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LIST OF TABLES, FIGURES, and MAPS

List of Reviewers and Participants

Review Team:

1. Dr. Shawn A. Mehlenbacher, Professor of Horticulture, Oregon State Univ.

2. Dr. Ronald L. Phillips, Regents' Professor of Plant Genetics, Univ. of Minnesota

3. Dr. J. P. "Hans" van Buijtenen, Professor Emeritus of Forestry, Texas A&M Univ.

Participants from The American Chestnut Foundation:

1. Dr. Frederick V. Hebard, Staff Pathologist

2. Dr. Paul H. Sisco, Staff Geneticist

3. Dr. J. Hill Craddock, Vice-President for Science, TACF Board of Directors

4. Dr. Albert Ellingboe, Science Director, TACF Board of Directors

5. Dr. Robert Leffel, Pennsylvania Chapter Breeding Coordinator

6. Dr. Robert Doudrick, USDA Forest Service and TACF Board of Directors

7. Mr. David Armstrong, Executive Director, Pennsylvania Chapter

The American Chestnut Foundation

Review of Scientific Programs, 12-15 August 1999

Agenda

Thursday, 12 August

Arrivals: Ron Phillips arrives at Tri-Cities Airport at 12:03 p.m.
Shawn Mehlenbacher arrives at 4:23 p.m.
Hans van Buijtenen arrives at 6:25 p.m.

Dinner at the Swiss Inn, 6:30-8:00 p.m.

Friday, 13 August

Breakfast at the Swiss Inn, 8:00-9:00 am.

Farm Tours, 9:00 a.m.to 12:00 noon. Tour conducted by Fred Hebard and Paul Sisco

Brown -Bag Lunch, 12:30 to 1:30 p.m.

Afternoon Sessions, Presentations by TACF Scientists:
Fred Hebard, TACF Staff Pathologist
Paul Sisco, TACF Staff Geneticist
Bob Leffel, TACF PA Chapter Breeding Coordinator

Dinner at the Swiss Inn, 7:00-9:00 p.m.

Saturday, 14 August

Breakfast at the Swiss Inn, 7:00-8:00 am

Review Team meets alone, 8:00 a.m.- 10:00 a.m.

Review Team poses questions to staff and participants, 10:00 - 11:15 a.m.

Review Team works on written report, 11:15 a.m. - 4:00 p.m.

Review Team presents oral summary of report, 4:00-4:30 p.m.

Reception with local residents at the home of Sue Payne, Emory, VA, 5:00-7:00 p.m.

Dinner at the Swiss Inn, 7:00-9:00 p.m.

Sunday, 15 August

Departures: Ron Phillips, Hans van Buijtenen and Shawn Mehlenbacher leave from Tri-Cities Airport at 7:00 a.m.on Delta Flights to Cincinnati and Atlanta.

I. Brief History of The American Chestnut Foundation

The American chestnut tree, *Castanea dentata*, was a dominant overstory tree of the Appalachian mountains from Maine to Mississippi, existing in pure stands in certain areas and comprising approximately 25% of the hardwood trees of the Eastern forests in 1900. It was a very fast-growing tree, able to send up shoots from crown buds around the stumps, and thus was valuable for wood production because harvested trees did not need to be replanted. Its wood was easily split, rot resistant (due to high tannin content), and thus used for fences, shingles, telephone poles, railroad ties, coffins, and furniture. Extracting tannin from bark and small tips was the basis of a large leather-tanning industry. Chestnut trees were a major component of the mast production of the Eastern forests, blooming in June (later than oaks and hickories) and having more consistent nut crops. According to "Chestnut and the chestnut blight in North Carolina":

> Chestnut is one of the most important commercial trees in North Carolina and probably the one for which it would be most difficult to find a substitute. It is by far the most abundant and widely distributed species throughout the mountain counties of this and other Appalachian States, and is used for a greater variety of commercial purposes than any other tree of the region. (Economic Paper #56 of the North Carolina Geological and Environmental Survey, Raleigh, 1925).

Chestnut blight, whose causal agent is an ascomycete fungus *Cryphonectria* [*Endothia*] *parasitica*, was introduced into the U.S. in the late 1800's, probably on Japanese chestnut trees. It was first seen on American chestnut trees at the Bronx Zoo in 1904 and by 1950 had killed most trees back to their stumps throughout the original range. Some organism in the soil, perhaps a *Trichoderma* fungus, counteracts the blight in the stumps, many millions of which remain alive and produce small shoots. When the canopy is opened up (as by a clearcut), these shoots can quickly reach ~30 feet and flower before being killed back by the blight fungus after 5 - 10 years. These surviving stumps provide an extensive genetic reservoir for preservation of the species.

However, the tree is threatened with extinction on rich sites, such as steep north-facing slopes, where few sprouts persist. On intermediate sites, where stumps are still common, clearcutting results in vigorous growth of the sprouts for 10 - 15 years, after which sprout clusters do not survive. Only on dry sites do sprout clusters survive the epidemic of blight that follows clear-cutting.

From the late 1920's through about 1960 an extensive effort was made both by the USDA and the Connecticut Agricultural Experiment Station to breed blight resistant timber-type chestnuts, but the effort was abandoned when the trees produced did not prove to be competitive in a

forest setting. The trees released by the early programs were mostly Chinese x American F_1's, BC_1's to Chinese and Chinese x Japanese x American trihybrids.

In 1981 Dr. Charles Burnham of the University of Minnesota concluded that backcross breeding could be successfully used to transfer blight resistance from the Chinese chestnut (*Castanea mollissima*) to the American chestnut (*C. dentata*). This conclusion was based primarily on a paper by Clapper (J. For. 50:453-455, 1952), the data from which appeared to show that there were two partially-dominant genes for resistance in the Chinese trees that had been used as parents in crosses with American chestnut.

> It was believed, and seemingly accepted without question for many years, that many genes must be involved in blight resistance to account for the wide range of blight reaction in hybrids and that the genes for blight resistance are recessive. Not until Clappers 1952 report was it shown that the inheritance may be relatively simple; that report also showed clearly that the genes are not recessive. . . . Thus, the usual procedure in early breeding programs was to make as many crosses as possible among promising trees, including interspecific crosses, hoping to obtain the one tree that combined adequate blight resistance with the desired timber-type growth form. . . That tree was then to be vegetatively propagated. . . . Another reason for the lack of success in the chestnut breeding programs was that selection of trees to be crossed was based on appearance alone. Early workers conducted no progeny tests. (Burnham, Rutter, and French, Plant Breeding Reviews Vol. 4, 1986, pp. 367 and 369)

Burnham recognized that backcrossing to an unimproved species was different from backcrossing to a crop plant.

> When applying this [backcross] method to an unimproved species, the variation in the recurrent parent must be sampled by using many individuals from different source populations. If the species being improved has a wide geographic range, a program may be needed for each major region.

He proposed that a program be started to transfer blight resistance from the Chinese species to American chestnut via 3 backcross generations, with selection for both blight resistance and the American type in each generation. This was to be followed by two open-pollinated generations -- one to create BC_3F_2 trees, some of which should be homozygous for the resistance genes, and the second to produce BC_3F_3 nuts in a seed orchard. Besides blight resistance, the other qualities of the American chestnut that were to be captured in the backcrossing were the American's timber-type growth habit, coldhardiness, competitive ability in forest-type plantings, and nut quality (sweetness). Burnham also suggested a program to backcross coldhardiness and nut

quality (sweetness) from American chestnut into Chinese chestnut (Burnham, Rutter, and French pp. 374-376.)

A group primarily of Minnesotans incorporated The American Chestnut Foundation in 1983 to raise funds to support the breeding program. A tireless contributor to the early fund-raising efforts was Phil Rutter of Badgersett Farm in Minnesota. A big breakthrough for the Foundation came in the late 1980's, when Rutter spoke at Scientists' Cliffs Conference Center in Maryland. In the audience was Cheri Wagner, who with her sister Jennifer and mother Anna Belle owned a family farm in southwestern Virginia. The Wagners offered a 30-year lease of 20 acres of their pasture land with the option to buy after 20 years. A second happy event was the hiring of Dr. Fred Hebard, a plant pathologist, as the farm superintendent. Fred had spent most of his adult life in training and work to restore the American chestnut tree. The Wagners agreed to include a barn and their original family house as part of their lease, and in 1989 Fred and his family moved into the house and began plantings on the farm.

In the mid-1990's, when the Wagner farm was almost full of trees, Mrs. Glenn C. Price, another long-time resident of Washington County, Virginia, provided the funds to purchase and endow a new farm of 93 acres, located only 2 miles from the Wagner farm, to honor her late husband. The first plantings on the Price Farm were in 1996.

In 1995 a 3-year grant from the Pew Foundation permitted the hiring of a second scientist, Dr. Yan Shi, who was a plant breeding graduate of the Univ. of Arkansas. When Dr. Shi left in 1997, he was replaced with Dr. Paul Sisco, a maize geneticist formerly with the USDA/ARS in Raleigh, NC. This spring a recent graduate of the Auburn School of Forestry, Peter Wood, was hired to be the Farm Technician.

The Foundation hired an Executive Director, John Herrington, soon after the Wagner Farm was leased. John chose to establish the administrative offices of the Foundation in Bennington, Vermont, which was his home. When John left to pursue other interests in 1997, the Foundation had another lucky break in getting a replacement. Marshal Case, an experienced fund-raiser for both the Audubon Society and the Crane Foundation, was just moving to the Bennington, VT, area and accepted the position. He has vigorously pursued the establishment of regional programs in several states and membership growth. The Foundation presently has 3270 members, with the largest concentrations in New York and Pennsylvania (about 500 members each).

Several state chapters are incorporated as separate entities, including New York, Pennsylvania, Maine, Indiana, and Connecticut. Pennsylvania, Maine, and Indiana have set up regional breeding programs in their own states, while New York has opted to support a genetic engineering effort based at the SUNY College of Environmental Sci. & Forestry at Syracuse (Drs. William Powell and Charles Maynard).

In 1998 a long-time major contributor to the Foundation, Mr. Bradford Stanback of Canton, NC, provided funds to set up a satellite office in Asheville to increase interest and membership in the southern Appalachian region. Recently a provisional chapter has been formed in that area.

1998 - 1999 also saw the development of a Strategic Plan for the Foundation.

The seven goals of the Foundation, as stated in that plan, are:

1. To breed genetically diverse blight resistant nuts for initial distribution in 2006.
2. To reintroduce the trees into the forest in an ecologically acceptable manner.
3. To develop funds and other assets from a variety of sources.
4. To diversify and strengthen leadership.
5. To educate the general public and increase awareness of and visibility for the foundation.
6. To generate scientific knowledge by conducting research, fostering science-based learning, and sharing with other disciplines.
7. To grow and enhance membership.

II. Breeding Strategies

A. Meadowview Research Farms, Virginia

As the first and primary site for the Foundation's efforts, Meadowview coordinates the work at all the other breeding sites. The breeding strategy has followed Burnham's plan precisely up to this point, with many trees of the BC_3 generation ready for disease screening and intercrossing within the next five years. The first plantings of BC_3F_2 trees in a seed orchard setting should occur in 2002, three years from now.

Sources of blight resistance:

Burnham, Rutter and French (1986) suggested several possible Chinese, F_1, and BC_1 sources of resistance for the breeding program (pp. 376-385). Fred Hebard, the staff pathologist who has conducted the breeding program at Meadowview since its inception, chose three for his main effort -- Clapper, Graves, and Nanking. Molecular marker analysis has indicated that the Graves source of resistance has at least some resistance genes different from those of the Nanking source. Mapping of loci correlated with resistance from the Clapper source is now underway at the U.S. Forest Service's Southern Institute of Forest Genetics in Saucier, Mississippi. (See the Molecular Marker section below).

1. **The Clapper Tree**. This is a BC_1 ([C x A] x A) tree named after R.B. Clapper of the USDA Bureau of Plant Industry and was from a test planting in the Crab Orchard Wildlife Refuge, Carterville, Illinois (Burnham et al., 1986, p. 378). It was a good forest type tree, and although it eventually died from blight, grafted progeny are still living at the Lockwood Farm of the Connecticut Agricultural Experiment Station. Clapper is partially inbred, since the same American tree (Forest Pathology 555 at Glendale, MD) was used in both the initial cross and the backcross. Its Chinese parent is listed as PI 34517, M16 tree, Bell, MD, collected in Tientsin, China, in 1912. This Chinese parent is no longer available, as far as we know. The full pedigree of the Clapper Tree is ([PI 34517 x FP 555] x FP 555). McKay and Jaynes describe its origin and characteristics as follows:

> [The Clapper Tree] was the outstanding planting of nearly 2,000 planted in 15 cooperative test plantings. The Clapper variety averaged 2.6 feet of height growth the first 17 years and the trunk was 7.3 inches in diameter 4.5 feet from the ground. (McKay and Jaynes, Chestnuts, Chapter 19 in R.A. Jaynes, ed., *Handbook of North American Nut Trees*, North Nut Growers Association, 1969).

2. **The Graves Tree.** This is a BC_1 ([C x A] x A) tree named after Arthur Graves of the Connecticut Agricultural Experiment Station (CAES). The full pedigree of Graves is ([Mahogany Chinese x FP 551] x Bowman Tree, Clinton Corner, NY). FP 551, like the FP 555 parent of the Clapper Tree, was a wild American chestnut tree growing near the Glendale, MD, USDA breeding station. The Graves tree is located at the CAES SGSP West Red Pine Lot R13T1. Its F_1 parent was also at the CAES -- SLotR2T8. Graves is from cross 37-53 made in 1953 and was selected from 9 nuts. The Mahogany Chinese grandparent of Graves is available and was used as the parent of a genetic mapping population at the Meadowview Farm.

3. **Nanking** Chinese. Fred Hebard chose Nanking as the most highly resistant graft-propagated Chinese cultivar based on previous work by Gary Griffin (Va Tech) and his students, as well as by Hebard's own studies (Hebard et al., 1984, Phytopathology 74:140). According to Jaynes:

> Nanking, perhaps the most widely planted of all Chinese chestnut cultivars, originated from seed collected by Peter Liu at Hang Chow, China, and [was] imported in 1935. . . After propagation and testing [as Seedling No. 7930] Nanking was named and released by the USDA in 1949. Nanking is second only to Crane in precocity, grafted trees frequently bearing nuts in their second year. The tree bears heavy crops annually. The Chinese cultivar Kuling, also released by the USDA in 1949, was from the same seed lot. (Chestnuts, Chapter 9 in R. Jaynes, ed., *Nut Tree Culture in North America*, Northern Nut Growers Assoc., 1979)

4. **Other Chinese sources of resistance.** Although cultivars and accessions of several *Castanea* species are planted at the Meadowview Farm, breeding work has concentrated on resistance from the Chinese (*C. mollissima*) species. Resistance levels vary widely among Chinese chestnut trees, but the most resistant Chinese are the best sources of resistance known. Currently at the Meadowview Farms there are eight sources of resistance advanced to the BC_2 generation, eleven to the BC_1, and 18 to the F_1. In addition to the Clapper, Graves, and Nanking sources, other Chinese advanced to at least the F_1 are Meiling and Kuling, Selections #72-211 and 65-4 from Greg Miller, Empire Chestnut Co., Orrin, a named cultivar, and Chinese Acquisition 99 (from a group of *C. mollissima* collected in China by Phil Rutter in 1989). However, because we have attempted to get at least 20 independent American lines for each source of resistance and because space at the Meadowview Farms is limited, the bulk of the breeding at Meadowview has been with Clapper, Graves, and Nanking.

5. **Large surviving Americans**: In his Ph.D. work with Gary Griffin at Virginia Tech, Fred was able to show by progeny tests that some surviving American chestnut trees do have low levels of heritable blight resistance. Dr. Griffin and his colleagues have taken the tack of intercrossing large surviving American trees (LSA's) to build up blight resistance in a pure *C. dentata* population. Their organization devoted to this strategy is called The American Chestnut Cooperators

Paul Sisco –
Personal Copy

The Bark

NEWSLETTER OF THE AMERICAN CHESTNUT FOUNDATION • Fall 1990

Hello, Members of the American Chestnut Foundation,

It has been a great summer for the Foundation and I hope a great one for all of you as well. Thanks to all of you who have been kind enough to fill out the survey that was sent with the last issue of the *Bark*. For those of you who forgot, or lost it or whatever, we include another copy with this issue. Please, if you haven't filled it out, take a minute and do so. This survey is our only real way to make sure that we are headed in the right direction with respect to our members. We hope to have the results tabulated and discussed at the annual meeting in October (more on the meeting on the next page).

Mark Michaud
Secretary

Meadowview Report

Things are going great in Meadowview. We have had abundant rainfall the past two years. More than 640 seedlings planted this year are at least a foot tall now. About 140 trees planted last year are thriving, as are about 50 grafts made this year. I encourage you to visit and see our progress. We are located ¼ mile southeast of Exit 10 (not Exit 10A) on Interstate 81 in Meadowview, Virginia, across the street from the Meadowview Elementary School tennis courts.

We were fortunate to have help with the grafting from Tom Jayne, formerly of the U.S. Department of Agriculture's Plant Introduction Station in Glen Dale, Maryland. Glen Dale is where the original USDA chestnut breeding work was conducted. Tom also has been instrumental in helping us locate and map the remaining trees from the old USDA program.

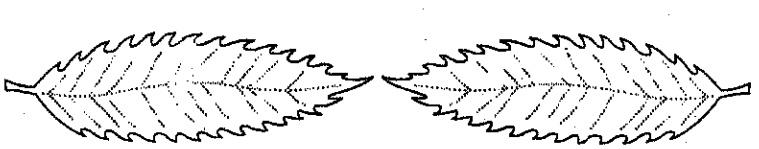
Our summer intern, Andy John, plant breeder Larry Inman and I made 900 controlled pollinations this year, 413 on American chestnut sprouts growing in the mountains near Meadowview, 355 at the Connecticut Agricultural Experiment Station in New Haven, and 132 at the Virginia Tech Horticultural Research Farm. Hopefully, we'll harvest at least 900 hybrid nuts from these pollinations. Mark Widrlechner, Paul Galloway and Phil Rutter made comparable numbers of crosses using pollen we sent them.

We were especially fortunate this year because Dr. Sandra Anagnostakis at the Connecticut Station discovered a large first backcross (¾ American, ¼ Chinese chestnut) in an overgrown area of Arthur Graves' old plantings. It has been named the Graves tree. It has good forest-tree form and is comparable in blight resistance to the Clapper tree, another first backcross. We used pollen from the Graves tree to produce ⅞ American chestnut second backcrosses. If we get a good harvest, some of these should be available to Foundation members. Remember that about a ¼ of these trees will be intermediate in blight resistance between Chinese and American chestnut and the remaining ¾ will have lower levels of blight resistance. We also intercrossed the Graves tree with the Clapper tree to demonstrate recovery of highly blight resistant chestnut trees from backcross trees and separation of high blight resistance from poor form.

Dr. Anagnostakis has been working very hard clearing out the old chestnut plantings in Connecticut, and determining the pedigree of the surviving trees. In addition to the Graves tree, she also has located several first hybrids of Chinese and American chestnut which have been incorporated into our breeding work. Her efforts have helped save many years of work, and I would like to take this opportunity to thank her.

Thanks to your support, the farm in Meadowview is in full operation, with a basic complement of equipment necessary to bring back the American chestnut tree. The farm is now the largest chestnut breeding facility in the country, both in terms of acreage and in number of seedlings grown per year. I hope you are encouraged to continue supporting our efforts.

Fred Hebard
Superintendent, Meadowview



Foundation (Web site: http://ipm.ppws.vt.edu/griffin/accf.html) Some of these LSA's have been crossed to normal, susceptible American chestnut trees and the progeny of these crosses then planted at Meadowview. The progeny of some crosses include trees with detectable levels of resistance (Hebard, unpublished).

6. **Irradiated American chestnut trees**: Certain M_2 progeny of irradiated American chestnut trees appeared to have blight resistance in experiments conducted by two chestnut pathologists: Dr. Dennis Fulbright of Michigan State and Dr. Bill MacDonald of West Virginia University. Some of these M_2 progeny have been planted at the Meadowview Research Farms, but so far their levels of blight resistance have not been impressive.

Sources of American chestnut germplasm:

1. Number of lines needed: Based on his reading of several papers by population geneticists (*e.g.* Namkoong, Annu. Rev. Phytopathol. 29:325-342, 1991), Fred decided that a minimum number of 20 lines would be needed per source of resistance to capture a reasonable amount of the genetic variation in a geographic region. Each American tree crossed to the original sources of resistance (Clapper, Graves, and Nanking) was defined as the ancestor of a line. Clapper and Graves BC_3 lines share no more than one common grandparent, whereas the BC_3 lines from Nanking will be even more distantly related.

2. American chestnuts for breeding: In 1989, his first year of crossing, Fred used American chestnut trees available at the Lockwood Farm of the Connecticut Agric. Expt. Station. to cross to grafted clones of the Clapper Tree. Seven of the 19 Clapper lines produced through 1998 are offspring of these Connecticut trees (which themselves were progeny of nuts from the Midwest.) After 1989 he began using American chestnut trees found in 4 to 10-year-old clearcuts in the Jefferson National Forest, which encompasses the highest mountains of Virginia and is within easy driving distance from Meadowview. Fred deliberately chose trees from various elevations in the national forest -- from 2,300 feet (approximately the elevation of the Research Farm) to greater than 4,000 feet. Chestnuts (and the related chinquapin -- *C. pumila*) proved to be abundant in the forest, and over the years Fred has used trees from over 30 different clearcuts. Another tree that is the ancestor of many progeny of the Graves tree is the Paul Galloway tree from Walpole, New Hampshire, an American chestnut about 70 feet tall with a very upright branching habit.

An important point about the Americans used at the farm is that for the most part they are either trees found wild in the mountains or trees grown at the farm from seed collected in the mountains. Thus there should be a minimum of selection for farm conditions.

New insights and problems that have arisen:

Nicking between the farm and the mountains: As trees planted at the Meadowview farm began to mature and flower, it became apparent that they were flowering earlier than their parent trees in the mountains. As early as the first year, 1989, Fred had started plantings at the Research Farm of nuts collected in mountain sites. These American trees at the farm have been used as parents in recent years, especially in production of the BC_3 generation. However, there are drawbacks to making crosses to Americans at the farms. In the mountains any contaminating pollen would be from pure American chestnut trees, whereas at the farm pollen can be from many genetic backgrounds. Thus pollen contamination at the farm generally destroys the value of a cross while contamination in the mountains often can be tolerated.

Male-sterility: Male sterility, most likely of the cytoplasmic/genic type, has shown up repeatedly. Analysis indicates that it is caused by an incompatibility between American cytoplasm and Chinese nuclear genes (Shi and Hebard, J. Amer. Chest. Fndtn., 1997, 11:38-47) The key observation is that male sterility shows up in crosses of American x Chinese, but not in the reciprocal cross between the same two parents. Both the Clapper and Graves BC_1 trees had Chinese cytoplasm and were male-fertile. When they were used as males on American trees, however, the BC_2 progeny had American cytoplasm, and a number of the trees were male-sterile. While the genetic analysis of fertility restoration is still underway, the data seem to indicate that nuclear restorer genes from the American parents are recessive to the Chinese non-restoring genes. In one set of BC_1 progeny from the Nanking source of resistance a Chinese nuclear male-sterility factor was localized by molecular marker analysis (See table in Molecular Marker section). A few of the most resistant trees in each line have been male sterile, particularly in progeny of the Graves tree, but we have been able to use these trees as females to advance to the next backcross generation. The indications so far are that there is no undesirable linkage between male-sterility and blight resistance.

The Clapper defect: A varying proportion of each Clapper BC_2 and BC_3 family has trees with symptoms that are known collectively as the Clapper Defect. These symptoms vary from extreme dwarfing and rough bark to milder necrotic or mottled lesions on the stem. This defect passes through pollen, so it is unlikely to be a virus. We do not know if there is a genetic cause. We also encounter varying proportions of rough bark with different symptoms in the progeny of other crosses, usually to a lesser extent than in Clapper progeny. These defects may be related to differences in lenticel morphology between the Chinese and American species.

Blight resistance - factors from American chestnut: In 1998 we obtained convincing evidence that some American chestnut parents yield backcross trees with better blight

resistance than occurs on average. This is evident for the cross CC1 x Clapper, which had significantly smaller cankers than the two other Clapper families in that test. There had been hints in previous years that some American parents yield progeny with improved resistance, but we generally have not had sufficiently large families to confirm it statistically, where pollen contamination was not also a potential confounding factor. Interestingly the improved blight resistance of the CC1 x Clapper family was much more evident when its members were screened for blight resistance at 4 years of age than at 1 or 2 years of age.

We obtained additional evidence that some American parents yield backcross progeny with better than average blight resistance from molecular mapping of one first backcross family. The family's source of blight resistance was an F_1 from the Nanking variety of Chinese chestnut. and its American parent the tree known as Mill Creek H. The most prominant marker associated with blight resistance in this family came from the American side and mapped to linkage group D.

Both the Musick first backcross family (also derived from Nanking Chinese chestnut) and the CC1 x Clapper family were noticeably lacking in progeny with defective bark as discussed above under The Clapper Defect. The Musick family, like the CC1 x Clapper family, had higher blight resistance than most other backcross families we have tested, of which there are perhaps 50. Most progenies in those other families had defective bark. It is unclear at present whether this phenomenon was related to the improved blight resistance of the Musick and CC1 x Clapper families.

A single, strong factor controls time of leaf emergence:

Molecular marker analysis in all three mapping populations showed a strong effect ($p<10^{-10}$) for leaf emergence, apparently a single gene on Linkage Group L. The Chinese allele from both Mahogany and Nanking is dominant and causes early leaf emergence. This was a surprise, since leaf emergence appears to be a continuous trait. However, at the half-way point of emergence (about April 29 in recent years) all the Chinese and F_1 trees have emerged leaves whereas almost all the pure American trees do not. Emergence scored at this half-way point had the strong effect seen in the marker analysis (Table in Molecular Marker Section).

Early generation testing for blight resistance:

F_2's and number of factors involved in blight resistance. In 1993 we screened about 700 F_2 trees for blight resistance. The data from the experiment are presented in Figure 1. A summary is also presented in Table 1. We found, as expected from numerous previous studies, that cankers on Chinese chestnut were significantly smaller than those on American chestnut, and that cankers on F_1 hybrids between American chestnut and the highly blight resistant cultivar of

Chinese chestnut Nanking were intermediate between the two parents. However, the cankers on the F_1s were closer in size to those on the American chestnut than those on Chinese chestnut.

We recovered about 12 highly blight resistant trees from 184 Mahogany Chinese x American F_2 progeny (Fig. 1). This result suggested that two incompletely dominant genes might control blight resistance. More important than the exact number of genes involved or their pattern of expression, the fact that we could recover highly blight-resistant progeny at such a frequency indicates that only a few factors control blight resistance and we should be able to backcross them into American chestnut.

The data also suggest that Nanking Chinese chestnut is homozygous for the genes for blight resistance, because the standard deviations of canker sizes in its F_1 progeny from hybridization with American chestnut were similar in size to those on the other parental types in the cross (Table 1). Numerous additional tests have supported this hypothesis, which would be expected if resistance to blight is not completely dominant and Nanking is highly resistant. Furthermore, as expected, the standard deviations of canker sizes in the F_2 progeny were larger than those in the parental types, while those in the BC_2 progeny were intermediate between the parental types and the F_2s. A simple Wright's analysis of these data suggested that 1 or 2 factors were controlling blight resistance. One defect of this test was that we did not have grafted clones of Mahogany Chinese chestnut nor F_1 hybrids of Mahogany with American. Those trees have been difficult to generate, due to pollen contamination and problems with vegetative propagation.

Table 1. Number, mean and standard deviation for the average length and width in August, 1993, of one canker each of two isolates of *Endothia parasitica* on parental types and crosses of Chinese and American chestnut

Source of Blight Resistance	Cross Type	Canker Statistics					
		Isolate SG2-3			Isolate Ep155		
		N	Mean cm	SD cm	N	Mean cm	SD cm
American	parental	10	6.1	1.2	10	6.2	1.2
Nanking x American	F_1	9	4.9	1.0	9	5.4	1.1
seedling Chinese	parental	12	3.4	1.0	12	4.3	1.0
Meiling	parental	4	2.2	0.4	5	4.2	1.0
Nanking	parental	5	1.3	0.2	5	2.1	1.0
Mahogany	F_2	184	4.3	1.5	184	5.4	1.6
Mahogany	BC_2	51	5.4	1.3	53	6.2	1.4
Clapper x Mahogany	BC_1-F_2	392	4.2	1.5	394	5.2	1.5

The parental checks had an average variance of about 1.1 cm, while the straight F_2s had an average variance of about 2.4 cm. Assuming the environmental variance was equivalent to the variance of the parental checks, these data indicate that the broad-sense heritability of blight resistance in this test was about 54%.

Homozygosity of blight resistance in F_2s. Some of the highly blight-resistant B_1-F_2s and straight F_2s were test crossed to American chestnut. There was considerable pollen contamination in some crosses, but not others. The more contaminated crosses were discarded. Resistance screening of the remainder revealed some trees almost as susceptible to blight as American chestnut, indicating segregation for blight resistance. Analysis of these trees with RAPDs indicated the occurrence of residual pollen contamination, so the results of the entire test are inconclusive. However, there was more evidence that progeny of the highly blight resistant Clapper x Graves B_1-F_2 were segregating for blight resistance compared to progeny of the straight Mahogany F_2s, some of which may not have been segregating for blight resistance. This suggests weakly that some of the Mahogany F_2s were homozygous for genes for blight resistance and that Clapper and Mahogany have different genes for blight resistance. That second highly tentative conclusion is bolstered by the fact that these two cross types differ in canker morphology on blight-resistant progeny.

Pollen contamination is now much less of a problem than formerly because we know better when to place bags on female flowers (as soon as styles begin to emerge), and we can monitor flowers more closely here at the Research Farms than at remote locations 100 to 700 miles away. We also have learned to increase our desired progeny numbers for test crosses from 50 to 100.

Backcrosses to Chinese. Results for blight resistance screening of some backcrosses of Chinese x American hybrids to Chinese are presented in Figure 2. Mean canker size for a backcross of a Mahogany F_1 to Mahogany was almost identical to that for a backcross of the Mahogany-derived Graves B_1 to Mahogany. This suggests the number of resistance factors in Graves is the same as in the F_1. However, a backcross of that F_1 to American may have yielded a few progeny with more blight resistance than we have found in most backcrosses of the Graves B_1 to American. Mean canker size on progeny from a backcross of a Nanking F_1 to Nanking was significantly larger than in the corresponding Mahogany backcrosses. However, there may have been pollen contamination in that cross and in the Nanking F_2. We did recover one highly-blight resistant tree from the Nanking F_2; it is unlikely there was contamination with Chinese pollen in those crosses.

Backcrosses to American. Results for a fairly typical screen of B_2 progeny of the Graves and Clapper trees are presented in Figure 3. This particular test was done in 1998; we have performed similar tests every year since 1994. Progeny with adequate levels of blight resistance were obtained in all families tested. The mean canker sizes of B_2 families were generally

significantly less than that for seedling American chestnut trees and F_1 hybrids between Meiling' or Nanking Chinese chestnut and American chestnut seedlings. Generally, Clapper families have slightly smaller mean canker sizes than Graves families.

There was one unusual result that was very clear, for the first time, in this 1998 test. Namely, the CC1xC family had a significantly smaller mean canker size than those from other families, including other Clapper families. This result suggests that the CC1 parent was contributing some factor that was modifying the phenotypic expression of blight resistance in this family.

The CC1xC family, by coincidence, happened to be the Clapper family Paul had chosen for genetic mapping of the Clapper tree, because we had large numbers of progeny in the family (these large numbers of progeny in the CC1xC family, as well as in the AC1xG and CC3xG families, were an additional reason why we referred above to this screen as fairly typical rather than just typical). This contribution of the CC1 parent unfortunately may obscure the mapping of blight resistance factors from the Clapper tree, but it could uncover other interesting loci.

Age at which chestnut can be screened for blight resistance. As presented in the attached paper, "The American Chestnut Foundation breeding plan: beginning and intermediate steps" (Appendix B), we originally thought that F_1 hybrids and other trees intermediate in blight resistance between Chinese and American chestnut would have canker sizes closer to American chestnut when they were young whereas they would have canker sizes closer to Chinese chestnut when they were older. Results from two separate experiments conducted in separate years which support this hypothesis are presented in Figure 4. Thus we initially planned to screen backcrosses, which have intermediate levels of blight resistance, when they were old and F_2 progeny, which have low levels of blight resistance, when they were young.

However, when we tested this experimentally using a randomized complete block design, we found the exact opposite result! This is illustrated in Figure 5. This would suggest that we should screen F_2 progeny when they are older and backcross progeny when they are younger, since we would more easily be able to distinguish the resistance classes important to identify in the experiment.

There are some flaws in this logic however. For one thing, in the experiment illustrated in Figure 5, the F_1 hybrids that were 2 years old or younger all died as a result of the inoculations, before we could have used them for further breeding. So we would be "throwing the baby out with the bath water" if we were to screen backcross progeny for blight resistance before they were 3 years old. Currently, we plant new first and second backcross progeny at spacings designed for screening them for blight resistance when they are 3 years old. Thus we have tightened spacing within rows from 7 feet to 4 feet, saving considerable room. We still maintain between row spacings at 20 feet to facilitate entrance of machinery. However, since screening backcross

progeny for blight resistance when they are 4 years old has worked up to this point, we are retaining that age for our third backcross progeny, trying to be prudent. With regard to test crosses to American not intended for further breeding, it may be prudent to screen them for blight resistance when they are 2 years old.

With regard to F_2 progeny, because we need many more progeny to recover homozygotes than heterozygotes, we need to keep them tightly packed so as not to use too much space, and also not to leave the selected trees widely scattered. Currently, we are planning on screening F_2 progeny for blight resistance when they are 3 years old, but we may cut that to 2 years, which worked in the past, as it allows us to pack trees in at 2 foot spacings within rows.

You may note in Figure 5 that cankers on F_1 hybrids are closer in size to those on American chestnut with the isolate Ep155 while they are closer to Chinese with isolate SG2-3. Use of both these isolates within an experiment allows us to discriminate between more blight resistance classes than use of either one alone, somewhat independent of the age at which trees are screened.

The general trend that canker sizes on Chinese American F_1s are closer to the American than to the Chinese parent in mosts tests suggests that Chinese chestnut, or other highly blight-resistant chestnut, may be a better test-cross parent in some instances than American chestnut, as long as it is the same source of blight resistance (i.e., the same or from the same Chinese chestnut tree).

Fig 1 - 1999 Sci. Review

Figure 1.

Mean Blight Canker Length & Width in Aug and Sept for:

Top Left: Control Trees

Top Right: F_2 progeny from the intercross of two 'Mahogany' Chinese x American F_1s.

Bottom Left: B_1-F_2 progeny from the intercross of the 'Graves' ('Mahogany'-derived) and 'Clapper' (non-'Mahogany'-derived) first backcrosses to American chestnut.

Bottom Right: B_2 progeny from a backcross of the 'Graves' B_1 to American

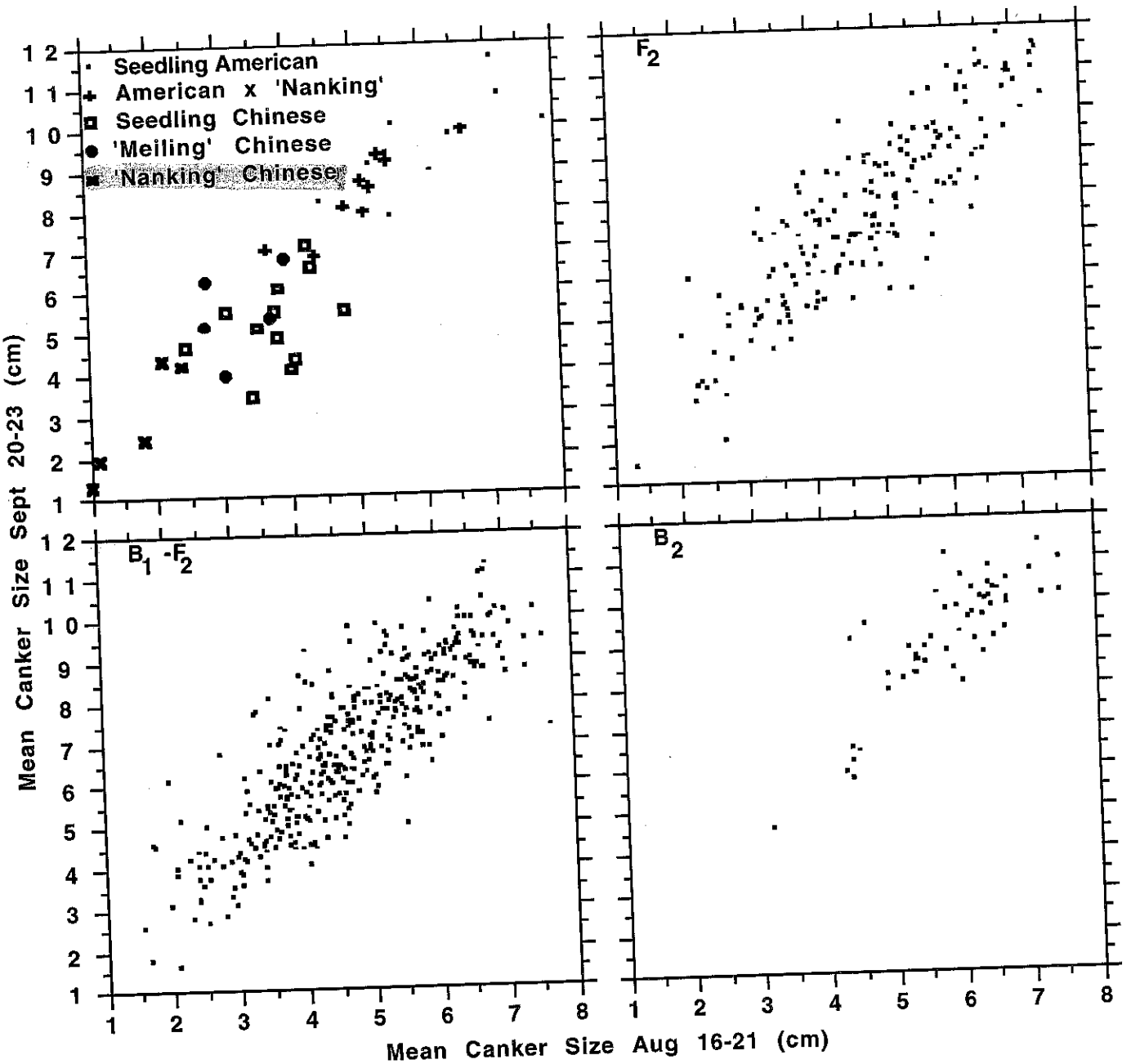


Figure 2. Segregation for disease resistance in backcrosses to Chinese chestnut

Canker size is the mean diameter (two measurements, length and width) of one canker each incited by the *Endothia parasitica* isolates, Ep155 and SG 2-3. The full-sib family pedigrees are:

MCB_1, Mahogany x (Mahogany x American)
MGr, Mahogany x [(Mahogany x American 1) x American 2] = Mahogany x Graves
NB_1, American x (American x Nanking)
NCB_1, Nanking x (American x Nanking)
NF_1, American x Nanking
NF_2, (American x Nanking) x (Nanking x American).

The parental checks are:

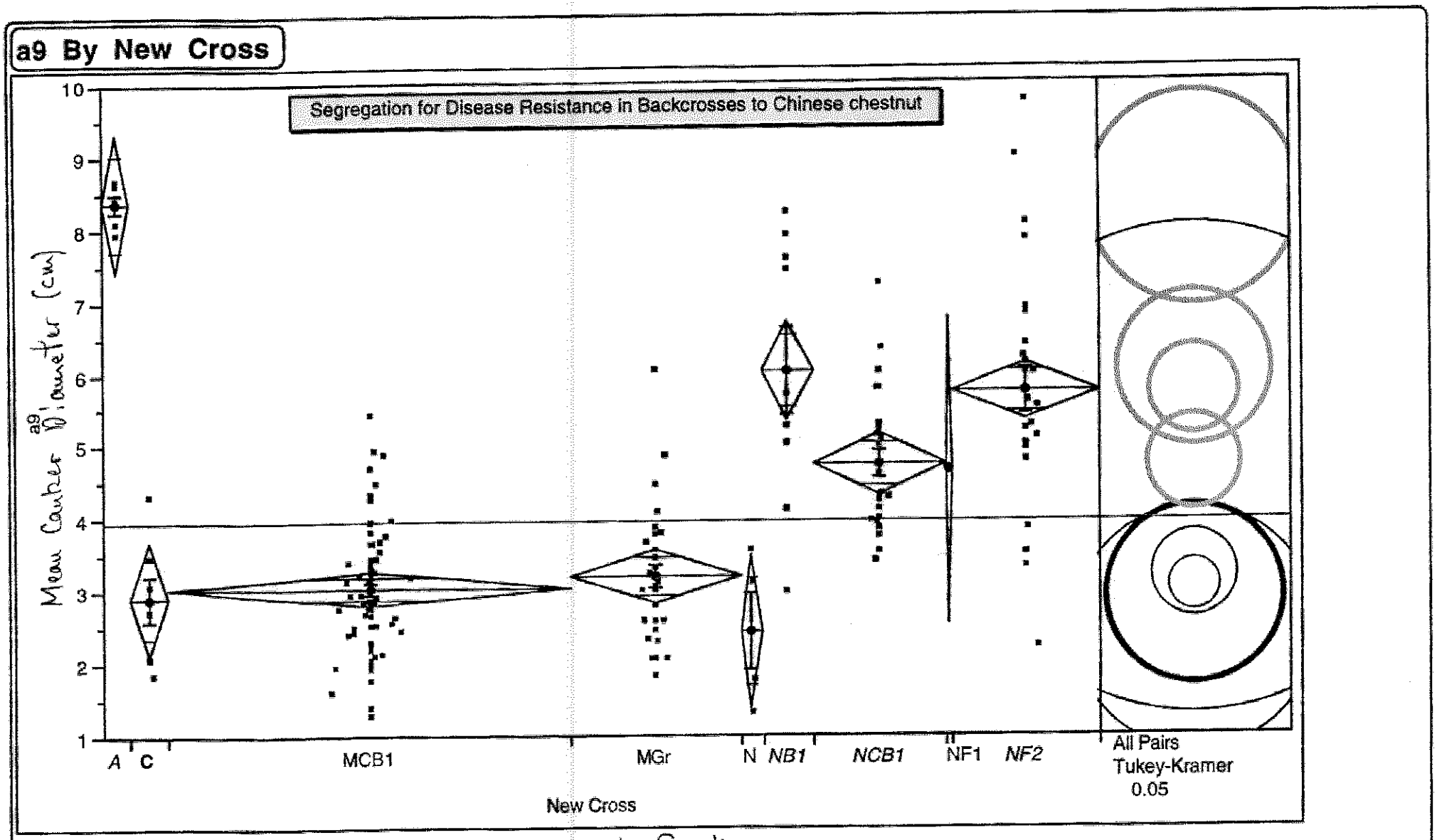
A, seedling American
C, seedling Chinese
N, Nanking Chinese.

The dots represent the mean value for a single tree. The color of a dot is the result of discriminant analysis classification of the canker size as being similar to:

Nanking, orange
seedling Chinese, green
Nanking F_1, blue
seedling American, red.

The means diamonds (in green) illustrate the number of replicates for each mean by their width and the 95% confidence interval by the little bars toward their tips. The blue bars, with a large mean dot, are standard errors of the mean. The circles on the right indicate mean separation; when circles overlap more than 40%, two means are not significantly different.

Figure 2.



Full-Sib Families

Figure 3: Segregation for disease resistance in full-sib BC_2 families

Canker size is the mean diameter (two measurements, length and width) of two cankers each incited by the *Endothia parasitica* isolates, Ep155 and SG 2-3. The full-sib families were fathered either by the Graves BC_1, in which case the male parent is indicated by a G, or else by the Clapper BC_1, indicated by a C. The dots represent the mean value for a single tree. The color of a dot is the result of discriminant analysis classification of the canker size as being similar to:

Nanking, orange
seedling Chinese, green
Nanking F_1, blue
seedling American, red.

The means diamonds (in green) illustrate the number of replicates for each mean by their width and the 95% confidence interval by the little bars toward their tips.

Figure 3.

Segregation for Disease Resistance in Full-Sib BC_2 Families

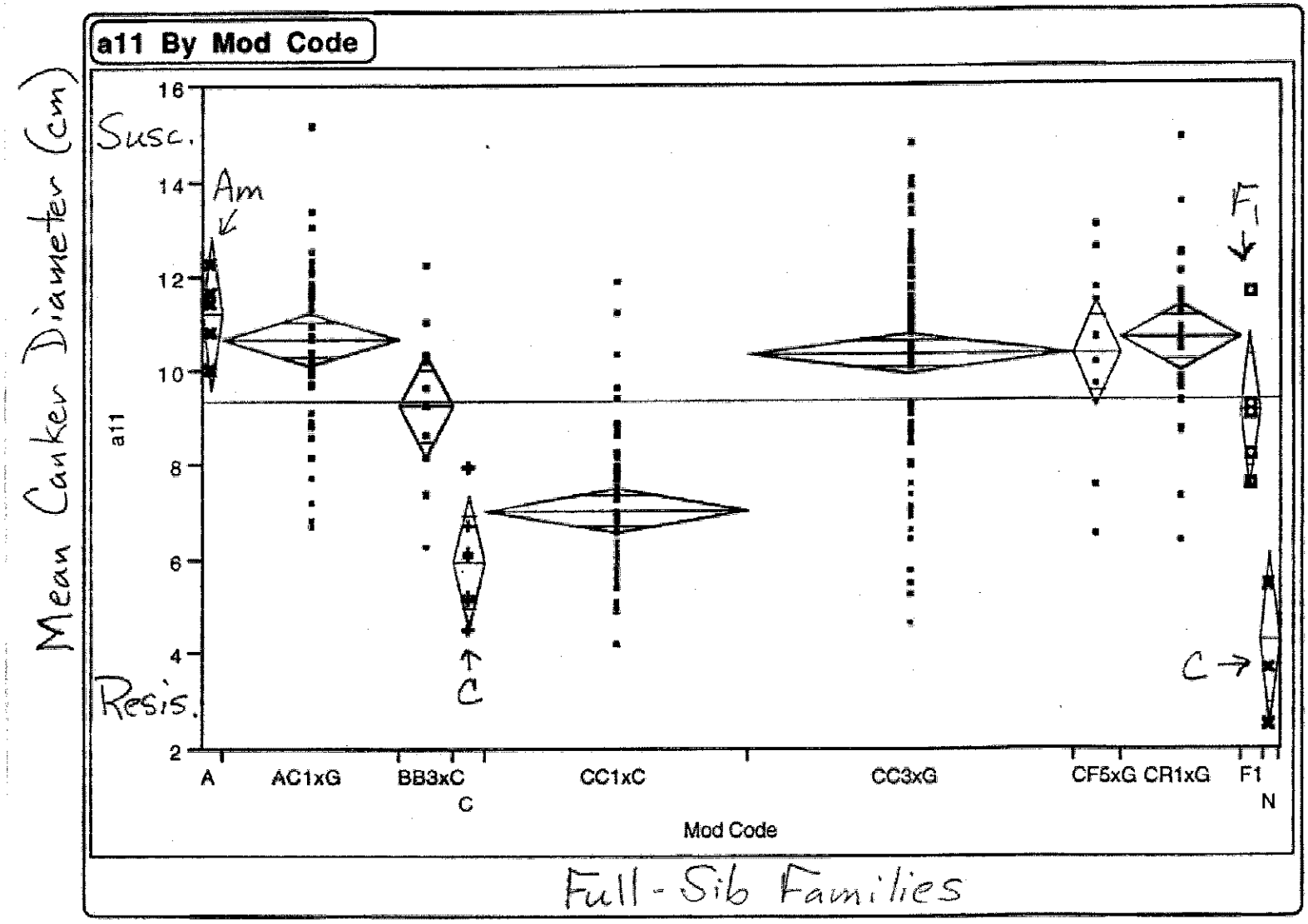
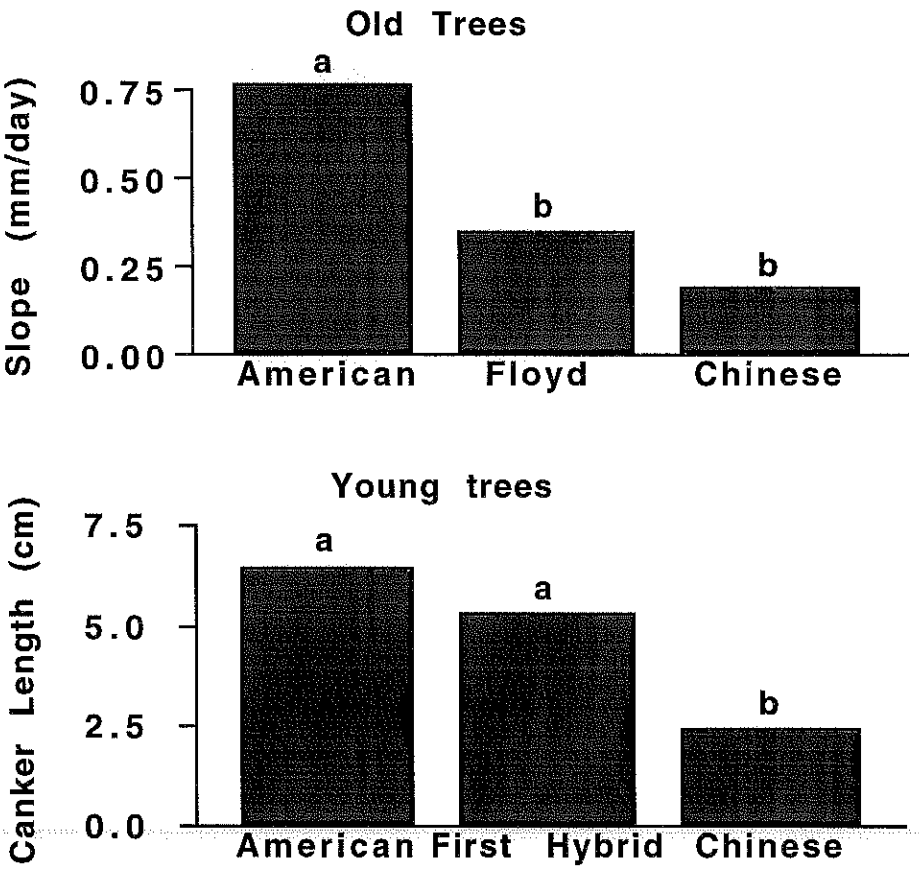


Figure 4.

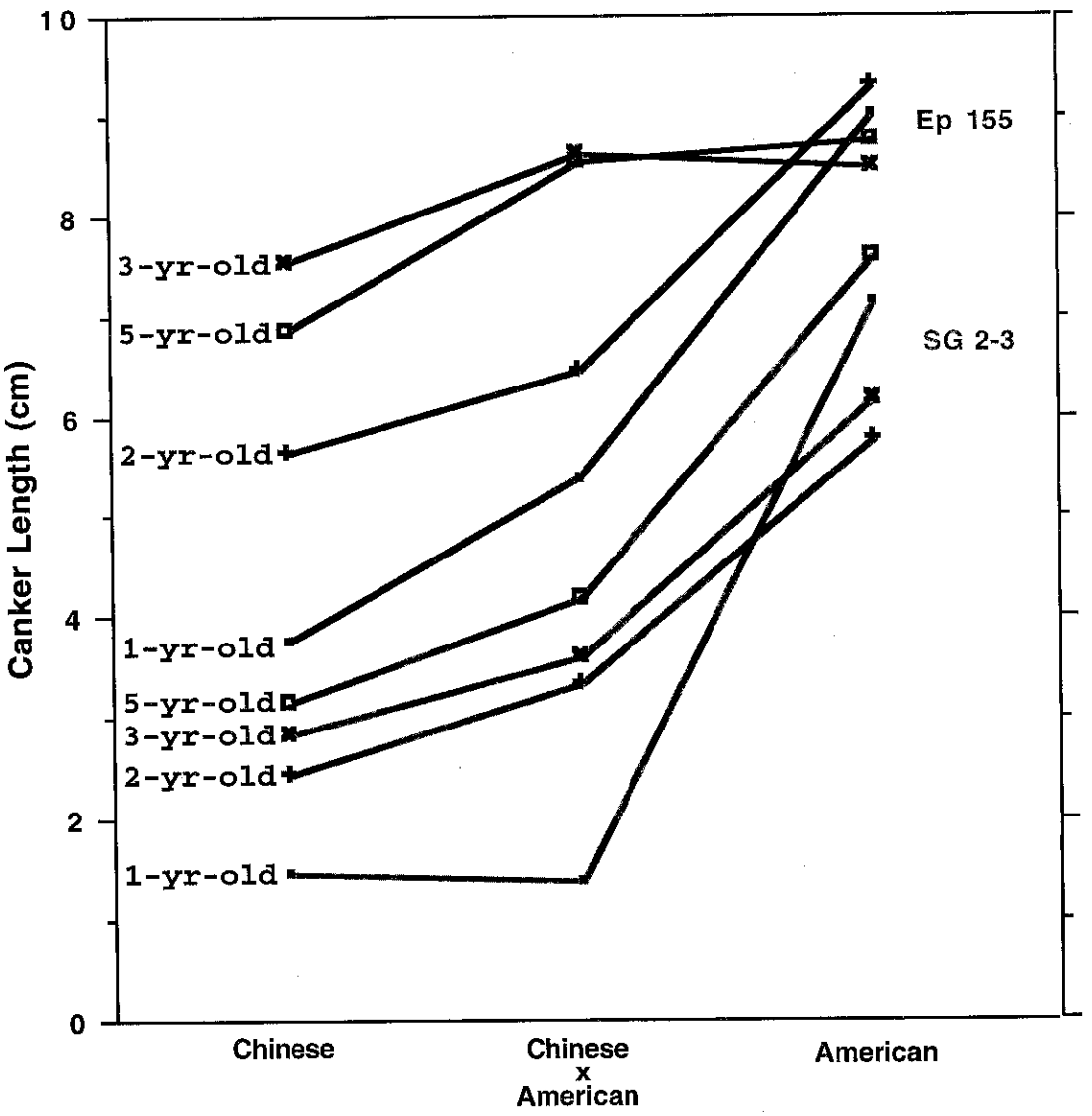
Canker growth statistics for young and old chestnut trees with low, intermediate and high levels of blight resistance.



Bars topped by the same letter are not significantly different ($p<0.05$) by multiple range tests.

Figure 5.

Mean canker length vs resistance for chestnut trees of different ages inoculated with two virulent strains of *Endothia parasitica*, one highly pathogenic (Ep155) and the other slightly pathogenic (SG 2-3).



Selection for American traits to accelerate backcrossing:

Among trees with similar levels of resistance, further selection is done for visual traits that distinguish American and Chinese chestnut trees. These include leaf, vein, and stem hairs, stipule size, bud shape, stem color, time of leaf emergence, and male sterility. The inheritance of these characters was scored by Hebard in F_1, F_2, BC_1 and BC_2 Chinese x American populations, and the results were published (Hebard, J. Hered., 1994, 85:440-446 -- Included in Appendix B). The results indicated that Chinese genes controlling interveinal hairs, twig hairs, vein hairs and stem color were linked, as were another gene for stem color and a gene for stipule size. Hebard concluded:

> The fact that genes for all traits but stipule size were linked to each other helps explain why the complex of hairy leaves and twigs and slightly greenish stems is so prevalent in trees with Chinese chestnut parentage. . . Taken all together, 3.4% of the BC_1s and 8.8% of the BC_2s had all American chestnut morphological traits -- namely, no interveinal hairs; a low density of vein and twig hairs; small stipules; red stems; and cylindrical, pointed buds. . . . Currently, we attempt to grow 75 trees in each backcross line. . . . We expect one-quarter or one-eighth of the 75 backcross trees to possess all major genes for blight resistance. Thus if we select among the blight-resistant progeny for all American chestnut morphological traits, we could only choose one or two individuals, on average, from a BC_2 population if one-quarter are blight resistant and 8.8% have all American traits.

Molecular marker analysis mapped genes controlling some of these traits to linkage groups, including a surprisingly strong single locus effect on time of leaf emergence (See the Molecular Marker section below).

Plans for the intercross generations and seed orchards:

Burnham's plan called for open-pollination of selected BC_3 trees to produce the BC_3F_2's, taking advantage of the fact that some type of incompatibility system prevents most self-pollination. These BC_3F_2's would then be planted closely-spaced in a seed orchard, where all but the most resistant trees would be eliminated (Burnham, Rutter, and French pp. 373-375.). Fred Hebard planned the plantings at the new Price Farm to allow for this open pollination -- BC_3 trees of a particular resistance group (*e.g.* Clapper or Graves) were planted in close proximity and somewhat isolated from trees of other resistance groups. However, Paul Sisco thinks it would be better to intercross the BC_3 lines by hand so that we would know the exact pedigree of each BC_3F_2 family. This would also ensure that any crosses are between lines rather than within lines and that no one line is overrepresented in the seed orchard. The current plan is to eliminate all trees at the Wagner farm and to use it as the seed orchard for the Clapper resistance group. The

place for the Graves seed orchard is still to be determined. The Nanking source is 1.5 generations behind Graves and Clapper.

B. Pennsylvania Chapter Breeding Program (by Bob Leffel)

Overall strategy:

Burnham (1986) stated that the ultimate goal of TACF is to establish breeding populations of blight resistant American chestnuts, each of which will be adapted to a different growth zone in the natural range as described by Inman in 1987 and 1989 in the Journal of The American Chestnut Foundation. In 1995, the reorganized Pennsylvania Chapter of The American Chestnut Foundation (PA-TACF) initiated a backcross breeding program to produce blight-resistant American chestnuts adapted to PA, coordinated locally by Dr. Robert Leffel (USDA-ARS Research Agronomist, retired) and his wife, Ann, with the guidance of TACF Staff Pathologist Dr. Fred Hebard. A nucleus of dedicated PA-TACF members has been trained by Dr. Hebard in hybridizing, mud-packing and inoculation techniques.

The PA-TACF breeding program, 1995-98, was summarized by Leffel and presented by Hebard at the 2nd International Chestnut Symposium, Bordeaux, France, October 1998. Procedures followed were those presented by Hebard (1994) as the beginning and intermediate steps of the TACF breeding plan. Maps 1 and 2 depict the locations of all backcross and American orchards seeded to date by PA-TACF.

The BC_3, BC_2, and BC_1 seeded by PA-TACF, 1995-99 is summarized in Table 1 by orchard location. Table 2 summarizes these seedings by source of resistance, and BC generation. Current stands in each orchard are requested of cooperators. In general, stands have been satisfactory. Exceptions include Longwood Gardens, where the BC_3 orchard is within the fenced nursery, a very level site, possibly inadequately drained for good performance by American chestnut, and Buffalo Mills BC_1 orchard which was seeded in pots but never transplanted into orchard by cooperator member because of family emergency.

Emphasis in the PA-TACF has been production of 20 BC_3 lines of Clapper origin' summarized in Table 3. Ten full and five partial lines were obtained through 1998 and the goal of 20 or more Clapper BC_3 lines is dependent on success of 1999 hybridizations. The total of 2264 Clapper origin BC_3 seed obtained through 1998 have been planted in nine orchards (Table 1). Leffel prepared a 14 April draft of "Breeding Blight Resistant American Chestnuts for Pennsylvania" a proposal and planting plan for a Pennsylvania seed orchard. This seed orchard would consolidate all BC_3F_2 of intercrosses among screened, selected trees of 20 BC_3 lines of Clapper origin (copy attached). The ten single-cross X 800 seed per single cross planting proposed is beyond the capacity of PA-TACF members and current cooperators.

Table 1 - Summary of Backcross Seedings by PA-TACF, 1995 - 99

Planting Year	Backcross Pedigree			Source Resistance	Source of Pollen	Cross Made in		# Nuts Seeded	Planting Location	
	Female	Male	Gen			Region	County		Nursery	# Alive
1995	NxLA	HWA	B1	Nanking	PA	Meadowview		133	Landisburg	
1996	Ort	CL287	B3	Clapper	Meadowview	SE	York	153	Brogue	
1996	Ort	CL287	B3	Clapper	Meadow	SE	York	100	Dornsife	
1996	Ort	CL287	B3	Clapper	Meadow	SE	York	14	CentralCty	
1996	Crouch	CL287	B3	Clapper	Meadow	SE	York	17	Brogue	
1997	Joliett	GR210	B3	Clapper	Meadow	Po	Schuylkill	82	Brogue	
1997	Joliett	GR210	B3	Clapper	Meadow	Po	Schuylkill	82	Dornsife	
1997	Joliett	GR210	B3	Clapper	Meadow	Po	Schuylkill	81	Longwood	
1997	Joliett	GR210	B3	Clapper	Meadow	Po	Schuylkill	82	CentralCty	
1997	Ort	GR226	B3	Clapper	Meadow	SE	York	64	Brogue	
1997	Ort	GR226	B3	Clapper	Meadow	SE	York	64	Longwood	
1997	Dorn2	CL53	B3	Clapper	Meadow	Po	Nor'umbrlnd	57	Brogue	
1997	Dorn 1,2	CL53	B3	Clapper	Meadow	Po	Nor'umbrlnd	59	Dornsife	
1997	Dorn 2	CL53	B3	Clapper	Meadow	Po	Nor'umbrlnd	57	Longwood	
1997	RC 4,6	GR210	B3	Clapper	Meadow	SW	Somerset	54	Dornsife	
1997	RC 2,9	GR210	B3	Clapper	Meadow	SW	Somerset	54	Longwood	
1997	RC3,5,7,8	GR210	B3	Clapper	Meadow	SW	Somerset	52	CentralCty	
1997	GoodSpring	GR210	B3	Clapper	Meadow	Po	Schuylkill	34	Longwood	
1997	Ort	GR137	B3	Graves	Meadow	SE	York	57	Hummel	
1997	Morrow	GR137	B3	Graves	Meadow	SE	Berk	2	Hummel	
1998	StuckT2	GR97	B3	Clapper	Meadow	SC	Snyder	26	Hummel	
1998	Hoover	CL	B2	Clapper	CT-AES	SE	York	9	MoSF	
1998	BSF1,2,4,8	CL	B2	Clapper	CT-AES	SE	York	45	MoSF	
1998	GL48 1,2	CL	B2	Clapper	CT-AES	SW	Bedford	35	MoSF	
1998	Su	CL	B2	Clapper	CT-AES	W	Westmrlnd	38	MoSF	
1998	Joliett	NHR2T2	B2	Japanese	CT-AES	Po	Schuylkill	32	MoSF	
1998	GoodSpring	NHR2T2	B2	Japanese	CT-AES	Po	Schuylkill	16	MoSF	
1998	TBOH	NHR2T2	B2	Japanese	CT-AES	Ohio		37	MoSF	
1998	RC 1	NHR2T2	B2	Japanese	CT-AES	SW	Somerset	3	MoSF	
1998	RC 6	NHR2T2	B2	Japanese	CT-AES	SW	Somerset	13	MoSF	

Table 1 - Summary of Backcross Seedings by PA-TACF, 1995 - 99 (continued)

Planting	Backcross Pedigree			Source	Source of	Cross Made in		# Nuts	Planting Location	
Year	Female	Male	Gen	Resistance	Pollen	Region	County	Seeded	Nursery	# Alive
1999	RC13	CL53	B3	Clapper	Meadow	SW	Somerset	102	Red Clay	
1999	Su 4-10	CL53	B3	Clapper	Meadow	W	Westmrlnd	40	Red Clay	
1999	Su 1,2,3	BE400	B3	Clapper	Meadow	W	Westmrlnd	107	BeechCrk*	
1999	Su 1,2,3	BE400	B3	Clapper	Meadow	W	Westmrlnd	94	Stahlstwn	
1999	GL 42	BE400	B3	Clapper	Meadow	W	Westmrlnd	100	BeechCrk*	
1999	GL 42	BE400	B3	Clapper	Meadow	W	Westmrlnd	88	Red Clay	
1999	GL 42	BE400	B3	Clapper	Meadow	W	Westmrlnd	16	Middlebrg	
1999	StuckT2	GR210	B3	Clapper	Meadow	SC	Snyder	44	BeechCrk*	
1999	StuckT2	GR210	B3	Clapper	Meadow	SC	Snyder	6	Middlebrg	
1999	StuckT1	GR210	B3	Clapper	Meadow	SC	Snyder	132	Longwood	
1999	StuckT1	GR210	B3	Clapper	Meadow	SC	Snyder	100	SouthPark	
1999	StuckT1	GR210	B3	Clapper	Meadow	SC	Snyder	100	Red Clay	
1999	StuckT1	GR210	B3	Clapper	Meadow	SC	Snyder	100	Stahlstwn	
1999	LuPe	AB185	B3	Clapper	Meadow	SC	Perry	6	Red Clay	
1999	GiYo	AB185	B3	Clapper	Meadow	NC	Lycoming	60	BeechCrk*	
1999	PeNo	AB185	B3	Clapper	Meadow	Po	Nor'umbrlnd	25	Stahlstwn	
1999	PeNo	AB185	B3	Clapper	Meadow	Po	Nor'umbrlnd	12	Middlebrg	
1999	Dehaas	BE395	B3	Graves	Meadow	NC	Clinton	152	MoSF*	
1999	Indiantwn Gap	BE395	B3	Graves	Meadow	SE	Lebanon	128	MoSF*	
1999	Michaux 10	BE395	B3	Graves	Meadow	SC	Franklin	79	MoSF*	
1999	Shnando-1	NHR3T5	B1	USDA104061	CT-AES	Po	Schuylkill	92	Quakake	
1999	BR6-138 F1	IG8- Am	B1	Meiling	PA	SE	York	2	Quakake	
1999	GL48-1,2,4	NHR3T5	B1	USDA104061	CT-AES	SW	Bedford	85	BuffaloMills	
								3222		

* Half of seed was potted for planting next spring as 1 yr old trees.

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Table 2 - Summary of Seedings by Source of Resistance and Generation

Source of Resistance	BC Generation	# Seeded	# Trees Remaining 7-20-99
Clapper	B3	2264	
Clapper	B2	127	
Graves	B3	418	
Japanese - NHR2T2	B2	101	
Nanking	B1	133	
Chinese MAU - USDA 104061	B1	177	
Meiling	B1	2	
		3222	

Proposal to Establish Seed Orchard at Penn State - Mont Alto (by Bob Leffel)

BREEDING BLIGHT-RESISTANT AMERICAN CHESTNUTS FOR PENNSYLVANIA
The first cultivar - from Clapper source of resistance

Dr. Charles R. Burnham (1981) proposed a solution to a century-old problem — the restoration of the American chestnut (*Castanea dentata*), eliminated as a forest tree in eastern U.S. by the fungal disease, chestnut blight (*Cryphonectria parasitica*). The solution proposed was the transfer of the blight resistance of other chestnut species to the American chestnut by the backcross method of breeding. Burnham and associates founded The American Chestnut Foundation (TACF) in 1983. Burnham et al. (1986) reviewed the history of breeding blight-resistant chestnuts, presenting the justification and steps for a backcross program to solve the problem. Burnham (1991) stated that The ultimate goal is to establish breeding populations of blight-resistant American chestnuts, each of which will be adapted to a different growth zone in the natural range. The Pennsylvania Chapter of TACF (PA-TACF), reorganized in 1995, initiated a backcross breeding program to develop blight-resistant American chestnuts adapted to Pennsylvania.

Hebard (1994) presented the beginning and intermediate steps of TACF's breeding plan and initiated a cooperative regional breeding program with PA-TACF in 1995, recently summarized by Leffel (1998). Breeders of corn and other agronomic crops have produced progeny from the third backcross that closely resemble the recurrent parent: thus a first release of a blight-resistant American chestnut cultivar is anticipated after three generations of backcrossing and two generations of intercrossing. Clapper, a BC_1 (first backcross) tree from the previous USDA/CT-AES program, survives via grafting at the CT-AES. Hebard hybridized local, SW Virginia American chestnuts at Meadowview Research Farm with Clapper, screened resulting BC_2 trees for blight resistance, and supplied pollen from screened BC_2 trees to PA-TACF, beginning in 1995. PA-TACF has produced BC_3 seed of ten full lines and five partial lines of its initial goal of 20 Clapper source of resistance' BC_3 lines with plans to complete its goal of 20 lines in 1999. The 2241 BC_3 seed from this program were seeded in orchards of approximately 100 to 400 trees each, on seven PA-TACF member properties and at Longwood Gardens, Allegheny County's South Park, and Red Clay Reservation. Plant stands are good at most locations. BC_3 trees seeded in 1996 are scheduled for inoculation with chestnut blight and subsequent screening for resistance in 2000, with each subsequent year's BC_3 trees following until completion of screening of the 20 Clapper BC_3 lines in 2004.

The next step in the program, the intercrossing of the selected moderately resistant BC_3 heterozygotes, is scheduled for 2001-2005, with subsequent establishment of BC_3F_2 trees from the intercrosses scheduled from 2002 -2006. The larger the intercross population, the greater the opportunity to select for optimum blight resistance, tree form, and adaptability to PA.

Intercrosses between one or more trees of a BC_3 line with one or more trees of another BC_3 line can be made most efficiently by ten controlled (bagged) singlecrosses, i.e.., 1x2, 3x4, ... 19x20, minimizing inbreeding. The resulting BC_3F_2 generation must be consolidated in a very large planting, far beyond the capacity of PA-TACF members and current cooperators.

The seeding of the BC_3F_2 orchard will require a minimum of 800 seed per singlecross X 10 singlecrosses = 8000 seed total. This orchard must be sufficiently compact to allow random interpollination after very stringent selection for blight resistance, tree form, and adaptability — a challenge in plot design! The 800 seed of each singlecross will be seeded in 10 replications of 80 seed each. The singlecross subplot of 80 seeds and the total 8000 seed planting are illustrated in Figures 1 and 2, respectively. This consolidated orchard requires 4.8 acres plus borders, must be isolated from all other sources of chestnut pollen, and free of all shading effects by other trees. Isolation within an open field of 20 acres or more will be ideal. The stringent selections for full blight resistance (equivalent to that of donor parent Chinese chestnut), tree form, and adaptability will reduce this 8000 BC_3F_2 tree orchard to as few as 100 trees, thus providing each surviving seed tree with an average space of 2088 (45.7^2) square feet, adequate for full expression of genetic potential for the life-time of trees. These remnant trees under open-pollination will produce seed of the first blight-resistant American chestnut cultivar for Pennsylvania, PA-TACF #1' !!

Robert C. Leffel
PA-TACF Breeding Coordinator

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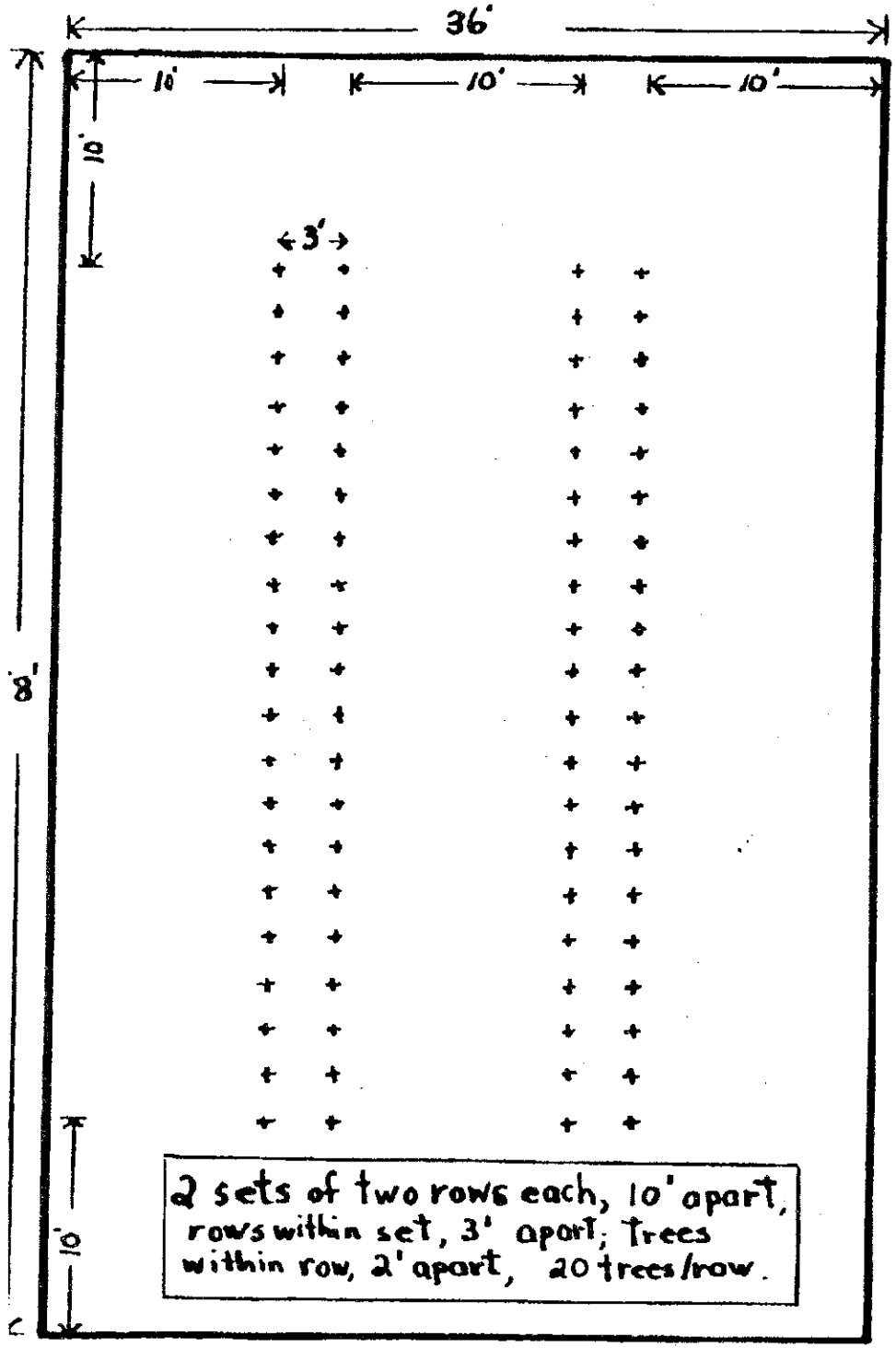


Fig. 1 Singlecross subplot - 80 trees

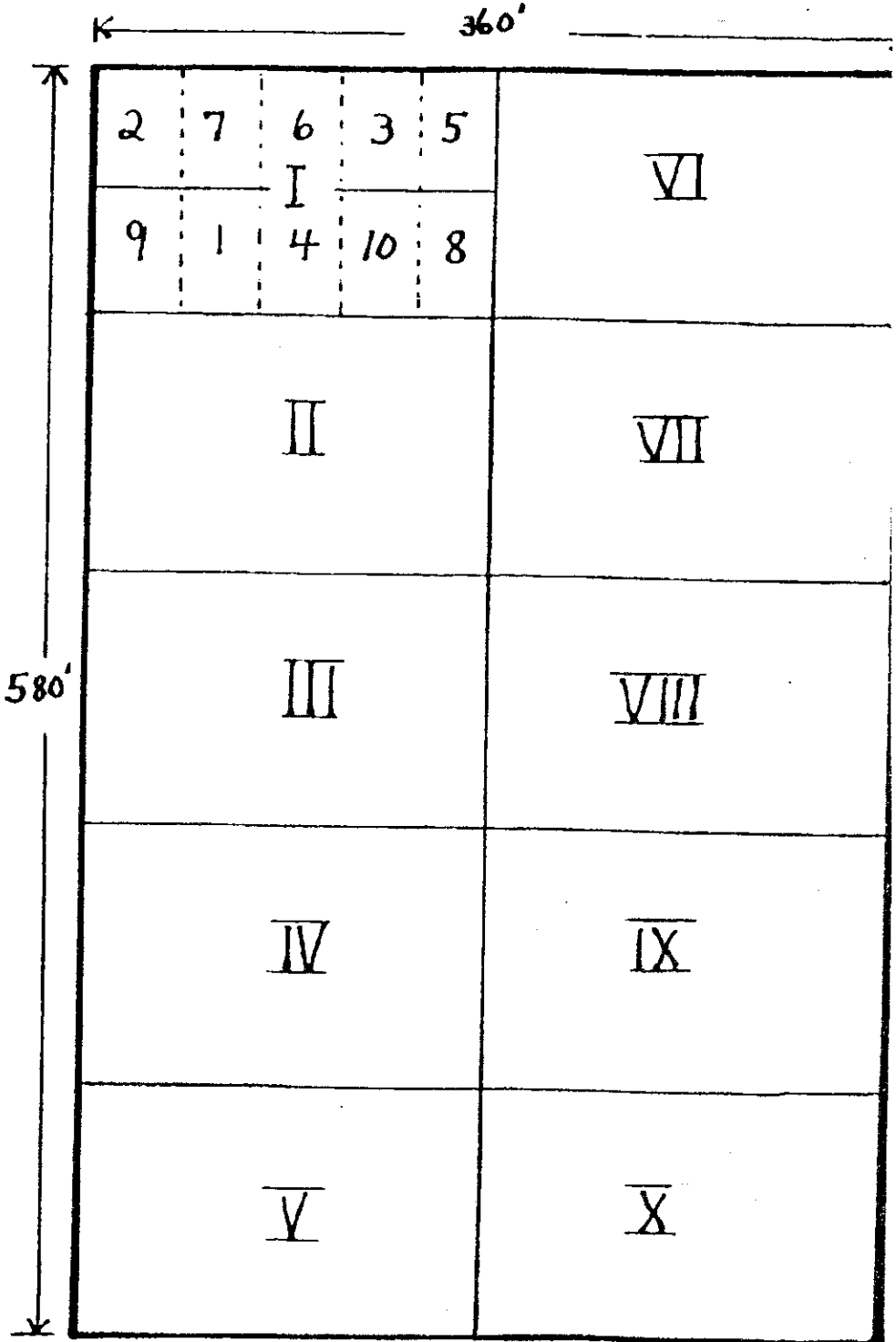


Fig. 2 - BC3F2 Seed Orchard Planting - 8000 tre

KEY To
Pennsylvania Chapter, TACF Breeding Program Orchard Maps

Map 1 - American Chestnut Orchards

American Symbol ★

1. Audubon Wildlife Sanctuary, Audubon, PA, 100 trees
2. Tyler Arboretum, Media, PA, 140 trees
3. Dave Armstrong, Hanover, PA, 107 trees
4. Barbara Bartusik, Zion Grove, PA, 50 trees
5. Forkston Property Association, Tunkhannock, PA, 30 trees
6. Kim Steiner, State College, PA, 210 trees
7. Phil Gruszka, Longwood Gardens, PA,135 trees
8. Bob Harrison, Tioga County, PA, 50 trees
9. Jack Laws, Bedford, PA, 50 trees
10. Bob & Ann Leffel, Brogue, PA, 220 trees
11. Lloyd Lupfer, Landisburg, PA, 50 trees
12. Bill Peifer, Dornsife, PA, 50 trees
13. Tom Pugel, Reels Corner, PA, 60 trees
14. Lee & Jean Saufley, Hummelstown, PA 25 trees
15. Lee & Jean Saufley, Renova, PA, 100 trees
16. Bob Summersgill, Stahlstown, PA, 60 trees
17. White Haven Sportsman Club, White Haven, PA, 100 trees
18. Ed Wilson, Clearfield, PA, 60 trees
19. Norm Wurzbach, Susquehanna County, PA, 30 trees
20. Merle Thorpe Trust, Thurmont, MD, 130 trees
21. Codorus State Park, Hanover, PA, 175 trees
22. Lancaster Environmental Center, Lancaster, PA 50 trees
23. Larry Kuhns, University Park, PA, 180 trees
24. Raystown Lake, Jeff Krause, Hesston, PA, 100 trees
25. Richard Norrie, McNette Township, Lycoming County, PA, 60 trees
26. Dave Prutzman, Stroudsburg, PA, 30 trees
27. Tom Pugel, Riegelsville, PA, 112 trees

Map 2 - Backcross Chestnut Orchards

BC1/F1 Orchards Symbol ◆

1. Reineman Wildlife Sanctuary, Landisburg, PA, 217 trees
2. Little Schuylkill Conservation Club, Quakake, PA, 193 trees

BC2 Orchards Symbol ▲

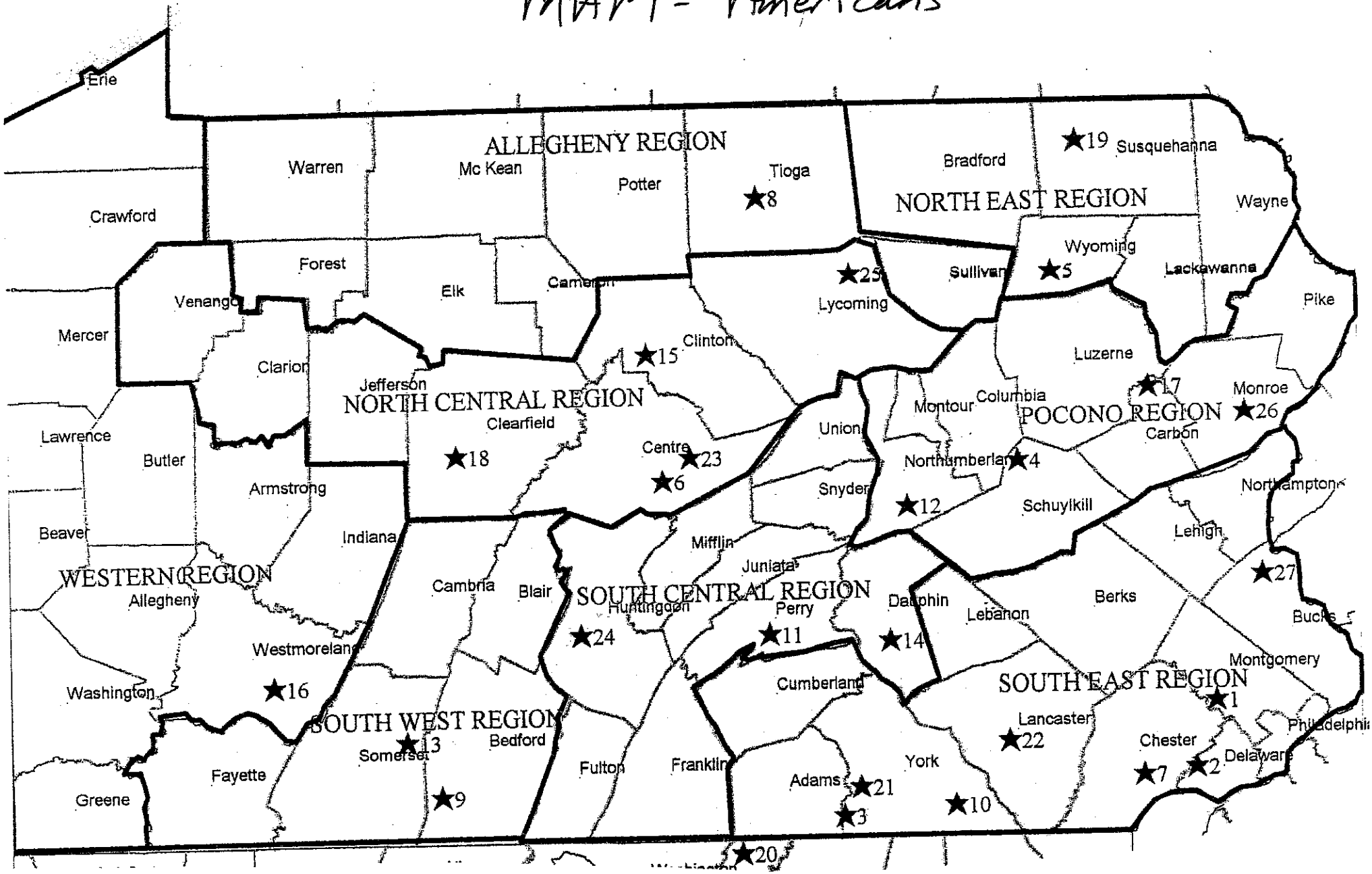
1. Moshannon State Forest, Clearfield, PA, 258 trees

BC3 Orchards Symbol ✚

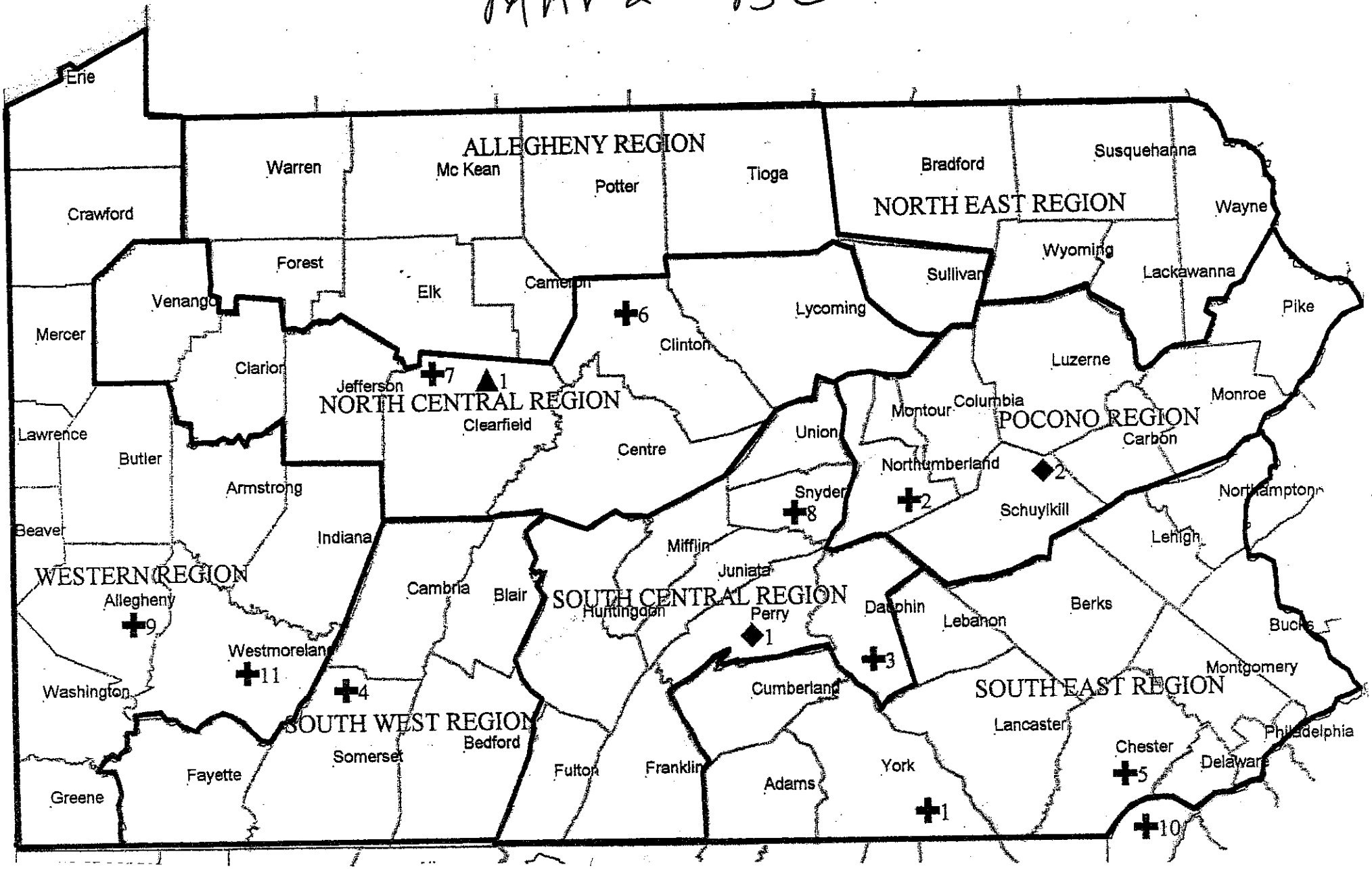
1. Bob & Ann Leffel, Brogue, PA, 428 trees
2. Bill Peifer, Dornsife, PA, 315 trees
3. Lee & Jean Saufley, Hummelstown, PA,75 trees
4. Tom Pugel, Reels Corner, PA,160 trees
5. Longwood Gardens, Kinnett Square, PA, 317 trees
6. Lee & Jean Saufley, Renova, PA, 353 trees
7. Moshannon State Forest, Clearfield, PA, 400 trees
8. Chandis Klinger, Middleberg, PA, 30 trees
9. Larry Patchel, South Park, Pittsburgh, PA, 113 trees
10. Red Clay Reservation, Greenville, DE, 378 trees
11. Bob Summessgill, Stahlstown, PA, 238 trees

Note: Tree numbers in backcross orchards are the total number planted at that site including check trees of American, Chinese and F1.

MAP 1 - Americans



MAP 2 - BC


Erie
ALLEGHENY REGION
Warren
Mc Kean
Potter
Tioga
Bradford
Susquehanna
NORTH EAST REGION
Wayne
Crawford
Forest
Elk
Cameron
Sullivan
Wyoming
Lackawanna
Venango
Lycoming
Pike
Mercer
Clinton
Clarion
Luzerne
Jefferson
NORTH CENTRAL REGION
Clearfield
Monroe
Montour
Columbia
POCONO REGION
Lawrence
Union
Carbon
Butler
Centre
Northumberland
Armstrong
Snyder
Northampton
Schuylkill
Beaver
Indiana
Mifflin
Lehigh
WESTERN REGION
Juniata
Cambria
Blair
SOUTH CENTRAL REGION
Berks
Allegheny
Huntingdon
Perry
Dauphin
Lebanon
Bucks
Westmoreland
Montgomery
Cumberland
SOUTH EAST REGION
Washington
Lancaster
Chester
Philadelphia
SOUTH WEST REGION
Bedford
Somerset
Delaware
Fayette
Fulton
Franklin
York
Adams
Greene
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11

C. Indiana Chapter Breeding Program (by Bruce Wakeland)

Clapper BC3 plantings - In the spring of 1996, following the example set by the Pennsylvania Chapter, Bruce Wakeland, consulting forester and Indiana chapter chairman, began the Indiana regional chestnut breeding program. Two American chestnut trees planted on the Wakeland home property in 1985 from seedlings purchased from Cold Stream Nursery in southwest Michigan were pollinated with BC_2 pollen from Meadowview (Clapper BC_2 tree: GR210). The two American chestnut trees pollinated are very good looking trees with one growing on a good site and one growing on a very sandy and poor site. These trees are labeled IW2, and IW3. These first pollinations resulted in 54 BC_3 nuts (34 IW2-GR210 and 20 IW3-GR210) which were planted in the spring of 1997 in an orchard on the Wakeland home property with 50 of them currently growing.

In the spring of 1997 American chestnut tree IW2 was the only tree found with flowers and it was pollinated with BC_2 pollen from Meadowview (Clapper BC_2 tree: GR226). In the fall 125 nuts were harvested and planted in the spring of 1998 in the Wakeland home orchard. About 110 of these are currently growing. Ten died for unknown reasons after leafing out this spring. (IW2 - GR226)

In the spring of 1998 American chestnut tree IW2 was again the only tree found with flowers that could be reached for pollination and it was pollinated with BC_2 pollen from Meadowview (Clapper BC_2 tree: BE325). In the fall of 1998 I harvested 93 nuts. (IW2 - BE325) In the spring of 1999 member Coy Willis of southeast Indiana planted 50 of these nuts on his home property and member Jack Seifert planted 25 on one of his properties in southern Indiana. I planted 18 in my home orchard. Approximately 75 of these trees are currently growing.

Tree IW2 is currently 14 years old, 7 DBH and 45 feet tall. It still looks very good and very well suited to northern Indiana. It was pollinated with American chestnut pollen this spring to produce nuts for general out planting.

In the spring of 1999, four new American chestnut trees were pollinated with BC_2 pollen from Meadowview (Clapper BC_2 tree: WV1). The Lake Maxinkuckee (Max) tree is 75 years old, 90 feet tall and 24 DBH, looks good and is completely healthy. The Delong tree (IW4) was a seedling purchased and planted at the same time and from the same place as IW2 but was planted at a different site on a property I own 15 miles away near Delong, Indiana. This tree, IW4, also looks very good and is about the same size as IW2. This 14 year old Delong tree IW4 is growing in competition with other trees and this is the first year it flowered. The Swihart tree (IS1) is 90 years old, is approximately 30 DBH, has the blight and is about 7/8 dead. It was planted in a yard by the current owner's father with its origins unknown. The Lloyd tree (IL1) is 6 DBH and 40 feet tall. It was planted by the owner from nuts from a tree that his grandfather planted. The owner believes that the parent tree came from a Pennsylvania source nearly 100 years ago. This

is the first year that tree IL1 has flowered. This tree has good form, growth rate and seem to be very well suited to Indiana.

Clapper BC_3 - Planted

Spring 1997 at Wakeland Orchard (GR210-IW2 30 trees) (GR210-IW3 20 trees)

Spring 1998 at Wakeland Orchard (GR226-IW2 110 trees)

Spring 1999 at Wakeland Orchard (BE325-IW2 15 trees)
at Coy Willis Orchard (BE325-IW2 40 trees)
at John Seifert Orchard (BE325-IW2 20 trees)

Spring 1999 Pollinated (WV1-Max, WV1-IW4, WV1-IL1, WV1-IS1)

American chestnuts planted at the Wakeland chestnut orchard - Also planted at the Wakeland Chestnut Orchard (6 acres available for planting) are American chestnut trees from several sources with the hope that they will be used for future pollinations. We have 8 seedlings planted in the spring of 1997 from the George Marshall (GMA) tree from Attica, Indiana. This tree was dying from the blight and died during the summer of 1997. The tree was 81 years old, 100 feet tall and 28 inches DBH and had excellent form. It was a single isolated tree and had never been known to produce viable nuts. We think the 12 nuts we collected and planted were self-pollinated somehow resulting because the tree was dying. The seedlings are mostly runts.

Other American chestnut trees planted in the Wakeland orchard include 7 trees from the Lauber woods northwest of South Bend, Indiana and 3 trees from the Sones woods west of South Bend. The Sones trees are too tall to pollinate and the larger Lauber trees have since died. I think that the Sones and Lauber trees represent trees which are native to northern Indiana but at this time I do not know how to prove or disprove this theory. The largest Sones tree from which we collected seed for the Wakeland orchard is 16 DBH and 90 feet tall and is one of nine found in a woodland. I also have 6 trees from the West Salem, Wisconsin grove.

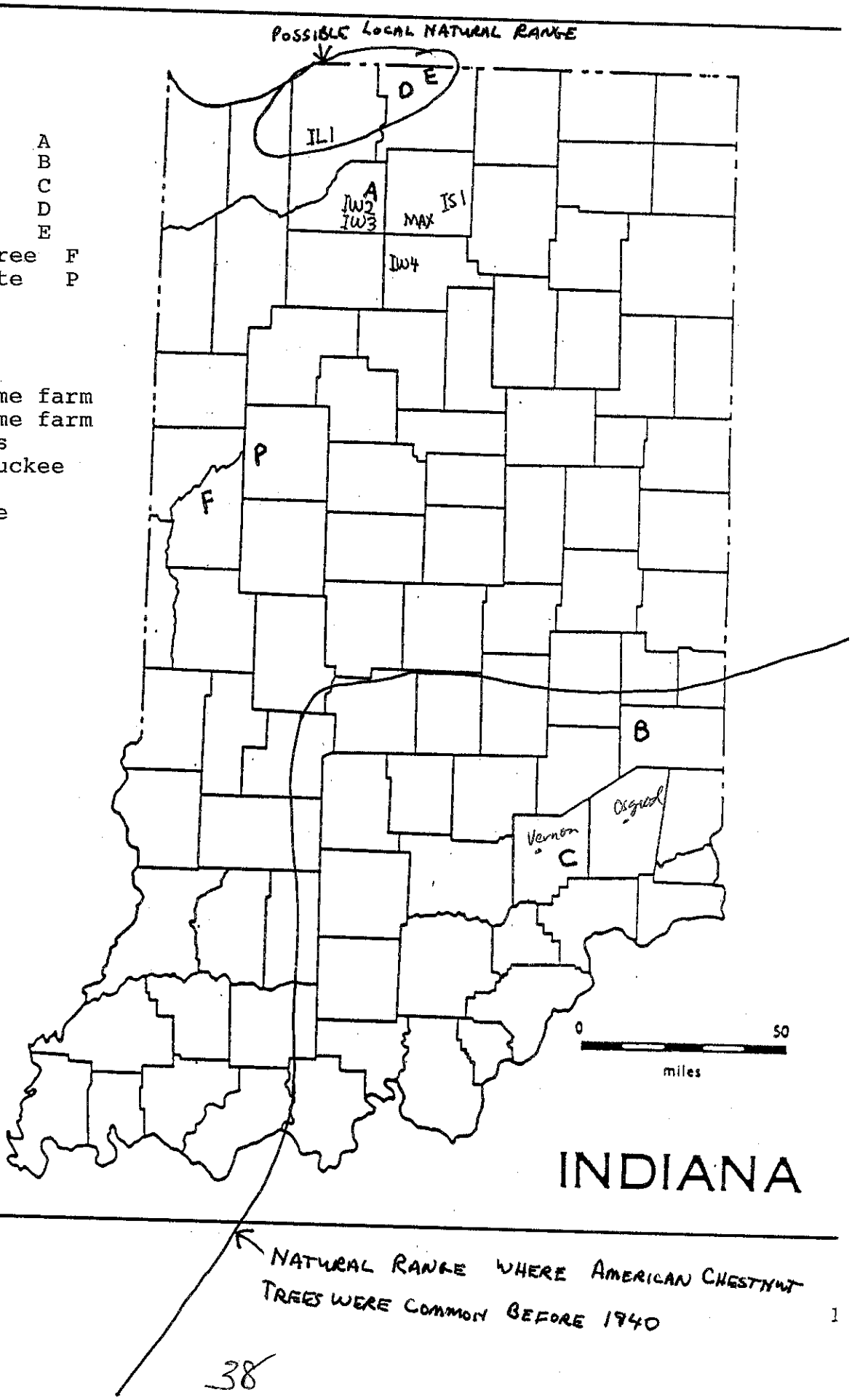
American chestnut tree locations - The natural range of the American chestnut tree was mostly in southeast and south central Indiana, with possible isolated pockets in north central and northwest Indiana. The current locations of American chestnut trees in Indiana which are large enough to produce nuts are nearly all in the northern 1/4 of the state. The blight seems to kill trees in southern Indiana before we can find them for pollination. We have been high profile in our efforts to locate trees for pollination in southern Indiana and have had little luck, However, I have a few leads to follow up at the present time. Many of the northern Indiana trees were planted by pioneers from New York and Pennsylvania in the 1800's and we are finding trees in yards or second and third generation trees in surrounding woods. Our efforts to find American chestnut trees anywhere in the state for pollination is ongoing.

Purdue University - The Hardwood Tree Improvement and Regeneration Center (HTIRC) has signed the TACF germplasm agreement and has a 4 acre area where, in the spring of 2000, we plan to plant most of the BC_3 nuts produced this summer. We are hoping for 400 nuts from the four trees pollinated this summer. HTIRC also hopes to be involved with the establishment of seed orchards when we get to that step of this breeding program. HTIRC plans to get involved with DNA level work and will be looking of the right time and opportunity to help us at that level.

Wakeland Orchard	A
Willis Orchard	B
Siefert Orchard	C
Sones trees	D
Lauber trees	E
Marshall Attica tree	F
Purdue Orchard site	P

BC3 mother trees

IW2 - Wakeland home farm
IW3 - Wakeland home farm
IW4 - Delong woods
MAX - lake Maxinkuckee
IL1 - Lloyd tree
IS1 - Swihart tree



D. Maine Chapter Breeding Program

Maine has undertaken a similar program of crossing the best BC_2 Clapper lines from Meadowview onto American chestnut trees in their region.

E. The Chattanooga Chestnut Tree Project (by J. Hill Craddock)

The goal of the Chattanooga Chestnut Tree Project is the restoration of the American chestnut to the Southern Appalachian and Cumberland Plateau Regions. The return of the chestnut to its place in the forest canopy requires a two-part plan of action: research on biological control of the chestnut blight disease and breeding the trees for disease resistance. A secondary goal of the Project is to provide support for the establishment of a commercial chestnut industry (for nut production) based on improved cultivars.

The Chestnut in Chattanooga. The Chattanooga community has a long history of involvement with the chestnut tree - historically - and most recently with restoration of the American chestnut tree. Primarily due to the effort and enthusiasm of one man, the late William G. Raoul of Lookout Mountain, Tennessee, the Chattanooga Chestnut Tree Project is now supported in part by the Summerfield Johnston Endowment for the Restoration of the American Chestnut and the Robert M. Davenport Professorship of Biology at the University of Tennessee at Chattanooga. Field work is ongoing at four locations; Bendabout Farm, The Lula Lake Land Trust, The Tennessee River Gorge Trust and Reflection Riding Arboretum.

Scope and Accomplishments to Date. The long-term goal of the project is the restoration of the American chestnut, *Castanea dentata*, to its former position as a component of the southern Appalachian hardwood forest ecosystem. A secondary goal is to provide support for the establishment of a commercial chestnut industry (for nut production) based on improved cultivars. The restoration effort relies on a two-part approach to solving the problem of chestnut blight: Breeding for blight resistance and biological control of the chestnut blight fungus. The breeding work, in turn, will depend on the continued availability of locally adapted American chestnut trees (southern germplasm) to use as parents. My role in the Project will be to establish University research objectives designed to accomplish the long-term goals. Research is planned or currently underway in all of the following project areas:

Germplasm collection and conservation of *Castanea dentata* genetic resources, in collaboration with University of Tennessee at Knoxville, Dept. Forestry Wildlife and Fisheries (UTK).

Back-cross breeding for blight resistance and timber form, in collaboration with the American Chestnut Foundation (TACF) and Bendabout Farm.

Biological control using hypovirulence - sampling local strains, introduction and inoculation of hypovirulent strains of the blight fungus, *Cryphonectria parasitica*, in collaboration with the Connecticut Agricultural Experiment Station, New Haven, CT (CAES) and West Virginia University, Morgantown, WV.

Cultivar evaluations, in collaboration with TACF and UTK.

Clonal propagation and the role of ectomycorrhizal fungi in adventitious root formation.

Ecology of restoration and reforestation, in collaboration with the Lula Lake Land Trust.

Gall wasp resistance breeding, in collaboration with UTK and CAES.

Inheritance of male sterility, in collaboration with TACF

Bendabout Farm. Bendabout farm is a privately owned property located about 40 miles east of Chattanooga in the Ridge and Valley domain, near Cleveland, Tennessee. The owners have been very supportive of the chestnut project and quite willing to enter into Memoranda of Agreement with UTC and TACF for long term cooperation. The chestnut plantation there now includes four separate orchards. The first, Orchard #1, was planted between November 1992 and March 1993. It is composed primarily of *Castanea dentata* (30 transplants) collected as saplings from a wild population found in Attala County, Mississippi. This central Mississippi population of American chestnut marks the extreme southwestern boundary of the native range for the species and may be very interesting from a genetic perspective. Fifteen other trees in the first orchard were transplanted from Lookout Mountain, Georgia and two trees were found locally at Bendabout Farm. Orchard #1 also includes one grafted tree of a clone from Lookout Mountain.

Orchard #2 includes about 120 trees that were grown from seeds planted at the site in May 1996 and March 1997. The seed nuts planted were the fruit of open pollination of second backcross (BC_2) trees at ACF Research Farm, Meadowview, Virginia. The resulting seedlings are designated BC_2F_2s. The BC_2F_2 planting will be measured for growth, form blight resistance and other segregating traits according to TACF guidelines. Orchards 1 and 2 are fenced and gated. Drip irrigation was installed and has been used effectively during summer drought. Orchard #2 also includes six native chinquapins (*Castanea pumila*).

Orchard #3 is a planting of our own seedling progeny from crosses made at Bendabout Farm. And orchard #4 is a small planting of 9 Japanese chestnut seedlings, *Castanea crenata* (from Connecticut).

Results of the backcross breeding work at Bendabout include 17 hybrid trees from the 1996 season, 40 progeny from the 1997 season and more than 200 hand-pollinated seedlings from the 1998 season. The 1999 pollination season went very well and fruit set appears very good (as of this writing, July 1999). Pollen from TACF-Meadowview was used all four years. In 1998 and again in 1999, I also employed pollen collected locally from two large Chinese chestnuts (FF-2-1 and FF-5-1), part of large planting of introduced chestnuts made in the 1930s by the Tennessee Valley Authority.

Reflection Riding Arboretum. The existing chestnut species collection at the Arboretum includes mature specimens of Chinese chestnut (*C. mollissima*), native *C. dentata* sprouts, some recently planted seedlings of *C. dentata* from Lula Lake, Japanese chestnut (*C. crenata*) and chinquapin (C. pumila). The Arboretum seems almost ideally suited for building a good germplasm collection (chestnuts grow well there) and progeny testing (particularly for gall wasp resistance).

Lula Lake Land Trust. The private, non-profit Trust manages and protects several thousand acres of land in the Rock Creek watershed on Lookout Mountain, in Georgia about 10 miles south of Chattanooga. The Lula Lake population of surviving American chestnuts represents a very important genetic resource and will be the source of much of the germplasm used in the Chattanooga breeding program. Surviving American chestnut clones are being mapped and labeled with metal tags. The term clone is used because most of the surviving specimens appear as clumps of multiple stems arising from a common root, although the connections are not always clearly evident. Several of the larger stems appear physiologically mature (they may bloom) and will be watched with particular attention during the growing season. These trees have survived probably because they have escaped blight infection to date, not because they are resistant to the fungus. The largest stems are all heavily cankered with blight and many stems are dead. The chestnut blight fungus (*Cryphonectria parasitica*) was isolated from bark cankers on three separate clones and is being grown in pure culture in the laboratory. The Lula Lake *C. parasitica* isolates have been converted to hypovirulent and will provide the basis for eventual biocontrol efforts (see below).

Two orchards were established in 1998 at Lula Lake. One is a BC_2F_2 seedling orchard planted using seed from Meadowview. 200 open pollinated seeds in three families were planted into an open forest setting. The canopy is a mix of oaks and pine. The other orchard is of 100 *C. dentata* seedlings from The American Chestnut Cooperators Foundation (Lucile Griffin).

Tennessee River Gorge Trust. A demonstration plot was established at the southern tip of Williams Island. Although the purpose of the planting is primarily educational, this planting will allow a direct comparison of growth habit, form, chestnut blight resistance and climatic adaptability for all of the different chestnut species. So far, four species are included: American chestnut (*Castanea dentata*), Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*), and Allegheny chinquapin (*C. pumila var. pumila*). Five seedlings of each species were planted on May 14 by UTC students. Wire cages were installed over the small trees for protection from deer-browse. Hybrids and other species will be added to the Williams Island planting in the future.

Campus Chestnut Nursery and Propagation Facility. Progress is being made on the development of a chestnut nursery/propagation facility to be located on the UTC campus. I have

a small greenhouse and a partly shaded container yard. All of the trees (seedlings and grafted trees) are grown in containers.

Germplasm Collection. Chestnut Project germplasm is listed in Table 1. Additional germplasm collections will be made from throughout the Southern Appalachian and Cumberland Plateau regions. The breeding orchard will be a diverse population of southern *C. dentata* types. This will allow us to choose an American parent for each new breeding line that is well adapted to the local growing conditions and will increase the likelihood that our future hybrids will grow well here. Rather than transplant from the wild, new additions to the breeding orchard will be made as grafts onto seedling rootstocks. Advantages to this approach include the possibility of earlier bloom for breeding, genotypic evaluations of the selections and lastly, from a conservation perspective, the parent clone will not be removed from its place in the wild. For example, scionwood (small twigs with dormant buds) were collected in late winter from the labeled clones at Lula Lake, within the Tennessee River Gorge Trust and from several other sites on Lookout Mountain and Walden Ridge. The scionwood was grafted onto rootstocks growing in the propagation greenhouseand then transplanted to the breeding orchard. In this way, the surviving Lula Lake trees will be multiplied without risking loss of the parent clone.

Exotic germplasm will be added to the breeding orchards in order to maximize the diversity of our resistant parents. The search for additional sources of blight resistance will include other species of chestnut not well represented in the TACF breeding program as well as diverse cultivars of European, Japanese and Chinese chestnut different than the parents used in the original TACF crosses. Recent germplasm aquisitions are listed in Table 2.

A new threat to the American chestnut. The oriental chestnut gall wasp *Dryocosmus kuriphilus*, is a tiny insect that lays its eggs in the buds of susceptible chestnut trees. The infestation causes a gall to form instead of a normal shoot, quickly affecting the productivity of the tree. Severely infested trees may weaken and eventually die. This pest is native to northern China and was first seen in this country in the 1970s after its accidental introduction to the State of Georgia. The gall wasp is credited with the near total collapse of the chestnut orchard industry in Georgia in the early 1980s. It is now moving northward throughout the native range of *Castanea dentata.* Chestnut species that possess resistance to gall wasp may include *C. crenata*, *C. mollissima*, and *C. pumila*. Inclusion of these species in the American chestnut breeding orchard will permit breeding for resistance to this serious pest.

Student Projects. One goal of the Chestnut Project as it was originally envisioned by William Raoul and the Late Provost Dr. Grayson Walker of UTC was that it should involve university students at every level. There are several student projects currently underway.

Survey of surviving Castanea dentata germplasm in the South Cumberland. The students are helping me on a census and mapping of *Castanea dentata* germplasm in the Chattanooga area. Specifically we proposed to survey the Tennessee River Gorge, Lula Lake Land Trust and similar adjacent areas for surviving American chestnut stems. We use a systematic survey technique

with the intent to map the location and densities of the living sprouts. Scionwood was collected for grafting into the breeding orchard now being established at UTC. Ideally we will be able to collect and identify a significant sample of southern *C. dentata* germplasm from the Cumberland Plateau. The survey will permit a detailed study of the chestnut blight fungus, *Cryphonectria parasitica* , for future inoculation with hypovirulent strains.

Biological control of chestnut blight. The students are working with me on the chestnut blight disease problem. Our hope is to apply some biological control methods to the fungus *Cryphonectria parasitica.* Biological control is based on hypovirulence, a phenomenon marked by the reduced virulence of the pathogenic fungus, making it less dangerous for its host. Hypovirulence is transmitted by a virus. The viral RNA can transform lethal cankers into slower-growing superficial bark cankers that do not kill the tree. Slowing the growth of the fungus allows the tree to live and bear fruit. We isolated the fungus from the native chestnut trees in the Tennessee River Gorge, Lula Lake Land Trust and from Bendabout Farm Orchard #1 . The isolates in pure culture were paired in compatibility group tests and converted to hypovirulent by Dr. Sandra Anagnostakis, Connecticut Agricultural Experiment Station (CAES). We are using two viruses - one from France and the other from Italy. Three hypovirulent strains were deployed in orchard #1 at Bendabout Farm. Different from the normal, lethal strains of blight fungus, the virus-containing hypovirulent strains cause a swollen, superficial canker with healthy bark tissue underneath. The first inoculations at Bendabout were done in early June 1998. At monthly intervals, we inspect all of the treated cankers, reinoculate some cankers and treat newly-formed cankers. We have seen some evidence that this biological control may be working; some of the treated cankers have ridges of callus tissue forming along the canker margins - a sign that the chestnut blight fungus was attenuated.

Table 1. Type and number of chestnut trees at Chattanooga[1], July 1999, with the number of sources of blight resistance and the number of American chestnut lines.

Type of tree	Trees	Number of Sources of Resistance	American Lines
American (Mississippi)	30		30
American (Northwest Georgia)	15		15
American (Southeast Tennessee)	2		2
American (Western Virginia)	100		?
Chinese[2]	>50	?	
Japanese	>10	?	
Chinkapin	>10		
American hybrids (BC_2F_1)[3]	38	3	3
American hybrids (BC_2F_2)	~250	3	8
American hybrids (BC_3F_1)	~60	1	1
Other hybrids[4]	~20	1	1

[1] Includes breeding germplasm orchards at Bendabout Farm, Lula Lake Land Trust, Williams Island and Reflection Riding Arboretum. Does not include native *C. dentata* sprout populations. Does not include recent accessions currently in propagation.

[2] Includes established, mature trees (30-60 years-old) at Reflection Riding and on TVA property at Ware Branch (Friendship Forest).

[3] Progeny of Bendabout Farm crosses

[4] Male sterility study

Table 2. Recent Germplasm aquisitions at Chattanooga.

Type of tree	Trees	Number of Sources of Resistance	American Lines
American (grafted clones)	5		5
Chinese (rootstocks)	~100	?	
Japanese	~50	?	
Chinkapin	~20		
American x Chinese hybrids (F_1)	15	2*	3
American hybrids (BC_2F_1)	66	3	3
Other hybrids and seedlings **	~200	1	1

* Sources of resistance are: FF-5-1 and FF-2-1 from the TVA planting at Warte Branch.
** Includes seed introductions from Europe, Australia and collaborative work with the Connecticut Agricultural Experiment Station.

F. Western Massachusetts Breeding Site (Sudbury Foundation)

Massachusetts is the first of several potential Regional Adaptability Programs deliberately modeled on the Pennsylvania chapter successes and for which we have raised money from local sources. We received an initial $25,000 from the Sudbury Foundation to establish a breeding program in Massachusetts. Our cooperator there is The New England Forestry Foundation. The Action Plan and Timetable are:

Year One - September, 1998 - August, 1999:

We will begin the program by locating and establishing a relationship with a cooperator in eastern [now western] Massachusetts. This cooperator must be willing to make a 30-year commitment to plant, grow, and maintain trees on its property.

During this first year, identifying mother trees in Massachusetts (a mother tree is a pure American chestnut that has reached reproductive maturity). Local volunteers, equipped with a complete identification guide, will assist us in locating and identifying trees; final verification will be made by our staff pathologist and geneticist. These discoveries will be recorded in a master database logging all surviving American chestnut trees in the country.

Once the mother trees have been identified, our scientists will select the best specimens to be used in the regional breeding. These trees will be pollinated in the spring with advanced hybrid pollen from our research farms in Meadowview, Virginia. Volunteers, with the help of staff, will perform the controlled pollinations.

Year Two - September, 1999 - August, 2000:

The second year will see the development of the training program for our regional volunteers. Over the course of approximately five planning meetings, our consultants and staff will create the training program, draft a training manual and outline the activities for the weekend seminars. The seminars will teach volunteers how to cultivate cooperator relationships, perform controlled pollinations, establish breeding stations, care for chestnut plantings, and evaluate and report on the progress of the plantings.

In the fall of 1999, we will harvest the BC_3 nuts produced in the spring. These chestnuts will be shipped to Meadowview for proper storage. The nuts will be shipped back to Massachusetts in the spring of 2000 to be planted and cared for by local volunteers. We continue to identify mother trees in Massachusetts and throughout New England for controlled pollinations in the spring of 2000 with pollen from selected BC_2 trees at the Meadowview Farm.

Year Three - September, 2000 - August, 2001:

In the fall of 2000, we will harvest the nuts produced from the controlled pollinations earlier that spring. These chestnuts will again be shipped to Meadowview for storage and shipped back to Massachusetts for planting in the spring of 2001.

G. Plantings at other testing sites

Early generation trees, primarily nuts from open-pollinated Clapper BC_2 trees, referred to as CL BC_2F_2's, have been planted at over 30 sites around the country. These plantings were partly for fund raising -- to please large donors to the foundation -- and partly for scientific reasons -- to test these trees in a number of different environments to see how they grew and whether local races of the blight pathogen might overcome the resistance genes selected at Meadowview. A list of these plantings follows on the next page. (Outplantings, BC_2F_2, 1996-1998):

Outplantings of Nanking BC2's and OP Clapper BC2's 1996 - 1998

To Whom Sent	# Nuts	Type	Year Sent	Location	Phone
Shelby Farms	196	Nank B2	1998	Memphis, TN (c/o Tim Martin)	(901) 382-0235
Merle Thorpe Trust	129	Nank B2	1998	Thurmont, Maryland (c/o Sam Carpenter)	(301) 271-2823
Richwood	1000	CL B2F2	1998	Richwood, WV -- Monongahela N.F.	
Carolyn Keiffer	25	CL B2F2	1998	US DOE Planting near Cincinnati	
Bernheim Forest	120	CL B2F2	1998	School Project, Kentucky	
Ron Meyers	654	CL B2F2	1998	NC Forest Service	
Hill Craddock	95	CL B2F2	1998	Bendabout Farm, TN	
Carl Mayfield	100	CL B2F2	1998	Springfield, VA	(703) 451-8540
President Carter	50	CL B2F2	1998	Plains, GA	
John Hoffman	50	CL B2F2	1998	Broad Run, VA	(540) 347-2970
Brad Stanback	50	CL B2F2	1998	Canton, NC	
Carolyn Keiffer	197	CL B2F2	1997	US DOE Planting near Cincinnati	
Brad Stanback	246	CL B2F2	1997	Canton, NC	
Bernheim Forest	1284	CL B2F2	1997	Kentucky	
Bendabout Farm	100	CL B2F2	1997	Tennessee c/o Hill Craddock	
Biltmore Estate	2198	CL B2F2	1997	North Carolina c/o Forrest MacGregor	
PA Chapter	2280	CL B2F2	1997	Pennsylvania c/o Bob and Ann Leffel	
President Carter	300	CL B2F2	1997	Plains, GA	
Woodstock Inn	100	CL B2F2	1997	Resort in Vermont	
Virgil Downs	300	CL B2F2	1997	Mansfield, OH	(419) 747-4077
DuPont Plant Parkersburg	300	CL B2F2	1997	c/o Bill MacDonald; Mike Wade, Forester	(304) 863-2803
John Hoffman	36	CL B2F2	1997	Broad Run, VA	(540) 347-2970
Gen. C. O. Totman	300	CL B2F2	1997	Waldoboro, ME (now deceased)	
Edwin Smoots	25	CL B2F2	1997	Burnsville, MN	(612) 890-2775
Albert Ellingboe	300	CL B2F2	1997	Wisconsin Dept. of Natural Resources	
Carl Mayfield	300	CL B2F2	1997	Springfield, VA	(703) 451-8540
James Ozanne	300	CL B2F2	1997	Darien, CT	(203) 656-1159
John Herrington	100	CL B2F2	1997	Location of plantings is unknown	
John Hoffman	100	CL B2F2	1996	Broad Run, VA	(540) 347-2970
Bernheim Forest	296	CL B2F2	1996	Kentucky	
Albert Ellingboe	127	CL B2F2	1996	Wisconsin Dept. of Natural Resources	
TVA	300	CL B2F2	1996	c/o Jimmy Maddox, Huntsville, AL	(205) 386-3096
Bob and Ann Leffel	210	CL B2F2	1996	PA Chapter	(717) 927-9557
Bendabout Farm	126	CL B2F2	1996	Tennessee c/o Hill Craddock	
DuPont Plant Parkersburg	300	CL B2F2	1996	c/o Bill MacDonald; Mike Wade, Forester	(304) 863-2803
William Lord	100	CL B2F2	1996	Park in Pittsburgh	(412) 793-0255
Ronald Parris	100	CL B2F2	1996	Judge Matthews' land in Grayson Co, VA	
Millikan Forestry Co	301	CL B2F2	1996	South Carolina	
Woodstock Inn	300	CL B2F2	1996	Resort in Vermont	

III. Molecular Marker Analysis

Two laboratories have been invaluable help to the Foundation in doing molecular marker analysis of blight resistance and other traits:

The USDA Forest Service's Southern Institute of Forest Genetics (SIFG) at Saucier, Mississippi (near Gulfport): Drs. Warren Nance, Robert Doudrick, and Thomas Kubisiak

Dr. Robert Bernatzky's lab at the University of Massachusetts, Amherst

The projects undertaken have been:

1) mapping blight resistance and other traits in both F_2 and BC_1 populations of Chinese x American. The Chinese cultivars mapped so far are Mahogany (grandparent of the Graves Tree) and Nanking.

2) chestnut genetic diversity study (all populations collected, most DNA extracted - plan to start collecting data later this fall).

3) mapping in an American x Clapper Tree backcross family (DNA extracted, RAPDs to be run this summer).

4) developing markers for species and hybrid identification (some diagnostic RAPDs already identified, pushing for other types of markers, RFLPs and organellar markers)

5) mapping work in *C. parasitica* -- genome-wide linkage map and markers linked to 5 of 7 *vic* genes (Kubisiak's work is complete, Michael Milgroom of Cornell still needs to collect some more data). *Vic* genes control vegetative incompatibility between strains of the fungus.

A. Analysis of traits using the Chinese cultivar Mahogany:

Loci associated with blight resistance and other traits were first identified in two mapping populations descended from Mahogany. An F_2 population of 185 trees was generated by crossing two Mahogany x American F_1's available at the Conn. Agric. Expt. Station. Of these 185 trees, 102 of the most susceptible and resistant were genotyped using isozyme, RAPD, and RFLP markers. The results were published in Kubisiak et al., Phytopathology 87:751-759, 1997 (copy attached in Appendix B).

A BC_1 mapping population was created at the same time as the F_2 by crossing the two F_1's to American chestnut trees. DNA from 57 BC_1 trees was analyzed with both RAPD and RFLP markers, and blight resistance ratings were available for 52 of those. Each F_1 was represented about equally, and 5 American trees were used as females for each F_1 (a different set of 5 for each F_1). The data from the BC_1 study are as yet unpublished.

The map presented in Kubisiak et al. (1997 -- See copy in Appendix B) showed 12 linkage groups labelled A through L, matching the haploid number of chromosomes in species of the genus *Castanea*. The map covered a total genetic length of 554 cM. Results from the BC_1 population showed that B and E were actually a single linkage group, which is labelled B in the table below.

The F_2 map confirmed the genetic linkages reported in Hebard's 1994 Journal of Heredity article (Appendix B). Loci affecting the number of interveinal hairs, vein hairs, twig hairs, and stipule size were within a 7 cM region on Linkage Group C. Another locus affecting stem color was linked but farther (about 12 cM) away.

B. Analysis of traits using the Chinese cultivar Nanking:

A BC_1 mapping population for the cultivar Nanking was generated from the following crosses:

KY110 F_1 Tree was from (Nanking x Lesesne Irradiated American)

Musick American x KY110 [Mu x N] 51 Trees Genotyped

Mill Creek H American x KY110 [MCH x N} 28 Trees Genotyped

Analysis of resistance loci coming from the two BC_1 families showed that they could not be combined, because both the Mill Creek H and the Musick American chestnut trees had strong positive contributions to resistance, but on different loci. There were also other peculiarities about the Mu x N cross: many loci were skewed in the direction of the Chinese parent, and no male-steriles were found among the progeny, even though the Musick tree had been used as the female. For purposese of this analysis, only the MCH x N backcross progeny are included.

The combined results from the Mahogany F_2 and BC_1 and Nanking (MCH x N) BC_1 mapping populations are presented in the following table, which shows single-point correlations between marker loci and traits measured, with the probability of rejecting the correlation.

Maximum Probability of No Correlation, Starting at p=.01, between Molecular Markers on Different Linkage Groups and Various Macroscopic Traits in Three Molecular Maps of Chinese and American Chestnut Hybrids[1].

Linkage Group	Stem Color		Stipules		Twig Hairs			Vein Hairs			Leaf Hairs			Linkage Group
	F_2	M B_1	F_2	M B_1	F_2	M B_1	N B_1	F_2	M B_1	N B_1	F_2	M B_1	N B_1	
A	-0.0001	-?0.01	0.01					0.01						A
BE	-0.001		0.0001	.01*				0.01						BE
C	-?0.001		0.0001		0.0001		.01	10^{-6}	.01*		10^{-11}	10^{-12}	0.0001	C
D				-?.001										D
F			.01*			0.01								F
G[2]							0.001							G[2]
H														H
I	-0.001													I
J					0.01			0.01						J
K														K
L	-0.01													L
M														M
Unknown														Unknown

Linkage Group	Time of Leaf Emergence in Spring			Lenticels	Bud Shape		Bud Tips		Male Sterile	Blight Resistance[3]			Height	Linkage Group
	F_2	M B_1	N B_1	F_2	F_2	M B_1	F_2	M B_1	N B_1	F_2	M B_1	N B_1	N B_1	
A										-0.01				A
BE					0.01	0.01	.01			10^{-7}**	0.0001			BE
C							.01	.01						C
D				0.01								-0.001		D
F	0.01				0.01					0.001	0.001	.01*		F
G[2]		0.01		0.01	0.01					10^{-6}*				G[2]
H										-0.01				H
I		0.01					.01							I
J								-.01				0.01		J
K														K
L	10^{-13}	10^{-9}	10^{-10}								0.01			L
M													-0.001	M
Unknown									0.0001					Unknown

Negative values indicate traits originating in American chestnut, positive, Chinese; when doubt exists, there is a question mark before the number. Stem color, the hair traits and blight resistance are the only traits for which most signs has been verified, except they also are verified for stipules in the F_2.

*Asterisks indicate segretation distortion. Each asterisk indicates distortion at powers of 10, starting with p<0.1, which differs from the conventional p<0.05.

[1] F_2 = Mahogany F_2 Mapping Population; M B_1 = Mahogany BC_1 Mapping Populations; N B_1 = Nanking BC_1 Mapping Population.

[2] There was no recombination on linkage group G in the M B_1 mapping populations.

[3] We practiced selective genotyping of the F2 population to increase the sensitivity of the tests for blight resistance.

IV. External Grant Program

Following is a list of our external grants for the last 10 years. The purpose has been to get scientific work done that we could not do in-house. Among the successes of the program have been:

1) The development of tissue culture techniques for propagating chestnut (Paul Read and Scott Merkle).

2) The development of genetic markers: isozymes, RFLP's and RAPD's (David Mulcahy, Robert Bernatzky, and Thomas Kubisiak).

3) Silvicultural studies to determine how best to return chestnut to the forest (Scott Schlarbaum, Jim Zaczek, and Greg Miller). Additional sivilicultural studies are being done with our cooperation by Kim Steiner, Henry Gerhold, David Loftis and Henry McNab, and a group of foresters in Kentucky may join the effort.

4) Studies to quantify the diversity in remaining American chestnut populations (Hong Wen Huang, Robert Bernatzky, and Thomas Kubisiak).

TACF Grants to Others 1989 - 1999

Year	To Whom	Institution	Amount	Total/Yr
1989	Gordon, Phil	New York Botanical Garden	1,000	
	Jensen, Keith	Evergreen Cloning Nurseries	300	
	Schlarbaum, Scott	University of Tennessee	2,000	
	Shain, Louis	University of Kentucky	2,400	
	Widrlechner, Mark	Iowa State University	400	6,100
1900	Gordon, Phil	New York Botanical Garden	500	
	Mulcahy, David	University of Massachusetts	2,000	
	Read, Paul	University of Nebraska	3,500	
	Shain, Louis	University of Kentucky	1,500	7,500
1991	Gordon, Phil	New York Botanical Garden	500	
	Inman, Larry	Private	900	
	Jensen, Keith	Evergreen Cloning Nurseries	150	
	Mulcahy, David	University of Massachusetts	2,000	
	Read, Paul	University of Nebraska	3,500	
	Shain, Louis	University of Kentucky	2,400	9,450
1992	Anagnostakis, Sandra	Connecticut Agr. Expt. Stn.	500	
	Gordon, Phil	New York Botanical Garden	500	
	Merkle, Scott	University of Georgia	3,000	
	Mulcahy, David	University of Massachusetts	3,000	7,000
1993	Anagnostakis, Sandra	Connecticut Agr. Expt. Stn.	1,000	
	Cutler, Rupert	Explore Park, Roanoke, VA	5,000	
	Gordon, Phil	New York Botanical Garden	500	
	Huang, Hong Wen	Auburn University	500	
	Merkle, Scott	University of Georgia	3,000	
	Mulcahy, David	University of Massachusetts	4,000	
	Read, Paul	University of Nebraska	3,000	17,000
1994	Cutler, Rupert	Explore Park, Roanoke, VA	6,000	
	Illinois Chapter	Research Grant	1,500	
	Leverone, Leslie	Cincinnati Zoo and Botanical Garden	1,000	8,500
1995	Bernatzky, Robert	University of Massachusetts	4,625	
	Byrne, Michael	Propagation Technologies	1,200	
	Read, Paul	University of Nebraska	6,000	
	Shain, Louis	University of Kentucky	5,000	16,825
1996	Byrne, Michael	Propagation Technologies	3,000	
	Kubisiak, Thomas	USDA/FS South. Inst. of Forest Genet.	3,000	
	Read, Paul	University of Nebraska	4,800	
	West Salem Project	Cummings-Carlson	1,500	
		Fulbright	1,500	
		MacDonald	2,500	
		Milgroom	1,500	17,800
1997	Huang, Hong Wen	Kentucky State University	8,200	
	Kubisiak, Thomas	USDA/FS South. Inst. of Forest Genet.	7,000	
	Zaczek, James	The Pennsylvania State University	7,761	22,961
1998	Bernatzky, Robert	University of Massachusetts	10,200	
	Miller, Greg	Empire Chestnut Company	4,928	
	Zaczek, James	Southern Illinois University	7,340	22,468
1999	West Salem Project	MacDonald	2,000	
	Caucasus Project		10,000	
	Dennis Fulbright	Andrea Vanini, visiting scientist	500	12,500
	GRAND TOTAL			148,104

V. Other Approaches to Chestnut Blight Resistance

The backcross breeding program of The American Chestnut Foundation is one of several approaches currently being used in an effort to combat chestnut blight. Among these other approaches are:

A. **Employing hypovirulence.** In Europe, where *C. parasitica* first invaded in the 1930's, the blight has not been as devastating to the European chestnut trees (*C. sativa*) as it was to the American species. At least part of the reason is the spread of hypovirulent strains of the fungus in Europe -- strains infected with a debilitating virus. Several laboratories are working to characterize the various viral strains and to test whether hypovirulence can be used as a means of disease control. One of the most interesting efforts to date is in a 30-acre stand of American chestnut in West Salem, WI, which has just recently been invaded by *C. parasitica*. Cankers of infected trees are being painted with cultures containing the viral strain Euro7. Euro7 is a moderate strain of the virus. The hope is that Euro7 will be strong enough to slow fungal spread without making the infected fungal strains non-competitive. Viruses that can weaken the *C. parasitica* fungus primarily travel from hypha to hypha in fusions that occur between compatible strains. In North America 40 different vegetative compatibility groups are known, thus reducing the effectiveness of hypovirulence as a control technique. In Europe, only a few vegetative compatibility groups are found.

B. **Intercrossing large surviving American trees.** The American Chestnut Cooperator's Foundation, headed by Gary and Lucille Griffin of Virginia Tech and John Elkins of Concord College is taking the approach of finding and intercrossing surviving American chestnuts in hopes of building up low levels of heritable resistance found in these trees.

C. **Genetically engineering resistance to the fungus.** The laboratories of William Powell and Charles Maynard at Syracuse are working to genetically transform chestnut with genes and peptides thought to have anti-fungal properties. Their work has the enthusiastic backing of the New York Chapter of TACF, which has secured $500,000 from their state legislature to help their efforts. So far they have two surviving trees in the field that are putatively transformed with a genetic marker (beta glucuronidase).

D. **Using naturally occurring fungal parasites, such as *Trichoderma* spp, to destroy the blight fungus in cankers.** *Trichoderma* spp. are implicated in the control of blight fungus in the roots of surviving trees and the occurence of healing cankers on some trees. Terry Tattar and Mark Mount at the University of Massachusetts, Amherst, are testing whether preparations of these fungi can control blight.

Line	BC2 Selec	BC3's/BC2	BC3 Amer Par	BC3 Orcd	Tot BC3's
op Amer Lckwd	CL326	28	Bu2-3,KH2,PL1	AG	28
R4T1	CL27	24	WB(RobtCv90,HHvn3)	CH	
	CL196	85	PL1	CH	109
R4T3	CL130	28	WB(RobtCv90)	CH	28
R4T9	CL98	96	HusseyBr	CH	96
R4T10	CL185	46	Bu1,2,3,Out	CH	
	CL285	5	CL356(QuarBr90)	CH	51
R4T12	CL81	2	KH4	AG	
	CL112	148	WB(RobtCv90)	CH, CLB3	
	CL234	52	WB,AP(RobtCv89,90)	Johns	
	CL242	11	CL356(QuarBr90)	CH	
	CL248	61	HHvn,Glade,Crip	CH	274
R4T14	CL50	51	BH2	CH	
	CL149	16	WB(RobtCv90)	CH	
	CL287	126	BH1,Crip,QB1,RC90,Bu3,HunMth1-2	CH	193
B2R1	BE72	30	Am28(HungMthr1-2 1991)	CLB3	
	BE400	14	KH4	CLB3	
	BE597	12	Am40(GladMtn1 1991)	CLB3	56
B3C1 (CC1xC)	FR194	31	WB(RobtCv90)	AG	
	FR353	65	WB(RobtCv90)	AG	
	BE137	11	HP3	CLB3	
	BE308	113	ClayBnk1	CLB3	
	BE325	181	QuarBr91	CLB3	401
HF2	AB393	170	WB(RobtCv90)	AG,QC	170
HW1	AB185	15	Am22(HungMthr1-2 1991)	HE	15
HW3	AB153	27	HP3,WB(RobtCv90,QuarBr92)	CLB3	
	AB427	115	AB(HuttBr)	AG,QC	142
QA1	GR122	1	WB(RobtCv90)	AG	
	GR194	36	ClayBnk2,3 and GladBig	AG	
	GR210	25	BH2,Glad3,4,WB(RobtCv90,QB1)	AG	
	GR300	18	HussBr2,4	AG	
	GR329	18	CL363(QB1),WB(RobtCv90)	AG,CLB3	98
QA3	GR350	3	WB(RobtCv90)	AG	3
QF2	GR331	48	WB22(RobtCv90),CL356,CL363(QB1)	AG,CLB3	48
QF3	GR97	123	BH2,KH4,HussBr2,QuarBr1	AG,QC	123
QF4	GR57	9	AB(HuttBr)	AG	
	GR86	34	Bu3,RobtCv90,QuarBr90	AG,CLB3	
	GR226	114	PL1,RobtCv90	AG,QC	157
RF1	AB124	58	Am22(HungMthr1-2 1991)	HE	
	AB307	56	WB(RobtCv90)	HE	
	AB311	25	Am22(HungMthr1-2 1991)	HE	139
RW2	AB39	140	Bu3,RobtCv90	HE,QC	
	AB77	53	RobtCv90	HE	193
TOT CL B3's					2324

Graves Pedigrees through 1998 Pollinations

Line	BC2 Selec	BC3's/BC2	BC3 Amer Par	BC3 Orcd	Tot BC3's
Paul Galloway	AB419	61	BH2,GM1,Bu3	BG	
	GR7	38	HutBr1,Bu3,BH2,HungM1-2	BG	
	GR137	94	RobtCv89,HungM1-2	BG,GB3	
	GR208	3	QB2	BG	
	GR239	3	GM1	BG	199
B3C3	SB25	73	CB1	BG	73
B3F3	BE134	101	Bu3,KH2,KH4	GB3	101
B3F4	BE295	97	Bu3,CripCk1	GB3	97
HF1	AB247	17	RobtCv90	BG,GB3	17
HW1	AB171	39	HungM1-2,QB1991,CripCk1	BG	
	AB224	31	HutBr1	BG	70
QF3	AB248	106	RobtCv90	BG,QG	106
RF5	GR24	108	RobtCv90	BG,QG	108
RF8	GR293	18	RobtCv90	BG	18
TOT GR B3'S					789

Additional Questions to Review Team

8/12/99

L. L. "Bud" Coulter, Former President of the TACF Board of Directors:

1. Are we giving sufficient attention to such genetic items as gall wasp resistance, tree characteristics (i.e., timber type vs. various other sorts), ability to compete under forest conditions (Chinese is somewhat lacking in this), nut production including pollination, character of wood, etc?

   Staff's reply: Our immediate goal at Meadowview is to recreate the American chestnut tree as much as possible with the sole addition of blight resistance. We are engaged in a species rescue effort. Thus we have focussed our limited resources so as to make the fastest possible advance in disease resistance while conserving as much of the American genotype as we can. Because each generation we cross to surviving trees in the Virginia mountains or their progeny, we feel as though we are we will come up with the characteristics of trees that survived in the forest before the coming of the blight. We make a visual examination for tree form (tall central leader), so in that sense we are selecting for a timber-type tree. Nevertheless there will be a tremendous amount of variability in the trees we release beginning in 2006 for everything except blight resistance.

2. Would we be acting responsibly to simply introduce a "resistant" tree into the forest without the proper consideration of the above?

   Staff's reply: We think we are doing the most responsible thing by capturing as much genetic variability as possible and letting Mother Nature make the selections among the released trees and their progeny. It is important to remember that we will not be releasing a single type of tree but a population of trees whose only common characteristic is blight resistance.

3. Should our genetic sources, particularly resistance, be expanded?

   Staff's reply: Definitely. We should use more sources of resistance as well as sample more of the surviving chestnut gene pool by giving our whole-hearted support to the breeding efforts in other areas such as Pennsylvania, Maine, Indiana, and Tennessee.

4. Are the genetic mapping studies we have underway adequate to help guide our program considering factors other than resistance?

> Staff's reply: Our mapping efforts are still in their early stages, but we have found the location of genes controlling several of the traits that distinguish the Chinese and American species, such as leaf and stem hairs, stem color, and time of leaf emergence in the spring. These studies should be continued.

5. Current programs include production of numerous breeding lines from different geographic regions. This is somewhat classic -- but -- are we evaluating the value of this program? Is the proliferation of this work more emotional than scientific?

> Staff's reply: We do not have enough data to know what are the important genetic differences in adaptive characters among American chestnut trees in their original range from Maine to Mississippi. Nevertheless, provenance tests in other species of wind-pollinated trees with a large, contiguous range have shown that such differences in trees from different regions are important. If we are serious about restoring the American chestnut tree, we MUST conduct or encourage breeding programs in several areas of the original range. Releasing only trees from Meadowview would be an invitation to disaster.

6. Are we properly examining the interaction of hypovirulence and tree resistance?

> Staff's reply. This interaction should definitely be studied by others using our released population of trees, but it is not an appropriate study for the Meadowview Farms. We do not wish to introduce hypovirulent strains of the fungus to the farm, because we want to screen our trees against the most pathogenic isolates we can find so that we can maximize the genetic resistance in our released trees.

7. This enormous project could become a disaster in the field if we do not understand chestnut tree establishment in nature. Are we making proper preparations to avoid this and making plans for considered choices of cooperators and followup?

> Staff's reply: We have funded several silvicultural studies recently through our external grants program. In addition, the U.S. Forest Service and several state forest services are providing us with information for free. The goal of all these studies is to see how best to get the chestnut trees coming out of the TACF program into the forest. We hope to have a good base of information before our release of nuts beginning in 2006.

Dave Armstrong, Executive Director, PA Chapter, TACF:

1. I know I'm just an amateur at this stuff, but I think TACF is ignoring or doesn't care about encouraging the major states in the American chestnut range to develop a breeding program like Meadowview's and PA's to breed for regional adaptability.

Planting, pollinating, and harvesting is a also great way to encourage interest by individuals and grow membership and develop chapters in other states. (But this is a management issue rather than a breeding program issue that us amateurs get concerned about.) If the TACF board were more effective, I think we could encourage more regional and state growth using the breeding program and getting more genetic diversity.

> Staff's Reply: Marshal Case (TACF Executive Director) sought out funding to get an effort going in Massachusetts and Kentucky. He got the funding, and we're proceeding with setting up regional programs there. He encouraged the Maine Chapter, which previously had been discouraged, and we have a breeding program on line there; i.e. they have been breeding for 3 years and are planting. He encouraged an Asheville Chapter and one is getting going. I expect they will start breeding. He also is encouraging a Massachusetts Chapter. So I think the recent record is clear that we are strongly encouraging regional breeding programs.

2. The Ort tree, widely used in the PA breeding program seems to be mostly American. But is it the best we could have used? By using Tom Kubisiak's DNA markers test on the few trees we use in the breeding program, why can't we get more reliable American species?

> Fred's Reply: I feel quite good about my identification of the Ort tree as American.

Stan Wirsig, New York Chapter, TACF:

1. Is the resistance of the homozygote high enough to protect a timber operation? If not, would TACF search areas in the orient (or its literature) for genes to raise the total resistance? Is there a resistance gene found by Kubisiak in *dentata* that would be useful?

> Staff's Reply: We don't know whether the resistance will be high enough under forest conditions. It is under orchard conditions. We expect it will be if the trees grow well. We are using several sources of resistance; there are probably enough introductions in the U.S. already without a need for further searches.

2. Is there a unit of measure for resistance, so that resistance can be read independently of site, planting space, and climate, or will these have to be compensated by recording and applying them as factors in tree performance?

Staff's Reply: There are environmental effects on resistance phenotype. The investigator can compensate for these to some extent. Numerical measures are not any more useful than ordinal measures except for specialized purposes.

3. What other factors should be considered in selection, and how can they be read?

Staff's Reply: Several of these were presented by Hebard in the *Journal of Heredity*. A more up-to-date version is in our document.

4. What control measures are being sought for gall wasp and other imported pests, also weevils?

Staff's Reply: None of these pests are considered to be of life-threatening importance to the tree at this time, so no major searches are underway. However, Sandra Anagnostakis and Scott Schlarbaum are working on gall wasp. Weevils can be controlled with currently available insecticides.

Kevin Scibilia, Arborist and TACF Board Member:

I have every confidence that the backcross breeding program for American chestnut will be reviewed favorably.

Questions I have for the reviewers or the Foundation itself:

A. Once we have a resistant American chestnut, how many outplanting sites are necessary to field test?

Staff's Reply: As many as possible throughout the former range.

B. How many trees should be planted at each site in A?

Staff's Reply: A minimum of 100 per location -- preferably more.

C. How long should the outplantings be maintained to "certify" true American characteristics and blight resistance?

Staff's Reply: Early on we we'll be able to monitor disease resistance, growth/year, and survivability, but we won't know whether the trees will compete, reproduce, and spread in the forest until they have had a chance to go through a complete "cycle" -- perhaps 100 years.

From an Anonymous Member of TACF:,

1. To have the best chance of selecting the very best plant for release to growers, you need the largest possible population of recombinants to select from. If you discard some of your plants for unimportant reasons, you are decreasing the size of your population and thereby decreasing your chances of recovering the best possible recombinant. . . . In weeding our some of the visual characters of the Chinese parent are we possibly eliminating a resistance gene or a gene that helps resistance, thereby producing trees with less than the maximum possible resistance to blight?

> Staff's reply: We select for American characters only among the most resistant trees of a set of progeny. Thus we select for resistance first, and the other characters only secondarily. Our molecular mapping studies have thus far only shown one linkage group where we have a Chinese character linked to a resistance gene -- this is Linkage Group "L" in the Mahogany backcross mapping population. Here one resistance gene is linked to a Chinese gene that causes early leaf emergence in the spring. It is possible that we may be able to get high levels of resistance without using the factor on "L", or we may need to grow out larger populations to break the linkage -- to get the resistance without the early leaf emergence.

APPENDICES

A. Questions for Review Team

TACF Staff: **Fred Hebard** and **Paul Sisco**:

1. Is our effective population size large enough for each source of resistance?

2 Are we using enough sources of resistance?

3. Should we control pollination in the production of the BC_3F_2's?

   Fred Hebard's argument: One worry I have is whether we will have enough personnel to do this. Open pollinations will give us more crosses. Some crosses may yield inferior plants while other crosses with the same tree may yield superior plants. Finally, I wonder how much loss of effective population size will occur if we do the crosses the way Paul has suggested.

   Paul Sisco's argument: I think it is important to control pollination in the making of the BC_3F_2's. Specifically, I think each of the 20 lines should be crossed with one other line to make 10 types of BC_3F_2 trees. At least two good trees from each line should be crossed to capture as many alleles as possible, but only 10 types should result from the intercrossing of the 20 (*e.g.* Line A x Line B, Line C x Line D, Line E x Line F, etc.) The use of 10 types resulting from crosses between 20 lines is outlined in more detail in Bob Leffel's plan for the PA Chapter Seed orchard. Only using a line once in a combination will help avoid inbreeding depression in the released population but may miss positive effects of specific combining ability between certain lines.

4. Would we suffer much inbreeding by setting up BC_3F_3 orchards for open-pollinated seed increase . What is the best plan for setting them up? (See an example in the PA Chapter Plan for a seed orchard at Penn State - Mont Alto).

5. Should we continue to the BC_6 generation after release of the BC_3F_3's?

   Fred Hebard's argument: The main reason for this is the long testing period needed to find out whether or not our trees will grow like the Amercan chestnut of old; we can accomplish the backcrosses before one cycle of testing is complete. Although corn and cattle breeders can recover the phenotype and breeding effect of a hybrid after three backcrosses and soybean breeders after four, we are starting with an extremely wide cross. Furthermore, our objective is to restore the species, plus reistance genes from other chestnut species, not to

get an acceptable cultivar. Because of that objective, I also don't think it appropriate to leave deliberately large blocks of Chinese in our trees for further natural or recurrent selection. An additional argument against that approach is that we know the recurrent parent was well adapted to Eastern American forests before blight arrived. Its restoration would represent a 100% gain in yield over replacement species, primarily oak of economic value. Again, because of the long testing, we do not know whether retention of large Chinese blocks would hamper the ability of our trees to compete in the forest; we do know that their elimination will, in all probability, result in a tree able to compete in the forest. Proceeding to BC_6 will not be detrimental to our other efforts. . Our progress toward elimination of large Chinese blocks might be monitored with markers. If we have sufficient saturation around the genes for blight resistance, these materials might help isolate the genes. Going to the BC_6 will also reduce the level of inbreeding within the Clapper and Graves lines. Also, there's no evidence that *C. dentata* suffers from a lack of genetic diversity.

Paul Sisco's argument: Given the limits of staff and resources at the Meadowview Research Farms, I think it would be better to focus on testing the levels of resistance in our BC_3F_3 Clapper and Graves lines, to advance other sources of resistance to the BC_3 level in 20 lines, and to do more theoretical work like genetic mapping. If there are any deleterious genes linked to disease resistance loci coming from the Chinese species, we are more likely to avoid them by using a variety of resistance genes, each associated with a different chromosomal segment, rather than by trying to break unfavorable linkages by advancing from the BC_3 to the BC_6. I also think it would be a good thing to introduce more genetic diversity into the American species by leaving in small random blocks of Chinese genes. (A former head of the USDA Forest Service speculated that *C. dentata* went through a genetic bottleneck during the ice ages that made it more genetically vulnerable to pests and pathogens and weaker as a species.) We could guard against deleterious traits by releasing as diverse a population as possible, letting Mother Nature do our selection for us in the wild.

6. If we do go on to the BC_6 generation, should we use the best BC_3F_2's or the best BC_3's?

Fred Hebard's argument: Briggs and Allard's classic presentation argues that making an F_2 increases the chance for breaking linkage blocks. We will need to test cross our BC_3F_2's; why not use these trees to proceed to the BC_6?

Paul Sisco's argument: If we continue backcrossing beyond the BC_3 level, I think we should go directly from our best BC_3 trees rather than the best BC_3F_2's. If we cross a BC_3 to an American, we can only reduce the size of the Chinese linkage blocks, whereas recombination in the BC_3F_2 could either reduce or increase the size of the Chinese block. If our purpose in further backcrossing is to reduce the size of the Chinese blocks, going straight from the BC_3

seems preferable. It will also avoid inbreeding effects from an intercrossed generation and help us to keep our 20 lines separate from each other.

7. How much should we use Marker Assisted Selection?

   Paul Sisco's argument: Probably not much at the moment, since many of our traits such as disease resistance appear to have complicated inheritance and our mapping populations have been small. If we could screen out trees in their first or second year of growth, however, it would certainly be to our advantage.

8. Should the Foundation try to protect its genetic material by germplasm agreements or patents, if our goal is the restoration of the species to the Appalachian mountains? If so, how can we do it, since we are planning to release a population of trees rather than a clone or series of clones? A copy of our current germplasm agreement is in Appendix C.

   Paul Sisco's argument: The germplasm agreement that the Board now requires to be signed is a legalistic document that offends many cooperators. It now specifically forbids breeding with our material, although this can be modified in special cases. Rob Doudrick of the US Forest Service has made the argument that we cannot really protect our material anyway, since we plan to release a heterogeneous population of trees whose disease resistance comes from public sources. Defenders of the germplasm agreement argue that if we do not try to protect our material, someone like Monsanto will take and patent it themselves, or an unscrupulous grower will release a small sample of the material that is not as resistant or as genetically diverse as we would like. If the reviewers have any insights that may help us think through this, it would be welcome, but expect some fireworks from those present.

9. Why was there no recombination on the G linkage group in the Mahogany BC_1, whereas there was in the F_2 populations from the same F_1 parents?

10. What is the best way to test whether chestnut has a gametophytic or sporophytic incompatibility system -- by looking at the point of abortion of pollen germination (stigma vs. style)? We know that the Clapper tree is supposed to be the product of a backcross to the same American parent. What does this say about the incompatibility system?

Rob Doudrick, U.S. Forest Service and TACF Board Member:

1. Additional pathogens/races: Are you worried about other races? How do you plan to evaluate "locally adapted pathogen races?" (Question on APHIS permit). How will you

identify those races? Will you need a permit to introduce these races to Meadowview for screening?

Staff's reply: We are concerned, but there has been no evidence to date indicating the existence of a race structure in *Endothia parasitica.*

One paper by Huang, Dane and Norton appeared to indicate the existence of races, but the experiment was flawed because there was a non-linear relationship between phenotypic resistance and the resistance metric used in the experiment. This led to a statistical interaction between the host and pathogen effects.

We have people planting our sources of resistance from *Castanea mollissima* at various locations in China, when they are available, to see whether their resistance breaks down, which would suggest the occurrence of races. Researchers in China led by Hongwen Huang are conducting additional experiments to search for races of the pathogen.

We have also initiated plantings of Chinese chestnut cultivars at Greg Miller's farm in Ohio that could serve as materials for testing the virulence spectra of putative races of the blight fungus. This would be in conjunction with a race survey and crossing of putative races.

We do not currently have any plans to evaluate "locally adapted pathogen races" at Meadowview, since none have been identified. Our current strategy is to deploy our plants at various locations to see whether any locally adapted races occur.

We could evaluate exotic races using laboratory pathogenicity tests, thus avoiding the regulatory problem.

2. Models for site colonization: How/what are the plans for dealing with pollen contamination and dilution in deployment. If our nuts are planted in clearcuts with surviving chestnut stumps, will the pollen from stump sprouts predominate?

Staff's reply: Almost all American chestnut trees that flower in the wild do so at a fairly young age, when they are fairly small (2-5 inches in diameter at breast height), and succumb to blight 0-3 years after flowering. So they produce small amounts of pollen in comparison to large trees. We expect our trees will grow large, and that pollen contributions from American chestnut will be relatively small to non-existent. Furthermore, we expect that any backcrosses of our trees to American chestnut will not have adequate levels of resistance to persist long in the forest.

3. Why have we not seen movement of Asians from the earlier plantings, even though they are blight resistant? What does this say about recolonization efforts?

   Staff's reply: There is some movement of Asiatic species away from plantings. It primarily occurs in disturbed areas, which is what we expect for all species of chestnut, based on the response of seedlings to light and weeds and the response of similar species such as oak.

4. What is our distribution of orchards and test plantings over the country?

   Staff's reply: Aside from Meadowview, we have major plantings of backcross generations in PA (thousands of backcross progeny). Small plantings are being initiated in ME, with larger plantings to follow. We have medium-sized plantings in IN (500-1000 backcross progeny), small plantings in TN (100-500 backcross progeny), SC (<100 backcross progeny), IL and Ohio (unknown number of progeny). There are plans to begin major plantings in western Massachusetts and Kentucky. Sandra Anagnostakis, in an independent program, has moderate-sized plantings of backcross progeny in CT.

   There are plantings of open-pollinated progeny from blight-resistant, second-backcross trees; susceptible second-backcross trees had been removed from the orchard. These plantings are located in numerous states, from MA to TN. Most are small, 100-300 trees, most of which will not have adequate resistance to blight. There are large plantings of those in PA and WV, and fairly large ones in NC and TN (See Table of Outcross Plantings of BC_2F_2s 1996-1998).

5. What additional characters, if any, do we need to monitor -- e.g., timber quality, wood quality, tannin content?

   Staff's reply: Currently we are hoping to begin monitoring form. Our trees are too small to monitor wood quality. We also could monitor nut size. In general, we do not plan to select within American chestnut for various traits, only to select against all Chinese traits except blight resistance.

6. What are our plans for resistance gene deployment? Will we pyramid all known genes or deploy mixed genotypes?

Staff's reply: Currently we do not have detailed plans for deployment, whether to plant blocks from single sources of resistance or to mix sources within a planting. We plan not to pyramid sources of resistance in controlled breeding.

7. Any evidence for breakdown of resistance in Chinese?

   Staff's reply: No

8. Is there enough genetic diversity in remaining sprout population? Has there been too much selection for survival?

   Staff's reply: Sprouts are abundant on moderately xeric to intermediate sites (>1000 stems/ha), so there does not appear to have been much selection among them yet. However, if the trees on those sites differed from those on relatively mesic sites, where few sprouts persist, then there has already been a profound shift in the genetic structure of the population. Since this is a wind-pollinated, obligate outcrosser, we expect, based on results with similar species, that there are not great changes in genetic structure over short distances.

9. Will there be a strong negative reaction from any groups to our planting trees with some Chinese genes? Rob heard last Thursday that an environmental group recently cut down a planting of transgenic poplars.

   Staff's reply: The Park Service has a policy that only "native" species are allowed to be planted or to persist on its properties. Our trees may be classified as a "non-native" species. However, I don't think our trees will encounter as much opposition as they would if they were transgenic plants.

10. Why is The American Chestnut Foundation the best group for restoring the American chestnut tree?

    Staff's reply: For one thing, we are the furthest along with backcross breeding and have accumulated considerable momentum. More generally, it was thought by our founders that a private foundation would have the most financial and administrative continuity to complete a breeding program that extended past the life of any single investigator. It appears we are achieving the financial continuity. The challenge will be to maintain administrative continuity within a small organization.

11. Devil's Advocate Question: What problems could arise that would sabotage our efforts to restore the tree? Are there backup approaches in case the initial one does not work?

Staff's reply:

1) Races of the pathogen

2) Inability of our trees to compete in the forest.

3) Inadequate levels of blight resistance in our trees.

4) Introduction of other defects in our trees from the Chinese.

5) New pests of chestnut arising (e.g., chestnut gall wasp and *Phytophthora cinnamomi* root rot, which destroyed chestnuts in many areas of the Piedmont from 1850 - 1910).

12. How much genetic diversity is enough, both for disease resistance and for the American characters?

    As much as we can get. We have approached it from the standpoint of what is the minimum with which we can get by.

13. What are the projected costs in staff, land acreages, administration, etc. to accomplish our goal?

    Staff's reply: Just for Meadowview, Fred would like to add two more parcels of 30 acres each at $150k each for seed orchards. Our farm operating budget of $100k plus $25k per year for capital is probably sufficient for a few years. Salaries of 2 scientists and 1 technician add another $85,000, and a planting coordinator to help our chapters will be needed soon. We can generate the genetic material to screen 200 trees per year for 150 markers. That might cost $15-30k. Administration (fund-raising, education, member sevices, accounting, etc.) add another $250k. Finally, external grants of ~$15k/yr provide information that we could not get in-house.

Dennis Fulbright, Mich. State Univ. and TACF Board Member:

1. Do we need to be breeding for yield? What does this mean in chestnuts -- tree type, straight trunk, tree height, or nut production? What is the optimum nut production -- fewer but bigger nuts for stronger seedlings or lots of nuts regardless of size?

Staff's reply: First, the objective is to restore the species, not to breed a high-yielding tree. American chestnut would represent about a 100% increase in timber yield, compared to the species that replaced it, if it were able to grow to its former size. So we expect this 100% yield increase to be a side benefit of successful restoration.

From everything we've seen with the trees over the years, the nut yield will be good if the tree can compete successfully in the forest, growing at the rate that gives the 100% increase in timber. Nut yield is highly dependent on the amount of sunlight the crown receives; only fast-growing trees will be able to compete successfully for sunlight to give a high nut yield. Because of the objective, we hope that our trees' nut size will be comparable to that of the American chestnut of old. We expect it will if we can remove enough of the Chinese germplasm, except for blight resistance.

We are currently trying to monitor tree form but not nut size. Perhaps parents with one tree form will give better yielding progeny than parents with another. Those parents could then be used for conventional tree improvement in the future. In our present program we are not engaged in conventional tree improvement -- rather in species restoration.

2. Are we selecting domesticated wimp trees by irrigating, fertilizing, and spraying for insect pests? Are we losing insect resistance, for example? Will these trees only be blight resistant under these ideal conditions? . . . I have planted Chinese chestnut in plots in southern Maryland where the young plants were just covered with blight in a nonfertilized, nonirrigated plot. The parents of these trees in Michigan resist blight when directly inoculated.

Staff's reply: Regarding pampering our trees: We try to minimize environmental variations in order to maximize genetic variation, especially in regard to blight resistance, which has a relatively low heritability (about 50%). The pampering is also necessary to speed the breeding cycle. Since we are constantly outcrossing to wild trees, or their first-generation domesticated descendants, we do not expect there will be a major shift in the genome.

We share your concern about resistance being inadequate in a stressful environment. Fred has seen numerous plantings of Chinese chestnut in the woods over the years, and most were severely damaged by blight. At that point, they had been overtopped by competing vegetation. If our trees are not able to compete successfully with other tree species, I expect they will be severely damaged by blight. In Nanking and our other pure Chinese parent selections we are using the highest levels of blight resistance we know. We used the Clapper and Graves trees because they had levels of resistance

comparable to our best F_1's and they were already advanced to first backcross to American.

We will not know whether our trees can compete successfully in the woods until they have done so. We are putting out some preliminary trials (the BC_2-F_2's), and will probably do the same with BC_3-F_2's, but the first decently sized trial will be with the BC_3-F_3's. And they will have to maintain their competitive edge for 100 years before we are certain we have succeeded (although their competitiveness at earlier ages will certainly give us some strong indications of whether we have succeeded). This is one reason Fred is urging that we continue backcrossing to B_6. That, almost assuredly, would closely restore the American genotype that was successful before the blight.

Regarding stem size and resistance, Fred did one study several years ago comparing trees of various ages (1,2,3 & 5 years old). The phenotypic blight resistance relative to F_1s and Americans (as measured by canker size), increased in Chinese with age.

Al Ellingboe, Univ. of Wisconsin and TACF Science Director:

1. I have given much thought to the question of races of pathogens and have done considerable research with organisms other than *Cryphonectria parasitica*.The genetics of host-pathogen interactions is far more complicated than most breeders are willing to consider. I think it is important that we get the reviewers tuned in to the really important questions about what we are doing and whether we can achieve our goal if we continue to proceed as we are now proceeding.

Bob and Ann Leffel, USDA/ARS retired and PA Chapter:

1. Burnham stated that the ultimate goal of TACF is to "establish breeding populations of blight resistant American chestnuts, each of which will be adapted to a different growth zone in the natural range." This goal may be greatly advanced by TACF providing pollen of its most advanced generation of BC trees screened for blight resistance. The resulting progeny of regionally adapted American chestnut trees provide progeny tests of the TACF pollen donor trees. Yet a minimum of 3 generations, 12 to 15 years, will be required for the cooperator to achieve a regionally adapted, blight-resistant American chestnut population. This assumes only one backcross to local germplasm is required for local adaptation. What germplasm agreement, if any, should TACF utilize for cooperative backcross breeding programs for regional adaptation with individual TACF members, State Chapters, State and Federal Agencies, and Private organizations? (Bob)

2. What is the most efficient method to breed blight-resistant American chestnuts adapted to a specified region? (Bob)

   Staff's reply: Do we need the chapters to make more than one backcross to their own adapted trees and/or should we increase progeny sizes in the F_2?

3. Should regional breeding programs use the same or different sources of resistance than Meadowview? What are the advantages and disadvantages? (Ann)

   Staff's reply: It would be nice if everyone could use different sources, but everyone wants the best and most advanced material for their crosses.

4. How American does a tree have to be to be included in the breeding program. If its leaves have a few glandular hairs from other species, does that remove it as a breeding tree if the general appearance and morphological characteristics are American? (Ann)

5. Are Meadowview data backed up anywhere? (Ann)

   Staff's reply: Yes

Greg Miller, Empire Chestnut Co. and Sci. Cabinet Member:

First of all, any strategic plan needs to include a "knowledge & ignorance" framework that lists the critical things we know and don't know about the problem. Once critical ignorance is acknowledged, then the decision needs to be made whether to pursue discovery of the unknown, or to work around it. In the case of chestnut blight resistance breeding, we have critical ignorance regarding both the mechanism(s) of resistance, and the inheritance of resistance. Of course, we have some knowledge in both of these areas, but the knowledge is incomplete.

When TACF began with the "Burnham" approach, the logic went (still goes?) something like this. 1) Backcross breeding has worked in lots of crops. 2) Backcross breeding has never been tried in chestnut. 3) No substantial evidence exists that backcross breeding will not work in chestnut. 4) Therefore, backcross breeding is the best approach.

Staff's reply: The logic also included Clapper's finding that a model of two incompletely dominant genes could explain the results of his backcross of F_1s to Chinese, so it was considerably tighter than the above scenario.

Of course, one of the "rules of thumb" of breeding strategy is that backcross is only practical if resistance is controlled by three or fewer genes (loci). There has been considerable discussion and

evaluation of evidence regarding how many genes are involved. However, the bottom line still is that we don't know. So, the evaluation team needs to consider the question, "How many loci are involved in chestnut blight resistance?" Because TACF has committed itself to the backcross method, there is a tendency for TACF scientists to look at the evidence with a biased interpretation, favoring two or three loci and no more.

> Staff's reply: Actually, Hebard was more committed to recurrent selection for phenotype among large, surviving American chestnut trees with low levels of blight resistance than to the backcross method, until the results discussed below indicated that the backcross method was a viable approach. He still has a soft spot for use of the large, surviving American chestnut trees and is starting to try to assemble populations suitable for tagging their loci for blight resistance.
>
> We recovered F_2s with resistance comparable to that of 'Nanking' at a frequency of 12 in 191. This is compatible with a two or three-gene model. We also recover backcross trees with intermediate levels of resistance at frequencies compatible with a two or three gene model. Test crosses to American of some of the highly resistant F_2 trees indicated they were homozygous for blight resistance. The molecular marker work indicated three loci were active in blight resistance in the F_2, and two in BC_1 (perhaps due to lack of recombination in one of the linkage groups from F_2).
>
> The results to date leave little doubt in our minds that we will be able to backcross enough blight resistance into American chestnut to yield highly blight-resistant trees. However, we are continuing to generate F_2 popuations of later generations of backcrosses, to continue to test the hypothesis that blight resistance is inherited relatively simply. A more important question at this point is whether that resistance will be durable.
>
> There undoubtedly are modifier genes involved. There may also be more than 2 or 3 genes for resistance in any one Chinese chestnut tree. Due to use of the backcross method, we may select a subset of the genes for blight resistance in any one line or set of lines. These may be more vulnerable to breakdown of resistance than the full complement of genes.

From the question of number of loci, other questions immediately arise, such as, "How can the number of loci be determined?" and "Is it worth the time and expense to find out?" It often happens in tree breeding that the genetic studies take as long or longer than the breeding program. In other words, by the time we discover the best breeding strategy, the breeding program is (or could have been) completed.

Parallel to the number of loci is the mechanism(s) of resistance. Again, we have lots of evidence but little hard knowledge. The review team should consider the question, "What are the

mechanism(s) of resistance?" Perhaps a more important question is "How can we screen for resistance?" If we had a good method to screen seedlings (the younger the better) for blight resistance, then we could work around a lot of the ignorance mentioned above.

Staff's reply: We believe we have a good method of screening for blight resistance and have been using it since 1980. The results of use of that method were discussed above. The mechanism of blight resistance and the number of genes involved and their mode of action is relatively unimportant as long as blight resistance is inherited in a fairly simple method. The mechanism of blight resistance has been the target of some of our external grants on the physiology of blight resistance. Also, we are proceeding to try to map with ultra high precision each gene for blight resistance. Cloning of those genes would be an important first step in uncovering the mechanism of blight resistance. Furthermore, we have been funding work on somatic embryogenesis and gene transformation that could lead to gene knockout studies, and similar work that should help uncover the mechanism of blight resistance.

Hebard, et al, investigated the histopathology of blight resistance and found that blight resistance resulted in an inability of the blight fungus to form mycelial fans quickly and in large numbers and an inability of those fans to expand rapidly. He also found occurrence of all the resistance reactions associated with resistance in other plants to biotrophs and semi-biotrophs, such as rusts and potato late blight, respectively. In other words, the mechanism of blight resistance appears to differ from that of biotrophs, where it appears to rely upon recognition of pathogen antigens by the host. Blight susceptible American chestnut trees recognize the blight fungus just fine, and react appropriately. Unfortunately, those reactions do not hinder formation and expansion of mycelial fans. This suggests, weakly, that blight resistance may not be vulnerable to new races of the pathogen.

Backing up to the "Burnham" approach, the reveiw team needs to ask, "Is the backcross method valid?" In view of our ignorance, it might be prudent to consider recurrent mass selection as an alternative or supplement to the backcross method. TACF breeding program is at a critical juncture. Up until this point, the crosses made and progenies generated are reasonable for either the backcross or the recurrent mass selection approach. From this point on, though, we need to pursue one or the other or both. An advantage of recurrent mass selection is that at any time any arbitrarily chosen population can be assembled and intercrossed, and then the resulting progeny subjected to selection. The selected progeny would then be intercrossed. I realize that this process is incorporated into the latter stages of the backcross program. Of course, the disadvantage of recurrent mass selection is that it slows the progress toward the nearly pure American chestnut. The catch is that it slows the progress only if resistance is controlled at three or fewer loci. If four or more loci are involved, then recurrent mass selection is the most practical (quickest, cheapest) approach. The greatest advantage of recurrent mass selection is that it will

work no matter what the inheritance, no matter what the mechanism, and regardless of how much we know or don't know. It only requires that we have reliable selection methods.

We can't handle the numbers of progeny required by recurrent selection, which are considerable larger than those of the backcross method, since one is usually selecting for homozygotes after the first step versus heterozygotes in backcrossing. Additionally, selection for American traits such as forest growth form and competitiveness probably should be conducted over a full rotation (40 years), as opposed to the 20 years (half rotation) used in pine, which is proving inadequete. Even with selection at half rotation, that would cut our generation turn-around time from 6 years to 22 years. Using the backcross method, we will recover automatically the traits of the recurrent parent. Finally, we get automatic progeny testing at each backcross generation, as opposed to phenotypic recurrent selection.

Recurrent selection may be one alternative for the later stages of the breeding program. However, currently our effective population number is fairly small. It probably will be better to rely on natural selection, since our goal is a naturally reproducing, wild population. At this time, we have a tightly focused, apparently successful breeding program. Tinkering with the fundamental breeding method at this point would be counterproductive, in Fred's view.

Other than the critical, central questions mentioned above, the review team needs to assess the value and cost/benefit of: 1) DNA studies, 2) laboratory modification & manipulation of chestnut DNA, 3) hypovirulence and other blight fungus studies, 4) other pests and pathogens, 5) the establishment & silvicultural studies, and all the other "side" issues involved in re-establishing the American chestnut tree to the Appalachian forests.

B. Publications resulting from TACF scientific efforts:

1. Hebard, F.V. 1994. Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. J. Hered. 85:440-446.
2. Hebard, F.V. 1994. The American Chestnut Foundation breeding plan: beginning and intermediate steps. J. Amer. Chestnut Fndtn. 8 (1): 21-28.
3. Kubisiak, T.L., Hebard, F.V., Nelson, C.D., Zhang, J., Bernatzky, R., Huang, H., Anagnostakis, S.L., and Doudrick, R.L. 1997. Molecular mapping of resistance to blight in an interspecific cross in the genus *Castanea*. Phytopathol. 87:751-759.

4. Shi, Y., and Hebard, F.V. 1997. Male sterility in the progeny derived from hybridizations between *Castanea dentata* and *C. mollissima*. J. Amer. Chestnut Fndtn. 9(1):38-47.
5. Leffel, R.C. Breeding blight-resistant American chestnuts for regional adaptation. 2nd International Chestnut Symposium, Bordeaux, France, 19-23 October, 1998, Acta Horticulturea, in press.

C. Facilities and equipment at the Meadowview Farms:

Three barns
Three tractors (old Ford 8N, Ford 6600, new John Deere orchard tractor)
Two mowers (bushhogs)
Orchard sprayer (new 1999)
Two used pickup trucks
Two bucket trucks (30', one person bucket; 48' extension, two person bucket)
Herbicide spray rig
Plow
Rototiller
6 computers (includes one server and one new notebook computer, all networked)

D. Germplasm Agreement (Next Page)

THE AMERICAN CHESTNUT FOUNDATION GERMPLASM AGREEMENT

This Agreement, is dated and effective _____, 199___, is between The American Chestnut Foundation, a District of Columbia nonprofit corporation with its principal facility in the State of Virginia (hereinafter referred to as "ACF"), and the entity executing this Agreement at the foot hereof (hereinafter referred to as the "Recipient").

The reasons for this Agreement: ACF is in the process of breeding hybrid chestnut trees closely resembling pure American chestnut trees but without susceptibility to the disease known as chestnut blight. The method of plant breeding being used by ACF is commonly referred to as the "backcross method" wherein lines of American chestnut stock are outcrossed once to other species of chestnut carrying genetic resistance to chestnut blight, and successive generations of such outcrosses are then repeatedly backcrossed to American chestnut to recover the desirable characteristics of the American chestnut tree while incorporating blight resistance It is in the interests of ACF and of the Recipient to be able to test and observe the characteristics of hybrids which are in the earlier stages of such backcrossing (*i.e.*, the original outcross and first through third backcrosses [and intercrosses between individual trees of the same generation of backcrossing] since *selected* offspring of third backcross trees are considered essentially an American chestnut type of tree). But ACF does not want the Recipient or others to use genetic material from such early stages for propagation purposes because: (1) ACF does not want large numbers of partially blight-resistant trees carrying significant numbers of non-American chestnut genes to be introduced into the landscape; (2) ACF wishes to preserve ACF's proprietary rights to such genetic material; and (3) ACF *most emphatically* does not want any person to take such material and market it, or to market any progeny from it.

The Terms of this Agreement: The Recipient has requested a sample of proprietary chestnut germplasm owned by ACF. This Agreement applies to all varieties of chestnut germplasm, and includes but is not limited to pollen, nuts, scion wood, sprouted seeds and small chestnut plants and rooted cuttings, all of which are hereinafter referred to as the "germplasm."

ACF agrees to supply samples of chestnut germplasm to the Recipient. In consideration of this action by ACF, the Recipient agrees to abide by the following terms and conditions as to said germplasm and any other germplasm which has heretofore been received or will hereinafter be received from ACF which is not otherwise covered by a prior or subsequent agreement, unless and until ACF specifically releases any condition imposed by this or any other agreement on the custody and use of any of said germplasm:

1. The Recipient understands and agrees that this Agreement conveys only a right to conduct research on the germplasm. None of the germplasm (or any material resulting in any manner from the germplasm) may be sold, offered for sale, given (by gift or otherwise), or in any other manner transferred or distributed to any third party whatsoever without first being covered by a specific written license from ACF describing the material sold or otherwise transferred, the conditions of the transfer, and other conditions acceptable to ACF in its sole discretion. ACF reserves the right to refuse transfer for any reason whatsoever. It is expressly understood that under this Agreement no implied or express license is granted by ACF to the Recipient for any transfer of the germplasm.

2. The sample of germplasm provided hereunder may be used for basic research, evaluation and/or field testing only. No transformation techniques will be used with the germplasm. No mutagenesis, tissue culture, or cellular techniques will be conducted with any seeds, plants, or plant parts of the germplasm, or of any plant material resulting from the germplasm, including pollen. No selection will be conducted within the germplasm.

3. Seed stock increases for evaluation are permitted. However no seed, plants, plant parts, seed parts, callous tissue or DNA of or resulting from the germplasm will be transferred or distributed to any third party.

4. The Recipient may conduct research and publish the results of research on the germplasm, but the Recipient agrees to acknowledge the contributions of ACF in the provision of the parental germplasm in all publications and in all descriptions of the research.

5. The germplasm is provided "as is." ACF MAKES NO WARRANTIES, EITHER EXPRESSED OR IMPLIED, AS TO ANY MATTER WHATSOEVER RELATED TO THE GERMPLASM INCLUDING WITHOUT LIMITATION THE CONDITION OF THE SAMPLE, ITS MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY WARRANTIES REGARDING INFRINGEMENT OF THIRD PARTY RIGHTS. The Recipient agrees to bear all risk resulting from the germplasm, either directly or indirectly.

6. This Agreement is nonassignable, is governed by the laws of the State of Virginia and may be amended only with the mutual written consent of both parties. This Agreement is effective when signed by the Recipient. Each individual signing for a corporate entity or any other entity hereby personally warrants his or her legal authority to bind that entity. If ACF prevails in any litigation alleging violation of this Agreement, ACF shall also be entitled to an award of attorneys' fees incurred in connection with such litigation. This Agreement is effective when signed by the Recipient.

THE AMERICAN CHESTNUT FOUNDATION	RECIPIENT
	NAME: _____
BY _____	BY _____
TITLE: _____	TITLE: _____
DATE: _____	DATE: _____

Frederick V. Hebard

14005 Glenbrook Ave., Meadowview, VA 24361. (703) 944-4631.
Born Philadelphia, Pennsylvania, U.S.A., March 24, 1948.

Education

B.S., Biological Sciences, Columbia University, New York, 1973.
M.S., Botany, University of Michigan, Ann Arbor, 1976.
Ph.D., Plant Pathology, Virginia Polytechnic Institute and State University, 1982.
Dissertation title: Biology of Virulent and Hypovirulent *Endothia parasitica* on American chestnut (*Castanea dentata*).

Positions Held

Staff Pathologist, American Chestnut Foundation, Meadowview, VA. 1997 - present.
Superintendent, Research Farm, American Chestnut Foundation, Meadowview, VA. March, 1989 - 1997.
Research Specialist, Department of Plant Pathology, University of Kentucky, Lexington. February, 1986 - February, 1989.
Research Associate, U. S. Department of Agriculture, Agricultural Research Service, Irrigated Agriculture Research and Extension Center, Prosser, Washington. Setember, 1982 - December, 1983.
Research Associate, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. November, 1980 - May, 1981; June - August, 1982.
Research Assistant, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. March, 1978 - November, 1980.
Research Assistant, Department of Botany, University of Michigan, Ann Arbor, Michigan, Ann Arbor. June - September, 1976.

Research Experience

1989 -	Chestnut blight: breeding for resistance.
1986 - 1989	Chestnut blight: physiology & epidemiology.
1982 - 1983	Verticillium wilt of alfalfa: histopatholgy and histochemistry.
1976 - 1982	Chestnut blight: histopathology, histochemistry, resistance, epidemiology, pathogenicity, hypovirulence, host taxonomy.
1977 - 1978	Cylindrocladium black rot of walnut: ecology.
1975 - 1977	Mode of action of gibberellin-promoted stem elongation in *Avena sativa* and geotropic response in the grass pulvinus.

1972 - 1973	High-energy irradiation response (HER) in phytochrome system of turnip seedlings.
1971 - 1976	Reaction of tissue cultures of *Castanea spp.* to *Endothia parasitica* and tannin content of *Castanea* tissue cultures.

Teaching Experience

1983	Organized and taught one week course on sample preparation and use of scanning electron microscope, Prosser, WA.
1981 - 1982	Occasional lecturer in Clinical Plant Pathology I, Blacksburg, VA.
1980	Teaching Assistant in Clinical Plant Pathology I, Blacksburg, VA.

Other Experience

1973	Carpenter, D. L. Hebard, Steamboat Springs, Colo.
1971	Campaign Aide, T. Longstreth, Philadelphia, Pa.
1970	Farm hand, H. M. Weingart Dairy Farm, Lebanon, Conn
1969	Clerk Supervisor, United Press International, New York, NY.
1967 - 1968	Intelligence Analyst, U. S. Army, Vietnam.

Professional and Honorary Societies

American Phytopathological Society
Society of Sigma Xi
Society of American Foresters

Publications

Hebard, F.V., Amatangelo, S.J., Dayanandan, P., and Kaufman, P.B. 1976. Studies on acidification of media by *Avena* stem segments in the presence and absence of gibberellic acid. Plant Physiol. 58: 670-67

Dayanandan, P., Hebard, F.V., and Kaufman, P.B. 1976. Cell elongation in the grass pulvinus in response to geotropic stimulation and auxin application. Planta 131: 245-252.

Dayanandan, P., Hebard, F.V., Baldwin, V.D., and Kaufman, P.B. 1977. Structure of gravity-sensitive sheath and internodal pulvini in grass shoots. Amer. J. Bot. 64: 1189-1199.

Griffin, G.J., Elkins, J.R., Tomimatsu, G.S., and Hebard, F.V. 1978. Virulence of *Endothia parasitica* isolated from surviving American chestnut trees. Pages 55-60 in: W.L MacDonald, F.C. Cech, J. Luchok and C. Smith, eds., Proceedings of the American chestnut symposium. West Virginia University, Morgantown.

Hebard, F.V., and Kaufman, P.B. 1978. Chestnut callus cultures: Tannin content and colonization by *Endothia parasitica*. Pages 63-70 in: W.L. MacDonald, F.C. Cech, J. Luchok, and C. Smith, eds., Proceedings of the American Chestnut Symposium. West Virginia University, Morgantown.

Rapoport, E.N., Heller, K.E., Dayanandan, P., Hebard, F.V., and Kaufman, P.B. 1978. Role of indole-3-acetic acid and gibberellin in the control of internodal elongation in *Avena* stem segments. Plant Physiol. 62: 807-811.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1979. Biological control of blight on American chestnut wit and American (naturally existing), hypovirulent strain of *Endothia parasitica*. Proc. W. Va. Acad. Sci. 51 2.

Kaufman, P.B., and Hebard, F.V. 1980. Plants at work: From seed germination to fruiting. In: P.B. Kaufman and D.L. LaCroix, eds., Plants, People and Environment. MacMillan Co., New York, NY.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1984. Developmental histopathology of cankers incited by virulent and hypovirulent *Endothia parasitica* on susceptible and resistant chestnut trees. Phytopathology 74: 140-149.

Hebard, F.V. 1981. Relationship between lesion development and disease progress: A more general mod for compound-interest disease. Phytopathology 71: 880.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1981. Relationship of sprout diameter to canker area and incidence of blight on American chestnut. Phytopathology 71: 224.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1981. An objective method for estimating biocontrol of hypovirulent and virulent cankers. Page 6 in: H.C. Smith, ed., U.S. Forest Service American Chestnut Cooperator's Meeting. General Technical Report NE-64, USDA Forest Service.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1981. Implications of chestnut blight incidence in recently clearcut and mature forests for biological control of blight with hypovirulent strains of *Endothia parasitica*. Page 12, H.C. Smith, ed., U.S. Forest Service American Chestnut Cooperator's Meeting. General Technical Report NE-64, USDA Forest Service.

Griffin, G.J., Elkins, J.R., and Hebard, F.V. 1982. Host-parasite interactions of *Endothia parasitica* on chestnut species. Pages 184-192 in: H.C. Smith and W.L. MacDonald, eds., Proceedings, USDA Forest Service American Chestnut Cooperators' Meeting. WVU Books, Morgantown, WV.

Griffin, G.J., Hebard, F.V., and Elkins, J.R. 1982. Blight resistance in American chestnut. Annual Repo Northern Nut Growers Association 73: 66-73.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1982. Summary research on biology of hypovirulent and virulent *Endothia parasitica* on blight-resistant and blight-susceptible chestnut trees at Virginia Polytechnic Institute and State University. Pages 49-62 in: H.C. Smith and W.L. MacDonald, eds.,

Proceedings, USDA Forest Service American Chestnut Cooperators' Meeting. WVU Books, Morgantown, WV.

Griffin, G.J., Hebard, F.V., Wendt, R.W., and Elkins, J.R. 1983. Survival of American chestnut trees: evaluation of blight resistance and hypovirulence in *Endothia parasitica*. Phytopathology 73: 1084-1092

Hebard, F.V. 1983. Histopathology of Verticillium wilt of alfalfa. Page 34 in R.N. Peaden, ed., Proceedings of Western Alfalfa Improvement Conference, 1983.

Hebard, F.V., Griffin, G.J., and Elkins, J.R. 1984. Developmental histopathology of cankers incited by virulent and hypovirulent *Endothia parasitica* on susceptible and resistant chestnut trees. Phytopathology 74: 140-149.

Scibilia, K.L., Hebard, F.V., and Shain, L. 1987. Field conversion of chestnut blight cankers initiated afte application of hypovirulent condidia. Phytopathology 77:1717.

Stolle, K., Zook, M., Shain, L., Hebard, F.V., and Kuc, J. 1988. Restricted colonization by *Peronospora tabacina* and phytoalexin accumulation in immunized tobacco leaves. Phytopathology 78: 1193-1197.

Hebard, F.V., and Shain, L. 1988. Effects of virulent and hypovirulent *Endothia parasitica* and their metabolites on ethylene production by bark of American and Chinese chestnut and scarlet oak. Phytopathology 78: 841-845.

Hebard, F.V., and Shain, L. 1988. Screening for blight resistance and pathogenicity in chestnut seedlings Phytopathology 78: 1533.

Hebard, F.V., and Shain, L. 1989. Partial purification of a metabolite of *Endothia parasitica* which induces ethylene production in chestnut and scarlet oak. Phytopathology 79: 685.

Hebard, F.V., and Shain, L. 1989. Screening young seedlings of chestnut for blight resistance. Phytopathology 79: 237.

Hebard, F.V. 1990. Some glue for Fortran. MacTutor, The Macintosh Programming Journal 6:46-54.

Hebard, F.V. 1991. Locating flowering American chestnut trees. Journal of the American Chestnut Foundation 6:98-100.

Hebard, F.V. 1991. Preliminary evidence that a single, dominant gene determines hairiness on leaves and twigs of Chinese chestnut. Journal of the American Chestnut Foundation 6:119-121.

Hebard, F.V. 1991. A rapid method of assessing whether a chestnut tree is surviving blight due to resistance or hypovirulence. Journal of the American Chestnut Foundation 6:122-124.

Hebard, F.V. 1994. An engineering approach to controlling chestnut blight with hypovirulence. Pages 169-170 in: Proceedings of the International Chestnut Conference, ed. by M.L. Double and W.L. MacDonald. West Virginia University Press, Morgantown.

Hebard, F.V. 1994. Effect of mortality in oaks due to Gypsy moth on the American chestnut populatio in Pennsylvania. Page 210 in: Proceedings of the International Chestnut Conference, ed. by M.L. Double and W.L. MacDonald. West Virginia University Press, Morgantown.

Hebard, F.V. 1994. Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. Journal of Heredity 85:440-446.

Hebard, F.V. 1994. The American Chestnut Foundation breeding plan: beginning and intermediate steps. Journal of the American Chestnut Foundation 8:21-28.

Hebard, F.V. 1995. Blight resistance in Chinese chestnut is inherited fairly simply. Phytopathology 85:1141.

Huang, H., Dane, F., Norton, J.D., Weaver, D.B., and Hebard, F.V. 1996. Linkage relationships of isozymes and morphological traits in interspecific chestnut crosses. HortScience 31:419-420.

Kubisiak, T.L., Hebard, F.V., Nelson, C.D., Zhang, J., Bernatzky, R., Huang, H., Anagnostakis, S.L., and Doudrick, R.L. 1997. Molecular mapping of resistance to blight in an interspecific cross in the genus *Castanea*. Phytopathology 87:751-759.

Schlarbaum, S.E., Hebard, F., Spaine, P.C., and Kamalay, J.C. 1997. Three American tragedies: chestnut blight, butternut canker and Dutch elm disease. In: Proceedings of Exotic Pests of Eastern Forests Conference, ed. by K.O. Britton and D. Brown. U.S. Forest Service, in press.

Shi, Y., and Hebard, F.V. 1997. Male sterility in the progeny derived from hybridization between *Castanea dentata* and *C. mollissima.* Journal of the American Chestnut Foundation 9:38:47.

Stiles, S., and Hebard, F.V. 1996. Backcross breeding simplified. Journal of the American Chestnut Foundation 10:35-39.

Paul H. Sisco

Occupation: Staff Geneticist
The American Chestnut Foundation Research Farms
14005 Glenbrook Avenue
Meadowview, VA 24361

Phone: (540) 944-4631

Email: paul@acf.org

Educational History:

College: Princeton University
Princeton, New Jersey

Dates Attended: 1963-1967; A.B. degree awarded June, 1967

Major Subject: European history

Academic Honors: Phi Beta Kappa
Danforth Graduate Fellowship

Graduate Schools:

1968-1972: Union Theological Seminary (New York) and Columbia University; M.A. (religion), Dec., 1971

1972-1973: City College of New York: mathematics (probability theory, linear algebra, differential equations)

1972-1973: Teachers College, Columbia: courses in teaching methodology

1977-1982: Cornell University (plant breeding and genetics); Ph.D., May, 1982

Academic Honors: Cornell University Fellowship

Employment History (Last position first):

1998-Present:	Staff Geneticist, The American Chestnut Foundation
1983 - 1997	USDA/ARS Research Geneticist, Department of Crop Science, North Carolina State University
1982 - 1983:	Postdoctoral Research Associate, Genetics, NCSU
1977 - 1982:	Graduate student and teaching assistant, Dept. of Plant Breeding, Cornell University, Ithaca, NY
1975 - 1977:	Teacher, Scarsdale High School, Scarsdale, NY
1973 - 1975:	Teacher, Passaic Valley H. S., Little Falls, NJ
1972 - 1973:	Intern teacher, Mamaroneck High School, Mamaroneck, NY
1970 - 1972:	Admin. Assistant in Financial Aid and Personnel at Union Theological Seminary, New York, NY
1967 - 1968:	VISTA Volunteer, McDowell County, West Virginia
Summers 1963-1965:	Research Assistant, Biophysical Labs, Univ of Tenn. Medical School, Memphis, Tenn.

Memberships:

Genetics Society of America
Crop Science Society of America
American Genetic Association
American Chestnut Foundation
Chair, 39th Annual Maize Genetics Conference, March, 1997.

Grants Funded:

$ 5,000 --	National Science Foundation, 1988. "Cooperative research at NCSU: a comprehensive integrated and documented linkage map for maize."

$ 180,000 -- USDA/NRICG, 9/89 through 9/92 (with C.W. Stuber). "Molecular marker assisted utilization of exotic germplasm in maize."

$ 164,000 -- USDA/NRICG, 8/93 through 7/97 (with R.E. Dewey and D.A. Danehower). "The *glossy15* gene of maize, a cell-specific regulator of leaf epidermal traits."

$ 300,000 -- National Science Foundation, 2/95 through 1/98 (with S. Chilton, M.D. Chilton, and J. Estruch). "Isolation of DIMBOA synthetic and regulatory genes from maize."

Refereed Publications:

1. Zilversmit, D.B., Sisco, P.H., Jr., and Yokoyama, A. 1966. Size distribution of thoracic duct lymph chylomicrons from rats fed cream and corn oil. Biochim. et Biophys. Acta 125:129-135.

2. Schuster, A.M., Sisco, P.H., and Levings, C.S., III. 1983. Two unique RNAs in *cms-S* and RU maize mitochondria, pp. 437-444. In Goldberg, R. (ed.) Plant Molecular Biology, Vol. 12 of UCLA Symposia on Molecular and Cellular Biology, Alan R. Liss, Inc., New York.

3. Levings, C.S., III, Braun, C.J., and Sisco, P.H. 1984. Plasmid-like DNAs of maize mitochondria, pp. 119-122. In Randall, D.D. (ed.) Current Topics in Plant Biochemistry and Physiology, Vol. 3.

4. Sisco, P.H., Garcia-Arenal, F., Zaitlin, M., Earle, E.D., and Gracen, V.E. 1984. LBN, a male-sterile cytoplasm of maize, contains two double-stranded RNAs. Plant Sci. Lett. 34:127-134.

5. Sisco, P.H., Gracen, V.E., Everett, H.L., Earle, E.D., Pring, D.R., McNay, J.W., and Levings, C.S., III. 1985. Fertility restoration and mitochondrial nucleic acids distinguish at least five subgroups among cms-S cytoplasms of maize (*Zea mays* L.) Theor. Appl. Genet. 71:5-15.

6. Braun, C.J., Sisco, P.H., Sederoff, R.R., and Levings, C.S., III. 1986. Characterization of inverted repeats from plasmid-like DNAs and the maize mitochondrial genome. Curr. Genet. 10:625-630.

7. Schuster, A.M., and Sisco, P.H. 1986. Isolation and characterization of single-stranded and double-stranded RNAs in mitochondria, pp. 497-510. In Weissbach, A. and

Weissbach, H. (eds.) Methods in Enzymology, vol. 118, Academic Press, Orlando, FL. 829 pp.

8. Sisco, P.H., Goodman, M.M., and D.L. Thompson. 1989. Registration of NC264 Parental Line of Maize. Crop Sci. 29:248.

9. Sisco, P.H. 1991. Duplications complicate the genetic mapping of *Rf4*, a restorer gene for *cms-C* cytoplasmic male sterility in corn. Crop Sci. 31:1263-1266.

10. Stuber, C.W. and Sisco, P.H. 1991. Marker-facilitated transfer of QTL alleles between elite inbred lines and responses in hybrids. Proc. Annual Corn and Sorghum Research Conf. 46:104-113.

11. Sisco, P.H., Cannon, R.E., and Goodman, M.M. 1993. *Catalase-3* (*Cat3*) gene mapped to the long arm of chromosome 4 in maize (*Zea mays* L.). J. Hered. 84:133-135.

12. Koester, R.P., Sisco, P.H., and Stuber, C.W. 1993. Identification of quantitative trait loci controlling days to flowering and plant height in two near-isogenic lines of maize. Crop Sci. 33:1209-1216.

13. Moose, S.P. and Sisco, P.H. 1994. *Glossy15* controls the epidermal juvenile-to-adult phase transition in maize. The Plant Cell 6:1343-1355.

14. Ragot, M.R., Sisco, P.H., Hoisington, D.A., and Stuber, C.W. 1995. Molecular-marker- mediated characterization of favorable exotic alleles at quantitative trait loci in maize. Crop Science 35:1306-1315.

15. Moose, S.P. and Sisco, P.H. 1996. *Glossy15*, an *APETALA2*-like gene from maize that regulates leaf epidermal cell identity. Genes and Development 10:3018-3027.

Patents:

1. Gracen, V.E., Sisco, P.H., and Bouthyette, P. 1986. LBN Cytoplasm. U.S. Patent # 4,569,152.

Robert C. Leffel

1925 Birth - reared on a 1600 acre Maryland estate managed by his father.

1943-46 U.S. Marine Corps

1948 B.S., University of Maryland: Agronomy-Crop Production
1950 M.S., Iowa State College: Crop breeding and plant physiology
1952 Ph.D., Iowa State College: Crop breeding, plant pathology, and genetics

1952-57 Research Agronomist, ARS-USDA and Univ. Maryland: Soybean breeding and culture
A. Assoc. Professor, Univ. Maryland: Forage Crop breeding and teaching plant breeding and other courses

1962-94 ARS - USDA:
- 1962-70 Investigations Leader: Clovers
- 1970-72 Investigations Leader: Clovers and Special Purpose Legumes
- 1972-76 Laboratory Chief, Plant Nutrition Laboratory
- 1976-83 Staff Scientist, National Programs Staff - Oilseed Crops Production

Research Agronomist, Soybean and Alfalfa Research Laboratory - Soybean breeding and genetics

1994 - Retirement: Farming and PA-TACF Breeding Program Scientist

A career devoted to cooperative multidisciplinary plant improvement programs with State, Federal, and Private organizations.

JAMES HILL CRADDOCK

University of Tennessee at Chattanooga, Department of Biological and Environmental Sciences, 615 McCallie Avenue, Chattanooga, TN 37403 • Telephone: 423-755-4341

PERSONAL

I was born in Louisville, Kentucky on 16 February 1960. I am married to Paola Zannini and we have one son, Emilio, who was born on 23 May 1994. I grew up in Woods Hole Massachusetts, on Cape Cod, son of a Marine Biologist father and an Emergency Room Nurse mother. I went to sea for the first time at age 16, working summers on commercial fishing boats. I have been a gardener since I can remember. I grew my first chestnut tree from a seed I planted when I was 15 and I am still a chestnut enthusiast. I moved to Italy in 1987 where Paola and I helped run her family's restaurant business. We moved to Tennessee in November of 1994.

EDUCATION

UNIVERSITA' DI TORINO
Dottorato di Ricerca in Colture Arboree, 1992
Thesis title: Indagini in *Corylus avellana* L. e *Castanea* spp. su: radicazione di talee tramite l'impiego di funghi ectomicorrizici; impollinazione e germoplasma di castagno (Investigations in *Corylus avellana* L. and *Castanea* spp. on: rooting of cuttings using ectomycorrhizal fungi, pollination and germplasm of chestnut).

OREGON STATE UNIVERSITY
Master of Science in Horticulture, 1987
Thesis title: Cryopreservation of pollen.

INDIANA UNIVERSITY
Bachelor of Arts in Fine Arts and Biology, 1983
Areas of concentration: Printmaking (etching and lithography) and Botany.

EXPERIENCE

UNIVERSITY OF TENNESSEE AT CHATTANOOGA - DEPT. BIOLOGICAL AND ENVIRONMENTAL SCIENCES
Robert M. Davenport Assistant Professor in Biology, January 1999 to present.
Assistant Professor, August 1996 to December 1998.
Teaching duties: My teaching responsibilities include Fundamentals of Biology (BIOL 121 and BIOL 122) and Introduction to Mycology (BIOL 351). **Research interests:** My current research is focused on the restoration of the American chestnut (*Castanea dentata*) to the Appalachian hardwood forest ecosystem and the establishment of a commercial chestnut industry in Tennessee. Project areas include: breeding for blight resistance and gall wasp resistance, genome analysis using molecular markers, investigations on the physiology and ecology of hypovirulence, and germplasm collection and evaluation. Other areas of interest include the role of mycorrhizal fungi in adventitious root formation and the rooting of cuttings.

TENNESSEE STATE UNIVERSITY - NURSERY CROPS RESEARCH STATION
Postdoctoral Research Associate, December 1994 to August 1996.
Project areas: Tree breeding, cultivar evaluations and germplasm collection. As a breeder, I was working on development of new *Cornus florida* cultivars with resistance to dogwood anthracnose disease. I used classical plant breeding techniques (emasculation, isolation and hand pollination or honeybee-mediated pollination of the flowers) as part of a large, multidisciplinary team that includes molecular biologists, plant pathologists and geneticists. The cultivar evaluations included a state-wide chestnut variety trial to evaluate clones for nut production and quality. My responsibilities for the chestnut germplasm collection involved the introduction, propagation (by grafting) and maintenance of *Castanea* clonal germplasm (in collaboration with University of Tennessee, Knoxville, Dept., of Forestry, Wildlife and Fisheries).

UNIVERSITA' DI TORINO, DIPARTIMENTO DI COLTURE ARBOREE
Postdoctoral Fellow, January 1993-December 1994
Teaching duties: University extension course for chestnut growers "Valorizzazione della Castagna in Valle D'Aosta." I was part of a five-member teaching team. I gave classroom lectures and field demonstrations on chestnut propagation and grafting, and on the ecology of chestnut forest soils including the ectomycorrhizal symbionts of chestnut. **Research projects:** *Castanea* germplasm and chestnut mycorrhizae. The *Castanea* germplasm project involved collection and propagation of endangered chestnut cultivars, establishment of cultivar evaluation orchards and international exchange of chestnut germplasm. The ectomycorrhizal fungi associated with chestnut were studied in the field and in the laboratory. I accomplished in vitro isolation and mycelial cultures of several species. Nursery inoculation techniques using spores, mycelial cultures and mycorrhiza fragments were investigated.

LICEO LINGUISTICO JEAN JACQUES ROUSSEAU
Lettore di Madrelingua in Inglese, September 1991-June 1992
Teaching duties: Conversational English, at the high school level. I taught six classes at all five grade levels (I had two senior classes).

UNIVERSITA' DI TORINO, ISTITUTO DI FRUTTICOLTURA INDUSTRIALE
Graduate Research Assistant, October 1989-October 1992
Teaching duties: University extension lectures and seminars.
Research projects: Rooting of chestnut and hazelnut cuttings facilitated by ectomycorrhizal inoculation of the rooting medium, international fruit and nut tree germplasm exchange, collection and propagation of endangered fruit and nut tree cultivars, breeding and selection of interspecific *Prunus* hybrids. The mycology work was done at the CENTRO DI STUDIO SULLA MICOLOGIA DEL TERRENO, C.N.R., which is located at the REALE ORTO BOTANICO DI TORINO.

USDA-ARS NATIONAL CLONAL GERMPLASM REPOSITORY, CORVALLIS, OREGON
Research Assistant September 1984-September 1987
In addition to my Masters thesis research on pollen storage, my responsibilities included propagation of new repository accessions by grafting and rooting of cuttings, pruning and fruit harvest in the collection orchards, virus indexing (ELISA) and virus elimination (thermotherapy and shoot-tip

micrografting) in the pear collection. I developed a simple protocol for the collection, handling and long-term storage of pollen for *Corylus*, *Fragaria*, *Rubus*, *Pyrus*, and *Vaccinium* which has been implemented as part of the Repository's mission to conserve genetic resources of asexually propagated fruit crops.

MEMBERSHIPS

American Society for Horticultural Science
American Chestnut Foundation (V.P. Science and Chairman, Science Cabinet)
Beta Beta Beta Biological Honor Society
International Society for Horticultural Science
Middle Tennessee Nursery Association
North American Fruit Explorers
Northern Nut Growers Association
Società Orticola Italiana
Tennessee Nurserymen's Association
U.S. Dept. Agriculture Regional Project NE-140 (Secretary)

PUBLICATIONS

REFEREED JOURNALS

Craddock, J.H. (1999) Chestnut Resources in North America. Annual Report of the Northern Nut Growers Association 89: in press.

Bounous, G., Paglietta, R., Bellini, E. and Craddock, J.H. (1995) Il miglioramento genetico del castagno: situazione, obiettivi e metodi. Frutticoltura 57(11): 63-73.

Meotto, F., Pellegrino, S. and Craddock, J.H. (1994) Funghi ectomicorrizici del castagno con particolare riferimento ai funghi eduli. Italus Hortus 1(2):58-64.

Craddock, J.H., Ferrini, F., Mattii, G.B., Nicese, F.P. and Pellegrino, S. (1991) Ricerche per l'individuazione di impollinatori del "Marrone di Chiusa Pesio." Frutticoltura 53(12):61-63.

Thomas L. Kubisiak

Plant Research Geneticist, USDA Forest Service, Southern Institute of Forest Genetics
23332 HWY 67, Saucier, MS 39574 Tel: (228) 832-2747

EDUCATION

Louisiana State University, Baton Rouge, LA, Ph.D. Forestry (Forest Genetics), December 1994. Ph.D. Dissertation: "Molecular marker linkage mapping in southern pine (longleaf pine and slash pine)".
University of Minnesota, St. Paul, MN, M.S. Forestry (Forest Genetics), December 1990. Master's Thesis: "Transformation of *Populus tremuloides* by *Agrobacterium tumefaciens*".
Michigan State University, East Lansing, MI, B.S. Forestry, August 1988.

HONORS AND AWARDS

American Legion Scholarship, 1985. Midland Foundation Scholarship (R. Cermak), 1985. Weyerhaeuser Company Foundation Poster Presentation Award, 1996. USDA Certificate of Merit, 1996; Invited Scholar Chinese Academy of Sciences, Wuhan Institute of Botany, Peoples Republic of China, 1998.

PROFESSIONAL EXPERIENCE

Plant Research Geneticist, November 1994-present, USDA Forest Service. Responsibilities include planning, designing, and implementing research programs on genetics of economically important traits in various forest tree species, as well as survey and conservation work on various threatened and endangered plant species. Research interests include; plant genome structure and organization with special attention to linkage analysis, molecular marker linkage to quantitative traits and marker assisted improvement, population level partitioning of genetic variation, and genetics of the host:pathogen interaction.
Graduate Research Assistant, January 1991-December 1994, Louisiana State University, School of Forestry, Wildlife, and Fisheries. Lead efforts to construct RAPD-based molecular marker linkage maps of longleaf pine (*Pinus palustris*) and slash pine (*Pinus elliottii*) for use in a backcross breeding program. Assisted in teaching a graduate level course - Research Methodology. Guest lecturer in various graduate level courses - Advanced Plant Genetics and Quantitative Genetic Plant Improvement.
Graduate Research Assistant, September 1988-December 1990, University of Minnesota, Department of Forest Resources. Developed a simple but efficient *Agrobacterium*-mediated transformation system for *Populus tremuloides*. Investigated the effects of various factors such as bacterial strain, host genotype, host tissue type, and presence of inducer compounds on the efficiency of transformation. Given sole responsibility for teaching dendrology laboratory for three consecutive fall semesters.

Publications

Stelzer, H.E., Doudrick, R.L., **Kubisiak T.L.**, and C.D. Nelson. 1999. Prescreening slash pine and *Cronartium quercuum* pedigrees for evaluation of complementary gene action in fusiform rust disease. Plant Disease 83:385-389.

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B_3-F_2 Plot Design at TACF's Wagner Research Farm: Design for Controlled Pollinations from a Circular Mating Used To Produce the B_3-F_2s.

Introduction

This document contains portions of the plan for the advanced and final stages of our breeding program. Some aspects of the plan have not yet been thoroughly explored, and not all details have been ironed out.

Biological Considerations

Production of B_3-F_2 Seed. This could be accomplished by controlled pollination or by open pollination. Currently, with much effort, our yield from controlled pollinations has been about 5000 to 6000 nuts per year. (We get about 1 nut per bag; personally, Fred thinks pine and elm breeding should be illegal, since they get about 200 and 1000 seeds per bag, respectively)!

With regard to open pollination, when our B_3 orchards were laid out, they were blocked by year of nut production rather than having the various years intermingled, so it will not be possible to achieve fully random mating among all lines by open pollination. However, to achieve fully random mating, even by controlled pollination in polymix crosses, we would have to wait until all lines were in production, and would then have a massive pollination, harvest, planting and culture effort. Currently, we are increasing our lines incrementally, so will begin production from B_3-F_2 seed much earlier than would occur were we to wait, with much less strain on our resources.

What we would effectively achieve from open pollination among select B_3 trees would be a series of disconnected full diallel crosses, if we could plant enough seed to capture resistance from most potential cross combinations. We have not explored how much seed might be needed. The number of lines in each of these blocks would have to assessed carefully to determine better the number of contributors to the effective disconnected full diallel crosses.

The number of seed needed per cross will vary with the minimum number of more-or-less homozygous trees we desire from each intercross. Currently, our data pretty much indicate that three incompletely dominant genes control blight resistance. If we desired two progeny homozygous for three factors from a single intercross, we would need to grow at least 423 trees to have greater than a 99% chance of obtaining those 2 progeny. Four homozygous progeny would require 640 trees, six, 835, and eight, 1021. So the mathematics indicates greater efficiency at higher numbers of desired homozygotes.

We have about 15 cross combinations for each of our two sources of blight resistance

that are about to be advanced to B_3-F_2. These will be collected over a 6 to 8 year period. We could probably generate a maximum of about 1000 nuts per cross during that period by controlled pollination.

Mating Design. A circular mating design is one where no tree is crossed with more than one other tree. It would be necessary to implement this design with controlled pollinations. Open pollination, as discussed above, would effectively result in a series of disconnected full diallel. This particular document is based on a circular mating design. The circular design would avoid inbreeding up to the F_2 stage. A principle design criterion for our F_2 seed orchards is to avoid inbreeding in production of F_3 seed.

Planting Capacity. We can pack emerged seedlings at a maximum density of about 5000 per acre, and might be able to plant and tend about 4 acres of such plantings per year. Over a 6-year period, that would be about 120,000 seedlings, or 60,000 per source of resistance. So our planting capacity would limit the total number of nuts per cross to 2000, about double what we could generate from controlled crosses.

Seed Yield from B_3-F_2 mother trees. We have little basis for determining what our seed yield will be, but crudely estimate that it might reach about 5000 nuts per tree per year for 10-year-old trees. (This estimate needs to be refined; the main unknown, however, is the effect on seed production of inoculation to screen for blight resistance; the inoculation is much more severe than is experienced in nature). We currently expect to generate about 30 lines from the Clapper source of blight resistance. If we were to generate one mother tree for each of these lines, we could harvest 150,000 nuts per year from one group of 30 trees. If the harvested nuts were planted at 200 per acre, one group of 30 mother trees could supply about 750 acres per year. Clearly, there is a need for seed increase.

Seed Increase. This might be accomplished by creating more than one group of B_3-F_2 mother trees for any source of resistance, by vegetatively propagating the single block, or by setting up F_3 seed orchards.

Regarding multiple groups of mother trees: we would lose a fair number of the alleles present in the straight B_3s if we kept only one group; the more groups of B_3-F_2 trees we plant, the more alleles we capture. We can avoid inbreeding by keeping groups separate (or half groups if we go to a circular mating design with controlled pollination). We are currently exploring the mathematical relationship between the number of groups and the number of alleles captured. Current results indicate that 3 or 4 groups would capture many of the alleles. Going to four would also increase seed production to the neighborhood of 600,000 nuts per year, enough for perhaps 3000 acres per year. Still not enough seed.

Regarding vegetative propagation: it appears from the above that it would be much better, at least up to a point, to increase the number of groups of mother trees by breeding than to increase one or two groups by vegetative propagation. In Fred's experience, grafting is much the best method for vegetative propagation of chestnut.

We have not yet evaluated the relative genetic merits, such as effective population size, of vegetative propagation versus going to F_3 for large seed increase. From an operational standpoint, seed are much easier to handle, quicker and more reliable. The F_3s would also give us some indications of the breeding value of their parents, and offer the opportunity for further selection at F_3, should that be necessary.

Physical Considerations

From the above biological considerations, it is clear that we should grow about 1021 B_2-F_2 trees from any single cross combination, if we adopt a circular mating design implemented with controlled pollinations. These 1021 trees would be subdivided into eight crossing blocks containing 15 cross combinations each for any single source of blight resistance. Although the 1021 trees would give us greater than a 99% chance of obtaining at least 8 trees homozygous for three factors, those 8 trees wouldn't necessarily be distributed one per crossing block. We probably would have to transplant a few trees to achieve balance.

Seed Germination. We currently obtain trees from about 80% of planted nuts, on average ground, with good seed. To get 1021 trees, we would need to plant about 1275 nuts or 160 per crossing block.

Sub-Plots. Each set of 160 nuts would be planted in one sub-plot within a crossing block. These would then be screened for blight resistance and our eight desired trees selected. Each crossing block would consist of 15 or so sub-plots with the circular mating design.

Seed Orchard. We need a minimum of 20 trees per acre to achieve effective cross pollination. When leaving one tree within each sub-plot, we would need plots no larger than 2178 ft^2. So we would need to pack 160 nuts into less than 2178 ft^2, or about 47 ft on a side.

Cultural. Seven feet is the minimum we can handle between rows. Double planting within rows is impractical because of difficulties with weeding.

Farm-Shape. The Wagner Research Farm has a long leg about 330 ft wide running west to east about 0.5 miles. The general slope runs from north to south, so rows should run east-west to minimize erosion. We would like to start planting at the back end of the farm as most desirable trees back there have been advanced to third backcross.

The 330 ft between the north and south fences could accommodate up to 44 rows at 7 ft between rows. For 20 trees per acre, it would be difficult to get square plots with 7' rows.

Plot shape. Square plots offer the greatest possibility of interpollination between all the trees they contain, except for circular plots, which are impractical for mechanical culture.

Combining Physical Considerations. Going to 30 trees per acre as a final density would let us establish a square block of 16 square subplots, with each subplot 38 ft on a side. An entire plot would be 152 ft on a side. Plots could be arranged in a checkerboard pattern across the long back leg of the farm, as indicated on the attached map. The checkerboard pattern would maximize distance between sibs in adjacent crossing blocks, if we retained the same placement of cross combinations within subplots between blocks (This has the disadvantage of favoring a particular subset of the possible matings). We would randomly assign cross combinations to particular subplots. We could omit trees from adjacent corners if we do not have 16 cross combinations. We could add a few extra cross combinations, beyond 16, if they existed, without seriously affecting the crossing block

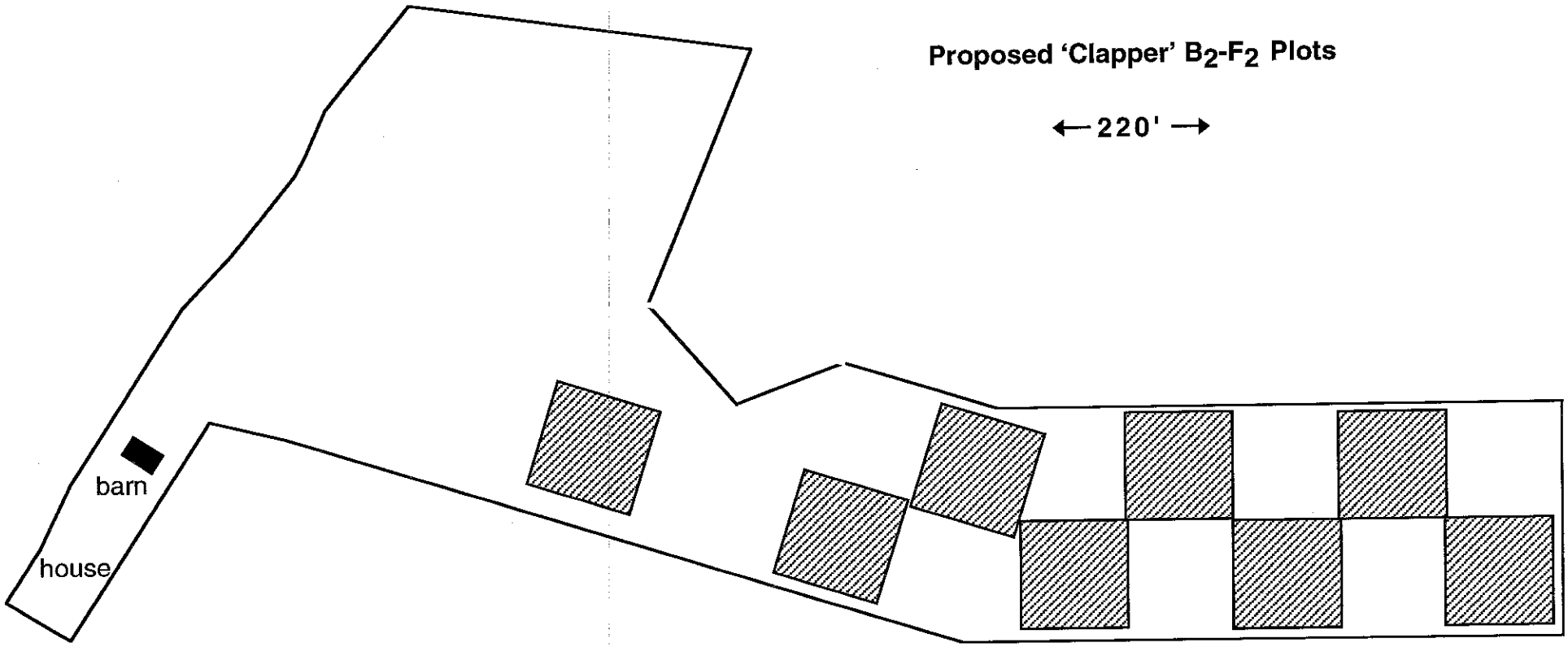
There would be five rows within each subplot, and a 10' border between rows of adjacent subplots. Each row would contain 32 trees planted 1 ft apart, leaving a 9 ft border between plots. There would be room for an access road on the lower side, and egress along the lower and upper sides.

American Chestnut Foundation

Wagner Research Farm

Proposed 'Clapper' B_2-F_2 Plots

← 220' →



Subplots / plot	Stacks / plot	Sub-plots / stack	Rows/sub-plot	Sub-plot height Ft between rows 7	Sub-plot width Row length for X trees p acre 20	30	Plot width 20 trees /a		Plot height	Plot width 30 trees /a	
15	3	5	8	59	37	25	111		295	75	
18	6	3	8	59	37	25	222		177	150	
18	3	6	7	52	42	28	126		312	84	
18	6	3	7	52	42	28	252		156	168	
21	3	7	6	45	48	32	144		315	96	
16	4	4	6	45	48	32	192		180	128	
16	2	8	5	38	57	38	114		304	76	
16	4	4	5	38	57	38	228		152	152	
20	2	10	4	31	70	47	140		310	94	
15	3	5	4	31	70	47	210		155	141	

Frequency of Selection for Blight Resistance in BC_2, American x {American x [American x Chinese]}, Chestnut Trees from Two Sources of Resistance in Five Years of Tests and Fit to a Three-Gene Model for Inheritance of Resistance.

Orchard	Year	Clapper B_2				Graves B_2			
	Measured	Selected	Not Selected	Chi-Sq (1:7)	p	Selected	Not Selected	Chi-Sq (1:7)	p
CL 90	1994	12	107	0.6	0.43				
GR 91	1995	11	50	3.4	0.07	10	71	0.7	0.40
AB 92	1996	14	123	0.7	0.42	6	53	0.3	0.59
Be 93	1997	13	88	0.0	0.91	7	60	0.3	0.61
WV 94	1998	15	64	3.0	0.08	18	146	0.3	0.56
VA 94	1998	6	56	0.5	0.50	21	92	3.8	0.05
JN 94	1998					1	31	2.6	0.11
Totals		71	488	0.3	0.60	63	453	0.2	0.69

Testing BC3-F3 Progeny of controlled crosses between BC3-F2s. The selections made in the BC3-F2 seed orchard need to be tested for resistance, for the absence of Chinese characteristics, and for growth. If suitable molecular markers are available, homozygosity at the putative resistance loci could be determined on the BC3-F2 selections. This might best be done on all the candidates that make the short list. The results would be a major consideration in making the final selection of BC3-F2s. It would also be desirable to make controlled crosses between BC3-F2 selections and test the BC3-F3 progeny for resistance by inoculation. Finally BC3-F3 seeds given to cooperators should be used to evaluate performance in the field based on a valid statistical design. One could visualize a very simple design where each cooperator is given three seedlots: one control and two lots from controlled crosses between BC3-F2 selections. Each cooperator would be a replication, and the results could be analyzed using a least squares program. It is important to follow up regularly with each cooperator to maintain their interest.

Here is an example of a design for using cooperators to test BC3-F3 progeny of controlled crosses:

Assume there are 20 trees left in the BC3-F2 orchard, numbered 1-20. Each cooperator receives one control lot (*e.g.* unimproved American or a slightly improved source of which you have a lot of seed) and a BC3-F3 seedlot from each of two controlled crosses between BC3-F2 orchard trees. It is important that everybody gets the same control lot. Each BC3-F3 seedlot to be tested needs to go to at least four cooperators. More is better. Each combination should be used only once. The scheme would look something like this, where Seedlot 1 = (Tree 1 x Tree2), Seedlot 2 = (Tree 3 x Tree 4), etc. Notice that each seedlot is represented four times.

		BC3-F3 Seedlots									
Cooperator	Control	1	2	3	4	5	6	7	8	9	10
A	x	x	x								
B	x	x		x							
C	x	x			x						
D	x	x				x					
E	x		x	x							
F	x		x		x						
G	x		x			x					
H	x			x	x						
etc.											

If there are not enough cooperators you could give them more seedlots. It would of course be even better if you could have cooperators that would be willing to take on an entire well designed progeny test. TACF staff would have to lay out and plant the test and take the measurements, and the cooperator would get to keep the surviving trees when the test is over.

The TACF Breeding Program Review
August 12 - 15, 1999

Introduction

The vision of The American Chestnut Foundation to restore the American Chestnut to its native habitat in the United States is being accomplished through the breeding program and various other activities. This report is mainly focused on the breeding program that has the goal of generating a chestnut blight resistant population with all the favorable characteristics of the American Chestnut.

A review team of individuals with expertise in different areas of tree breeding and/or genetics consisted of Dr. Shawn A. Mehlenbacher, Professor of Horticulture, Oregon State University, Dr. Ronald L. Phillips, Regents' Professor of Plant Genetics, University of Minnesota, and Dr. J.P. van Buijtenen, Professor Emeritus of Forestry, Texas A&M University. They were provided extensive documentation prior to the meeting on the procedures and accomplishments of the breeding program. A tour of the Price and Wagner farms acquainted the team with the various orchards, status of the availability of the various generations of trees, inoculation procedures, and many other aspects of the program. Participants from the The American Chestnut Foundation included Drs. J. Hill Craddock, Albert Ellingboe, Robert Leffel, Robert Doudrick, Messrs. David Armstong and Peter Wood, and the two staff scientists Dr. Frederick V. Hebard and Dr. Paul H. Sisco.The staff scientists made formal presentations about their progress and the underlying science. Dr. Robert Leffel also reviewed the breeding progress of the Pennsylvania Chapter and its interrelationship with the program in Meadowview.

The review team was impressed by the progress made to date in the breeding program. The scientists are focused on the goals as set forth originally by Dr. Charles R. Burnham and advanced through the many efforts of The American Chestnut Foundation. These efforts represent an exceptional example of how volunteers with a highly focused mission can accomplish a goal of broad interest to the American people but one for which federal and state funds are extremely limited. We commend the staff scientists for their dedication and sincere interest in achieving the goal. The overall goal of the Foundation is quite ambitious; that is, to restore the species in the United States. The review team is pleased that the Foundation recognizes the long-term effort required in the breeding program and that members are providing sound advice and support. We believe the backgrounds of the two staff scientists are complementary and were pleased to recognize their positive personal and professional interactions. Their understanding of the complexity of Chestnut tree backcross breeding for type and disease resistance is impressive and serving the program well. Their enthusiasm for the

project and dedication to keeping the program focused while interested in applying the most efficient scientific approaches are certainly commended by the review team. The key people in making this program successful appear to have a good relationship.

The focus of this review is principally on the breeding program and an examination of the underlying science. While the review team has several suggestions, we recognize that there is a restricted budget and that keeping focused on the primary objectives is quite important. We are pleased that The American Chestnut Foundation judged this review as an important activity; a regularly scheduled scientific review every 3 -5 years is appropriate. Of course, we recognize that the preparation for the review is an important step as well as the post-review decisions. The review team offers this document as a means for further discussion by the Board and staff, recognizing the fact that we spent only two days on-site.

Defining and revisiting objectives periodically is an essential part of a vibrant research program. In this regard, the objectives should be more explicitly stated relative to the breeding program *per se*, as opposed to the goal of restoring the Chestnut to its native range and perhaps to future goals of improving timber quality, nut production, and other characteristics. A clear flow chart of all aspects of the program would be useful. The current information, although immensely useful, left the review team with a less than perfectly clear picture of the overall program. For example, a clearer idea of the number of traits and the measurements that demonstrate recovery of the recurrent parent (American) in the various generations would have been helpful in assessing progress. Efficiency gained through early selection, progeny testing, clonal propagation to achieve replication and to assist in amplifying superior genotypes, and use of land all need further consideration. The number of sources and their genetic relationships at least in terms of disease resistance needs further testing.

This report is outlined around aspects considered in designing a breeding program; *i.e.* **Parents, Crossing, Selection, Evaluation, Release**, and **Equipment**. First, however, we list the general recommendations to The American Chestnut Foundation.

Recommendations:

1. We endorse the backcross strategy as appropriate for the stated objective of combining blight resistance with American timber-type growth habit.

2. We recommend the use of additional sources of resistance and the development of BC3F2 selections from each of these sources.

3. We recommend expansion of the number of American parents used in breeding.

4. We recommend incorporation of clonal propagation (by grafting or stooling) as a routine procedure in the breeding program.

5. We recommend investigation of methods to increase seed yields from controlled pollinations and seed orchards while minimizing contamination.

6. We recommend more rapid elimination of undesirable seedlings from plots. The clonal propagation of the best seedlings will allow wiser use of the available land.

7. We recommend improved weed control and use of herbicides other than Roundup.

8. We recommend the extensive use of stakes in plots and labels on trees.

9. We recommend additional research on methods to determine levels of blight resistance, particularly methods applicable to young trees.

10. We recommend that seed orchards be established using clonally propagated clones, and that such orchards be established on well-drained sites where chestnuts will thrive.

11. We recommend that as superior selections are identified and seed (or seedlings) from seed orchards become available, that trials be established to determine adaptation of this chestnut germplasm.

12. We recommend that the current TACF Germplasm Agreement be replaced with two separate types of agreements. The first type would be a memorandum of understanding or material transfer agreement. This type of agreement would allow cooperators to use TACF selections in breeding and for evaluation purposes. A second type of agreement should be developed to cover the propagation and marketing of new cultivars developed by TACF.

13. We recommend the establishment of an attractive, well-manicured, and well-labeled collection of parents and advanced TACF selections at the entrance to the Wagner Farm as part of TACF's efforts to educate the public about its activities.

14. We recommend the purchase of a freezer (-80C) for storage of leaf and pollen samples.

15. We encourage the use of DNA markers, primarily through grants or contracts with outside agencies, for the detection of pollen contaminants, mapping resistance loci, recovery of the recurrent parent genome, and possible patent protection of advanced selections.

The TACF breeding program is developing blight-resistant chestnuts with American-type timber growth habit. Active plant breeding programs constantly evolve, redefining objectives as new

information becomes available, seeking out and using new parents, improving pollination techniques, improving propagation methods, solving problems as they are encountered, and adopting improved methods as appropriate. Objectives need to be prioritized, and attention focused on the most important objectives.

Parents

Choosing parents. The backcross method being used requires identification of sources of blight resistance (donor parents) and susceptible American chestnuts (recurrent parents).

Sources of resistance. Most of the advanced selections in the TACF breeding program now incorporate resistance from one of three sources: Clapper, Mahogany/Graves, and Nanking. Additional sources would be highly desirable. We encourage the TACF scientists to assemble a collection of potentially resistant parents (25-30) and to evaluate their blight resistance and other characteristics, and to use the best 15 or so as parents. The review team is concerned about the Clapper defect.. This problem may become even more serious in the future, and illustrates the need for use of additional sources of resistance. The possible existence of pathogenic variation in the chestnut blight fungus is an additional reason to use additional sources of resistance. Continued collaboration is encouraged with the USDA Repository in Somerville, TX (Dr. L.J. Grauke, curator) for the importation of chestnut germplasm from Asia.

American parents. The number of American parents used in each backcross has been limited in past years. Their inherent disease susceptibility leads to early death, although mud packs can prolong their lives somewhat. Efforts are now being made to broaden the genetic base on the American side, and we commend these efforts. The American parents are most critical in the last backcross, as half of the genes will come from the American parents used in crosses. By using cooperators in Pennsylvania, Tennessee, and other states, diversity in the American chestnut can be incorporated. Also, because chestnuts bloom first in the south and later in the north, travel during the pollination season could lead to the use of more American parents. Similarly pollen collection from early-blooming trees at lower elevations can be used to pollinate American trees in clearcuts at higher elevations, thus extending the pollination season. The continued use of large surviving American trees in breeding is encouraged, as some of these trees do indeed appear to transmit some resistance.

Seed stratification. Procedures for seed stratification appear to be adequate. Refrigerated storage facilities have been adequate in past years, but additional space may be needed in the future.

Crossing

Pollination. The large number of outcrosses (pollen contamination) in past years is a concern. We encourage the staff to further investigate different materials (bagging materials, bag sizes, *etc.*) and procedures (time of bagging, pollen collection and storage, *etc.*) to reduce the incidence of outcrossing. Removal of adjacent trees and some pruning would be expect to result in increased flowering and set on selected trees. Male-sterility, presumed to result from the interaction of American cytoplasm and dominant nuclear genes from the Chinese chestnut, can be viewed as both a blessing and a curse. Male-sterility dictates the direction of crosses but makes possible the release of hybrid cultivars from seed orchards containing grafted trees of two clones. In the absence of contamination by outside pollen, seed harvested from the male-sterile clone would be entirely the result of pollination by the other selected clone. Such a cultivar would have a narrow genetic base, however, and thus less desirable for reforestation.

Research on pollen-stigma incompatibility. Pollen-stigma incompatibility is known to exist in chestnut, but it is not known if it is of the gametophytic or sporophytic type. At this time, we feel that this research problem is of low priority. Determination will involve examination of seed set following crosses in a very large number of combinations, including reciprocals, among full sibs and backcrosses to the two parent clones. We would assign a lower priority to this research.

Breeding strategy. The backcross method appears to be working, as advanced selections from the TACF breeding program combine desirable growth habit with a moderate level of resistance. A higher level of resistance is expected in the BC3F2 generation. We support continued use of this method (see Figure) and the development of additional BC3F2 selections. These BC3F2 selections could be clonally propagated (by grafting or stooling) and used as parents of a seed orchard. BC3F2 selections could be released as clones for limited use (homeowners, small farmers), but the vast quantities of trees needed for reforestation would be from seed orchards. These same BC3F2 selections (or their BC3F1 parents) could be used as parents for additional generations of backcrossing. Additional backcrosses may be needed to achieve adaptation to local conditions, and State chapters could play a very important role. Likewise, State chapters and TACF members could play a very important role in the evaluation of promising selections and seed lots. The use of additional sources of resistance and advancing them to the BC3F2 generation by staff at Meadowview should receive a higher priority than advancing the Clapper, Mahogany/Graves, and Nanking sources to the BC6. Time and experience will determine the number of backcross generations needed, and the importance of genotype x environment interaction. The linkage map developed for the Mahogany F2 population indicates that many Chinese characters are on linkage group C and independent of blight resistance genes (in this cross). In the absence of selection, the fraction of the genome from the American parent will increase from 1/2 to 3/4 to 7/8 to 15/16. Accompanying this increase in the American

contribution, we expect to recover American-type growth habit. The Chinese x (Japanese x American) hybrids developed in Connecticut many years ago reached a height of 40 ft and stopped growing. The pedigrees of TACF selections, with the large American contribution to their genomes, would be expected to behave like American chestnuts.

Figure

Chinese x American 1

F1

x American 2

BC1F1 (Note: Clapper and Graves are BC1F1 selections)

x American 3

BC2F1

x American 4

BC3F1

x siblings (controlled pollinations)

BC3F2

clonally propagate the best selections

--> recurrent selection (disease resistance, growth rate, form, wood quality)

--> further backcrossing (if needed)

Establish seed orchards --> seed or seedlings for distribution

Progeny tests (or further evaluation of parent clones)

Remove inferior clones from the seed orchard.

Argument against going to BC6. At the BC3, it appears that the desired combination has been obtained of blight resistance and American type trees. Many examples can be cited in fruit and nut crops where the objectives were met long before the BC6. If the BC3 is the last generation, then locally adapted American parents should be used to produce this generation. Given a choice between advancing the Clapper, Mahogany/Graves, and Nanking sources to the BC6 or starting to use additional resistance sources, the latter is clearly more important in at least one reviewer's opinion. Furthermore, there would be more to gain in using BC3F2 selections to start a recurrent selection program rather than continue with backcrossing. Remember that for forest establishment, a heterogeneous population of heterozygous trees should be the objective. It seems that the use of unevaluated American parents in the backcross generations is equally (if not more) likely to introduce undesirable traits than the relatively few Chinese genes that would remain. Additionally, resistant seedlings from the BC3 generation would be a very significant improvement over the current cultivars (= dead trees). As more is learned in future years, it may even be possible to reduce the number of backcross generations to two, and establish seed orchards with BC2F2 selections.

Arguments for advanced backcrossing: There is a debate among the staff and others concerning the value of continuing the backcross program to more advanced generations. This is a legitimate discussion with no simple answers and, in fact, the review team is divided on the seriousness of the question. The importance of the advanced backcross program depends on many factors that are largely unknown at this time and revolves around how deleterious the 6-7% Chinese genes remaining in the released germplasm will be to the ultimate survival and uses of the material.

Although theory dictates that the recovery of American genes in the BC3 will be 93-94%, this is an average figure derived assuming no selection and normal genetic behavior. The actual percent American in any one selected line may be rather different. Significant deviations can occur due to sampling, selection for and against traits, meiotic behavior (recombination and transmission frequencies) in the interspecific hybrid material, and other factors. Even with expected levels of recovery of the American genes, the remaining Chinese genes could lead to problems in the future.

A trade-off exists between utilizing breeding time and resources for advanced backcrossing versus starting over with additional sources of resistance. The review team favors the development of useful materials with other sources of disease resistance since we know that susceptibility to the blight can devastate the species and that the pathogen will undoubtedly undergo change over the years and could well overcome any specific resistance genes bred into the American germplasm. However, there are advantages of further backcrossing as a means to protect the future use of proven material. We would recommend that further backcrossing, if performed, be done with the aid of molecular markers to maximize the recovery of the recurrent parent and perhaps reduce the number of backcross generations needed. These materials may be especially useful as timber quality, yield of timber, nut production and other possible characteristics become important. If

further backcrossing is performed, we would recommend that BC3 trees be used based on BC3 F3 progeny tests that will be available. The State chapters of The American Chestnut Foundation may play a role here since many will be crossing the released germplasm to locally adapted American trees. If two generations of backcrossing to these trees are achieved, BC5 materials will be generated. If it is learned that there are relative low levels of genotype x environment interaction in nearby states, this might be a reasonable approach.

Selection

Seedling growth. An average of 80% survival from direct planting of stratified seeds in the orchard would be adequate. Growing seedlings in the greenhouse and then transplanting the seedlings to the field is an alternative that the staff should consider. Greenhouse facilities and transplanting will be necessary if marker-assisted selection is adopted. The planting of seedlings in the field in such a way that collected data lends itself to statistical analysis is commended. The inclusion of resistant Chinese checks is part of the field plot layout. The regular use of stakes and tree labels is necessary. Weed control in the seedling plots needs to be improved. We encourage the use of herbicides other than Roundup, as this herbicide can easily kill a chestnut tree. The precocity shown by many selections was striking, allowing a breeding cycle of only six years. This is truly remarkable for a timber crop species. By planting on well-suited sites, controlling weeds, and providing regular irrigation and fertilizer applications, further shortening of the cycle may be possible. It has been repeatedly shown that rapid seedling growth is a key to early fruiting (precocity) in fruit and nut crops.

Selection for blight resistance. The currently-used methods of evaluating levels of resistance are the result of years of effort on this subject and we are confident that genetic resistance is being identified through these tests. Investigation of alternative methods that would allow selection at an earlier age, either in the field or greenhouse, or using detached shoots, is encouraged.

Gall wasp and other pests. At this time, TACF staff should keep abreast of the current location of the gall wasp in the southeastern states. By encouraging plantings by cooperators in areas where the gall wasp is present, sources of resistance to this pest might be identified.

Evaluation

Evaluation of growth habit. In seedling blocks, a range in growth habit is evident. The vigorous, upright growth of the best BC3F1 selections is striking. A wide range in precocity is also

evident, and there may be an undesirable relationship between desirable growth habit and lack of precocity. Experience over the next few years will reveal if there is need for concern.

Evaluation of morphological traits. The leaf hairs, green twigs, and large stipules of the Chinese species mapped to linkage group C in the Mahogany F2 population, and these loci were independent of the three identified resistance loci. Thus, in this population, it appears that there would be no disadvantages to selecting against these Chinese traits. Since so many loci are located on linkage group C, there would be no selection pressure against Chinese alleles at loci on other linkage groups. This may not be the case in other populations.

Clonal propagation of selections. Clonal propagation by grafting can be done routinely in chestnut. The graft incompatibility commonly encountered when one species is grafted on another would be expected to be much less common when working with material that is 75% or greater American chestnut. Hill Craddock has extensive experience in grafting chestnuts and would willingly provide his services to TACF for this purpose. Grafted trees of advanced selections could also be used to establish trials to determine their region of adaptation. Also, grafting would allow the establishment of seed orchards from selected parent clones. Stooling is an alternative method to grafting and has been used widely in France. At this time, tissue culture does not appear to be a cost-effective means of clonally propagating chestnut selections.

Clonal propagation would also allow more efficient use of prime chestnut land by the breeding program. Effective breeding strategy requires rapid advance through the generations, and with limited land and resources, this means a need to eliminate seedlings that do not meet the stated objectives of the program. Selected seedings could be clonally propagated, and then the block of land cleared and prepared for a cover crop or replanting. Wise use of land will become even more critical in future years, as more resistance sources are used and population sizes increase. We recommend the adoption of clonal propagation of selections as a routine part of the breeding program.

Molecular Markers: From a genetics standpoint, the goal of the Chestnut breeding program is to recover from an interspecific hybrid the chromosome segments carrying genes of the American chestnut except for those that confer blight resistance. That is currently being accomplished based on using the American chestnut as the recurrent parent in sequential crosses and selection for disease resistance and readily visible American morphological traits. Since several of the traits readily distinguishing American and Chinese types are now known to be located on the same chromosome, selection on the basis of morphological traits alone may be problematic.

Molecular markers are "neutral markers" in the sense that they have no effect on the phenotype of the plant. Hundreds and even thousands can be monitored in a single cross. The DNA fragments used as molecular markers distinguish American from Chinese and the outcome is that information can be generated documenting whether American or Chinese chromosome segments

are present. Scoring segregating progenies (mapping populations) for the markers and traits of interest allows associations to be made between the markers and the genes controlling the trait. This leads to a better understanding of the genetics of traits of interest and provides a DNA marker for following the trait through crosses. For example, stipule size appears to be genetically associated with disease resistance in current crosses. Use of stipule size and appropriate markers might enhance the frequency of recovering disease resistance.

Such molecular markers could be immediately used in The American Chestnut Foundation breeding program for detecting pollen contamination and documenting pedigrees. Markers linked to major loci for disease resistance could be used to help in the early identification of seedlings with disease resistance and in determining if new sources of resistance represent different genes. Molecular markers can be used to identify the BC3 material with the greatest recovery of American genes and could allow the selection of material more suitable for release. In newly initiated programs with different sources of disease resistance or in advanced backcrossing programs, molecular markers can be used to advance the rate of recurrent parent recovery. If a generation could be saved via such selection, considerable savings would be realized. Molecular markers are also used to fingerprint germplasm for future identification, patent applications, and protection of patented materials.

Although scoring for quantitatively inherited traits in the mapping populations and determining linkages with molecular markers is desirable, we do not recommend a "marker assisted" breeding approach at this time (except for disease resistance). However, we do favor the use of markers in the ways mentioned above. To accomplish this task, the review team recommends that a proposal be developed that would lead to useful molecular markers for the chestnut. This proposal can be discussed with the U.S. Department of Agriculture and other agencies, foundations, and individuals. Utilizing the resources of the USDA Forest Service in Saucier, Mississippi, and the University of Massachusetts is highly recommended. The foundation also should explore the relative costs and efficiencies of contracting out the molecular marker work. Although we do not recommend a molecular marker lab at this time for The American Chestnut Foundation, equipment and facilities should be provided to foster and facilitate the work, especially since one of the staff scientists has expertise in this area. We also recommend that the staff give serious consideration to maintaining their mapping population on a long-term basis.

Release

Seed Orchard Design. The design proposed by Hebard consists of 8 orchard blocks of approximately 5 acres. Each block is divided into 16 sub-plots consisting of one BC3-F2 family. Within each subplot the 160 full-sib seedlings are arranged in 5 rows of 32 trees each. Spacing is one foot within rows and 7 foot between rows. After testing for blight resistance only one tree

will be left per subplot. This will result in a final stocking of about 30 trees per acre. The total size of the orchard will be approximately 4 acres. Production at age 10 is estimated at 1500 lbs per acre or 6000 lbs for the whole orchard. This is equivalent to about 480,000 nuts. The blocks are laid out in a staggered pattern to minimize crossing between related individuals.

An alternate layout could be achieved by laying out the subplots in rows and shifting the crosses two positions to the right each time a new row is started. This would give adequate spacing between related individuals and would not require staggering the blocks thus leading to a more efficient utilization of the available land. Even when using the square blocks of 16 blocks, staggering the blocks is not necessary. It is important to maintain an adequate isolation zone around the orchard.

Expansion of Orchards. The 4-acre orchard is probably adequate while the orchard is being progeny tested, since the early releases will be primarily for the purpose of evaluation. Once some or all of the selections have proven resistant, the orchard could be expanded quickly by grafting the resistant parents. Not enough information is available to estimate the size of the expansion needed. If the need for seed is substantial, serious consideration should be given to contracting out the work. A state organization might be a logical choice.

Seedling seed orchards are generally inefficient, because they are at once a progeny test and a seed orchard. The two require different management methods, so at least one of the two will be managed less than optimally. This may be acceptable when small quantities of seed are needed, but becomes impractical if there is a substantial demand for seed.

The availability of one or more economical vegetative propagation methods has other benefits. They can be used to assemble breeding materials in one place so extensive travel can be avoided, they can be used to preserve valuable genotypes to improve the accuracy of testing, or to do destructive testing on genotypes that need to be preserved.

Progeny Testing. The selections made in the BC3-F2 orchard need to be tested for resistance, for the absence of Chinese characteristics, and for growth. If suitable markers are available, homozygosity at the putative resistance loci could be determined on the selections themselves. This might best be done on all the candidates that made the short list. The results would be a major consideration in making the final selections. It would also be desirable to make controlled crosses on the selections and test them for resistance by inoculation. Finally the seeds given to the cooperators should be used to evaluate performance in the field. This should be done according to a valid statistical design. One could visualize a very simple design where each cooperator is given three seedlots: one control and a lot from each of two individual selections. Each cooperator would be a replication, and the results could be analyzed using a least squares program. It is important to follow up regularly with each cooperator to maintain their interest.

Germplasm Agreement

The panel suggests that the following modifications be made to the agreement:

Last sentence of paragraph 2: Drop "or to market any progeny from it"

Paragraph 3, item 2: Keep only the first sentence "The sample of germplasm provided hereunder may be used for basic research, evaluation and/or field testing only."

In addition the panel believes that it would be useful to have two different agreements. One would cover materials provided to cooperators for research purposes; the other would be for contractors, who agree to mass produce propagules for the marketplace.

It would also be prudent to seek patents for appropriate materials such as clones of selections in the BC3-F2 generation and beyond.

Equipment

Basic equipment is available, but somewhat old. It might be wise to replace one of the trucks in the near future. A smaller tractor will be needed to be able to work in the narrow rows in the BC3-F2 orchard. A -80° C freezer will be needed for storage of pollen and samples for DNA extraction. Facilities for seed stratification are limiting and an additional refrigerator will be needed. Better office space is needed and plans are being made to make some improvements on the house at the Wagner farm and turn it into office and laboratory space. A handheld computer would be very handy for recording of measurements, storing maps, and storing information on germplasm, tests, and pedigrees.

Response to 1999 TACF Science Review

Frederick V. Hebard, Staff Pathologist

Specific recommendations

1. They endorse backcrossing. This comment needs no response

2. They recommend use of additional sources of resistance and advancement of those to B3-F2. We believe this is already in progress. Currently, we have two sources of resistance we are advancing to B3 in at least 20 lines each, and one source being advanced to B1 in at least 20 lines each. We also have 33 sources of resistance as pure Chinese, 18 sources in F1, 11 in B1, and 8 in B2, including the three prime sources. These are being advanced as fast as the three prime sources of resistance, but not in 20 lines each. We could not possibly advance more than 1-5 lines for each of these sources of resistance, in addition to the three prime lines. Perhaps the review team was not aware of these resources; we did provide a copy of our latest progress report ("Meadowview Notes for 1999" in the latest TACF Journal), but perhaps did not refer prominently enough to this document from within the main text of our review packet.

3. They recommend expansion of the number of American parents used in breeding. It is difficult to imagine how this might be done without considerably more resources than currently available.

4. They recommend routine use of clonal propagation. We have been routinely using clonal propagation in the program since 1990. With regard to one suggestion in the text following the recommendations, we do not feel that clonal propagation by grafting will reveal the full range of adaptation of advanced selections, as roots will not be tested. However, clonal propagation would be the preferred method of increasing the yield of B_3-F_3 seed. It may also prove useful for consolidating selections to reuse land; however, rent of a tree spade might also accomplish that goal with much less effort.

5. They recommend investigation of methods for increasing seed yields from controlled pollinations. We plan to have students investigate this. We cannot possibly do it ourselves during pollination season, as we are too busy making crosses. Regarding pollen contamination, that has been fairly minimal the past 5 years.

6. They recommend more rapid elimination of undesirable seedlings from plots, and clonal propagation of the best seedlings to allow wiser use of the available land. If there has been any genius involved in the development of this breeding program, it has been to grow the trees at adequate spacing to the point where they can be reliably screened for blight resistance. Therefore we are approaching this topic with caution. Additionally, the use of clonal propagation will slow

our generation turnover time. See also the comments below under recommendation #9.

7. They recommend improved weed control and use of herbicides other than Roundup. Regarding Roundup, it is the only herbicide which covers the entire spectrum of weeds encountered at the farms. Spot applications of assorted herbicides are impractical. We have not encountered undo problems with chestnut mortality from Roundup. Dupont has recently introduced a new pre-emergent that should be registered this winter. It is reported to give much better control than Princep, which we have been using. We intend to try it. If this is not successful, we probably should request proposals for herbicide research as part of our grant program. We might add that this was one of the worst summers for weeds we have encountered; we had plenty of sunshine and plenty of rain, and temperatures in the mid 80s --ideal conditions for weeds.

With regard to the suggestion that our breeding cycle can be shortened further, we expect that wider spacing between trees in plots would be more efficacious than better weed control and irrigation. This is suggested from our experience with trees that experience varied degrees of crowding due to uneven germination within and between years. Regardless, the review team, and other hardwood geneticist who have visited our farms commented on the "striking" precocity of our trees. We do not feel that we can profitably push our seedlings too much more, except that irrigation may prove very valuable, especially in dry years.

8. They recommend extensive use of stakes in plots and labels on trees. Most of our trees are labeled as needed, with temporary labeling (which lasts 2-3 years). Since most of the trees will not be in the ground more than 5 years, more permanent labeling appears unnecessary to us, except for more permanent plots of trees, none of which exist at this point. More extensive labeling would require additional resources.

9. They recommend additional research on methods to determine levels of blight resistance, especially in young trees. One of us (Hebard) has conducted and published numerous experiments on screening seedlings of various ages for blight resistance, including screening callus tissue cultures, excised stems, excised twigs, and grafted scions as well as intact trees down to 0 years old in the field and in the greenhouse. The Swiss pathologist, Bazzigher, also investigated various methods of screening chestnut for blight resistance. The current methods used at this farm are the best that Hebard could devise based upon those experiments. They are similar to those employed by Griffin at Virginia Tech and Anagnostakis at the Connecticut Agric. Expt. Station, as well as Bazzigher.

The great failing of some past chestnut breeding programs in the U.S. was excessive searching for early methods of screening for blight resistance, rather than testing trees 2 to 5 years old. We are pursuing aggressively use or molecular markers to screen for blight resistance, which appear to us to be the best avenue for further research on this topic. The particular recommendation goes

hand-in-hand with recommendation #6 on "wiser" use of land; we feel extensive pursuit of these two recommendations would be detrimental to the progress of our breeding program. However, we are planning to initiate a study on greenhouse screening of young seedlings for blight resistance with Carol Young of the U.S Forest Service's Bent Creek Experimental Forest in Asheville.

10. They recommend that seed orchards be established using clonally propagated trees. We intend to test this with some B2-F2s which currently are being screened for blight resistance. Clonal propagation of our B3-F2s is our current plan for seed increase. The alternative is B3-F3 seed orchards, which might also allow a round of recurrent selection.

11. They recommend trials to determine adaptation of our superior selections. We plan to conduct such tests.

12. I defer discussion of the germplasm agreement to the Board.

13. They recommend establishment of what we have termed a "demonstration plot" at the Wagner Farm. We intend to establish such a plot in the garden at the Wagner house.

14. They recommend purchase of a freezer for storage of leaf and pollen samples. We concur.

15. They encourage use of DNA markers. We also encourage this, with the caveat that we would not want to see the basic breeding program suffer for pursuit of DNA markers.

In general, we are very pleased with the review, and agree with most of the points raised by the team, with the exceptions noted above. We greatly appreciate the hard work put in by the reviewers, and their sharing of their considerable experience.

Response To Science Review

Paul Sisco
TACF Staff Geneticist

I felt that the three reviewers gave us a sympathetic, conscientious, and thorough review, based on their own extensive experience in the fields of genetics and tree breeding.

Here is my interpretation of what they said and how I think it impacts our present and future program:

I. Appropriateness of the Backcross Breeding Program for incorporating chestnut blight resistance into American-type chestnut trees.

I think the reviewers gave us strong support for the basic strategy of our backcross breeding program. They were impressed by the size and blight resistance of our best BC2 trees and by the quick generation time -- as little as 6 years from seed to seed.

II. Modification of the Burnham, Rutter and French plan

A. Controlled pollinations rather than open-pollinations:

1. Controlled pollinations among the BC3's to produce the BC3F2's:

   Instead of open-pollination of the BC3's to produce the BC3F2's, as outlined by Burnham, Rutter, and French (Plant Breeding Reviews, Vol. 4, 1986), the reviewers recommended controlled pollinations among specific BC3 lines, so that we would know the complete parentage of each BC3F2 selection. These BC3F2's, which will be selected for their high level of resistance, will be parents of the first American-type highly resistant chestnuts that we will release beginning about 2006. The reasons for the use of controlled pollinations include:

   a. Controlled pollinations will ensure equal representation of each BC3 line in the BC3F2 seed orchard, so that we will maintain as much genetic diversity as we can among the seed we release to the public.

   b. Controlled pollinations will allow us to identify superior and inferior BC3 lines with regard to their contribution to resistance, their percentage of American germplasm, and with other characters such as tree size and shape,

nut production, time of leaf emergence and time of flowering, etc. Inferior lines can then be eliminated from the seed orchard.

2. Controlled pollinations among BC3F2's to produce BC3F3 seed for widespread testing for adaptation and disease resistance.

   A new insight for me was the reviewers' comment that the primary purpose of the initial BC3F2 seed orchard at Meadowview would be to produce seed for widespread testing for local adaptation and longevity of disease resistance. Thus controlled pollinations are also appropriate among the BC3F2's to produce these BC3F3 seed for testing, again so that we can identify and eliminate inferior BC3F2 plants based upon their full-sib progeny tests.

III. Need for Replication of the Seed Orchards

The reviewers suggested that TACF strongly consider replicating the BC3F2 seed orchards by clonal propagation (grafting or stooling) for two main reasons:

A. The initial BC3F2 seed orchards will not be ideal as seed orchards, because they will be progeny testing orchards converted to seed orchard production. Thus the spacing of the trees will be irregular. Example:

1 2

3 4

5 6

If these six trees in the initial orchard were then graft-propagated, they could be planted in a regular fashion with a randomized design.

1 2 3 5 1 4 6 2 3

4 5 6 6 3 2 1 4 5

B. Replication of the seed orchard will increase our total seed production. We could contract out this work, so that large-scale seed production does not distract us from the work of evaluating additional sources of resistance.

IV. Need for more sets of BC3F2 based on different sources of resistance.

The reviewers felt that the blight disease would eventually overcome our Clapper, Mahogany/Graves, and Nanking sources of resistance, so that we need to collect and evaluate more sources of resistance and advance these to the BC3F2 level with at least 20 American lines. This will obviously take a lot of land and resources. THUS:

V. Need to consider more efficient methods for screening and maintenance of lines.

They specifically suggested we investigate methods for screening the trees at the seedling stage before planting, so that only the most resistant ones would be planted and thus take up valuable space. They also suggested graft propagation of the best selections, so that they could be consolidated into one place and also replicated for testing and preservation at other locations.

VI. Importance of the State Chapter Breeding programs to develop trees with local adaptation and to carry on the backcross program beyond the BC3.

VII. Importance of DNA markers to assess types and levels of disease resistance, pollen contamination, and percent American genotype in released trees.

Dr. Phillips in particular suggested that we use a combined grant writing/ political approach to gain money for molecular marker research in chestnut. Recently a laboratory working with loblolly pine has gotten a $4 million grant for marker research. Researchers around the country are lobbying for a similar amount to be used on hardwood tree marker research. I have made contacts with the USDA Hardwood Genetics lab at Purdue, the Molecular Forest Genetics lab at Penn State, and our collaborators at the SIFG in Saucier, MS (Tom Kubisiak) and at the Univ. of Mass. (Robert Bernatzky) about a multi-institutional grant on disease and insect resistance of chestnut, oak, and walnut. We are contacting senators and congressmen and plan a meeting in Washington, D.C. in early January.

Response to "The TACF breeding program review August 12 - 15, 1999" by Drs. Mehlenbacher, Phillips, and van Buijtenen

by **R.C. Leffel**, Coordinator, PA-TACF Breeding Program

The Review Team's report is mainly focused on the breeding program and the overall goal of the Foundation "to restore the species in the United States" ----"keeping focused on the primary objectives is quite important." Amen!!

In regard to the general recommendations by Review Team:

Recommendations 4, 6, and 10 include clonal propagation of improved germplasm. I've cited Tucker Hill's survey on chestnut grafting (Rutter, Grimo, Anagnostakis, and Miller) and think a "reasonable doubt" exists for the use of clonal propagation as routine procedure in the breeding program. I suggest that the more feasible methodology is formulation of synthetic varieties and their subsequent generations of seed increase. Otherwise, I generally agree with the recommendations and emphasize:

Recommendation 12 of the Review is essential: there must be a distinction made between exclusivity of TACF's releases from Meadowview and the availability of pollen from TACF and the germplasm subsequently developed from such pollen by cooperative programs. The cooperative programs utilizing BC2 pollen will require a minimum of 6 years for BC3 generation, 6 years for BC3F2 generation, and 4 years for progeny testing of BC3F2 trees — a total of 16 years. Too much work to remain in the sole domain of TACF!! In short, remain exclusive — or develop the cooperative program envisioned by Dr. Burnham!

Recommendation 7,8, and 13 include the tidiness of plantings, grounds, etc. — the "Arlington Cemetery" appearance, especially important to the public eye! But this requires increased SUPPORT! The Scientific Staff at Meadowview is in great need of increased technical help and labor, and improved facilities and equipment!

In regard to the Report's text under its headings of:

Parents: Sources of resistance: With reasonable seed set in 1999, PA-TACF will have obtained adequate BC3 seed of 20 'Clapper' lines, our first objective, attained in 5 years. In 1999 PA-TACF attained the ability to produce 20 lines from a source of resistance in a single year!! Also, we can cross each of 20 PA Americans to a different source of resistance (20 sources). Where to go from here?

Crossing — Pollination: To date, we've had minimum production of seed in our control bags. Yesterday, I made the following observation on bagging: We placed 32 Lawson No. 421 white bags and 5 Lawson No. 401 'Showerproof' brown bags on a PA-American tree this spring. No. 401 is slightly larger. Hurricane Floyd removed all but paper clip and its fragment of bag for 31 of 32 No. 421 bags, but all 5 No. 401 bags are intact. Size of bag may be a factor, as developing burs obviously burst many of the white bags. Does color of bag affect seed set?

Research on pollen-stigma incompatibility: A lower priority - because of the tremendous task required to determine, I assume. But it can be important, especially if a lot of crosses fail because of cross-incompatibilities. Surely, an area for scholarly pursuit with a big Grant!

Breeding strategy - BC6? - Advanced Backcrossing?: The BC3F3 seed, via open-pollination, produced for release (screened and selected BC3F2 x BC3F2) may not be complete recombinations among all BC3F2's because of spatial relationships within the BC3F2 orchard. Utilizing BC3F3 seed (produced by controlled pollinations or by compositing equal amounts of seed from each maternal tree) for a larger BC3F3 orchard to produce BC3F4 seed for reforestation may be more efficient than clonal propagation and can be the source of a synthetic variety and recurrent selection for the future.

I think the use of a BC tree homozygous for resistance and two generations of backcrossing for local adaptation advantageous (my draft of 8-10-99). The Reviewers suggest "it may even be possible to reduce the number of backcross generations to two, and establish seed orchards with BC2F2 selections"

Lush (*The Genetics of Populations*, 1948) states that "mating of cousins less closely related than first cousins produces inbreeding so slight that they are scarcely worth considering as inbreeding". Each State Chapter should use a different source of resistance.

Selection - Seedling Growth and Selection for Resistance

A forest breeder has expressed some concerns to me on nurturing seedlings and transplanting versus direct seeding and survival of the fittest. This is similar to forage crop seedings, where only a fraction survive and establish stands. More efficient inoculation techniques, seed or early seedling screening, etc. Will be a very significant contribution!! Why hasn't the academic community, spending $2 million annually on chestnut and chestnut blight research, developed such techniques? Too practical, I assume.

The Review Team has served us well and substantiates our need for a more effective scientific discipline, TACF Science Cabinet consisting of Forest Tree Breeder, Forest Tree Pathologist, Forester, and Geneticist (Population, Molecular). Other disciplines such as Ecologist, Forest Tree Entomologist, Forest Tree Physiologist, and Soil Scientist, can be included or consulted as needs arise.

Response to TACF Science Review

by Henry Gerhold
Forest Geneticist, The Pennsylvania State University

Paul,

In response to your request I'm sending my thoughts about the TACF Breeding Program Review.

I regard this as an excellent report with many useful comments and recommendations. I do not find fault with any of them, but would like to elaborate especially on issues that pertain to Recommendations 2, 3, 6, 9, and 11. Certain kinds or research are needed to implement these properly, and breeding objectives need to be stated more explicitly than the objectives that I am aware of (although perhaps they already exist).

Selection currently is based on incidence and size of cankers after inoculation of saplings, I believe. Is this sufficient to quantify level of resistance and classify type of resistance? Eventually we would like to identify each resistance gene and understand how it acts.

Ecotypic adaptation of chestnut is not well defined to my knowledge. We can assume that genetically based variation exists in the geographic and elevational range. But we need better information related to American provenances and breeding populations.

An important question has been raised about breeding strategy that involves switching from backcrossing to recurrent selection, recognizing that there is a trade-off between resistance level vs. rate of progress. So this is a third research topic that deserves attention as the work proceeds. I would maintain flexibility by preserving two options until there is convincing evidence to make a decision.

These thoughts lead me to suggest two additional recommendations that I'll call 16 and 17:

16. Develop explicit research objectives regarding methods of selection for resistance, ecotypic adaptation, and breeding strategy; prioritize them along with others; and outline procedures to pursue these objectives.

17. State explicit breeding objectives including types of resistance genes and frequencies, range of ecotypic adaptation of varieties and heterozygosity, and other tree traits such as trunk form, growth rate, and fruiting. Objectives will need to be review and revised periodically, of course, as research and breeding progress.

Reply to TACF Science Review

Gary Griffin
Virginia Technological and State University

Fred and I worked on the problem of inoculation of seedlings at a young age when he was here. The bark phloem is so thin on small, young seedlings that it does not take much inoculum to cause necrosis to the vascular cambium at that stage. Since the stem diameter is also very small, stem girdling and death can occur quickly. That is why we have been waiting for longer periods to inoculate, just as you have in the past. It is possible a technique could be worked out, but it probably will take a good deal of experimentation to do it. To detect high levels of resistance, it should be somewhat easier. Greenhouse inoculations would be ok if the trees grow as rapidly as they do in the field. The root systems are likely to be larger in the field.

Clonally propagating your best selections is a good idea, I believe. However, not all clones propagate equally well and for American chestnut this can be a frustrating endeavor at times. Just when you think the clone is growing well it may suddenly die. If it makes it through the first winter, that is a good sign. In grafting, rootstocks and grafting method play a big role in determining success.

I hope these opinions are of some help to you.

Regards, Gary

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