NE-140 Technical Committee Meeting
Biological Improvement of Chestnut (*Castanea* spp.) and Management of Pests

IMAX Theater, Chattanooga, Tennessee
October 27-28, 2000

Attendance:

Alabama:	Fenny Dane (Auburn University) James Maddox (Tennessee Valley Authority)
California:	Pam Kazmierczak, Massimo Turina, (University of California-Davis)
Connecticut:	Sandra Anagnostakis, NE-140 Secretary, John Anderson, NE-140 Administrative Advisor (Connecticut Ag. Experiment Station) Phillip Gordon (NY Botanical Garden)
Georgia:	Scott Merkle (University of Georgia)
Kentucky:	Sunshine Brosi, Chuck Rhoades (University of Kentucky)
Maryland:	Becky Bierman, Angus Dawe, Donald Nuss (University of Maryland Biotechnology Institute)
Massachusetts:	Timothy McKechnie, Robert Bernatzky (University of Massachusetts)
Michigan:	Dennis Fulbright, Andy Jarosz (Michigan State University)
Mississippi:	Tom Kubisiak (USDA Forest Service)
Missouri:	Michael Gold (University of Missouri Agroforestry Center)
New Jersey:	Bradley Hillman (Rutgers University)
North Carolina:	Catherine Clark (North Carolina State University) Robert Doudrick (USDA Forest Service)
Tennessee:	Catherine Bock (Tennessee Aquarium) Herb Burhenn (Dean), J. Hill Craddock, NE-140 Chair, Tom Fuller, Pearl Huang, Charles Nelson (Deptartment Head), Alysia Vrallis (University of Tennessee-Chattanooga) Scott Schlarbaum (University of Tennessee-Knoxville)
Virginia:	Fred Hebard, Peter Wood, Paul Sisco (The American Chestnut Foundation)
West Virginia:	William MacDonald, Mark Double (West Virginia University)

A reception was hosted at the IMAX Theater on October 26, 2000 from 7:00-9:00 pm. The meeting was called to order at 8:30 am on October 27, 2000 by Chairman Craddock. He welcomed everyone and then introduced Herb Burhenn, Dean of the College of Arts and Sciences, University of Tennessee at Chattanooga. Burhenn lauded the efforts of William Raoul, a Tennessee native, who sparked interest in chestnut in the Chattanooga area. Christine Bock welcomed NE-140 participants on behalf of the Tennessee Aquarium.

John Anderson, Director, Connecticut Agricultural Experiment Station

Anderson read a letter from Colin McKeen of the Canadian Chestnut Council. McKeen sent along his best wishes for a productive meeting and offered regrets for his absence. Anderson noted that the NE-140 report is due within 60 days; this comment was directed to Chairman Craddock and Secretary Anagnostakis. The new format for reporting accomplishments requires language that is understandable to the lay person. The minutes should be kept short but include: meeting minutes, accomplishments and impacts and publications. Anderson noted that NE-140 began in 1981 and he read the milestones that were set forth in that report. He indicated that milestones need to be put into the 2000 report.

Effective October 1, 2000, the USDA moved from regional research projects to multi-state research projects. Anderson read the mission statement of the multi-state project. A few changes accompany the multi-state project concept. First, Anderson went over committee governance. The guidelines suggest the election of a chair, a chair-elect, and a secretary; all officers elected for a 2-year term. Second, all decisions by a multi-state project will be free and open. Finally, there will be one vote per entity.

Anderson then encouraged the group to develop their own home page on the world-wide web. He noted this is not a requirement, but it would enhance the visibility of the group.

With a number of new participants in 2000, there was some discussion as to how individuals become formal members of NE-140. Anderson stated that joining the project requires the support of the agency head who will indicate how much time a PI will contribute to the project. A one-page form is required for membership, and research must fit into one of the two project objectives.

<u>Objective 1.</u> To improve chestnut trees for timber and nut production, and determine the cultural requirements of chestnut seedlings in nursery and natural settings.

James Maddox, Tennessee Valley Authority, Environmental Research Center

The TVA continues to provide land sites for outplanting American chestnut. He has been working toward real-world situations. There are many problems involved with restoring chestnut, mostly at local levels. His interest in establishing chestnut is in seedling vigor and seedling survival.

There have been two severe droughts in Alabama. Water potential in 2000 is nearly indiscernible in Alabama soils because it is so dry. There are 1/2" cracks in the soil.

He is working with mycorrhizal infections on chestnut and he noted that there are no endomycorrhizal data available on chestnut. Chestnuts are raised in artificial nursery beds and transplanted to land that previously grew cotton.

His planting beds were:

-6 to 8" spacing

-3 sets of beds with 24 compartments, measuring: 25' (L) x 2' (W) x 1.5' (D)

In the field experiments, succession crops, cover crops and biotype root inoculants created 80 treatments (800 seedlings). Three transplant field experiments were conducted on land that had been continuously farmed for 200 years (mostly cotton). The

trees were planted in 15' rows, with 6' tree spacing. The intrarows were hand-planted with rip furrow-drilled pines.

Methods from Experiment 1:

Treatments (32)

- 1996 VAM inoculants (separately applied) [Vesicular Abuscular Mycorrhizae]
    - *Glomus etunicatum*
    - *Glomus etunicatum* 329
    - *Glomus diaphanum*

- 1998 Fall cover crops
    - Crimson Clover
    - Vernal Alfalfa

- 1999 Biotype Root Inoculants
    - Mycotree (Pt)
    - Azokote (Azospirillum)
    - Mycotree + Azokote
    - None

Seven treatments had better than 80% survival and twelve treatments had better than 70% survival. *Glomus etunicatum* was in 9 of the 12 treatments. He continues to try and engineer good (fibrous) root systems.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

Chestnut roots (in conjunction with Scott Schlarbaum). In order to determine if woody roots of a chestnut seedling are good predictors of survival in the forest, chestnuts were collected in 1997 and planted in 1998. Seedlings included Japanese, American and two hybrids. In 2000, 550 trees were dug; heights, woody roots and crown collar were measured. She found no correlation between the number of woody roots and height. The trees have been outplanted in Kentucky (by Sunshine Brosi for her M.S. Thesis work), at a sub-station in Windsor, CT and in CT forests. Height growth will be recorded each year for the next five years to try and correlate the number of woody roots and tree height. To keep trees alive, cankers are being treated with CHV1.

Phillip Gordon, New York Botanical Garden

Riparian Project. He has been looking at chestnuts for a long time, but was unsure what he was looking for; he is a visual scientist. He has not appreciated what a chestnut needs to be a major canopy tree. Chestnut blight has impacted the entire forest ecosystem, so the question must be asked, "What good qualities have arisen from chestnut blight?"

The root system of chestnut is unimpaired by chestnut blight. Chestnut roots are huge and since the tree grows so rapidly, chestnut transpires more than any other hardwood. It needs oxygenated water for the root system. Roots keep on growing despite a dead top and they function to hold down the soil. Chestnut grew best on

riparian areas (along stream banks). Since chestnut grows so well, it cleans the water from manure and fertilizers in the nearby fields.

He noted the function of leaf types:
-Sun leaves collect sunlight.
-Shade leaves function to transpire.
-Floral leaves feed the growing nuts.

The USDA Natural Resource Conservation Service and the Connecticut chapter of The American Chestnut Foundation have combined efforts to look at chestnut as a forest riparian buffer species. The project is being conducted on the Connecticut River.

Scott Merkle, University of Georgia

Cryopreservation. His overall goal is still to develop embryogenic cultures for mass propagation and gene transfer applications. Merkle's lab switched its emphasis from gene expression a few years ago, as Daniel Carraway (a Ph.D. student) graduated. In addition, researchers at SUNY have experienced good results in this area.

Cryopreservation Rationale:

- Embryogenic American chestnut cultures lose plantlet ability quickly, compared to other hardwood species.
- Cryostorage can aid with preservation of American chestnut genetic diversity.
- Protocols are already tested with other embryogenic cultures.

Cryogenic Protocol consists of three phases:

1. Preconditioning
   -pretreatment with 0.4M sorbitol for 24 hr
   -DMSO at either 5% or 10% (to prevent dehydration and freezing injury)
2. Freezing
   -Nalgene controlled descant-freezing containers (-80° freezer)
   -liquid Nitrogen (-196°)
3. Recovery
   -2 minutes in 40° C water bath
   -cell clumps collected on nylon mesh

Two embryogenic lines were tested (A and B). Fred Hebard at TACF in Meadowview, VA provided immature nuts used to start these cultures. The cultures were maintained on semi-solid woody plant medium, containing 2,4-D. Evaluation of recovery and regrowth of cryostored material was based on visual assessment and fresh weight gain. The cell clusters appeared to be brown-to-black immediately after thawing. Within 2-3 weeks, heart, torpedo and cotyledon stage embryos formed and were later transferred to a medium lacking 2,4-D to mature further. While both DMSO treatments yielded 100% recovery, the 5% DMSO cultures recovered more rapidly. Thus, embryogenic American chestnut cultures appear to be excellent material for long-term storage via cryopreservation.

Defined medium to improve somatic embryo quality. Merkle reported on the work of Rodney Robichaud who tested amino acid supplements in the germination frequency of American chestnut somatic embryos. Previous work used autoclaved amino acids; this test examined amino acids that were filter-sterilized. All the media in previous experiments used casein hydrolysate, an undefined nitrogen source.

Six organic nitrogen treatments were examined. Three embryogenic culture lines were included in the experiment. Organic nitrogen treatments significantly affected production of embryos and cotyledon-stage embryos. The highest number of embryos was derived from clumps cultured on 1 g/L-glutamine. Compared to the control, glutamine-treated embryos tended to produce well-defined cotyledons and a pronounced radical.

Embryogenic culture initiation. In order to provide fresh embryogenic cultures for somatic embryo development and maturation, culture initiations were begun in 1999. Burs were collected in mid-August from 3 parent trees growing at TACF's farm in Meadowview, VA. Two levels of 2,4-D were tested (2 mg/l and 4mg/L). A total of 16 embryogenic cultures were initiated for an overall rate of 2.4%. The 4 mg/L level of 2,4-D yielded a higher frequency.

Scott Schlarbaum, University of Tennessee at Knoxville

His seedling planting project began in 1998. The cooperators in this project are: TACF, University of Kentucky, Pennsylvania Bureau of Forestry, Berea College and the Daniel Boone National Forest.

Seedlings were produced at the Flint River Nursery in southern Georgia. After one year, the tallest tree was 6'6" in height. There were a few dead trees, as a result of chestnut blight. The seedlings were lifted in early February and graded into three grades; premium, medium and cull. They also evaluated 1st order lateral roots, height, root collar diameter and existence of chestnut blight.

The seedlings were designed for two planting sites.

Sunshine Brosi, University of Kentucky

She is looking at chestnut in the knobs (rolling mountains) and eastern coalfields of Kentucky. Chestnuts were outplanted at two sites at each of two areas (Morehead in eastern KY and Berea College in the knob area). A total of 810 seedlings were auger-planted on March 3-4, 2000. Initial growth did not vary among the families that were tested.

There was significant mortality attributed to *Phytophthora cinnamomi*. The *P. cinnamomi* did vary across sites, but did not vary across initial seedling characteristics. Mortality at the Morehead site was 65% and 72%, while mortality at the Berea site was 74% and 22%. Once mortality was recognized, a soil drench of Metalaxyl was used; it was successful in controlling the disease. Three-hundred seedlings are still alive; the tallest is 7'. Growth of remaining trees did vary across sites. There was more increase in girth at Berea sites compared to Morehead.

Chuck Rhoades, University of Kentucky

Chestnut establishment. He is a new faculty member at UK. He is examining chestnut establishment; sites to be planted next spring include:

1. Tygarts State Forest (northeast of Morehead)
2. Morehead District
3. Stanton District (southwest of Morehead)
4. Berea College Forest (southeast of Berea)

5. UK at Robinson (north of Hazard, KY)

Ectomycorrhizal study. He is looking at ectomycorrhizae and *Phytophthora*. He will be doing challenges with several soil types in nursery and forest plantings.

Michael Gold, University of Missouri (Center for Agroforestry)

His interest is in developing alternatives for farmers in Missouri. Work is conducted with black walnut in the Center for Agroforestry and they would like to develop chestnut to compliment walnut and pecan for local nut farmers.

He has begun an experiment with 28 cultivars. The prime factor in a successful nut crop seems to be size, rather than taste. He is also looking at marketing along with nutrition of chestnuts.

Dennis Fulbright, Michigan State University

In 1992, he was asked by the Michigan extension service if MSU could respond to chestnut growers; their trees were small and had little yield. At the time, the growers were using mostly Chinese chestnut seedlings. As a response, a variety trial was put out in 1992. The following are data from nuts collected from grafted chestnuts (data from 2000):

Variety	Trial	Wt (lb)
'Colossal' (92)	1	25.7
'Colossal' (92)	2	49
'Colossal' (97)	1	1
'Colossal' (97)	2	1.8
'Dunstan'	1	24
'Dunstan'	2	22
'Dunstan' Revival	1	19
'Willamette'	1	7.5
'Willamette'	2	12
'Eaton'	1	9

Fulbright advised the farmers to plant grafted trees, as orchardists have been using seedlings with very limited success. Orchardists were growing Chinese chestnut seedlings and the deer continued to browse the seedling since they were small initially. Nut growers want trees that will grow and bear nuts in a short time frame, but they are somewhat unwilling to bear the initial cost. Seedling can be purchased at $1/each, while grafted seedlings may cost $10-$12/each. His advise remains—use grafted seedlings.

Hill Craddock, University of Tennessee at Chattanooga

Chattanooga chestnut tree program. Tom Fuller is working on grafting experiments to test isozymes using different combinations of scions and rootstocks. He has had good grafting success with B_2F_2s from Meadowview. It might be informative to send these trees to China to see if they are overcome by blight.

Pollen project. Christine Bock is working on a pollen project; she is trapping pollen around a breeding orchard. This study is in progress.

Gall wasp. Anagnostakis gave Craddock some chestnut crosses (*C. pumila* x *C. crenata*) to see if they will segregate for gall wasp susceptibility. Craddock found some

trees last year in northern Georgia that were heavily infested. He showed slides of a parasitoid wasp that is used as a control agent of gall wasp in Japan.

Hypovirulence. Craddock showed slides of trees at Hooper Ridge, NC with very superficial cankers and exposed wood. In his chestnut orchards, Craddock uses hypovirulent isolates as a biocontrol. He isolated *C. parasitica* isolates from the orchard and sent them to Anagnostakis. She converted the virulent isolates with CHV1 agents and sent them back to Craddock. Cankers are excised from the tree, painted with a slurry of hypovirulent isolate and taped to prevent desiccation. Trees are treated two times per year. This program has been successful such that he has trees that are large enough to use in a breeding program.

Phytophthora. Craddock has some trees that died within a few weeks. He attributed this mortality to *Phytophthora* sp. The trees did not resprout from the base.

Pollen bags. Tennessee did not have a good fruit set in 2000. Bob Leffel of the PA chapter of TACF did a test with white versus brown bags for bagging trees. He saw no difference; he noticed the brown bags did not rip as easily as the white bags.

Fred Hebard, The American Chestnut Foundation

Large surviving chestnut. He showed slides of a large (15") surviving chestnut in Wayah, NC, located in the Nantahalah National Forest. There are many cankers on the tree; none have exposed bark.

Breeding program update. Hebard noted that different American parents have been used at each step to avoid inbreeding. Three sources of resistance from Chinese chestnut have been used. The final step in the breeding program is to intercross B_3s and release B_3F_3 nuts. The timeframe is:

B_3 x B	2001-06
B_3F_2 x B_3F_2	2006

There are 14,000 trees growing at the farms in Meadowview. At each backcross step, trees can vary. They have been selecting against Chinese traits (large stipules, green stems, hairy stems), and selecting for American traits (small stipule, red stems, lack of hairs). If there are only a few genes controlling resistance, then the backcross method should be straightforward. If there are more than a few genes, then trees may be produced that are resistant to chestnut blight, but retain many Chinese characteristics. The evidence for the exact number of genes controlling blight resistance is still inconclusive, but partial data suggest that three (incompletely dominant) genes are responsible.

In addition to screening trees for blight resistance, molecular mapping of crosses has been another means that has been used to investigate the number of genes controlling chestnut blight. The preliminary data indicate that different Chinese trees may contain different genes for resistance, while they may have a few resistance genes in common. Hebard stated that it is clear that the molecular mapping of blight resistance genes would be more informative if the precision of measurements of blight resistance is increased—if larger groups of trees are mapped and more genetic markers are added. In 1999, a orchard was planted for that purpose. Results are expected in three years from that planting.

Correlation of markers on various linkage group with blight resistance

Linkage Group	Mahogany F2	Mahogany BC1	Nanking BC1	Clapper BC2
B/E	0.00001	0.0001		0.01
F	0.001	0.001	0.01	
G	0.00001			
C			0.001	
I			0.01	
L		0.01		

In the BC_1 Nanking crosses in 1997, there was pretty good separation in the virulence assay (canker size) using Ep 155 and SG2-3. Hebard noted that environmental conditions also play an important role in variance of canker sizes (i.e. cool weather does not allow for canker expansion).

Cytoplasmic male sterility. Male sterility occurs when a Chinese chestnut male is crossed to an American chestnut female (but not when the Chinese chestnut is the female). In conjunction with Tom Kubisiak, one gene controlling male sterility was mapped. The Chinese chestnut cultivar, 'Nanking', was used. Timothy McKechnie and Michelle Phipps took additional data on a large Clapper BC_2 family. Two genes appear to control male sterility in the large Clapper family.

Male sterility, the failure of a plant to produce pollen, is not a trait that is desirable in the breeding program. However, this trait can be very useful. First, this is a trait that definitely comes from the Chinese parent, so it is a marker that can be selected against in the effort to eliminate as much of the Chinese genome as possible. Secondly, it can help control the pollen parent in crosses. An isolation block of male-sterile F_1 trees can be planted to surround pure American chestnut trees to allow for open pollination. The seed harvested from these F_1 trees would be guaranteed to be BC_1 seed.

Paul Sisco, The American Chestnut Foundation

As indicated by Hebard, Sisco noted that the mapping population of chestnut is inadequate. The American Chestnut Foundation is expanding their breeding program; he is working on establishing a plantation at Warren Wilson College in Asheville, NC. While Hebard concentrates on breeding, Sisco is working on genetics with outplantings. He noted that leaf emergence is the most heritable characteristic.

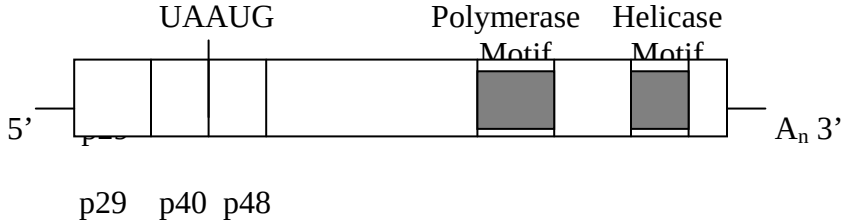
Bob Leffel of the Pennsylvania chapter of TACF has done 10 reciprocal crosses to look at male sterility. This could be very useful information for the breeding program.

Transposon (transposable element). A transposon is a DNA sequence that "hops" (transposes) from one site in a DNA molecule to another in a reaction catalyzed by a transposase enzyme. Sisco noted that there are different classes and families of these elements. When a transposon is excised, it forms a loop and a subsequent "footprint" is left. In corn, transposons are very common. They develop clonal sectors and affect kernal color, waxy leaves, etc. Last July, Sisco saw a seedling with variegated leaves. If this seedling lives, we may be able to get pollen. This may be evidence of a transposon in chestnut. A variegated chestnut may be of horticulture value.

<u>Objective 2.</u> To better understand the interactions and ecology of this host/ pathogen/parasite system at the molecular, organismal and environmental levels to develop effective biological controls for chestnut blight.

Don Nuss, University of Maryland Biotechnology Institute

Virus-encoded replication elements. He reported on Nobuhiro Suzuki's work. He prefaced his comments with a brief overview of the molecular understanding of CHV1-Ep 713.



There are two open reading frames, ORF A and ORF B; UAAUG is the junction. The UAA portion serves as the termination codon of ORF A and the AUG portion is the 5'-proximal translation initiation codon of ORF B. N-terminal portion (codons 1-24) of the 5' proximal end of ORF A are found to be required for virus replication. The remaining 598 codons of ORF A are completely dispensable.

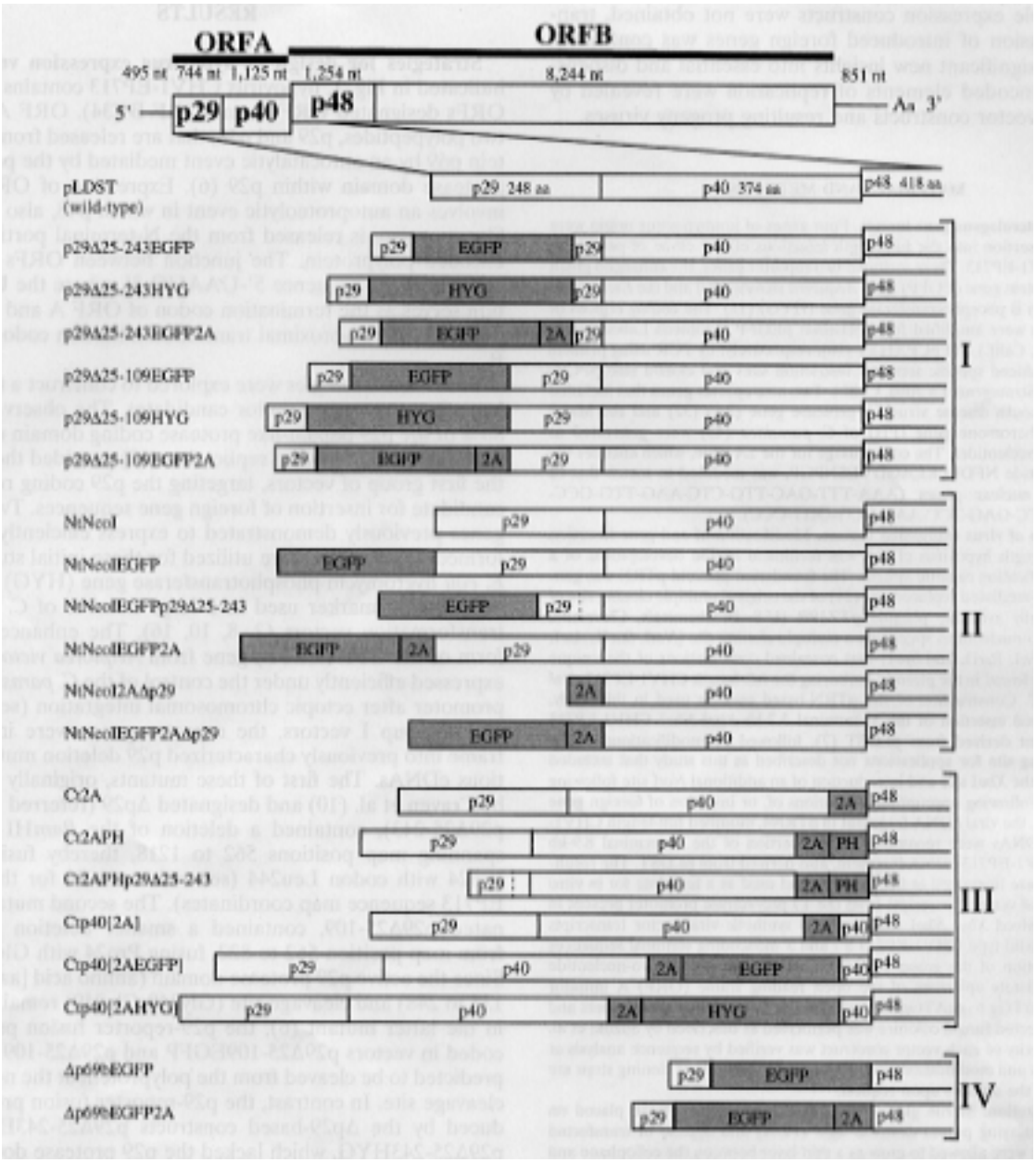
Suzuki examined strategies for hypovirus expression vectors. He constructed 20 vector candidates in four groups and modified infectious CHV1-Ep713 cDNA.

Group 1: Six constructs; inserted EGFP or HYG genes in various regions of p29.

Group 2: Four constructs of insertions at the precise N terminus of ORF A.

Group 3: Six constructs of gene insertions in the 3'-terminal portion of p40.

Group 4: Two constructs in which the EGFP gene was inserted in place of most of



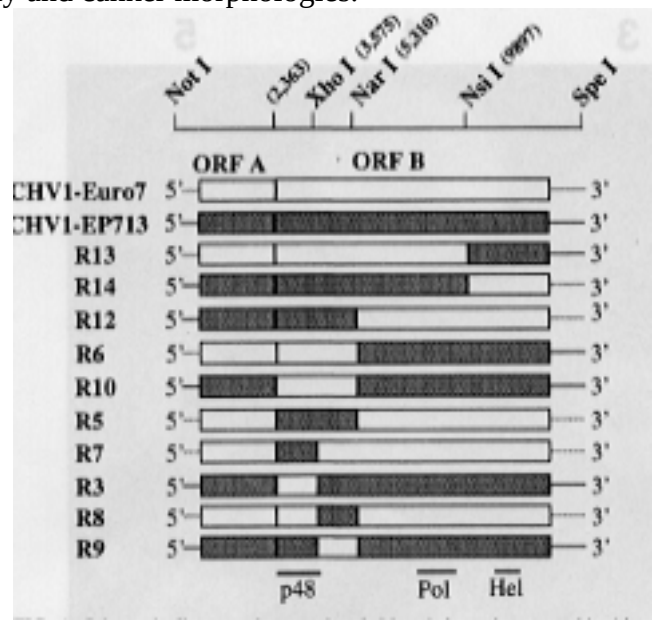
ORF A.

Conclusions:

- The 5'-terminal portion of the p29 coding domain seems to be required for RNA replication.
- The 5'-UAAUG-3' pentanucleotide is altogether dispensable for virus replication.
- p40 is not required for virus replication.
- p29 has been shown to contribute to virus-mediated reductions in host pigment production, asexual sporulation and laccase production.
- Portions of ORF A influence the efficiency of virus transmission to asexual progeny.

Use of chimeric hypoviruses. Nuss reported on the work of Bao Chen who used chimeric hypoviruses to fine-tune the interaction between *C. parasitica* and its host.

Chen used infectious cDNA clones of mild (CHV1-Euro 7) and severe (CHV1-Ep 713) hypovirus strains. Differences in virus-mediated alterations of fungal colony morphology, growth rate and canker morphology were mapped to a region of ORF B that extended from nucleotide 2,363 to 9,904. By swapping domains within this region, it was possible to generate chimeric hypovirus-infected isolates that exhibited a spectrum of defined colony and canker morphologies.



Since chimera R13 (composed primarily of CHV1-Euro 7) and R14 (CHV1-Ep713) both caused colony formation similar to the parental viruses, neither the 3'-terminal portion of the ORF B coding domain nor the 3'-non-coding region appears to contribute to differences in colony morphology. This suggests that the portion of ORF B responsible for the differences in virus-induced colony morphology resides between the N terminus (nt 2363 and nt 9897. The resulting data for the chimeras (canker expansion on excised chestnut stems [cm^2] and stromata per canker) are as follows:

	Canker Size	*Stromata*
Strain	**Day 30**	**Day 30**
Ep 155	23.8	130
Ep 155/CHV1-Euro 7	3.7	59.6
Ep 155/CHV1-Ep 713	1.1	6.6
Ep 155/R13	4.5	68.6
Ep 155/R14	1.2	1.8
Ep 155/R12	1.4	13.6
Ep 155/R6	1.5	33.2
Ep 155/R10	1.7	38.4
Ep 155/R5	1.2	14.8
Ep 155/R7	1.9	7.2
Ep 155/R3	1.2	28.2
Ep 155/R8	1.4	31.8
Ep 155/R9	1.6	47.6

Reciprocal chimeras, R6 and R12 resulted in colony morphologies that were very similar to each other and to that each other and to that exhibited by CHV1-Ep 713-

infected isolates. This indicates that severe CHV1-Ep 713 colony morphology phenotype is dominant. Little difference in colony morphology resulted from swapping of ORF A portions of the R6 and R12 chimeras to form R5 and R10. The conclusion is that ORF A domain does not make major contributions to the differences in phenotypic changes caused by the two viruses. Differences in conidiation map to the region extending from nt 2363 to 5310, while determinants responsible for differences in canker size reside on both sides of the *Nar*I site.

Data from R7 indicates that the CHV1-Ep 713 coding domain contains a dominant determinant for suppression of pustule formation. Results from R9 indicate that CHV1-Ep 713 p48-mediated suppression of pustule formation depends on some sort of interaction with proteins encoded within the region from nt 3575 to nt 5310.

These data suggest that it may be possible to uncouple canker size from suppression of pustule formation, an encouraging implication for biological control. A small canker with dense pustules production (as in chimeras R6, R10, R8 or R9) may be useful biological control agents.

Becky Beirman, University of Maryland Biotechnology Institute

Transgenic field study. Permission was received in 1998 to deploy transgenic strains of *C. parasitica* for population replacement studies in the Meshomesic Forest in CT. Specific risk assessment parameters are:

- Surveying nontarget woody species for infection by transgenics
- Surveying insects for recovery of transgenics
- Determining the effect of transgenic strains on the *C. parasitica* population structure
- Examining the phenotypic variability of recovered transgenic strains

Two plots were established in the Meshomesic Forest, one control and one transgenic-treated. All vegetation in the control plot was sprayed with a backpack mistblower using either water or transgenic conidia (10^{11}-10^{12} conidia per ml). The areas were sprayed 4-6 times per year.

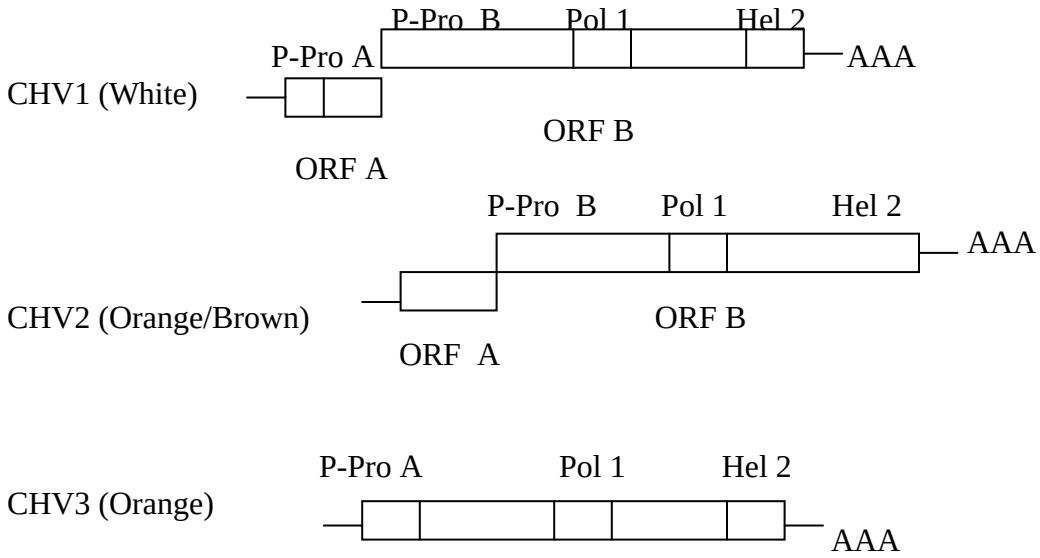
	1998	1999	2000
Cankers sampled	32	114	
v-c types	25	13	unknown
Transgenic Ascospores		161/1401	unknown
Strains containing Hyg ^{R}	0	5 Hyg ^{S}	1/156

Around 3,300 insects were cultured in 2000, representing 7 orders of insects, spiders and mites. From these, 156 isolates of *C. parasitica* were recovered; one isolate was hygromycin resistant.

Non-target woody species (i.e. beech, oak, maple, hickory) samples have yielded no *C. parasitica* isolates. Thus, it appears that transgenic hypovirulent isolates behave no differently from natural *C. parasitica* strains in terms of host specificity. Non-target species will continue to be sampled once each year.

Bradley Hillman, Rutgers University

Viral organization. The genomic organization of the 3 species of the genus, *Hypoviridae* is as follows:



The proper nomenclature of the virus is:

Family: *Hypoviridae*

Genus: *Hypovirus*

Species: *Cryphonectria Hypovirus1*

CHV3-is specifically GH2. It is comprised of multiple RNAs. There is defective RNA and a satellite RNA. The RNA sequence variability within a virus may be great. The defective RNA of CHV3-GH2 varies 10% with the parent RNA.

CHV1—found in Europe, China and Japan
CHV2— found in southern China and New Jersey
CHV3— found in North America (Michigan, Ontario and south-western Pennsylvania)

SR2-type RNA does not cross hybridize with CHV3-GH2, but this RNA is pervasive in the eastern U.S. Hillman hypothesizes that SR2 will probably be its own species; it is 50% similar with CHV3-GH2 dsRNA. This however, will require completing the sequencing.

Speciation of this family (*Hypoviridae*) is difficult because there is no serology available. Setting hard and fast rules is difficult, because there is no precise answer as to what the threshold should be (i.e. 70%). Thus, speciation requires some soul searching on part of the researcher. Hillman theorizes that CHV3 may be the progenitor of this family.

Transposon. A transposon is defined as a piece of DNA that can move about the genome. His lab is looking at a transposon that occurs as several-to-many copies in the nuclear genome in *C. parasitica*. This element has been used as a repetitive probe for fungal population studies. While this probe has been used for genotype confirmation, it has only recently been characterized.

The nuclear transposon used by Milgroom and Hillman is characterized as 3,563 base pairs. It falls in line with a set of transposons from maize (activator).

Hillman speculated that a transposon is not causing the flat condition in *C. parasitica*. There is an extra band in the transposon that is not present in Ep 67 or Ep 155. This transposon, Crypt1-CN is interrupted (or infected) by a FOT1-like transposon. If southern China is the cradle for *C. parasitica,* then you would look for a transposon-free isolate may occur in that region. Hillman and Milgroom wanted to get a transposon-free isolate so a transposon could be inserted, to study its function. Even Ep 155 has the unique transposon.

Transposons may be valuable in examining *C. parasitica* fungus/virus evolution. Do viruses interact with transposons? Horizontal transfer of transposons may be similar to cytoplasmic viruses.

Pam Kazmierczak, University of California at Davis

Chestnut orchards in California. She showed slides of a chestnut orchard south of Stockton, CA. Chestnuts were planted around the time of the gold rush. Some small orchards are in production; mostly with the variety, 'Colossal.'

There was an interception of *C. parasitica* in San Francisco in 1914.

Outbreaks in California occurred in:

- 1934 San Joaquin County
- 1935 Alameada County
- 1942 Butte County
- 1946 Elorado County

Annual inspections for chestnut blight have been conducted and the state was declared disease-free in 1960. New outbreaks occurred in 1986. In one orchard, DeMartini, a clonal population of the fungus has been reported.

Massimo Turina, University of California at Davis

MAP Kinase. He is trying to understand how a virus can cause the hypovirulent phenotype. He is looking at differences at the molecular level, specifically at the proteins that are down-regulated. CHV1 dsRNA inhibits transcription of both mating type pheromone genes. What is the mechanism of that down-regulation? To look at how pheromones are generally down-regulated, he looked at the yeast system as a model; it is controlled by a single transduction pathway.

His cloning strategy has been:

-amplification of a conserved region through PCR, using randomized primers

-use of PCR products

He found that the gene in *C. parasitica* was similar to genes in *Saccharomyces cerevisiae* (Mkk1 and Mkk2). Could the *C. parasitica* kinase genes compliment those of *S. cerevisiae*? Functionally, it can compliment yeast.

The kinase gene in *C. parasitica* is Cpk1. The gene in *S. cerevisiae*, Ste7, codes for MAP kinase and it is involved in the signal transduction pathway regulating mating and vegetative growth in yeast. Attempts to delete Cpk1 in *C. parasitica* have been unsuccessful. Cpk1 is able to compliment the mating defect of Ste7 yeast deletion strains.

His conclusions were:

-Cpk1 is a Ste7 homologue.

-The phosphorylation state of Cpk1 in liquid cultures does not seem to be related to
pheromone stimulation of the MAP kinase cascade.

-There are differences in hyperphosphorylation levels and time course expression of Cpk1 in viral infected and uninfected *C. parasitica*.

Sandra Anagnostakis, Connecticut Agricultural Experiment Station

Chestnut pathogens. She found five fungi that appear to be causing bark disease on chestnut trees in CT. Only one of the five fungi caused a canker; it was identified as *Pezizza* sp.

Rocky Hill plot. Trees in this plot were treated with CHV1-Ep 147 from 1982-86. In addition, another 50 trees in the plot were sprayed with a mixture of Hv strains in 1987 and 1988. Survival of chestnut has been much better in the Rocky Hill plot than in a nearby State forest. Thirty-six cankers were sampled in 2000. While all canker isolates were orange, 27/36 isolates yielded dsRNA.

Fenny Dane, Auburn University

She is looking at the diversity of American and Chinese chestnut species. Her work is in conjunction with Hongwen Huang (Director, Wuhan Institute of Botany, Wuhan Province, China).

Genetic diversity if dependent on evolutionary history and population history. Her two main objectives are:

- Assess the level of genetic diversity within and among American and Chinese chestnut
- Study evolutionary relationship

In addition to *C. dentata* and *C. mollissima*, she is studying chinkapins. She looked at isozymes of populations of: *C. dentata, C. mollissima, C. seguini, C. henryi*, and *C. pumila*. Genetic variability of Allegheny chinkapin is higher than that of American chestnut as shown in the following table.

Species	H_e
C. mollissima	0.311
C. henryi	0.261
C. seguini	0.186
C. dentata	0.167
C. pumila	0.228
C. pumila	0.277

She found there are continent-specific loci. The Changjiang River Valley in China seems to be the center of diversity. She hypothesizes that American chestnut came from China to North America across the Bering Straight.

Robert Bernatzky, University of Massachusetts

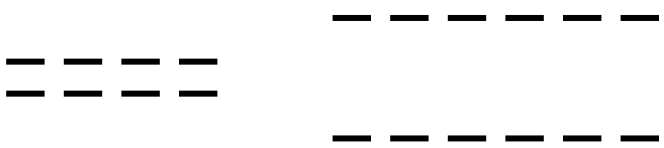
He is trying to characterize cDNA clones from leaves for the following purposes:

- for use in population studies
- for use in genetic diversity studies

- for use in identifying material from unknown tree species

He is working with 4 Chinese populations ('Mahogany', 'Nanking', CA99 and 72-211), six American chestnut, 3 F_1 hybrids and 2 BC_1 crosses ('Clapper' and 'Graves'). He is looking at 20-25 markers and has found that there are some markers that discretely distinguish species. There are some problems associated with markers in that many have shared alleles among species. He showed autoradiograms of the four Chinese and six American populations. A representation is shown below:

C. mollissima *C. dentata*



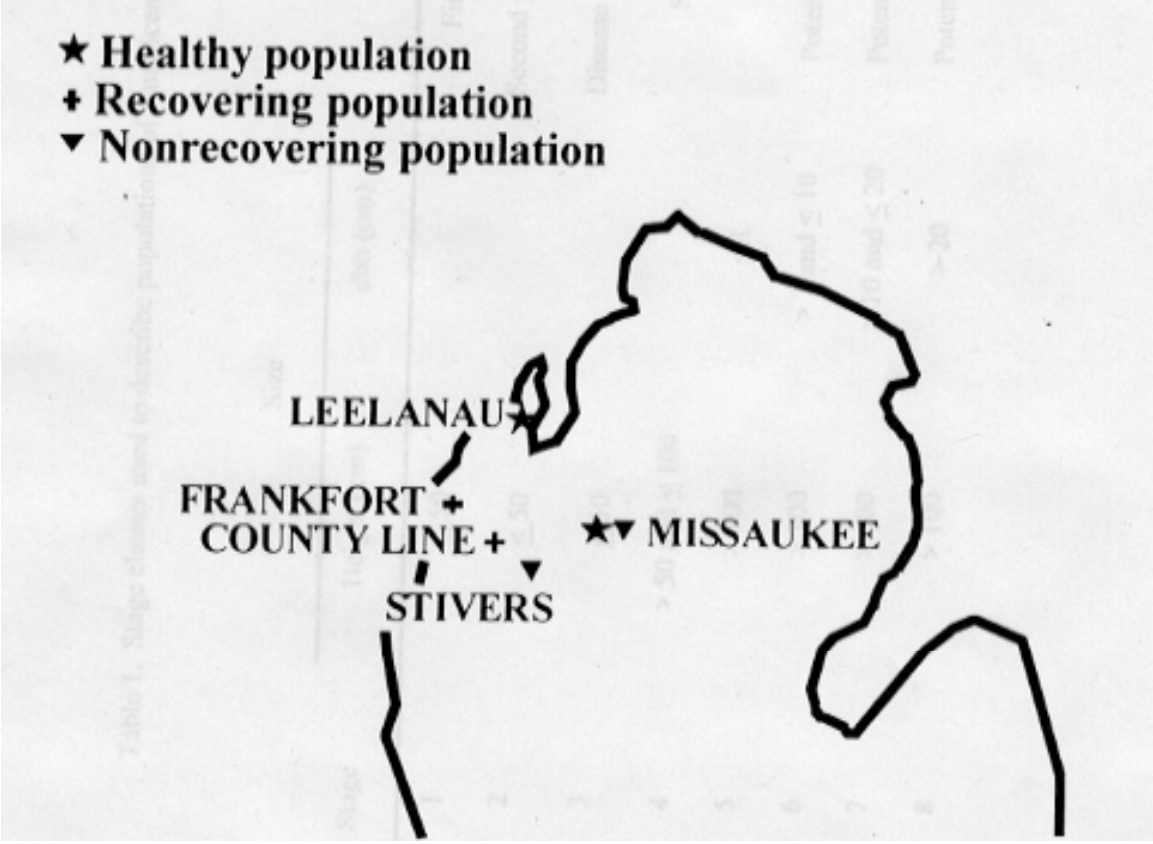
The above marker would be a good candidate for distinguishing species. He noted that a marker is useful if it is always present in one species and absent in other species.

He chose 8 markers that are simple, single-locus markers. He would like to test his markers and he asked NE-140 members to send him material, of known origin, to see if he can distinguish the material.

Andy Jarosz, Michigan State University

Chestnut demographics. He and Anita Davelos have looked at the interaction between blight and dsRNA in several populations of American chestnut in Michigan. They have examined the following populations:

- Leelanau (healthy)
- Missaukee (healthy
- Frankfort (recovering)
- County Line (recovering)
- Stivers (non-recovering)
- Missaukee (non-recovering)



They have asked several questions. What is occurring in Michigan in areas without disease? What does the disease do? When dsRNA is present, what are the effects?

This the 5th year of the study. Trees have been divided into eight size classes:

	Size		
Stage	**Ht (cm)**	**DBH (cm)**	**Category**
1	<50		First year seedling
2	<50		Second year seedling or older
3	<50		Disease or herbivore damaged
4	>50 and <100		Small juveniles
5	>100	<1	Juveniles
6	>100	>1 and <10	Potentially reproductive
7	>100	>10 and <20	Potentially reproductive
8	>100	>20	Potentially reproductive

General characteristics after five years:

Missaukee (healthy)

-reproduction is limited to large trees

-once a tree reaches size class 7, they don't get smaller

Stivers (diseased)

-smaller trees begin to show reproductive effect

-bigger trees are getting smaller

Missaukee (diseased)

-similar trends to Stivers

County Line (recovering)

-some large trees still die back

-young trees are reproductive

-there is robust growth from stage 7 to stage 8

Frankfort (recovering)

-similar trends to County Line

The dynamics of a healthy population are very different from diseased sites. Under Michigan conditions, a recovering site is similar to a healthy site. If we were to predict, we would state that recovering populations are in good shape for the long-term.

The question remains, "If we want to get a recovering population, where do we begin?" The largest effect in a stand comes from "bumping up" stage 6 trees to stage 7. Therefore, the stage 6 trees should be the area of concentrated effort.

Mark Double, West Virginia University

He reported on a field experiment that examined the influence of canker age and time of hypovirus introduction on the development of hypoviruses in cankers. An orange-pigmented strain of *C. parasitica* was used to initiate 160 cankers on 40 American chestnut stems; four cankers per tree. Cankers were initiated in May or July 1999 and outlined with a permanent marker at 8-week intervals after canker initiation. Cankers were treated with vegetatively compatible isolates containing one of two hypoviruses (CHV1-Euro 7 or CHV3-COLI 11-1). Sets of cankers were challenged in July, September and November, 1999 and April and July, 2000. Each hypovirus was introduced into the advancing canker margin (top and bottom) of eight replicate cankers/date. Cankers will be extensively sampled one year following challenge. The rate of hypovirus colonization will be evaluated by removing bark samples within all rings and evaluating colonies that arise.

Preliminary data from cankers that were initiated in May 1999 and challenged two months later in July, indicated that isolates from outer ring samples decreased markedly in the November ring, compared to September and April rings. This finding was similar for both hypoviruses. Outer ring data from May 1999 initiated/April 2000 challenged (collected in July 2000), indicate good movement of the CHV1-Euro 7 hypovirus (60%) compared to CHV3-COLI 11-1 (22%).

William MacDonald, West Virginia University

County Line, Michigan. He showed slides of the County Line site, beginning in 1984 and continuing until 2000. The recovery of the site was remarkable; dead trees in 1984 have given rise to a healthy stand in 2000. Some trees at this site at 10-12" in diameter and 90% of the trees contain hypovirus.

He also showed slides of the Stivers site (a non-recovering site) to contrast the County Line site.

West Salem, Wisconsin update. New findings for 2000 include:

- 490 trees are now infected with 1637 cankers; 228 new trees were discovered in 2000 harboring 394 new infections
- 730 cankers were sampled in 2000 including 279 of the 394 discovered in 2000
- CHV1 (Euro 7) continues to be the most commonly identified hypovirus.
- Five isolates were recovered from cankers that were vegetatively incompatible with the three hypovirulent v-c types (Bockenhauer, Schomberg and Rhyme).
- Hypovirulent isolates were recovered from approximately 80% of all cankers that occurred on trees treated from 1993-97.
- Hypovirulent isolates were recovered from about 24% of cankers discovered on trees that have not received any hypovirulent treatment (1998-2000).
- A subjective rating of cankers shows progressive improvement in canker morphology from 1994-2000.

Catherine Clark, North Carolina State University

She is looking at AFLPs (Amplified Fragment Length Polymorphisms) for genetic linkage mapping. When AFLPs work, they are very robust. To date, she has:

- 78 primer-enzyme combinations (PEC) screened
- 90 F_2 progeny derived from an interspecific Chinese x American F_1
- 12 PECs produced 276 polymorphic marker bands

The gels have been scored by Paul Sisco using AFLP-Quantar. She has 59 BC_1 progeny screened with 12 PECs. Tom Kubisiak is working with the genetic data to produce linkage maps.

Tom Kubisiak, USDA Forest Service, Southern Institute of Forest Genetics

Mapping resistant loci. He is focusing on three sources of resistance (F_2 and BC_1 populations), and he has been able to map in all three sources. Grossly, he has been able to identify areas of resistance and characteristics of chestnut. He has been trying to follow Chinese markers and overall, he has been fairly successful. He is investigating if traits such as twig color, stipule shape, stem hairs, and leaf hairs, are linked to resistance. Maps are not complete; there aren't enough markers on the map. He continues his work to fine-tune the maps. Catherine Clark generated another 276 markers; the TACF contracted with NC State University for this work. Kubisiak noted that AFLPs are more robust than RAPD markers, especially if you want to say a particular linkage group in one population is the same as the linkage group in another population.

He is looking at dominant markers. The approach has been to construct a combined map. He will use the program, 'Mapmaker" to construct a better map. To date, he has constructed: single parent maps, combined maps and framework maps.

These have provided good gross-order maps but not good local-order. He mentioned that robust is comparable to reproducible.

The maps, thus far, seem to be 'boiling down' to 11 large linkage groups. Thus, he would like someone to examine chromosome numbers in chestnut.

About 20% of the markers are distorted (100 of 500+).

Chestnut diversity project. He has been examining chestnut populations from across the U.S. The assays for eight populations are complete; 14 populations are not complete. He is developing SSR markers (simple sequence repeater). To date, he has 10-12 SSR loci.

Business Meeting

The business meeting began with discussion about the multi-state project. Craddock felt the items Anderson discussed were guidelines, not mandates. There was discussion about the chair-elect office, and the 2-year officer term. MacDonald suggested that the group maintain its current status of a chairman and secretary (elected for 1-year terms). Anagnostakis made the suggestion to include a chair-elect. It was finally decided that officers will be elected for a 1-year term. Fulbright nominated Pam Kazmierczak as chair-elect and Michael Gold as secretary (for one-year terms). The vote was unanimous. The officers for 2000-01 are: Sandra Anagnostakis (chair), Pam Kazmierczak (chair-elect) and Michael Gold (secretary). Anagnostakis agreed to host the 2001 meeting in Connecticut and Kazmierczak agreed to host the 2002 meeting in Davis, California.

The remaining discussion centered around the web page for NE-140 that Anderson suggested. Craddock stated that he did not feel comfortable putting the web site together. Hillman noted that designing a web page is only part of the work; maintaining the site takes significant effort. Anagnostakis stated that she knows a professional web site designer and is willing to make inquiries. Doudrick said that since he is with the Southern Research Station, he may be able to assist with design and maintenance. Hillman felt comfortable with Doudrick's offer as the Southern Research Station is a stable entity that can maintain a web site. Anagnostakis agreed to send an official letter of inquiry to Doudrick for a web page design; she will pursue the professional web page designer as a backup.

Craddock then passed on the USDA-Secretary of Agriculture's *Honor Award for Excellence* plaque to Anagnostakis, With regard to the official NE-140 minutes, Anagnostakis asked for suggestions as to what milestones should be included in the report. Craddock suggested Double's minutes and the TACF report should serve as sources of information.

The meeting was adjourned at 12:00 noon on October 28, 2000. Following the meeting, tour of the Lula Lake Land Trust was conducted by Craddock.