OFFICIAL

NORTHEAST REGIONAL RESEARCH PROJECT

PROJECT NUMBER: NE-140 (Rev.)

TITLE: Biological Improvement of Chestnut and Management of Chestnut Pathogens and Pests

DURATION: 1 October 1998 through 30 September 2003

STATEMENT OF THE PROBLEM

Some of the most damaging biological invaders that have become established in North America are plant pathogenic fungi, especially those introduced into our forest ecosystems. Most significant of these is *Cryphonectria parasitica*, the fungal pathogen responsible for the blight of American chestnut (*Castanea dentata*). This canker organism, native of Asia, was introduced before the turn of the 20th century, and within 50 years of its discovery had infected and killed most American chestnut trees growing in eastern North America (8, 24). American chestnuts would almost certainly be extinct or endangered if not for their ability to produce sprouts from the base. These grow, become infected, die, sprout, and repeat the cycle again and again. The loss of this important forest tree had unparalleled ecological, sociological, and economic consequences.

Restoration of timber chestnuts and improving orchard chestnuts in the United States are the long term goals of the members of this regional project. In the shorter term, we expect to build on our understanding of two biological approaches to the control of chestnut blight: use of biocontrol agents to control the fungus populations and tree breeding to improve the fitness of the trees. The members of this group are strongly dependent on each other for the success of their individual projects Working together, they hope to reverse the effects of this devastating pathogen, and find ways of dealing with new imported pests such as Oriental Chestnut Gall Wasp.

JUSTIFICATION

The former value of American chestnut cannot be overstated. The wood was extremely rot resistant, as well as straight. It was one of the most versatile woods in the forests of eastern North America with uses that ranged from telegraph poles and firewood to building materials and fine woodwork. Records of early wood use in Connecticut (23,44) and Maryland (59) give remarkable figures of the annual board footage of lumber milled from chestnut. The loss of a species that once occupied over 25% of many sites in our eastern forests was ecologically devastating. The repercussions of this loss are still manifest in the declines of oak and other species that now occupy sites once populated by chestnut. Restoration of chestnut timber trees has the potential to increase all forest productivity, but this is especially true on poor, dry sites. The oak-hickory forest on these sites does not produce stems large enough to be marketed. In addition, the increased production of mast by adding chestnuts to these areas is expected to cause a shift of wildlife populations, primarily white tail deer, away from more mesic sites where they are having negative impacts on forest regeneration due to increased browsing pressure.

The nuts produced by chestnut trees also are valuable for mast and for human consumption. The loss of this mast had a severe impact on populations of large mammals such as black bear, and wild birds such as American turkeys. Although the discipline of wildlife biology had not been fully developed in the early 1900's, many accounts indicate a catastrophic decline in these species, presumably because the mast was critical for surviving the winter season.

As a human food, chestnuts are easily digested, filling, high in potassium, low in fat and generally closer in food value to grains and potatoes than to nuts (47). They are a staple in Asian diets and extensively used in Europe. More than 10 million pounds of chestnut are imported into the U.S. every year and wholesale for $2.00–$4.00 per pound (information from the Chestnut Marketing Association, Greg Miller, President). Since orchards of good chestnut varieties can yield 2,000–3,000 pound of nuts per acre, this is an important economic opportunity for growers.

National Priorities for the fiscal year 1997 (USDA/JCFAS report to the Secretary of Agriculture) include three major sections that are addressed by this project:

1. Develop Improved Agricultural and Forest Management Systems that are Compatible With Natural Ecosystems
   - further developing and promoting environmentally safe techniques of integrated pest management (IPM)
   - incorporating pest and disease resistance into plants with the goal of enhancing their performance and quality while reducing dependence on chemical control
2. Develop and Transfer Information on Management for Increased Productivity and Sustainability of Natural Resources on Public and Privately Owned Lands
   - developing basic information on ecological interactions and incorporating this data into ecosystem management strategies that ensure the land's capacity to provide multiple values and uses and conserve biodiversity
   - restoring disturbed forest ecosystems and protecting forest health
3. Improve Global Competitiveness of U.S. Food, Agricultural, and Forest Products
   - provide research base for new and differentiated products

Since 1982, the research on chestnut blight in North America has been coordinated under a single USDA-Regional Research Project. This organization was awarded the Department of Agriculture Secretary's Honor Award in June of 1997, demonstrating the value of previous work.

Now we are ready to start the next phase of the work, and the following general topics link this project to the Priorities listed above:

1.

- further develop strains of the chestnut blight fungus with hypovirulence viruses and defective mitochondria for use in IPM programs to protect timber and orchard chestnut trees, and find environmentally safe techniques for control of chestnut gall wasp
- incorporate (by breeding) chestnut blight resistance and gall wasp tolerance into chestnuts for planting in forests and orchards

2.

- conserve the diversity of our native *Castanea* populations for future use
- study the developmental biology and cultural requirements of chestnut seedlings in nurseries and natural settings

3.

- produce new chestnut varieties, and information on existing varieties, that will allow orchards of all sizes to produce nut crops competitive with those imported into the U.S. for the fresh market

In addition, the potential usefulness of fungal viruses as biological control agents for other plant pathogenic fungi is being explored. Research by the NE-140 committee is setting the tone, and much of the direction, for this entire area of biological investigation.

A large number of publications have been authored by committee members, with many jointly authored between or among committee members. Over the last five years, publications included 169 research papers, book chapters, reviews and popular articles (a list is attached).

RELATED CURRENT AND PREVIOUS WORK

Breeding work was begun with chestnuts at the turn of the last century, but the only program that has continued without interruption is that at The Connecticut Agricultural Experiment Station (4). Some studies of the disease and the fungus were done in the early 1900s, but interest waned with the lack of success in controlling the disease. The issue was reborn with the discovery of chestnuts in Italy that were surviving in spite of their chestnut blight cankers (11). The recovery phenomenon, termed "hypovirulence", usually involves viruses that cause a disease of the fungus, reducing its ability to kill chestnut trees (36). This discovery reawakened the scientific community to the long-standing issue of chestnut blight, a topic that had not undergone the scrutiny of modern scientific investigation. Plant and fungal physiology projects were begun to study the enzymes involved in the interactions of tree host, fungal pathogen, and viral parasite. Two additional institutions began breeding chestnut trees for blight resistance using modern genetic principals; The University of Tennessee in Knoxville and The American Chestnut Foundation in Meadowview, Virginia (4). The large collection of species and hybrids of chestnut that had been maintained in Connecticut was made available to all who needed material. When it became clear that the oriental chestnut gall wasp (*Dryocosmus kuriphilus*), introduced into Georgia in 1974, was spreading northward, the breeding programs included screening for resistance to this pest in their plans.

NE-140 had it beginnings in 1978 when approximately 200 people attended a symposium held in Morgantown, WV at West Virginia University. There were 34 papers given, and the discussion that ensued convinced the group that a Regional Research Committee was needed. In 1982, five experiment station scientists agreed to participate. Within a few short years, the committee grew to include 13 experiment stations and other participating academic institutions and governmental units. As a result of this group's activities, the following events have occurred:

- Connecticut has improved the records of holdings and continues to maintain the finest collection of species and hybrids of chestnut in the world (6).
- New York (Cornell) confirmed, by making genetic tests of Asian and American populations, that the blight fungus came to the US from Japan (43).
- Connecticut imported hypovirulent strains from Dr. J. Grente in France (who first described the disease of the fungus) and showed that these strains could control chestnut blight cankers on American chestnut trees (8).
- Michigan described the spread of American hypovirulence viruses through the blight fungus population in a stand of trees planted in Michigan (35).
- Connecticut described a genetic system in the blight fungus that prevented strain fusion and the transmission of hypovirulence viruses (1, 2, 3).
- Michigan presented a physical and genetic map of the mitochondrial genome of a strain of the blight fungus (10).
- Michigan described a type of hypovirulence determined by genes in mitochondria (42).
- Michigan, New Jersey, and West Virginia described and compared three American hypovirulence viruses (19, 54)
- New Jersey, Maryland, Michigan, and Texas spearheaded the naming of hypovirulence viruses as a new genus, now recognized by the International Committee on Taxonomy of Viruses (37, 38)
- Maryland transformed virulent strains of the blight fungus with cDNA copies of hypovirulence virus RNA genes, and produced stable, transgenic hypovirulent strains with virus genes and fungal genes together in the nucleus (14, 15)
- Maryland, Connecticut, and West Virginia were granted permission from USDA/Plant Quarantine to test transgenic hypovirulent strains of the chestnut blight fungus in the forests of Connecticut and West Virginia. This was the first permit granted to test transgenic organisms for their ability to spread and effect biological control of a plant disease (7).
- Kentucky, Michigan, Texas, Maryland, Virginia, and Connecticut have made detailed studies of enzymes produced by the blight fungus that may be related to its

ability to kill chestnut trees (5, 12, 13, 16, 17, 18, 20, 21, 22, 25, 27, 28, 31, 33, 34, 39, 40, 42, 46, 53, 55)

- Kentucky and Connecticut have studied enzyme systems in chestnut trees that may be related to their ability to resist the blight fungus (26, 29, 30, 45, 48, 49, 50, 51, 52)
- Virginia, Connecticut, and Tennessee have been breeding chestnut trees and selecting progeny with resistance to chestnut blight disease, and with the timber form of American chestnut trees (4). These will be ready for release within five years.
- Mississippi, Virginia, Massachusetts, and Connecticut prepared a genetic map of chestnuts and found three molecular markers associated with resistance to chestnut blight disease (41)

OBJECTIVES

1. *To improve chestnut trees for timber and for nut production, and determine the cultural requirements of chestnut seedlings in nurseries and natural settings.*

2. *To better understand the interactions and ecology of the host/pathogen/parasite systems at the molecular, organismal, and environmental levels to develop effective biological controls for chestnut blight.*

PROCEEDURES

Objective 1: To improve chestnut trees for timber and for nut production, and determine the cultural requirements of chestnut seedlings in nurseries and natural settings..

1. Traditional breeding programs are being carried out in Connecticut, Tennessee, and Virginia. All three programs share chestnut pollen and seed as needed. Crosses are made in the spring (early July), seeds harvested in the fall (October), and are planted the following spring. Tests for blight resistance are made when the seedlings are 3 to 5 years old by inoculating branches with pure cultures of the blight fungus and noting canker enlargement rate. All three programs will soon be able to speed up selection using molecular markers. Assessment for form and nut quality may be done on 5-year-old trees.
2. Identification of molecular markers is being done in Mississippi with plant material supplied by all NE-140 members. Genetic input is provided by Virginia. The work to date has concentrated on identifying Chinese chestnut-specific markers for resistance to chestnut blight disease and is now being expanded to examine Japanese chestnut markers as well. Seven 10-mer RAPD primers are used with extracted and purified tree DNA in PCR to identify three regions of DNA correlated with chestnut blight resistance, flanking regions, and "control" regions (used in Mississippi and Connecticut, so far). The next major step in this work will be to use bulk segregant analysis for fine-scale mapping of the three genetic regions associated with blight resistance.

3. Plant tissue culture laboratories in Georgia and New York (SUNY) will maintain and multiply important clones of chestnut hybrids produced by Virginia, Connecticut, and Tennessee. In addition, Georgia will develop cultures of trees in native populations of American chinquapins (*C. pumila* var. *pumila* and var. *ozarkensis*), threatened by chestnut blight and ink disease (caused by the root pathogen *Phytophthora cinnamomi*), to insure that important germplasm is not lost. Chinese chinquapins (*C. Henryi*) are available in Georgia, and will be included in the program. All three chinquapin types seem to have valuable resistance to the Oriental Chestnut Gall Wasp (*Dryocosmus kuriphylus*), which is currently found in Georgia, Alabama, Tennessee, and North Carolina. New York (SUNY) efforts include the development of five constructed genes for resistance to blight that will be put into somatic embryos of chestnut using Agrobacterium-mediated transformation.
4. Cultivar trials (of commercially available chestnut cultivars for nut production) are underway in Connecticut and Tennessee, and commercial plantings in Michigan are being monitored. The most commonly planted cultivar in the U.S. (mostly on the west coast) is currently 'Colossal', which is a European X Japanese hybrid with some resistance to chestnut blight, but winter hardiness has not been documented and resistance to ink disease is unknown. We assume that it will be susceptible to infestation by gall wasp. The trials in Tennessee of this, and other commercial cultivars, will test for resistance to blight, ink disease, and gall wasp, and the Connecticut trial will test for blight resistance and winter hardiness. Plantings of 'Colossal' in Michigan will be checked for survival in that climate and for blight resistance.
5. Plantings of new hybrids for assessment of resistance to gall wasp, chestnut blight, and ink disease have been made and will continue to expand. This work is being done by Tennessee (plantings in Tennessee), and by Connecticut (plantings in Georgia in cooperation with Jerry A. Payne, U.S.D.A./ARS, Tree Nut Research Laboratory, Byron, and in North Carolina in cooperation with W. Henry McNab, U.S.D.A./Forest Service, Bent Creek Research Forest, Ashville). Tennessee is using Japanese stock plants with reported resistance to gall wasp, and Connecticut

is using American chinquapins and Chinese chinquapins with reported resistance. The breeding programs will seek to identify the two genetic bases of resistance and then combine their lines if that seems useful.

6. In addition, we must protect existing populations of American chestnut trees for future inclusion in the breeding programs and for studies of genetic diversity. This is being done by planting seed collected from natural populations, and by introducing hypovirulence viruses and transgenic hypovirulent strains of the blight fungus into natural populations and seed orchards. Native populations currently being protected are in Connecticut, New York, Massachusetts, Maine, New Jersey, Virginia, Pennsylvania, Maryland, West Virginia, North Carolina, Tennessee, and Georgia. Valuable plantings in Wisconsin are being maintained, as well. In Michigan and Ontario plantings of American chestnut trees are being protected from blight apparently by debilitation of the fungus with a cytoplasmically-transmissible, mitochondrial disease.
7. Trees grown from irradiated American chestnut seed that were planted at the National Colonial Farm in Virginia have been evaluated for blight resistance. Seedlings of these trees have been planted in Michigan for further testing (by Michigan, Virginia, and West Virginia)
8. Chestnuts imported by the U.S.D.A. and planted prior to 1960 are being located and evaluated for resistance to chestnut blight, ink disease, and gall wasp by Connecticut and Tennessee.
9. Natural populations and orchards in Connecticut and West Virginia will be examined for the effect of competing vegetation on tree survival (with the cooperation of Gary J. Griffin, VPI &SU, Blacksburg).
10. Forest and orchard planting techniques will be studied by Connecticut, Tennessee, Virginia, and Michigan.

Objective 2: 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.

1. Spread of the chestnut blight fungus is being studied in natural populations of American chestnuts and orchards of species and hybrids. This work is in progress in Connecticut, New Jersey (cooperating with New York), New York, and West Virginia (with Michigan). These projects include work on the vegetative incompatibility system of the fungus, and specific *vic* genes used as markers.
2. The spread of hypovirulence viruses is being monitored in natural and planted populations in Connecticut (with Maryland), New Jersey (with New York), New York, West Virginia (with New Jersey, Michigan, and New York), and Tennessee (with Connecticut and West Virginia). American chestnut trees planted in West Salem, Wisconsin at the end of the last century now have chestnut blight disease. Hypovirulent strains of the fungus are being used to try and establish a biological control. The treatments are being supervised and monitored by Michigan, West Virginia, New York, and the Wisconsin Department of Natural Resources (Jane Cummings-Carlson). Trees planted in Michigan, where natural hypovirulence viruses developed, are being monitored by Michigan (with West Virginia).
3. The survival and spread of transgenic hypovirulent strains is being evaluated in Connecticut and West Virginia, both working with Maryland where the strains were developed.
4. Alternative controls for chestnut blight disease are being sought in Massachusetts, using naturally-occurring bark fungi, such as *Trichoderma*. This work will continue with studies of the nature of the control mechanisms.
5. The molecular biology of the host/pathogen/parasite system of *Castanea/C. parasitica/Hypovirus* is beginning to yield valuable information about the basis of the interactions. Sequencing and comparisons of the Hypoviruses will be done by New Jersey, Michigan, Maryland, West Virginia, all working closely with each other. Studies of the effect of specific fungal genes on Hypovirus replication and gene expression will be done in Maryland, New Jersey, and Texas. The function of the

fungal genes as sex pheromones, and their involvement in virulence will be examined in Texas. The use of Hypoviruses as gene expression vectors, and the introduction of Hypoviruses into other fungal pathogens (for biological control of other plant diseases) will be studied in Maryland.

6. Hypovirulence caused by mitochondrial mutations and mobile genetic elements will be studied in Michigan.

DESCRIPTION OF COOPERATION

1. ***Shared Biological Materials, and Expertise***

EXPERTISE

Anagnostakis: fungal ecology and genetics, and field tests of hypovirulence, maintenance of a collection of species and hybrids of chestnut, and native populations of *C. dentata*, chestnut breeding program

Craddock: management of orchard plantings and mychorrhizal organisms, chestnut breeding program

Doudrick & Kubisiak: plant molecular biology and cytogenetics

Fulbright: plant pathology, native Hypovirus systems and mitochondrial hypovirulence, molecular biology, maintenance of the National Colonial Farm irradiated trees, and Michigan and Wisconsin plantings of *C. dentata*

Hebard: plant pathology, management of the breeding material and breeding program, for the American Chestnut Foundation

Hillman: virology and molecular biology of native and introduced Hypoviruses

MacDonald: plant pathology, mycology, studies with hypovirulence of the cytoplasmic and transgenic type

Merkle: plant tissue culture and gene-gun transformation

Milgroom: fungal population genetics

Nuss: virology, fungal transformation systems, and molecular biology

Powell: plant tissue culture and Agrobacterium-mediated transformation

Schlarbaum: forest tree genetics, and maintenance and evaluation of orchards, chestnut breeding

Tattar: plant pathology, biological control

Van Alfen: plant pathology and molecular biology

PLANT MATERIAL:

Castanea native populations N (*dentata*, *pumila* var. *pumila*, and *pumila* var. *ozarkensis*), *Castanea* species imported from elsewhere I (*crenata, mollissima, Henryi, seguinii*, and *sativa*), and *Castanea* hybrids H, are maintained and studied by NE-140 scientists. These trees are in: Connecticut [N,I,H], Alabama [I], Florida [N], Georgia [N,I,H], Louisiana [N,I,H], Maine [N], Maryland [N,I,H], Massachusetts [N], Michigan [I *dentata*, H], Mississippi [N,I,H], New Jersey [N,I,H], New York [N], North Carolina [N,H], Pennsylvania [N], Tennessee [N,I,H], Virginia [N,I,H], West Virginia [N], and Wisconsin [I *dentata*]

FUNGAL MATERIAL:

Strains of *Cryphonectria parasitica* are shared by the members of NE-140, and important strains are deposited with the American Type Culture Collection. Strains with genetic markers are available, and information on the genetic determinants of vegetative incompatibility (*vic* genes) is available for use in population studies. A review of some of the strains available is in (3).

VIRUS MATERIAL

Hypovirus types from France, Italy, Michigan, West Virginia, Kentucky, and China are studied and shared by NE-140 members.

2. *Past Shared Experience*

This group has worked together productively for several years, as evidenced by the accomplishments listed in RELATED CURRENT AND PREVIOUS WORK above, and the fact that they were awarded the 1997 Department of Agriculture Secretary's Honor Award.

EXPECTED OUTCOMES

In accordance with the Fiscal Year 1997 Priorities Report (USDA/JCFAS), our expected outcomes are:

1. More efficient use of natural resources to meet diverse social needs, by restoring chestnut as a timber crop
2. Increased options for controlling pests and diseases of chestnut trees
3. Greater availability of improved chestnut varieties for use in agricultural production; as orchard trees for nuts and timber trees for lumber and poles
4. Strategies for planting chestnuts in harvested and disturbed ecosystems
5. Development of chestnuts as a new, US-produced product for the fresh market to replace imported nuts

ORGANIZATION

The organization of regional research project NE-140 was established in accordance with the format suggested in the "Manual for Cooperative Regional Research". One person at each participating agency is designated, with approval of the agency director, as the voting member of the Technical Committee. Other people at the agencies are encouraged to participate as non-voting members of the committee. Members vote each year for a Secretary, and the Secretary becomes the Chair the following year as a new Secretary is elected.

SIGNATURES:

Regional Project Title: Biological Improvement of Chestnut and Management of Chestnut Pathogens and Pests

Administrative Advisor - John F. Anderson — 4/7/98 Date

Chair, Regional Association of Directors — 4/7/98 Date

for Administrator, Cooperative State Research, Education, and Extension Service — 6/25/98 Date

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ATTACHMENTS

A. <u>PROJECT LEADERS AND RESOURCES</u>

REGIONAL PROJECT NE-140

Biological Improvement of Chestnut and Management of Chestnut Pathogens and Pests

(1 October 1998 - 30 September 2003)

INSTITUTION	LOCATION	LEADER (OTHER PARTICIPANTS)	SPECIALTY
Texas A&M University	College Station, TX	Van Alfen, Neal K.	Molecular biology, plant pathology
University of Maryland	College Park, MD	Nuss, Donald L.	Molecular biology, virology
Connecticut Agricultural Experiment Station	New Haven, CT	Anagnostakis, Sandra L.	Mycology, genetics, biocontrol, plant breeding
Southern Institute of Forest Genetics, USDA	Saucier, MS	Doudrick, Robert L. (Thomas L. Kubisiak)	Molecular biology, plant genetics, cytogenetics
Michigan State University	East Lansing, MI	Fulbright, Dennis W. (Frank Ewers, Andrew Jarosz, Helmut Bertrand)	Plant pathology, biocontrol, molecular biology (ecology, pathogen dispersal)
American Chestnut Foundation	Meadowview, VA	Hebard, Fred V. (Yan Shi)	Plant breeding, plant pathology
Rutgers University	New Brunswick, NJ	Hillman, Bradley I.	Virology, molecular plant pathology

West Virginia University	Morgantown, WV	MacDonald, William L. (Mark Double)	Plant pathology, biocontrol
University of Georgia	Athens, GA	Merkle, Scott A.	Plant tissue culture
Cornell Univ.	Ithaca, NY	Milgroom, Michael G.	Population genetics
State University of New York - CESF	Syracuse, NY	Powell, William A. (Charles Maynard)	Plant pathology, plant tissue culture
University of Tennessee	Knoxville, TN (Chattanooga, TN)	Schlarbaum, Scott E. (J. Hill Craddock)	Forestry, genetics (plant breeding)
University of Massachusetts	Amherst, MA	Tattar, Terry A. (Mark S. Mount, Phyllis Berman, Robert Bernatzky)	Plant pathology, (microbiology, biological control, molecular biology)

B. AGENCY COMMITTMENTS

INSTITUTION	SY	PY	TY
Texas A&M University	0.1	0.2	0
University of Maryland	0.3	5	2
Connecticut Agricultural Experiment Station	0.75		0.5
Southern Institute of Forest Genetics, USDA	0.4	0.2	0.2
Michigan State University	1	0	0
American Chestnut Foundation	1		0.75
Rutgers University	0.2		0.2
West Virginia University	0.35	0.75	
University of Georgia	0.1		0.1
Cornell Univ.	0.3	0.8	0.2
State University of New York -CESF	0.2	1	
University of Tennessee	0.35	0	0
University of Massachusetts	0.3	0.5	0.75