an expression of increased stress on the fungus. It is hard to image that viruses affect cell death, unless the virus is somehow interacting and playing a role in cell contact.

Fuad Thana worked with Ep 155 and P74-3, two strains that differ at vic3. Smith highlighted the following table.

Strain combination	**Cell Death Frequency**	
Allele 1 Allele 2	Allele 1	Allele 2
Ep155 + P74-3	0.59	0.71
Ep155 Ep713+P74-3	0.35	0.20
2-tailed T-test	0.07	0.002
Ep155 + P74-3	0.55	0.73
Ep155 Euro7+P74-3	0.52	0.41
2-tailed T-test	0.79	0.007

For Ep713-infected P74-3, there was a slight decrease in allele 1, but a large decrease in allele 2. For Euro7, there was a slight reduction in allele 2 and no reduction in allele 1.This indicates that there is a difference between Ep713 and Euro7 viruses in relation to cell death frequency.

Smith used chimeric constructs from Don Nuss (Ep713 and Euro 7) and data indicate that with Ep713, there was significant reduction in contacts at both alleles while Euro7 produced only reduced contact with allele 2. Using construct R1 (OrfA from Ep713 and OrfB form Euro7), there was a significant reduction in contacts at both alleles. Thus, R1 was similar to action by Ep713. Smith concluded that the factor responsible for cell death lies in OrfA. Using R2, the reciprocal cross (OrfA from Euro 7 and OrfB from Ep713), there was no loss in cell death frequency, so OrfB must play some role in this process. Smith has some preliminary data that p29 (from Don Nuss) reduced the frequency of cell death. Smith speculated that viruses may interfere in a general sense with cell death or they may specifically react with the incompatibility protective system.

Alice Churchill—Cornell University

Churchill is studying pigments of the chestnut blight fungus. The chestnut blight fungus produces a family of orange and yellow pigments that exhibit diverse biological activities. Churchill posed several questions, “Are these pigments debilitating the chemical defenses of the host tree? Do they play a role in maintaining fungal virulence?

Can a pathogen be just as successful with or without the pigments? Or, do the pigments play a role in enhancing fungal development? These pigments have antimicrobial, antiviral and antioxidant activities *in vitro*, as well as perturbing normal cell death and signaling pathways. Some of the known anthraquinones are the dimeric compounds, skyrin and oxyskyrin (orange pigment) and rugulosin (yellow pigment). Some of the monomer precursors to the three mentioned pigments are: emodin, chrysophanol and aloe-emodin. The roles that the pigments or their precursors play in the biology of *C. parasitica* as it interacts with its tree host are unknown. By using genetic approaches that prevent or interfere with the expression of genes predicted to contribute to pigment production, the role of pigments in the biology of this devastating tree pathogen can be determined. Specifically, her approach is to compare the development and disease-causing ability of a wild type, pigment-producing strain of the fungus with genetically identical strains that differ only by their inability to make pigments due to a site-specific mutation in the pigment biosynthetic machinery. In this way, the pigments produced by *C. parasitica* and their contribution to pathogenicity or development in chestnut trees might be determined. The major pigments produced by *C. parasitica* are classified as aromatic polyketides. The first step in the biosynthesis of polyketides in fungi requires the activity of a large multifunctional, multidomain enzyme, a polyketide synthase (PKS). Previously, she 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*; 60 unique cosmids that contain identical or similar PKS sequences were identified. One cosmid was chosen for further analyses since it encoded a PKS gene whose expression was clearly correlated with orange pigment production by *C. parasitica* cultures. These and other results suggested that this gene could be involved in polyketide pigment biosynthesis. 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 and transport. In addition to a PKS gene, the gene cluster contains genes similar to those that encode a protein folding enzyme, two transcription factors, a transcription enhancer, two monooxygenases, an oxidoreductase, a hydrolase, and part of a toxin transporter. These are the types of genes that are predicted to be present in a pigment biosynthesis pathway. Targeted disruption of the Cp-*PKS1* gene has begun to determine if altering the function of this gene by mutation affects pigment production and/or the ability of *C. parasitica* to cause disease in American chestnut. She reported that *PKS*1 is differentially expressed in Ep155 when grown on a medium that supports orange pigment production. Using PKS fragments, she sequenced cosmids and ended up with a putative gene cluster, containing cosmid ctB5 (~46 kb). In her initial attempt to disrupt this gene, she utilized constructs in which the *PKS*1 gene was interrupted by the Tn7 transposon. The whole cosmid, containing Tn7-tagged *PKS*1 gene was introduced into Ep155. Churchill showed slides of the phenotype of the *PKS*1 knockout transformation. After 31 days in high light, the knockout isolate had a white phenotype. In the second experiment, cotransformation of linear Tn7-tagged *PKS*1 gene and an empty cosmid vector conferring hygromycin resistance was introduced into Ep155 protoplasts. Again, the white phenotype was evident. She was really encouraged by this finding. Molecular analyses (PCR and Southern blotting) did NOT confirm that *PKS*1 was disrupted by Tn7. The PKS knockout experiment summary is:

- Occasional mitotic instability of hygromycin resistance gene and pigment-deficient phenotype (leakiness), as well as molecular data, suggest that Tn7 may be unstable *in C. parasitica* transformants, making molecular detection of disruption event difficult.
- New disruption vector with Hygromycin R cassette inserted into PKS1 was constructed.
- PKS1 binary vector knockout construct was introduced into Ep155 by *Agrobacterium tumefaciens*-mediated transformation. Characterization of putative PKS1 knockout strains are in progress. She has one confirmed *PKS*1 knockout that is reduced in pigmentation but it is not white. She also has five additional putative knockouts (by PCR).

Future activities include:

- Confirm knockout of *PKS*1
- Characterize phenotype in vitro and *in planta*
- Knockout of aflR-like regulatory gene in Ep155
- Identification of pathway products if not anthraquinones
- Characterization of other candidate PKS genes for function
- Determine if altered pigmentation by single gene disruptions affect fungal virulence, development, or fitness.

Business Meeting

A list of NE-1015 meeting sites (from 1982-2005) was shown. Bill MacDonald, chair-elect, extended an invitation for NE-1015 to meet in West Virginia in 2006. Consensus of the group was to hold the meeting in mid-October, following nut harvest.

Chairman Churchill asked for nominations for chair elect in 2006. Angus Dawe (New Mexico State University) volunteered to chair the meeting in 2007 and offered to host the meeting in Santa Fe, NM. MacDonald expressed concern that attendance might be limited due to the distance from the East coast. Hebard indicated that Hungry Mother State Park in VA would be a possible site for the 2007 meeting; that would facilitate a tour of the TACF farm. Vayda also expressed concern about a New Mexico meeting site.

Discussion followed as to those in attendance who are "official" members—those receiving money from their experiment station. Only a few individuals in attendance receive support for work associated with NE-1015. Churchill remarked that those in attendance were working with chestnut because of their interest in the work, not funding associated with experiment stations.

Anagnostakis nominated Angus Dawe as vice chair for 2006. The motion was seconded by Bradley Hillman and the vote was unanimous. It was the consensus of the group that Dawe chair the 2007 meeting, but that Hebard will be local arrangements chair and the meeting will be held at Hungry Mother State Park.

Vayda indicated that the NE-1015 report is due 30 days following the meeting so that SAES 422 can be completed. Mark Double agreed to provide Alice Churchill with a complete set of meeting minutes by the end of October.

Dawe asked about a NE-1015 website. He indicated that the current NE-1015 website is difficult to access. The USDA/CSREES/CRIS network is confusing. Dawe indicated that those individuals in attendance at the NE-1015 meeting represent the core of individuals that are working on chestnut and they should be the central resource for the

country. Warmund indicated that she is a member of a north-central regional project and each state downloads their state report to the managed website. Official members have passwords to access information. As members, Warmund indicated that each station pays $50 to help maintain the website. She indicated that a well-run website is good advertisement for the project. She explained that the 'paid' website is separate from the USDA system and they have had a lot of 'hits' from the paid website. Hillman expressed concern about getting websites linked. Dawe agreed to act as the webserver and agreed to host the site initially. Hillman agreed to assist. Double asked if there is anything that can be done to assist Dawe with this initiative. Dawe suggested that investigators that have their own website should send them to Dawe so that they can be linked. Dawe indicated that an on-line registration form for future meetings might be possible on the proposed website.

MacDonald indicated that he has extra copies of the 1978 and 1992 chestnut proceedings that he is willing to distribute to individuals that may want a copy.

Hebard suggested that it might be time to think about organizing another international chestnut meeting.

The meeting was adjourned at 12:00 pm on October 15, 2005. Following the scientific exchange, two field trips were conducted. Members had their choice of: (1) trip to SUNY-ESF to see Powell and Maynard's transgenic chestnut trees with a stop at Chittenango Falls; or (2) a field trip to the Cornell University orchard with a stop at the King Ferry winery. A Thanksgiving dinner, held at the White Eagle Conference Center, following the field trips, concluded the meeting.

Respectfully submitted,

Mark Double
October 2005

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)

NE-1015 Publication List 2004-2005

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