



CONTENTS

NOTES

From the Editor <i>Dale Kolenberg, Communications Director</i>	5
We Thought You Might Be Interested.. Recently Published Books Reviewed by <i>Dr. Fred Paillet, Ph.D.</i>	7
Cathedral Peace Window Honors Dr. Norman Borlaug and Jimmy Carter <i>By Joe Bjordal</i>	9
Many Challenges Still Ahead <i>by Marshal T. Case, President & CEO</i>	11
Research Objectives of the American Chestnut Foundation, 2004-2014 Summary of TACF's 10-Year Plan <i>By Frederick V. Hebard, Ph.D., Staff Pathologist</i>	13

FROM THEN TO NOW

The American Chestnut Has Earned its Place <i>By Chris Ditlow, Oak Park Cabinetry, Inc., Harrisburg, PA</i>	22
Creating Artwork from American Chestnut is Both a Joy and a Challenge <i>By Gary P. Carver, Ph.D., CarversCarvings, Ijamsville, MD</i>	25

SCIENCE AND NATURAL HISTORY

Leather, Tannin and the Chestnut Tree <i>By Dr. William G. Lord</i>	30
Notes from Meadowview Research Farms 2003-2004 <i>By Frederick V. Hebard, Ph.D., Staff Pathologist</i>	38
Designing Chestnut Orchards to Control Pollen Parent Without Hand-pollination The use of self-incompatibility and cytoplasmic male sterility <i>By Paul Sisco, Ph.D., Regional Science Coordinator</i>	46
Cytoplasmic Male Sterility and Chestnut Breeding Programs <i>By Robert C. Leffel, Ph.D., Research Agronomist, ARS-USDA, Retired</i>	53



n o t e s



FROM THE EDITOR

In this issue of *The Journal*, we begin a series of articles that will explain and illustrate the implicit characteristics of the American chestnut tree. When describing TACF's mission to restore the American chestnut to its native woodlands, the stature that this tree maintained within its ecosystem is always noted. Its contribution to the integrity of contiguous forest species, along with its economic value to humans is undisputed.

But what is it about the American chestnut tree that elevates it to this level of superlatives? We've asked experts from chestnut-related occupations, and researchers focused on distinct aspects of the tree's biochemistry, to tell us why this tree is so unique.

We start this new series with chestnut lumber. Chris Ditlow and Gary Carver each tell us about being a woodworker who creates items from American chestnut lumber. Chris puts the wood into its historic perspective, showing how American chestnut lumber has grown into a prized commodity, valued for its unique beauty, from its prior status as rough-cut attic framing or inexpensive filler between more popular wood veneers. Gary carves birds from American chestnut. His creations bring the wood to life, conveying as much about American chestnut's personality as they do about the birds he is depicting. It's no easy task, he says, but well worth the effort.

In Science and Natural History, TACF Regional Science Coordinator Paul Sisco, and PA-TACF member Bob Leffel have written companion articles on the use of cytoplasmic male sterility (CMS) in backcross breeding. Paul introduces the concept, which he and Bob see as a potential alternative breeding method that will eliminate the need for hand pollination. Bob explains how the method is being used by the PA Chapter in their new breeding program for regional adaptability. As of 2004, 2,488 trees have been planted in 23 CMS orchards. Data will soon be available on the initial results of the method which, as Bob points out, is still experimental.

In this issue we again celebrate the enormous contributions to peace and humanity made by TACF founder Dr. Norman Borlaug and Honorary Board Member Former President Jimmy Carter. Their work has been commemorated in a stained glass Peace Window at St. Mark's Cathedral in Minneapolis. TACF Secretary and General Counsel Don Willeke participated in the development of this project for the Episcopal Diocese of Minnesota.



An update on progress at Meadowview Research Farms from Staff Pathologist Fred Hebard is included in Science and Natural History. Fred has also written a summary of the 10-year plan for continuing our research, which will be published in full in a special issue of *The Journal*. Bill Lord has contributed an interesting historical perspective on the contribution of American chestnut to the leather production industry in the United States, and Fred Paillet reviews some interesting new books about the American chestnut's status in our northeastern forests.

We hope you are inspired.

A handwritten signature in cursive script that reads "Dale". The letter "D" is large and stylized, with a long horizontal stroke that curves under the "a" and "l".

Dale Kolenberg is Communications Director
for The American Chestnut Foundation

WE THOUGHT YOU MIGHT BE INTERESTED...

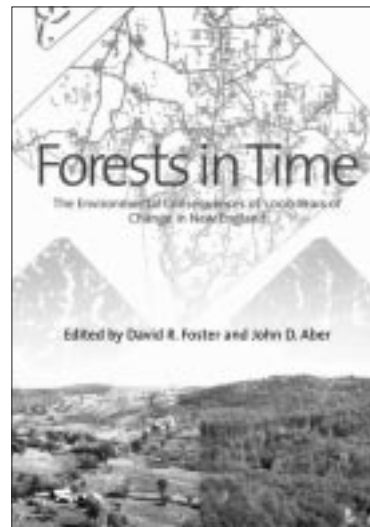
Recently Published Books
Reviewed By Fred Paillet, Ph.D.

Forests in Time, Edited by D. R. Foster and J. P. Aber, 2004,
 475 p (hardcover; Yale University, New Haven)

This readable and well-illustrated monograph reviews all of the recent ecological studies at the Harvard Forest in north central Massachusetts. Chestnut and the history of chestnut in New England are major subjects in several chapters. This reference serves as a real landmark in chestnut ecological studies by being the first such study to state explicitly that the abrupt increase of chestnut pollen about 2,500 years ago most likely represents a sharp increase in chestnut from a previously existing population. This simple statement effectively refutes the common belief that the relatively late rise of chestnut to co-dominance in Connecticut and points north was caused by late arrival from a glacial refuge. Chestnut was likely here early with other nut-bearing species, and then something happened to tip the balance in its favor. A number of us have harbored this suspicion, but here is the first definitive statement in print - backed up by an impressive set of evidence. Foster and Aber do not hazard a guess as to what that event was, but show that the response of the forest to disturbance after that time was completely altered.

The rest of the book has chapters dealing with the history of New England's forests, where changes induced by European settlement are embedded in other changes that were already underway, and where today's regenerating forests still reflect events that happened long ago. Some experiments quantify expected relations, such as the rate at which carbon is being stored in accumulating forest soils and biomass, while nitrogen fertilization experiments suggest that an as yet undefined mechanism(s) for the accumulation of nitrogen must exist in the forest.

All in all, this is a pretty good overview of what is known and what is not known about the long-term history of the northeastern deciduous forests where chestnut was once—and will one day again be—a prominent part of the landscape.





“...the ghost of chestnut and the spirit of Thoreau constantly haunt the pages.”

Stone by Stone—the Magnificent History of New England’s Stone Walls, Robert M. Thorson, 2002, 287 p (paperback; Walker and Company, New York)

The complete story of New England’s stone walls, starting with the geologic history of the original bedrock. Although chestnut does not figure prominently in this entertaining and occasionally even poetic tribute to one of the dominant features of New England, this is a great read for anyone interested in our northeastern forests.

The frontispiece of the book shows a typical stone wall in oak woods with what appears to be a slab of old chestnut wood leaning against the wall. So the ghost of chestnut and the spirit of Thoreau constantly haunt the pages. One of the most significant secrets divulged in this book is that the perpetual yield of stony “products” from these fields had nothing to do with plows. Stones were being raised from their repose in glacial till at a rate of several millimeters per year by a process of frost heave in soils deprived of their organic insulation layer. Thus stones were coming up relentlessly even as the overlying blanket of humus was melting away.

This ties in with one theme of the Foster and Aber book in that the rebuilding soils of recovering forests will still be accumulating organic matter for decades or even centuries to come. Another prominent fact is that almost all fences involved wood posts and rails; stone walls of themselves were primarily “geologic waste disposal features” plain and simple. This book presents many more such gems of geologic and historic wisdom woven into a fabric made from personal anecdotes, historic diaries, and serious economic reports.

The only thing missing from this unpretentious little book is a set of photographs that do justice to our stone wall legacy, showing the delightful play of seasons: the contrast of grey stone, green moss and white snow in the winter, or the many subtle shades of lichen-encrusted rock embedded in leaf litter on a sunny spring day. Besides, most New Englanders already know that stone walls, chestnut sprouts, and the decaying skeletons of former chestnut monarchs just seem to go together.

Dr. Fred Paillet is retired Project Chief, Borehole Geophysics Research Project, U.S. Geological Survey. He is a frequent contributor to The Journal whose expertise on the history of American chestnut has been of tremendous value to TACF.

CATHEDRAL PEACE WINDOW HONORS DR. NORMAN BORLAUG AND JIMMY CARTER

By Joe Bjordal, Courtesy of the Episcopal Diocese of Minnesota

Two chapters in Don Willeke's life came together last May, resulting in the visit of a world-renowned scientist and Nobel Peace Prize winner to St. Mark's Cathedral in Minneapolis.

Willeke, a member of the cathedral congregation and a Minneapolis attorney, is also chair of the cathedral property committee, and oversaw the design and creation of seven new stained glass windows, two and a half years in the making. One window depicts "peace makers" of the 20th century. As chair, Willeke was instrumental in including Minnesota's two Nobel Peace Laureates in the window: U. S. Secretary of State and former Minnesota Senator Frank W. Kellogg and Dr. Norman Borlaug, the 1970 recipient of the Nobel Peace Prize for his work in revolutionizing agriculture, most notably the production of wheat. (As a result of his work, Borlaug is credited with saving more human lives than all other Peace Prize winners combined.)

The World Peace Window depicts 13 peace makers of the 20th century positioned around a stylized river flowing from the Tree of Life. In addition to Borlaug and Kellogg, the window includes Martin Luther King, Jr., Albert Schweitzer, Dorothy Day, Desmond Tutu, Eleanor Roosevelt, the Dali Lama, Mahatma Ghandi, and Mother Theresa, with a triptych of Jimmy Carter, Anwar Sadat and Menachim Begin. A banner in the center of the window reads "Blessed are the Peacemakers."

Willeke is also a person who cares about trees. He has previously headed Minnesota's Urban Forest Council, the National Urban Forest Council, the Tree Trust and American Forests. In 1983 he was among the founders



PHOTO BY JOE BJORDAL, ELECTRONIC COMMUNICATIONS
MANAGER, EPISCOPAL DIOCESE OF MINNESOTA

Nobel Peace Prize Laureate, Dr. Norman Borlaug, speaks inside St. Mark's Cathedral in June, after receiving a photograph of the stained glass window in which he is depicted with other 20th century peace makers. He is flanked by the Very Rev. Spenser Simrill, Dean of the cathedral and by TACF Board Secretary and General Counsel Don Willeke, who arranged the visit.



World Peace Window,
St. Mark's Episcopal Cathedral,
Minneapolis, MN

of The American Chestnut Foundation [along with] Dr. Borlaug, [who] continues to serve as an honorary director of TACF, along with another person depicted in the window, former United States President Jimmy Carter. Willeke is now TACF's Secretary and General Counsel.

Thus the chapters intertwined and an historic visit took place.

Following a luncheon, Dr. Norman Borlaug spoke to a group in the Whipple Chapel about his life work and passions. Termed the "founder of the Green Revolution," it is estimated that Borlaug's work has saved over one billion human lives.

Borlaug, who has resided in Mexico for many years and, at the age of 90, still teaches international students, was in Minneapolis to deliver the commencement address at his alma mater, the College of Agriculture, Food and Environmental Sciences at the University of Minnesota. Borlaug accepted an invitation from Willeke to visit St. Mark's and view the World Peace Window. About 40 persons were on hand to greet the Nobel Laureate, witness the presentation of a photo of the window to him and listen to the respected teacher talk about his life and work over a luncheon that included chestnut crepes for dessert.

A generous gift of over \$200,000 allowed the cathedral to complete the clerestory stained glass windows, many of which contained only amber-colored glass since the Cathedral's construction was completed in 1910. Willeke's committee oversaw the design and installation of the remaining seven windows in time for last summer's General Convention of the Episcopal Church in Minneapolis.

As Borlaug thanked Willeke and the Cathedral Dean, Spenser Simrill, for the honor, he noted that, as far as he was aware, this was the only place his likeness appeared in stained glass and he considered it a true honor.

MANY CHALLENGES STILL AHEAD

By Marshal T. Case, President and CEO

Since its founding in 1983, The American Chestnut Foundation has been maturing into a research and networking organization, with science options and opportunities expanding at a rapid pace.

Six years ago, TACF was an 1,800-member organization with four state chapters. Today, it is composed of more than 5,000 members, 11 state chapters, and two provisional chapters. Active members volunteer many thousands of hours each year, from growing chestnuts in backyards and regional orchards to pollinating flowers and harvesting nuts from regionally important wild trees, in addition to their important financial contributions. Volunteer chapter officers and committee members conduct membership meetings, publish newsletters, work with local and regional news media, and represent TACF at statewide regional events.

TACF staff serve two primary functions: conducting science at the Virginia research farms, with support work at other planting locations, and providing membership services, including our regular informational publications, and field support and funding resources for the growing “army” of chestnut volunteers.

A fast-expanding component of the growing organization is our partnerships. In 2002, a major event occurred when Pennsylvania State University signed a formal, long-term agreement with TACF. The Pennsylvania chapter is part of the partnership, enabling us to hire a tree breeding coordinator and orchard manager, stationed at Penn State. The University has designated ten acres of their new Arboretum for chestnut plantings and research. In 2004, another major event happened when Peabody Energy signed the first phase of a five-year agreement with TACF to conduct research with mined land reclamation. This was our first, very significant, corporate collaboration.

Foundations have provided growing and significant support since 1998. In particular, National Fish and Wildlife Foundation and National Forest Foundation have contributed major support for specific research and partnership programs. Park Foundation, of New York, funded an irrigation system at the Virginia research farms, to which American Electric Power provided a \$10,000 power line. Sudbury Foundation, of

“...the challenge to us of TACF’s mission...is equaled only by the challenge to keep pace with the many opportunities presented on an almost daily basis.”



Massachusetts, has sustained six years of critical regional funding for New England operations.

Another milestone in 2004 was the first financial support received from the US Department of Agriculture Forest Service. As a result of working with the Forest Service State and Private Forestry (forest health section) and Congressman Charles Taylor of North Carolina, \$250,000 was allocated in the Federal budget for TACF research in the southern Appalachians.

Rapid growth brings new challenges. TACF board, chapters and staff have been able to respond to the many new opportunities, starting with the first strategic plan in 1998 as the membership and network began to expand. This was an organization-wide effort, with seven regional meetings conducted over a one-year period, with full document review opportunities for the entire membership.

To keep pace with this tremendous growth on all fronts, and to position TACF to better take advantage of growing opportunities (as with the USDA Forest Service), I asked senior staff scientist Dr. Fred Hebard to formulate a 30-year science plan, in three 10-year phases. The summary of the full document published here is the result of a first-draft presentation to TACF's Science Cabinet and full Board of Directors at their April, 2004 meetings. There was a four-month time period for review and comment and this is a summary of the approved full document. A special issue of *The Journal* will be published containing the full 10-year plan: "Research Objectives of The American Chestnut Foundation, 2004-2014." It will be available to all members, by request.

Finally, the challenge to us of TACF's mission—solid, successful science resulting in the restoration of the American chestnut tree to its native woodlands in the eastern United States—is equaled only by the challenge to keep pace with the many opportunities presented on an almost daily basis.

These research objectives are the driving force behind our very existence as an organization. Members, state chapters, and our networking partners and collaborators make up the key components for success in restoring the American chestnut. Board, cabinet members and staff provide leadership and coordination to help make "the impossible comeback" a reality.



RESEARCH OBJECTIVES OF THE AMERICAN CHESTNUT FOUNDATION 2004-2014

By Frederick V. Hebard, Ph.D., TACF Staff Pathologist

A comprehensive 10-year plan for TACF's research has been prepared. We intend to publish the entire version in a future issue of *The Journal*. Below is a summary of the plan, starting with the research objectives, which are arranged in order of priority for our Meadowview Research Farms, for our state chapters, and for further breeding and testing of trees.

— *Frederick V. Hebard, Ph.D., TACF Staff Pathologist*

OBJECTIVES

A. Meadowview

- 1) Complete planting of two seedling seed orchards and selection of trees reasonably true breeding for blight resistance. One seed orchard will be for the 'Clapper' source of blight resistance, and one for the 'Graves' source.
- 2) Advance a third source of resistance derived from the Nanking cultivar of Chinese chestnut to third backcross in 20 lines of American chestnut.
- 3) Determine what additional sources of blight resistance might be useful in restoring American chestnut and obtain first or second backcross F₂s homozygous for the genes conditioning blight resistance.
- 4) Continue breeding of large, surviving American chestnut trees that have shown low levels of heritable blight resistance, to determine whether that blight resistance might be increased to a usable level.
- 5) Evaluate traits of chestnut that might be related to its ability to grow as a dominant forest tree.
- 6) Begin advancing one source of blight resistance to sixth backcross, in anticipation of comparing its field performance to that of third backcross trees.





“A key question is whether or not backcrossing will work, whether or not we can recover highly blight-resistant chestnut trees able to compete in our native forests.”

B. State Chapters

1) Finish advancing the ‘Clapper’ and ‘Graves’ sources of resistance to third or fourth backcross, by crossing 20 separate pollens for each onto 20 separate American chestnut trees from each of NC, ME, MA & PA, and complete planting of two seed orchards in each of those states. Selection of trees homozygous for the genes conferring blight resistance would be completed in the following 10 years.

2) Complete advancing one source of blight resistance as above in KY (‘Graves’), TN (‘Clapper’), MD (‘Clapper’), IN (‘Clapper’), and VT (‘Graves’), and complete planting one seed orchard. Selection of trees homozygous for the genes conferring blight resistance would occur in the following 10 years.

3) Form new state chapter centered near northern WV or eastern Ohio and initiate backcrossing, and likewise in VT and AL. Revitalize chapter in CT and initiate backcrossing. These four chapters would more-or-less complete the infrastructure for our regional breeding program.

4) Continue supporting research at Syracuse University and the University of Georgia aimed at transforming chestnut with DNA plasmids containing genes for blight resistance (NY Chapter and national).

5) Initiate backcrossing onto 20 American chestnut trees of additional sources of blight resistance obtained under Objective 3 in Meadowview, with each chapter using a separate source of blight resistance.

C. Testing & Further Breeding

1) Organize a symposium to discuss results from cooperators on establishing American chestnut in the forest, with a view to formulating planting guidelines.

2) Initiate testing in the forest of trees obtained from Meadowview seed orchards. Supplement with trees from chapter seed orchards where feasible and appropriate.

3) Initiate testing in orchard settings (rather than forest) of trees obtained from Meadowview seed orchards, with a view to continuing improvement of the breeding population and creation of B₃-F₃ seedling seed orchards. Improvement would be achieved by both family-level selection and selection of individuals within families. Repeat with trees from chapter seed orchards as these come into full production from all breeding lines.

4) Initiate a longitudinal demographic and epidemiological survey of American chestnut sprout populations in areas likely to be undisturbed for the foreseeable future, such as National Parks and National Forest Wilderness Areas.

5) Initiate provenance tests (common garden studies) of chestnut from our regional seed orchards.

6) Initiate testing of blight-resistant backcross trees in the presence of hypovirulent strains of the blight fungus, to assess whether combining the two control methods gives better remission of disease than either alone.

7) Initiate wide scale planting and monitoring of blight-resistant American chestnut in the Appalachian Mountains with a goal of planting 200,000 acres over the next 30 years.

BRIEF DISCUSSION

A key question is whether or not backcrossing will work, whether or not we can recover highly blight-resistant chestnut trees able to compete in our native forests.

Blight Resistance

The prospects for success in our backcross breeding program are bright if only a few genes control blight resistance. However, if numerous genes are needed to confer resistance, then some of these will either be lost during backcrossing so that we will not have enough resistance or else so many associated Chinese chestnut traits will be retained that the trees will not resemble American chestnut.

Results over the last 10 years indicate that only a few genes control blight resistance, and that we should be able to backcross it into American



chestnut. First, we were able to recover highly blight-resistant progeny when we intercrossed with each other three types of crosses; these were: $\frac{1}{2}$ -American, $\frac{1}{2}$ -Chinese trees; $\frac{3}{4}$ -American, $\frac{1}{4}$ -Chinese; and $\frac{7}{8}$ -American, $\frac{1}{8}$ -Chinese. Secondly, we have genetically mapped some of our trees using molecular and morphological markers, and blight resistant mapped to only a few gene locations.

American Type

It is a hypothesis that three backcrosses of a Chinese x American first hybrid to American chestnut will be sufficient to restore the American type to our progeny, albeit an hypothesis based on experience in the breeding of many different crop plants and farm animals. To accelerate the recovery of American type we follow standard practice in backcrossing by selecting for American traits at each step, from among the trees that have adequate levels of blight resistance.

Currently this selection is done using morphological traits. Under Objective A.5, we also follow other traits for ecological adaptation, such as the time of bud flush in the spring. We also are placing these traits on molecular genetic maps where possible.

We will know with certainty that our trees will thrive like the American chestnut of old only after they have done so. It will take 50-100 years for our trees to reach 100 feet in height and diameters in excess of 2-3 feet, if they can. It will only take about 20 years, on the other hand, to advance one source of blight resistance to the sixth backcross, which should be enough to restore the American type as much as is possible. This we intend to do, as outlined in Objective A.6.

We must balance the possibility that more backcross generations might be needed against other needs of the breeding program, primarily the possibility that one or more of our sources of blight resistance could break down.

Breakdown of resistance

Once highly blight-resistant, American-type chestnut are restored into the forest, it is possible that the blight fungus could evolve means of overcoming their resistance. The resistance is then said to have “broken down.” One encouraging sign that our blight resistance might not break down is that no Chinese chestnut trees have been found in the U.S. that



are as susceptible to blight as American chestnut, despite widespread planting. All Chinese chestnut that have been examined have cankers typical of intermediate to high levels of resistance, and the highly resistant cultivars have retained that trait, being canker free for the most part. So their resistance has not broken down. However, blight resistance from Chinese chestnut might break down after it has been backcrossed into American chestnut. The purpose of a number of our objectives is to help avoid breakdown of resistance, as well as testing for its occurrence.

Our primary strategy for avoiding breakdown of blight resistance at this point is to use more than one Chinese chestnut tree as a source of resistance. In addition, we are evaluating the merits of Japanese and American chestnut as a source of blight resistance, the latter under Objective A.4. Since blight resistance is not known to have broken down yet, we cannot isolate races of the pathogen specific to the resistance they break, which precludes a lot of experimental approaches used in pathosystems where resistance has broken down.

To implement our strategy, each breeding location is advancing three sources of blight resistance, two from our most advanced sources, which they share in common, and one separate source for each chapter. These activities are encompassed in Objectives A.1, A.2, B.1, B.2 and B.5. If we can develop breeding populations from each, we will have used about 12 separate Asian chestnut trees as sources of blight resistance. But which Asian trees should be used, and how can we tell they have different genes for blight resistance? Such questions will be addressed under Objective A.3, which is discussed in the full version of this plan. Briefly, we intend to address this question using both classical and molecular techniques.

Testing

Most plant pathologists believe that deploying resistant plants over a wide area for several years is the only firm indication of durable disease resistance. It is the one method that can exert selection pressure on the pathogen severe enough to uncover almost all races capable of breaking the resistance. This is a principle reason we wish to plant over such a wide area under Objective C.7; this will be our ultimate, best test of resistance stability.

We do not envision that planting on such a scale will begin until approximately 2015, and acknowledge that it will require much effort and further improvements to planting techniques. The 200,000-acre figure



“Most plant pathologists believe that deploying resistant plants over a wide area for several years is the only firm indication of durable disease resistance.”



“In a common garden test, the seed from each chapter would be planted together in one location, so that we can compare their performance. The common garden tests also will help determine the range of adaptability of chestnut...”

is also an upper limit on the number of acres of trees needed to exert significant selection pressure on the blight fungus. We are specifically recommending that no more than 200,000 acres be planted to our trees over the next 50 to 100 years until they have shown they can be dominant forest trees. Specific plans for monitoring these plantings will be formulated 10 years from now.

The extensive planting envisioned under Objective C.7 is designed, in part, as the ultimate test of the stability of our blight resistance. But there are plenty of things that could go wrong before our resistance breaks down. Foremost among these would be that the trees fail to grow as well as the American chestnut of old or that their blight resistance is insufficient. The two traits, forest competitiveness and blight resistance are intertwined. Chinese chestnut cannot compete well in our native forests against trees like yellow poplar; it gets overtopped and shaded out. However, while Chinese chestnut trees are being killed by competing trees, the severity of blight on them increases. Cankers, on the other hand, weaken trees. So, even if our trees could compete successfully in the forest when blight is absent, in the presence of blight, trees with insufficient resistance might be weakened to the point where they couldn't compete. With a slight deficiency of either forest competitiveness or blight resistance, our trees probably will fail. The tests under Objectives C.2, C.5 and C.6 are designed to help determine whether our trees have adequate forest competitiveness and blight resistance.

We wish to set up common garden tests of our B₃-F₃s from the seed orchards at our different chapters under Objective C.5. In a common garden test, the seed from each chapter would be planted together in one location, so that we can compare their performance. Several tests would be planted at different locations, such as one in MA, one in PA, one in VA, etc. The common garden tests may serve as a negative control, if trees bred in one region fare well in their own region but do not fare well in others. The common garden tests also will help determine the range of adaptability of chestnut and help in the choice of seed for distribution. Finally, they may help test the effectiveness of the breeding done by different chapters.

Further Breeding

Release of B₃-F₃ plants into the environment is not envisioned to be the last step of the breeding program. Rather it is the first release in an ongo-

ing breeding program. The selection of B_3 - F_2 parents of the first release probably will be imperfect. Some parents probably will not be homozygous for blight resistance at all loci. We will want to cull these. We could detect them by test crosses to American chestnut or by the performance of their B_3 - F_3 progeny. We plan to evaluate the performance of B_3 - F_3 progeny as outlined in Objective C.4, essentially switching the breeding method from backcrossing to recurrent selection. We would select both for blight resistance and American type in seedling seed orchards of B_3 - F_3 trees, among both individuals and open-pollinated families. Then the B_3 - F_3 seed orchard would replace the B_3 - F_2 seed orchards.

It probably would be best if B_3 - F_3 seed orchards were planted on new land rather than trying to interplant among existing B_3 - F_2 seed orchards. Our current vision would be to put them on public lands under the management of state and federal forestry agencies. We would not want to start planting these until most trees in our B_3 - F_2 seed orchards were in production, which will not occur before 2015. By then, the tests initiated under Objective C.2 as well as other plantings will have indicated whether our trees were worthy of further breeding and effort by public forestry agencies.





f r o m t h e n t o n o w

THE AMERICAN CHESTNUT HAS EARNED ITS PLACE

By Chris Ditlow

Oak Park Cabinetry, Inc., Harrisburg, PA



“Chestnut was a common, easily dried and processed wood that was not appreciated until its demise.”

If I walked into my home town of Harrisburg, Pennsylvania in 1890, where would I find American chestnut being used? Well, it wouldn't be in the governor's house as finished trim work. You might see it in the governor's attic, however, used as rough framing, or as shingles on the roof. Or you may find it in the carriage house out back. More likely, you'd find it in the wood pile, waiting to fuel the kitchen woodstove.

If chestnut wood was used for finished trim work, it was most likely in the small-town train station or the Victorian-era row house. I've seen varnished chestnut millwork in some of these old homes, on finished doors and paneling. Next door would be identical trim made of pine with a coat of paint on it. The person with the chestnut preferred an inexpensive wood. Chestnut was just pennies more than clear pine.

Chestnut was plentiful. However, the wormholes commonly found in chestnut degraded its value, and made it less desirable than most other hardwoods. I've seen old tally sheets with various woods offered for sale. When chestnut is listed, you'll also see it marked "clear" in brackets, indicating no visible worm holes. Indeed, today, when hardwood is graded, insect damage in a board will not allow for sale by grade rules.

In those days, only chestnut showing minor bug damage was considered appropriate for millwork. It was never used in a home as finished flooring because of its softness. It was used as flooring in the barn, however, or as rough flooring in the attic or in a warehouse, and it was frequently used as sheathing boards on the roof.

Because of its abundance in the pre-blight days, chestnut was taken for granted. I have read many vintage woodworking publications on millwork and cabinet making, and chestnut is rarely mentioned. It would have been a favorite of the less skilled country cabinet maker who specialized in utility cabinets such as jelly cupboards, because it was so easy to hand rip and plane. But if a client wanted a quality cabinet, it would be made of walnut or cherry.

In the early machine-furniture industry, clear grade chestnut boards

DILLARD GRAVELY, COURTESY OF JIM WILSON



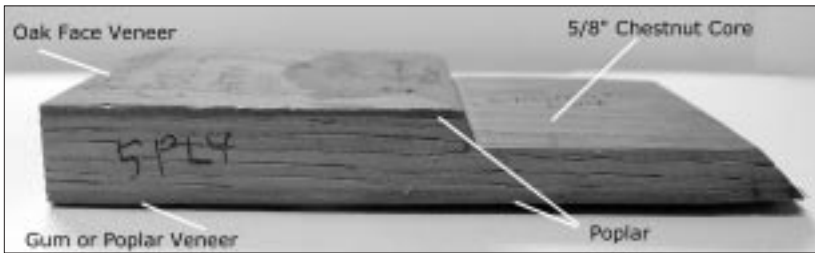
Jim Wilson, next to the reclaimed American chestnut cabinet built for him by Chris Ditlow.

This reclaimed American chestnut cabinet was built by Chris Ditlow for Jim and Esta Wilson, TACF members from Martinsville, VA. But it started its journey in Connecticut when an old barn was razed. Esta's son Brian and his wife Jean Ann, of Norwich, CT, purchased 4 of the barn's beams as a Christmas present for Jim, knowing he wanted to build a cabinet to fill a very limited space next to his fireplace. Too large to deliver to Virginia by car, the boards sat in Connecticut for nearly a year, until a trucker-friend of Brian's was able to carry them to his home in Christenburg, VA.

Several months later, on a truck run to Harrisburg, PA, he was able to deliver the boards to Chris, another of Brian's friends. Using building plans that Jim supplied, Chris built Jim's cabinet, after removing over a quart of handcrafted nails from the beams.

"This beautiful cabinet fits perfectly into our small space," said Jim. "Chris is a very talented craftsman and can build anything, especially if it's chestnut wood. He has made many craft and furniture items for the Foundation, free of any costs for materials or labor. He really loves the chestnut tree."

were manufactured into inexpensive consumer goods such as iceboxes or bedroom furniture. But chestnut was most useful at that time for lumber core plywood, because it was easy to dry and very stable. The plywood had a solid chestnut core, $\frac{5}{8}$ inches thick, covered with a more desirable face veneer such as quarter-sawn white oak, a favorite style around 1900. This technique was used in most "oak" furniture from the



(top) An advertisement for a 19th century New York City furniture manufacturer that utilized American chestnut in its products.

(bottom) Chestnut was most useful during the late 19th century as lumber core plywood, because it was easy to dry and very stable. The plywood had a solid chestnut core, 5/8 inches thick, covered with a more desirable face veneer such as quarter-sawn white oak, a favorite style around 1900.

1890's until World War I, when the styles went to colonial revival. The chestnut "sub parts" were still common until the 1950's, when dead chestnut began to play out of supply.

In my opinion, if the blight never occurred, the abundance of chestnut trees would cause it to still be a secondary wood, used more for pallets than for finished goods, similar to poplar's use today.

But that's all changed. I've bought and sold salvaged chestnut, and the

ironic thing is that the first question the buyer asks me is how many bug holes does the wood have. Bug holes! A character never considered in a bygone age. Chestnut has very nice workability features that today's craftsmen like. Also, its high tannic acid content makes it easy to darken by fuming with ammonia, a feature traditionally used in mission oak styles.

CONCLUSION

Chestnut was a common, easily dried and processed wood that was not appreciated until its demise. We always reflect on the past, and the warmth of chestnut's brown color, bug holes and all, adds to its charm as it ages like a fine wine. Let's all pray that TACF can restore it to its proper place in our eastern forests

Chris Ditlow creates custom-designed kitchen cabinets for architects and builders. His interest in American chestnut was sparked by his earlier work in the antique business, repairing hundreds of pieces of furniture.

CREATING ARTWORK FROM AMERICAN CHESTNUT IS BOTH A JOY AND A CHALLENGE

By Gary P. Carver, Ph.D.

CarversCarvings, Ijamsville, MD

Reclaimed American chestnut is my favorite wood for carving. I love its beauty and significance. But as a wood, chestnut has few of the properties that any carver (or “Carver”) would consider ideal. Without a doubt, American chestnut is one of the trickiest and most difficult woods to carve.

The main challenge is that it is reclaimed from old structures. Any used wood (especially if it is 100 to 200 years old) is likely to be uneven and non-uniform. Any particular beam could have been in a barn, inside the kitchen of a cabin, or under the floor of an outhouse. As a result, the color may vary from a golden white, to a grayish brown, to an almost chocolate brown, even within a single beam. I have also smelled some strange odors when working with chestnut.

The hardness of the wood also may vary. A weathered beam may be brittle and riddled with deep cracks, wormholes, rusted nails or nail holes surrounded by black iron stains, hidden flaws and rotted areas, as well as other surprises, such as internal cavities and even bullets. Except for color variations, any of these defects can sabotage a carving project, if not cause an injury to an unsuspecting carver.

Carving American chestnut is more challenging than carving other reclaimed woods. Two intrinsic properties compound the difficulty. The first is its “ring-porous,” sometimes called “open-grained,” nature, with large pores in its earlywood. “Earlywood” and “latewood,” also called “springwood” and “summerwood,” form a tree’s growth rings. The growth rings, which manifest themselves as the grain of the wood, are dramatic in American chestnut, and, because it is a fast growing tree, can be widely spaced. Although this type of grain gives chestnut its exceptional beauty, it also gives the wood a “coarse” character.

The intrinsic coarse-grained character is an impediment to carving intricate detail and delicate features. Add the huge difference in hardness between



Merganser, carved from reclaimed American chestnut by Gary Carver



Chestnut's widely spaced growth rings are part of the challenge for Gary.

the earlywood and the latewood, which is partly due to the extremely large earlywood pores, and chestnut becomes a major challenge for carvers.

Chestnut's earlywood attracts (almost sucks in!) cutting tools — they tend to dig into the open-pored part of the grain. No matter how steady you try to hold a tool and cut across the grain, especially widely spaced grain, a wavy surface results. Even sanding chestnut can produce a wavy surface. The best approach to getting smooth, well-controlled surfaces is to work in the direction of the grain. This means frequently repositioning the tools and the item being carved and being careful not to cut too deeply when working where the earlywood (or any flaw) is prominently exposed.

The second property that makes American chestnut difficult to carve is that it splits easily. It has very thin, nearly invisible “rays,” the ribbons of wood cells that grow in a radial direction outward from the center of the tree to the bark, cutting across the circular annual growth rings. Wood that has prominent rays, such as oak, is difficult to split.

Because chestnut wood has thin, small rays, it is great for making split rail fences. However, it splits too easily for carving features that jut out. Unless small protrusions, such as the beaks of birds, are in the direction of the lines of grain, which is the stronger direction in any type of wood, they are likely to break off at the slightest provocation.

If American chestnut is so unpredictable, contains many types of faults, is not amenable to intricate detail or delicate features, is coarse, splits easily, and has extremely friable earlywood, how do I carve it? The answer is that I design my carvings to be smooth and stylized and I “go with the grain” and with any flaws in the wood. It sometimes takes me a long time to determine how to orient a carving within a piece of chestnut so that the shape works with the grain and the defects.

Fortunately, this approach complements my intent that the shape of a carving reveals the life and character of each piece of wood, and that it draws people to touch the carving. In my mind, a person reaching out to touch a chestnut wood carving infuses life back into what was once part of a magnificent living tree. The carvings thereby enrich the connection among people and the memory (and future promise!) of the American chestnut tree.

I have been carving vintage American chestnut wood for about five years. I use mainly hand-held power tools, both rotary and reciprocating, called “flexible shaft carving tools” and “power gauges.” I like to carve bird shapes, especially parrot-like birds. Maybe that is because I live with

In my mind, a person reaching out to touch a chestnut woodcarving infuses life back into what was once part of a magnificent living tree. The carvings thereby enrich the connection among people and the memory (and future promise!) of the American chestnut tree.

— Gary Carver

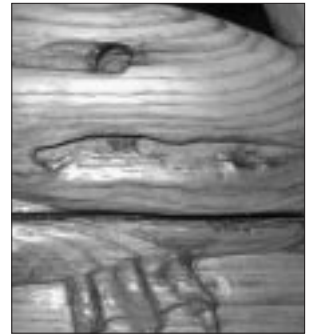
a flock of cockatoos that consider me their pet and sometimes chew toy.

That may be *how* I carve American chestnut wood, but given all the difficulties, *why* would I (or anyone else) want to carve American chestnut wood? Maybe for the same reason that we want to give back this once-magnificent tree its rightful glory in our eastern forests.

I see American chestnut wood as uniquely beautiful. I believe that anything made from historic American chestnut wood is special because it preserves a bit of our natural world that may have been lost forever. It feels like every chip I cut away loses a part of an ancient something that is more important than my carving. That is why I never throw the chips and waste pieces in the trash; instead, I scatter them in the woods so that they return to the environment.

Conversely, every molecule of wood fiber that remains in the carving memorializes past generations of trees and helps bring back the greatness of the American chestnut. At my first craft fair, a gentleman told me how he and his brothers cried when they asked their father why the big old play-tree in their front yard was not looking good and their father told them that it was dying, that “all the chestnut trees are dying.” He was clutching the chestnut carving he had bought from me to his chest and, as he turned to leave, said, “Thank you. You made my day.”

Gary Carver is a retired physicist who experienced a “left-brain, right-brain” transition that turned him into a woodcarver, in accordance with his name. He has combined his love of carving and of the American chestnut tree by creating birds out of wood reclaimed from old cabins and barns. CarversCarvings, which include his chestnut birds, are featured on TACF’s Virtual Gallery on www.acf.org, and on Gary’s site, www.carverscarvings.com.



Reclaimed chestnut wood’s texture and flaws are enhanced by Gary’s designs.



science and natural history

LEATHER, TANNIN AND THE CHESTNUT TREE

By Dr. William G. Lord

Leather is made from a variety of animal hides by a process known as tanning. On average, fresh hides consist of water and protein. Tanning involves the replacement of a large portion of the water content with tannin; bonding with the protein and giving it strength and stability. Tannin is a complex phenolic substance produced by many plants, effective as a defense mechanism against predators and parasites. In America, from Colonial times until well into the 19th century, leather was produced by small industrial units known as tanneries, modeled after methods prevalent in Europe since the 15th century.^[1]

Tanning was both laborious and odious, and a trade that stubbornly resisted change. The grist mill, the blacksmith and the tannery were an essential part of every developing community. Whereas the miller and the smithy were receptive to advanced techniques, the tanner was never sufficiently goaded by circumstance to change. He acquired his most essential ingredient, tannin, from the bark of hemlock, oak and chestnut, all in plenteous supply.^[2]

The initial process of removing hair from the hides required soaking in lime pits and required up to a year to complete. A tradesman known as a beamer then scraped the hide clear of flesh and remaining hair in preparation for the tanning pits. His strength rivaled that of any blacksmith. "This was exhausting work and in the course of a day one man normally beamed only a dozen hides."^[3]

The "tan bark" was ground to the texture of a coarse saw dust. The de-haired hides were placed between alternate layers of ground bark in an elongated pit and then immersed in water. Tannin leached from the bark and slowly replaced the water in the hides. According to the wisdom of the tanner, the process was repeated by laboriously removing the hides by means of long poles with hooks into adjacent pits for further treatment with the alternate layers of tan bark. This process required several months after which the hides were dried and then worked with various oils, soaps, dyes and other ingredients to perfect a finished product. The entire process might take two years.^{[2] [3]}



The Story of Chestnut Extract,
Illustrating the Resources of the
Champion Paper and Fibre
Company
copyright 1920, The Champion
Paper and Fibre Company

Hemlock was the preferred tanbark in the North; oak in the Mid Atlantic and the South. However, tanners generally used a blend of hemlock, chestnut or oak. Tanners rated their tanbark like gourmets grade coffee beans. “For instance, if one hide was tanned entirely with chestnut, the leather would be very hard, and would crack easily. Oak would be the most satisfactory if only one had to be used. The leather would be too soft if just hemlock were used.”^[3]

It is obvious that chestnut, although its bark was utilized, was not essential to the tanning process. Events in France brought a dramatic change wherein a method was developed to extract tannin from the wood of the European chestnut in concentrated form. Commencing in the early 1870’s extract plants could produce tannin that yielded a superior quality of leather. “The principal characteristics of the tannin of chestnut wood extract are its quality of imparting firmness and solidity to leather, and its capacity to make weight.”^[4]

In America, rights to this process were purchased by the Champion Fibre Company. In 1908 it installed a huge combined tannin extract and paper mill in Canton, western North Carolina, amid a mountainscape of hardwood forest. The choice was made because of the abundance of native chestnut which was found equal to the European chestnut as a source of tannin and also made a superior pulp for paper.

At this time the chestnut blight was a known presence, discovered in New York City in 1904, but Champion officials perceived it as a distant threat that would never endanger the vast chestnut reserves in the Southern Appalachians.

Chestnut trees were taken down and sawed into logs which were hauled by ox teams or washed down the mountain slopes in water fed flumes to landing sites. Here the logs were hewn and split into uniform five-foot lengths and transported over narrow gauge rails to the main line and onto the plant’s storage yards.

The storage yard presented a vast 75-acre area with a row upon row aggregate of 15,000 cords; a two months reserve supply. Wood was consumed at a rate of twelve cords per hour. At the plant the split logs were fed by a conveyer into, “five giant revolving chippers, reducing them, “to small chips



In clean, leak-proof, labeled barrels, sealed against evaporation, large quantities of Champion chestnut extract are shipped to tanners in domestic and foreign markets. From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company.



"Piled high in the seventy-acre woodyard at the Champion mills, are fifteen thousand cords of reserve chestnut, two months' supply, a protection against delays in production." From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company.



“Chestnut wood was found to possess characteristics suitable for exceptionally soft, clean, white wood pulp. Also, the Chestnut of western North Carolina was discovered to be exceptionally high in percentage of tannin. These two desirable factors inspired a cycle of operations that has gone far to place the Champion mills in a position of leadership in chemically prepared products.”

—From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company

almost as quickly as one sharpens a pencil.” The chips were screened to a uniform size, conveyed to overhead bins and then poured into a set of, “thirty-six huge steam-tight metallic cauldrons, called autoclaves. Here by use of boiling water, tannic acid is removed from the wood.”^[5]

The extract was piped to evaporators; huge air-tight metal tanks, for concentrating the extract and the tannin-leached chips were conveyed to the pulp mill, there to make the “soft, clean, white wood pulp for paper manufacture.”^[5]

The French method of extracting tannin from chestnut wood prompted change. A superior tannin product was dependably available as a liquid or as a powder and greatly shortened the time required to produce leather. This efficiency over the tan bark method was a major factor in the establishment of much larger tanneries that gradually replaced the small scale, individualistic tanner.

The Champion Fibre Company began its operation in 1908 and gave no apparent consideration to the destructive impact of the blight. R. W. Griffith, a company official, wrote an article on the company’s operation. No date is given, but somewhere close to 1915 seems reasonable. Regarding the blight he considered forest fires as the greatest present danger. “The blight, which destroyed so many of the chestnut trees in the eastern parts of the country, fortunately never reached this area, and there is no immediate prospect of its doing so. The



Twelve cords per hour are required to produce Champion extracts; here is seven minutes’ supply. From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company.



Two hundred and seventy-five cords of chestnut from timberlands or woodyard reserve are carried along this ramp into the extract plant each twenty-four hour working day. From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company

area, however, is not immune from the ravages of forest fires, but in the timberlands operated by the Champion Fibre Company,the underbrush is always cleared as the first precaution to be taken against fires, and this permits the development of a second growth [coppice], which becomes available for cutting in about twenty-five years.”^[4]

Griffith may have been whistling in the dark even as he wrote. The blight flood was inundating the chestnut of western North Carolina by the early 1920’s. E. H. Frothingham of the U. S. Forest Service estimated the total stand of chestnut in the Southern Appalachians in 1924 at 33,700,000 cords. This resource was vital to the extract plants and by extension, to all aspects of the leather industry. A study was made to “determine the value of blight killed chestnut for extract purposes.” The very heartening results and conclusions were published in 1929 by R. M. Nelson and G. F. Gravatt of the USDA.^[6]

In essence, blight-killed chestnut would provide a profitable source of tannin. “....there is no appreciable loss in tannin content in trees that have been dead for as long as 20 or 30 years. Chestnut trees killed by the blight, therefore, can be used for the production

of extract for 20 or 30 years after death or until the wood is badly decayed.” The bark, with its relatively high tannin content, [7-12%] and the thin sapwood layer with 2-4%, would weather and waste away within a few years after the tree died, but the standing heartwood, [7-12%] contained by far the greater aggregate of tannin.^[6]

In western North Carolina, and throughout the Appalachians, death claimed the chestnut giants, rendering the landscape bleak with their sad, twisted, leafless forms. But they prolonged the life of Champion’s extract plant for many years until the last ghosts were harvested in 1951. One notable change came as trucks and better roads gradually replaced the slow, patient toil of the ox team.

As a post script, here is something for conjecture: An interesting feature of the Nelson-Gravatt study showed that the roots and root bark of chest-

nut have a very high percentage of tannin. "...root bark varies in tannin content from 25 to 37 percent and root wood from 9 to 23 percent." It was further shown from tests conducted by the Pennsylvania Blight Commission [circa 1912] "...that the tannin content in the [blight] infected bark is considerably higher than that in sound bark" It is well established that the roots of chestnut are strongly blight resistant, enabling the tree to persist by sprouting. Is the blight resistance of the roots related to its higher tannin content compared to that of the above ground bark and wood? Did the higher tannin content in infected bark indicate a defensive effort by the chestnut? The Commission's comment on its data was a limp equivocation. "No satisfactory explanation has been offered as to this tannin increment." ¹⁶¹

Within a radius of one hundred miles from the Champion mills the chestnut trees are cut into logs, barked and split to be trucked to railroads for shipment to the mills. From *The Story of Chestnut Extract*, copyright 1937, The Champion Paper and Fibre Company

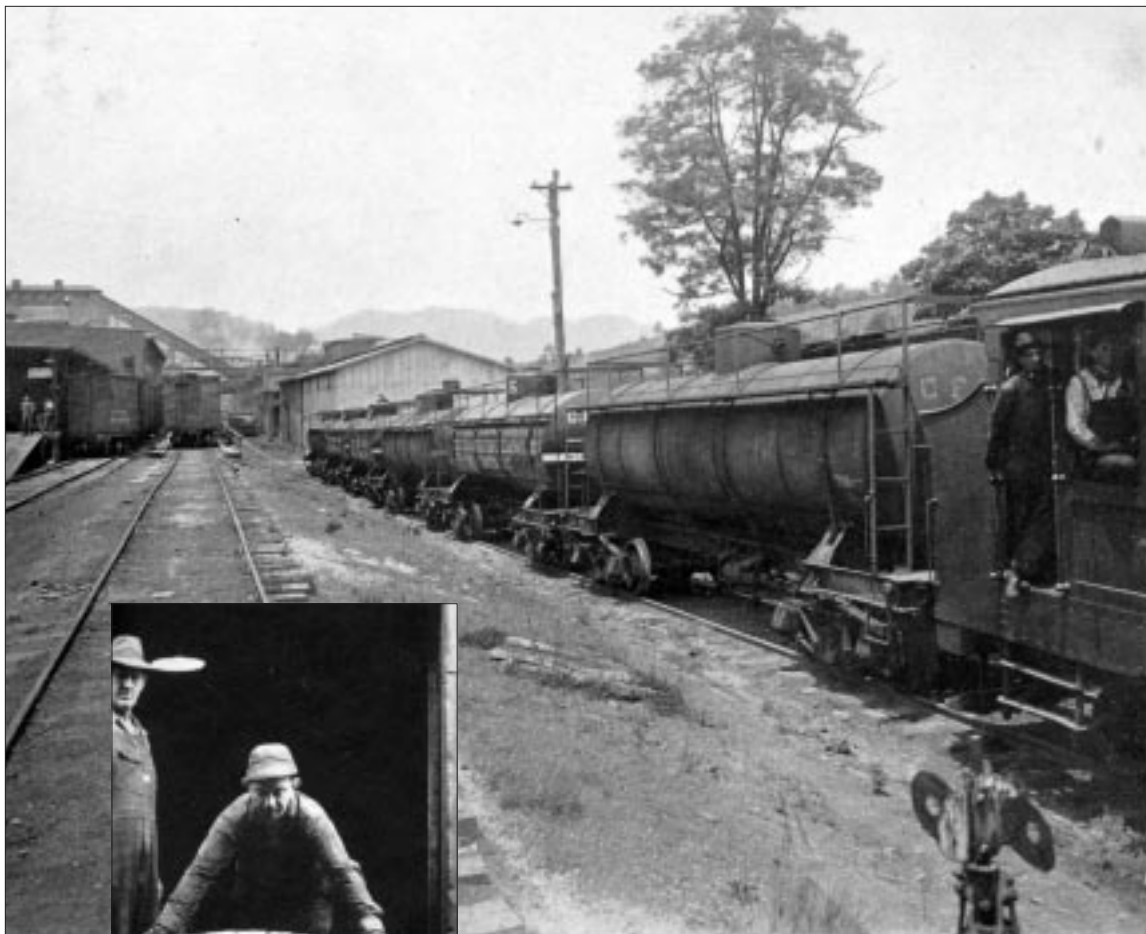
Appreciation is extended to Dr. Paul Sisco for providing the resource publications and for reviewing the text.

Dr. Bill Lord, a retired veterinarian, holds a seat on the Board of Directors of The American Chestnut Foundation, and is a member of its Development Cabinet.

Dr. Lord's article documents the death knell that the demise of American chestnut dealt to the tannin industry in the Appalachians. An interesting note is that the use of vegetable tannins was already being marginalized by mineral salt tanning during this period. By the time Champion and other tannic acid plants were running out of chestnut during the 1940's, the use of mineral salts, primarily chromium, was already the primary method of tanning. This was likely due to economics (the chromium process was cheaper), supply (a long period of heavy harvest depleted the supply of oak and hemlock, as well as chestnut, for tannins), and quality (chrome-tanned leather is much softer). However, chestnut tanning was still prized for some purposes, including tanning of leather for shoe soles.

Mineral salt tanning is the primary method used today, with minor amounts of tannic acid still utilized





A train of tank cars loaded with Chestnut Extract. *From The Story of Chestnut Extract*, Illustrating the Resources of the Champion Paper and Fibre Company, copyright 1920, The Champion Paper and Fibre Company

Canton, NC, 1951 — The last barrels of chestnut extract produced at Canton are being rolled into a box car by Buster Wood, son of Canton Old Timer S. C. Wood. Jim Medford, Champion Old Timer, stands in storage room doorway. The last production report was made out by W. V. “Vent” Haynes, assistant superintendent of Extract Department, on the morning of June 11, 1951.



“Canton NC _ The closing chapter was written June 11 [1951] in the epic story of one of the most colorful phases of the 43-year operation of Champion’s Canton Division.

The Extract Department,...was closed down for the first time since the combination of Western North Carolina men and Western North Carolina chestnut wood began supplying the world with a high grade of chestnut extract in 1908.

The end of the story has been written not by choice but by necessity. More than a decade ago a blight attacked and killed the chestnut trees. Since then Champion’s Extract unit has continued to utilize the dead trunks that stood throughout the mountains like so many monuments to the past glory of the chestnut...

There was a time and not too long ago that Champion’s Extract Department ran full capacity (300 barrels every 24 hours) and was the world’s largest single-unit producer of twenty five percent chestnut extract.”

(tannins can be derived from a variety of plant materials found throughout the world.) Other current methods utilize emerging new organic compounds. A new silicate process is also apparently gaining ground in the market.

—Hugh Irwin, *Conservation Planner*
Southern Appalachian Forest Coalition

Hugh Irwin is Vice Chair of TACF’s Board of Directors and Head of its Science Cabinet

NOTES

1. Encyclopedia Britannica, 15th ed., vol. 7, p 225
2. Welsh, Peter C., *The Craft that Resisted Change*, Technology and Culture, vol. 4, pp 299-317, 1963
3. Cragie, Carter W., *Tanning in Chester County*, Pennsylvania, 1711-1850, a 15 page article written in 1968 and published by the Pennsylvania Dutch Folklore Center, Lancaster, PA
4. Griffith, Robert W., *The Story of Chestnut Extract*, 21 pages of text and photographs, 1915, Champion Fibre Co., Canton, NC
5. Author unknown, *The Story of Chestnut Extraction*. 35 pages of text and photographs, 1937, Champion Fibre Co., Canton, NC
6. Nelson, R. M. and Gravatt, G. F., *The Tannin Content of Chestnut Trees*, The Journal of the American Leather Chemists Association, vol. XXIV, no. 9, 1929, pp 479-499

NOTES FROM MEADOWVIEW RESEARCH FARMS 2003–2004

by Frederick V. Hebard, Ph.D.
Staff Pathologist

During 2003, Meadowview had an excess of rainfall compared to normal years. The rain was especially heavy during pollination in June. On one day we could not use even the small bucket truck for bagging because of the sloppy conditions; usually, we only see sloppy conditions in winter and early spring. The rain also caused standing water in one low spot; before we could get it drained, the ground soured (became anaerobic), killing the trees along that low spot. Additionally, we experienced wind-driven hail, which left numerous pock marks on the north side of most trees and shredded the leaves on several. Hopefully, that weather pattern will not repeat itself in the next 100 years!

It was moderately wet again in the winter and spring of 2004, delay-

ing plowing until March. But we were able to get the planting done by early April, partly because we now have sufficient equipment to prepare orchards quickly once the weather breaks, thanks to the generous support of TACF members. Thank you!

Our current holdings are in Table 1, and changes from 2003 to 2004 are indicated in Table 2. We have almost 21,000 trees, not too many more than last year (Table 2). The addition of B₃-F2 trees has been offset by the removal of straight third backcross trees as we have made selections in B₃ orchards and rogued the rejects. We also rogued deselected B₂'s



Left to right, Jason Mann and George Sykes cover chestnut plants with styrofoam cups to protect them from rain and freezing temperatures, while Leon Porter positions aluminum cylinders around the nuts being planted by Benji Cornett.

at the Wagner Farm as part of converting it to a seed orchard for Graves B₃-F₂'s. The first Graves B₃-F₂ seeds have now been planted there, about 1,100. They are doing well to date. We have begun testing the blight resistance of 2-year-old Clapper B₃-F₂'s this year; preliminary results will be in by the Annual Meeting in October. Some of those Clapper B₃-F₂'s are flowering, and should start producing nuts in 1 or 2 years.

Table 3 presents the current holdings of 'Graves' and 'Clapper' third backcrosses in the various state chapters. The number of trees, lines and chapters continues to grow. This year, we count 13,904 third and fourth backcross trees and planted nuts in the various chapters. This count is down a bit from last year because the totals do not include B₃-F₂ trees growing at the Penn State arboretum, and because some of the totals previously had included planted nuts for which mortality had not been determined.

Overall, I was very pleased with the 2003 nut harvest (Table 4). Despite the wet weather, we managed to make most of the desired crosses, and many of them gave decent yields, with little pollen contamination. There were the usual disappointments in some crosses: nut yields were below average, less than 1 nut per bag. But we made progress on most fronts.

I would like to thank Lou Silveri, Ron Myers, Dave Lazor, Mason Jeffries, Gene Whitmeyer, and Harry Norford for helping out with pollination this year. They came down on their own and stayed at Emory and Henry College. We also had a group come down under an Elder Hostel program. Sam Fisher, Neil Rich and Chrystle Gates of the Southwest Virginia 4-H Center have been very helpful managing the Elder



(top) Graves orchard, at Wagner Research Farm. In the background are selected second backcross trees from which pollen is collected for TACF chapters.

(bottom) George Sykes, left, and Fred Hebard at the Graves seed orchard on Wagner Research Farm, preparing the soil for planting.

Hostel program, which would not occur without their initiative. Thank you -this wouldn't get done without your help.

If you would be interested in helping to pollinate in June, 2005, call us at Meadowview Research Farms in early June: (276) 944-4631. If you would be interested in the Elder Hostel program, call 617 426-8055 or write 75 Federal St., Boston MA 02110.

TACF members are welcome to visit Meadowview Research Farms. Take Interstate 81 in Virginia to Exit 24, Meadowview. Take VA Route 80 southeast for 1/3 mile. We are in the white house on the northeast side of the road. We generally are open during normal business hours, but it might be good to call ahead: (276) 944-4631.

A Quick Guide to Chestnut Breeding Terminology

PARENT	=	OFFSPRING
American x Chinese	=	F ₁ , "F-one"
F ₁ x F ₁	=	F ₂ , F-two
F ₂ x F ₂	=	F ₃ , F-three
F ₁ x American	=	B ₁ , first backcross, or B-one
B ₁ x American	=	B ₂ , second backcross, or B-two
B ₂ x American	=	B ₃ , third backcross
B ₃ x American	=	B ₄ , fourth backcross
B ₁ x B ₁	=	B ₁ -F ₂ , B-one F-two
B ₁ -F ₂ x B ₁ -F ₂	=	B ₁ -F ₃ , B-two F-three
B ₂ x B ₂	=	B ₂ -F ₂ , B-two F-two
B ₂ -F ₂ x B ₂ -F ₂	=	B ₂ -F ₃ , B-two F-three
B ₃ x B ₃	=	B ₃ -F ₂ , B-three F-two
B ₃ -F ₂ x B ₃ -F ₂	=	B ₃ -F ₃ , B-three F-three

TABLE 1

Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2003, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	Number of		
	Nuts or Trees	Sources of Resistance	American Lines*
American	2116		210
Chinese	669	49	
Chinese x American: F ₁	617	23	91
American x (Chinese x American): B ₁	778	14	31
American x [American x (Chinese x American)]: B ₂	1532	10	87
American x {American x [American x (Chinese x American)]}: B ₃	5275	8	73
Am x (Am x {Am x [Am x (Chin x Am)]}):B ₄	86	1	1
(Chinese x American) x (Chinese x American): F ₂	710	5	5
[Ch x Am] x (Ch x Am) x [Ch x Am] x (Ch x Am):F ₃	6	1	1
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B ₁ -F ₂	688	3	3
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B ₂ -F ₂	343	4	4
[A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B ₃ -F ₂	5836	2	17
Chinese x (Chinese x American): Chinese B ₁	142	3	3
Chinese x [American x (Chinese x American)]	41	1	1
Japanese	3	2	2
American x Japanese: F ₁	11	2	2
(American x Japanese) x American: B ₁	79	2	2
Castanea seguinii	48	1	1
Chinese x Castanea pumila: F ₁	9		
Large, Surviving American x American: F ₁	262	9	9
(Large, Surviving American x American) x American: B ₁	631	6	9
[(Large, Surviving American x American) x American] x American: B ₂	37	1	2
Large, Surviving American x Large, Surviving American: l ₁	385	12	12
Large, Surviving American: F ₂ = F ₁ xF ₁ , same LS parent	467	5	5
Large, Surviving American Other	59	2	2
Irradiated American x American: F ₁	3	1	1
Other	24		
Total	20,857		

* The number of lines varied depending on the source of resistance. We will have to make additional crosses in some lines to achieve the desired number of 75 progeny per generation within a line. In keeping with past practice, the number of lines for each source of resistance are added separately; thus, progeny from two sources of resistance that share an American parents would be counted as two lines rather than one line (this only occurs rarely).

science and natural history

TABLE 2

Changes between 2002 and 2003 in the number of chestnut trees and planted nuts of different types at TACF Meadowview Research Farms, including changes in the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	Increase or Decrease* in Number of		
	Nuts or Trees	Sources of Resistance	American Lines
American	-4		23
Chinese	-249	8	
Chinese x American: F ₁	-79	-2	-4
American x (Chinese x American): B ₁	-286	-3	-5
American x [American x (Chinese x American)]: B ₂	-118	1	-9
American x {American x [American x (Chinese x American)]}: B ₃	-1150	2	2
Am x (Am x {Am x [Am x (Chin x Am)]}):B ₄	0	0	0
(Chinese x American) x (Chinese x American): F ₂	0	0	0
[Ch x Am] x (Ch x Am) x [Ch x Am] x (Ch x Am):F ₃	0	0	0
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B ₁ -F ₂	0	0	0
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B ₂ -F ₂	-38	0	-1
[A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B ₃ -F ₂	2384	0	9
Chinese x (Chinese x American): Chinese B ₁	0	0	0
Chinese x [American x (Chinese x American)]	0	0	0
Japanese	0	0	0
American x Japanese: F ₁	-3	0	0
(American x Japanese) x American: B ₁	-119	0	0
Castanea seguinii	0	0	
Chinese x Castanea pumila: F ₁	0		
Large, Surviving American x American: F ₁	11	-4	-18
(Large, Surviving American x American) x American: B ₁	-137	-1	-3
[(Large, Surviving American x American) x American] x American: B ₂	37	1	2
Large, Surviving American x Large, Surviving American: I ₁	191	6	6
Large, Surviving American: F ₂ = F ₁ xF ₁ , same LS parent	-236	0	0
Large, Surviving American: Other	0	0	0
Irradiated American x American: F ₁	-38	0	0
Other	-2		
Total	164		

* The decreases in B₁, B₂, B₃, and Large, Surviving American B₁ & F₂ trees reflects roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting. The decreases in the number of American lines in backcrosses reflect changes in how these are counted.

TABLE 3

Number of third-backcross chestnut at TACF Chapters in 2004, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Chapter	Number of		
	Nuts or Trees	Sources of Resistance	American Lines*
Maine	1445	2	29
Massachusetts	3076	2	28
Pennsylvania	5350	2	36
Maryland	33	1	1
Indiana	1496	1	11
Kentucky**	150	2	2
North Carolina	1049	2	9
Tennessee**	745	5	6
Alabama	560	1	5
Total	13,904		

*Numerous B₃-F₂s also have been planted but these are not included in this table.

**Data for 2003

TABLE 4

The American Chestnut Foundation Meadowview Research Farms 2003 nut harvest from controlled pollinations and selected open pollinations.

Nut Type	Female Parent	Pollen Parent	Pollinated			Unpollinated Checks			Number of American Chestnut Lines*
			nuts	bags	burs	nuts	bags	burs	
Am x Am	American	American	33	98	257	0	14	18	1
B ₂	American	B ₁ Nanking	94	214	272	0	25	23	11
B ₁	F ₁ 72-211	American	41	114	175	1	15	24	2
B ₁	F ₁ mollissima7	American	6	90	168	0	9	15	1
B ₁	F ₁ mollissima10	American	45	90	177	1	9	19	1
B ₂	American	B ₁ MusickChinese	11	18	33	0	3	3	1
B ₂	B ₁ MusickChinese	American	58	111	170	0	9	13	2
B ₂	American	F ₁ mollissima13	12	67	119	0	5	8	3
B ₂	B ₁ Nanking	American	242	377	1117	0	40	144	6
B ₂	B ₁ Meiling	American	75	115	328	7	13	44	2
B ₂ -F ₂	B ₂ R1T7	B ₂ R1T7	111	213	639	2	23	72	2
B ₂ -F ₂	B ₂ Clapper	open	1227	open pollinated					6
B ₂ -F ₃	B ₂ -F ₂ Clapper	open	994	open pollinated					3
B ₃	American	B ₂ Mahogany	14	65	77	0	10	0	2
B ₃	B ₂ Mahogany	American	14	14	24	0	1	3	1
B _v	B ₂ Graves	American	66	385	1016	1	43	116	6
B ₃	American	B ₂ Nanking	196	93	197	0	15	25	6
B ₃	B ₂ Nanking	American	3	40	107	0	5	12	1
B ₃	American	B ₂ R1T7	22	42	35	1	7	3	4
B ₃	B ₂ R1T7	American	5	18	37	0	2	8	1
B ₃	American	B ₂ R11T14	6	72	88	0	11	14	2
B ₃	B ₂ R1T4	American	137	103	264	0	7	33	1
B ₃ -F ₂	B ₃ Clapper	open	4099	open pollinated					11
B ₃ -F ₂	B ₃ Graves	open	1125	open pollinated					5

(Continued on next page)

TABLE 4 (continued)


Nut Type	Female Parent	Pollen Parent	Pollinated			Unpollinated Checks			Number of American Chestnut Lines*
			nuts	bags	burs	nuts	bags	burs	
F ₁	Chinese Kuling	American	31	68	119	0	8	18	1
F ₁	Chinese Mahogany	American	67	121	196	1	9	28	4
F ₁	Chinese Nanking	American	341	114	223	1	9	28	3
Isa B ₁	F ₁ Ort	American	48	88	252	0	8	27	1
Isa B ₂	B ₁ ScientistsCliff	American	75	50	89	4	7	11	3
Isa F ₁	American	Hill4565	153	77	125	1	7	8	1
Isa I ₁	F ₂ Gault	opDaresBeach	116	96	226	1	6	13	1
Isa I ₁	opDaresBeach	F ₂ Gault	22	58	106	0	7	9	1
Total Controlled Pollinations			2044	2812	6349	21	293	686	

*The number of American lines for this table is restricted to the number of American chestnut trees that were direct parents, not grandparents, of progeny.

DESIGNING CHESTNUT ORCHARDS TO CONTROL THE POLLEN PARENT WITHOUT HAND-POLLINATION

The use of self-incompatibility and cytoplasmic male sterility

Paul Sisco, Ph.D., TACF Regional Science Coordinator



In most methods of plant breeding, such as the backcross method being used by The American Chestnut Foundation, it is important to control both parents in a cross — to know the identity of the male parent as well as the identity of the female parent. If nuts harvested from a chestnut tree are labeled correctly, it is easy to know the female parent. It is just the tree from which the nuts were harvested. Controlling the male parent is much more difficult. To date, our chapter breeding programs have used bagging and hand-pollination to control the pollen parent. This is labor-intensive, expensive, and potentially dangerous. Female flowers must be bagged at just the right time to prevent contamination, and ladders and bucket trucks must be used to get to the flowers. Dr. Robert Leffel, retired plant breeder and scientific advisor to our PA Chapter, has asked a good question. At the chapter level, where there are small farms with small orchards, why not set up our orchards so that hand-pollination is not necessary. In this article and the accompanying one by Dr. Leffel, methods are described for setting up small orchards in such a way that the identity of the male parent can be known without the use of hand pollination.

Some plants, like most animals, have separate male and female individuals. These are known as dioecious plant species. Asparagus, spinach, persimmon, date palm, and many holly species are examples of dioecious plants. If a single male holly is planted in a grove of 100 female hollies, both the male and the female parents can be easily determined without any attempt to control pollination. The female is the tree from which the berries are harvested, and the single male tree in the grove has to be the male parent.

Most plants, however, have both male and female flowers on the same individual. These are known as monoecious plant species. Monoecious plants have the potential for self-pollination, and in some crop species, such as peas, wheat, lettuce, and tomatoes, self-pollination happens very often. The flowers of these plants are structured so that pollen almost always falls on the female flower of the same plant. These plant species

tend to be inbred and uniform — well-adapted to stable environmental niches but ill-adapted to environmental change.

Other monoecious species have developed methods for preventing self-pollination and encouraging outcrossing. Such methods include: (1) the male and female flowers on a single individual can bloom at different times; (2) the female flower can be physically distant from the male flower, making it difficult for pollen to travel to a female flower on the same plant; (3) genetic mechanisms can prevent the germination of pollen, or pollen tube development, on a female flower of the same plant; and (4) pollen may simply not develop — the plant has male flowers, but they are sterile, while the female flowers remain fertile. Individuals of outcrossing monoecious species are genetically diverse and thus better able to cope with environmental change.

All chestnut species are monoecious, having both male flowers and female flowers. Chestnuts are also outcrossers. They mostly use method #3 to prevent self-pollination — a method known as self-incompatibility. An isolated chestnut tree will rarely set nuts, because its female flowers recognize its own pollen and prevent the pollen tubes from reaching the egg cells (McKay, 1942). Chestnut trees can also be male-sterile, as in method #4.

DESIGNING ORCHARDS TO ELIMINATE THE NEED FOR HAND-POLLINATION

Chestnut orchards can be designed to take advantage of these mechanisms of pollen control so that both male and female parents can be known without the necessity of hand-pollination. Below I have described several such designs. In the accompanying article, Dr. Robert Leffel relates how one of these designs has been adopted by the Pennsylvania Chapter of TACF to breed for multiple sources of blight resistance and for regional adaptation.

Causes of pollen-sterility in plants: Pollen- or male-sterility is a common phenomenon in plants. Pollen can fail to develop fully for several reasons:

- (1) stress factors, such as drought
- (2) the effect of nuclear genes that are inherited in a normal Mendelian fashion (Albertsen and Phillips, 1981)
- (3) a non-correspondence between nuclear and mitochondrial genes — so-called cytoplasmic male sterility (CMS).



Design #1*

Two chestnut trees in isolation from all other chestnut trees



Tree #1



Tree #2

If two chestnut trees are close to each other, but isolated from all other chestnut trees, no hand pollination is necessary to control the parents. Because of the natural self-incompatibility of chestnut, almost all the nuts of tree #1 will be pollinated by tree #2, and vice-versa. The rare "selfed" nut will produce an inbred tree that will either die early or be defective in some way. Caution: This will only work if the blooming times of the two trees overlap.

Design #2

Several chestnut trees with a single designated female parent



Americans used as males



F₁ Female

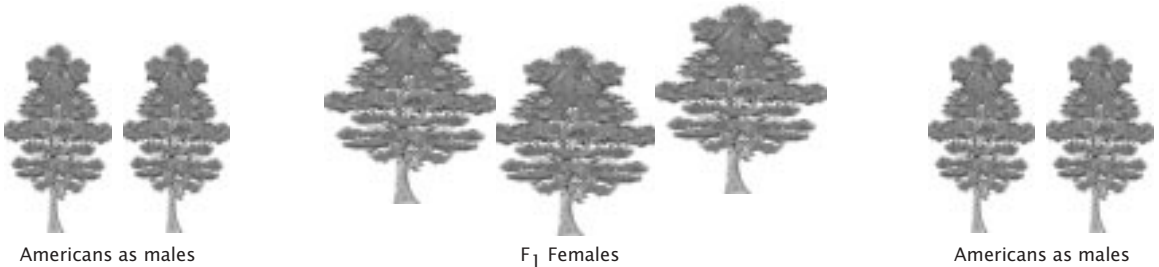


Americans used as males

One can also design an orchard so that the male parents of a certain desired type will cross with a single female of another type. In the example diagrammed above, the female is an F₁ hybrid created by crossing a pure Chinese and a pure American chestnut. The males are all from pure American chestnuts collected in a certain region. The progeny of the F₁ female will almost all be of the desired BC₁ type, if the flowering times of the males and the single female tree overlap, and if this orchard is grown in isolation from other chestnut trees. Like Design #1, this design will not require hand-pollination because of the natural self-incompatibility of the female. The progeny of any selfed nuts on the female would likely yield defective trees, easily eliminated. Unlike Design #1, however, nuts collected from the males would not all be of the same type. Some would be pure American and some would be BC₁. In this design, nuts would only be collected from the single female.

*Tree diagrams reproduced from drawings by Bruce Lyndon Cunningham

Design #3
Several chestnut trees with multiple designated female parents



If more than one female of a desired type is planted in an orchard, one cannot depend on self-incompatibility to produce offspring of the desired type. This is because the female trees could potentially cross with each other as well as with the desired male trees. In this case, one must use some form of male-sterility to prevent pollen formation in the trees designated as females.

The phenomenon of cytoplasmic male-sterility (CMS): For recent reviews on the mechanisms of CMS in plants, see Newton et al. (2004) and Chase and Gabay-Laughnan (2004). Mitochondria are bacterial-like “organelles” in a plant cell that produce energy, and in fact they are probably descended from bacteria that became associated with higher organisms eons ago (Palmer et al., 2000). Most of the proteins in a mitochondrion are encoded by the plant’s nucleus, but a few mitochondrial genes are encoded by DNA in the mitochondrion itself.

In the following article, Dr. Leffel discusses how CMS can be used to eliminate the need for most hand pollination in a breeding program for local adaptation. Some might question whether CMS should be used at all, because (1) pollen sterility would seem to be a defect, (2) it necessitates selecting for additional Chinese nuclear genes during the backcrossing program and (3) it requires larger BC₁ and BC₂ orchards.

Here are my thoughts about those three objections to the use of CMS in a chestnut breeding program:

Pollen sterility is a defect: Careful studies on the effect of CMS in corn showed that CMS did have a small negative effect — it reduced yield about 3%. But this was offset by the energy saved from not having to produce

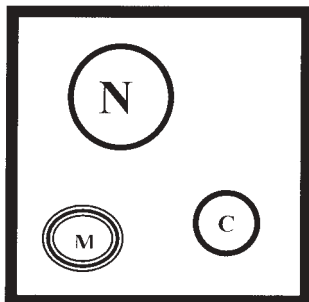


Fig. 1

A stylized plant cell with a cell wall (black box), nucleus (N), mitochondrion (M), and chloroplast (C). In plant cells the DNA of the nucleus encodes most of the genes, but mitochondria and chloroplasts also have DNA that encode a few genes. In most plants, such as chestnut, mitochondria and chloroplasts are inherited strictly maternally — a plant receives all its mitochondria and chloroplast DNA from its female parent. Chloroplasts capture energy from sunlight, and mitochondria convert energy into a type that can be used by the plant. Mitochondria, in other words, are the “powerhouses” for both plants and animals.

Proper function of the mitochondrion requires that the proteins encoded by the nucleus and the proteins encoded by the mitochondrial DNA work together correctly. Sometimes when a cross is made between species, such as Chinese and American chestnut, the nuclear genes of one species and the mitochondrial genes of the other species do not work efficiently together, because they did not evolve together. The mitochondria of the hybrid work well enough for most plant functions, but for pollen formation a lot of energy is required. The hybrid mitochondria cannot produce enough energy, and pollen abortion results.

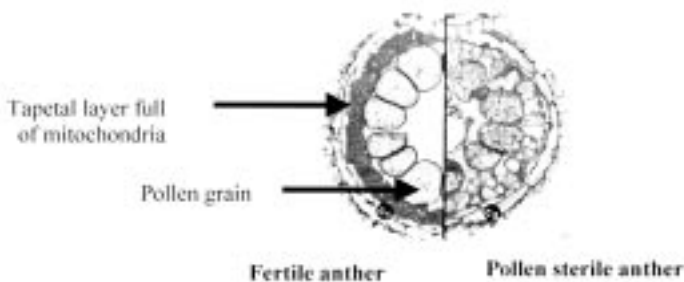


Fig. 2

Light micrograph of half-section of a fertile (left) and sterile (right) corn anther. In the fertile anther, the tapetal layer is full of mitochondria producing energy for the developing pollen grains. In the sterile anther, the tapetum and its mitochondria are defective and the pollen is aborting. From Warmke and Lee (1977).

In chestnut, CMS occurs when American chestnut is used as female and Chinese chestnut as male to make an F₁ hybrid (Shi and Hebard, 1977). The F₁ is pollen sterile. Thus there must be some interaction between certain genes in the Chinese nuclear genome and the American mitochondrial genome that results in pollen abortion. This sterility can continue on to subsequent generations, appearing in a certain percentage of the trees, until the Chinese nuclear gene or genes associated with the sterility are replaced by American nuclear genes.

pollen — a gain in yield of about 3%. So the two effects cancelled each other out, and CMS corn plants had the same yields as male-fertile corn plants. (Duvick, 1965).

One particular type of sterilizing mitochondria in corn — *cms-T* mitochondria — proved susceptible to a fungal disease. In the case of chestnut, however, the mitochondria are normal, American chestnut mitochondria.

Data from two crosses at our Meadowview Research Farms indicate that there may be differences among American chestnut mitochondria with respect to the sterilizing reaction with Chinese nuclear genes. An F₁ tree was crossed as male to two different American chestnut trees as female. The progeny of the cross to one American female — Mill Creek H — included both male-sterile and male-fertile trees. The progeny of the cross to the other American female — the Musick tree — were all male-fertile.

Selecting for sterility will carry along more of the Chinese nuclear genome: It is true that selecting for male-sterility in the early generations will carry along a greater proportion of the Chinese nuclear genome. Any BC₁ or BC₂ trees selected for male-sterility as well as for blight resistance will on average be more Chinese than trees selected for blight resistance alone. However, these Chinese genes for sterility will be eliminated immediately in the final generation in which the trees are selected for male fertility. So in the long run, a breeding scheme using CMS should reach the

TABLE 1

Results of the cross of an F₁ male to two different American female parents.

Female Parent	Male Parent	Male-fertile BC ₁ progeny	Male-sterile BC ₁ progeny	Total Progeny
Mill Creek H American Chestnut Tree	F ₁ ('Nanking' x Lesesne Irrad. Amer.)	18	12	30
Musick American Chestnut Tree	F ₁ ('Nanking' x Lesesne Irrad. Amer.)	61	0	61

same percentage American genome as a breeding scheme that does not use CMS in the early generations.

The BC₁ and BC₂ orchards will have to be at least twice as large: Because the MF trees will be rogued out of the BC₁ and BC₂ orchards, those orchards will need to contain at least twice as many trees as for a MF-only orchard, so that sufficient trees remain after roguing to select for blight resistance and American characters. There should be plenty of seed available to plant these orchards, however, since the seed will be produced by open-pollination. So the only extra requirement will be for more land, and BC orchards, being fairly small, do not take up much space.

It will be very interesting to follow the year-by-year results of the Pennsylvania Chapter in this new and exciting enterprise.

REFERENCES

Albertsen, M.C. and Phillips, R.L. (1981). Developmental cytology of 13 genetic male sterile loci in maize. *Can. J. Genet. Cytol.* 23: 195-208.

Chase, C.D. and Gabay-Laughnan, S. 2004. Cytoplasmic male sterility and fertility restoration by nuclear genes. In: Daniell, H. and Chase, C.D. (Eds.). *Molecular biology and biotechnology of plant organelles*. Kluwer Academic Publishers: London, U.K., in press.

Duvick, D.N. 1965. Cytoplasmic pollen sterility in corn. *Adv. Genet.* 13:1-56.

McKay, J.W. 1942. Self-sterility in the Chinese chestnut (*Castanea mollissima*). *Proc. Amer. Soc. for Hort. Sci.* 41:156-160.

Newton, K.J., Gabay-Laughnan, S., and De Paepe, R. 2004. Mitochondrial mutations in plants. In: Day, D.A., Millar, H., and Whelan, J. (Eds.) *Plant mitochondria: from genome to function*. Kluwer Academic Publishers: London, U.K. p. 121-142.

Palmer, J.D., Adams, K.L., Cho, Y., Parkinson, C.L., Qiu, Y.L., and Song, K. 2000. Dynamic evolution of plant mitochondrial genomes:

mobile genes and introns and highly variable mutation rates. Proc. Natl. Acad. Sci. USA 97:6960-6966.

Shi, Y. and Hebard, F.V. 1997. Male sterility in the progeny derived from hybridizations between *Castanea dentata* and *C. mollissima*. J. Amer Chestnut Found. 11(1):38-47.

Warmke, H.E. , and Lee, S-L.J. 1977. Mitochondrial degeneration in Texas cytoplasmic male-sterile corn anthers. J. Hered. 68:213-222.



CYTOPLASMIC MALE STERILITY AND CHESTNUT BREEDING PROGRAMS

By Robert C. Leffel, Ph.D.,
Research Agronomist, ARS-USDA, Retired

Leffel (2001) proposed cytoplasmic male sterility (CMS) as a potential methodology for breeding blight-resistant, timber-type chestnuts. As explained in the previous article by Dr. Sisco, CMS is sometimes found in the progeny of interspecific crosses, where the nuclear gene(s) of one species is put into the cytoplasm of another species. Cytoplasm, the part of the cell other than the nucleus including the mitochondria and chloroplast DNA, is usually inherited from the female parent only, via the egg cells. Thus the inheritance of CMS is maternal, whereas the restoration of fertility is controlled by nuclear factors from both parents inherited in a regular, Mendelian fashion. CMS in chestnut provides a method of emasculating plants genetically to aid in the control of the pollen parent, eliminating the need for hand pollination.

TERMINOLOGY—CYTOPLASMIC GENOTYPE

(cms-Amer): cytoplasmic genotype of American chestnut that confers male-sterility in the presence of Chinese alleles (forms) of certain nuclear gene(s) *S* but confers fertility in the presence of the corresponding American alleles of these genes. This appears to be the most common cytoplasmic genotype in American chestnut.

(Amer^F): cytoplasmic genotype of American chestnut that confers male-fertility in the presence of Chinese alleles of nuclear gene(s) *S*. One example of this cytoplasm, that of the 'Musick' tree, is shown in Table 1 of Dr. Sisco's article.

(Chin^F): cytoplasmic genotype of Chinese chestnut trees. So far as is known, trees with Chinese cytoplasm are male-fertile regardless of the nuclear genotype.

TERMINOLOGY — NUCLEAR GENOTYPE

S: The Chinese allele of a nuclear gene that confers male-sterility when in combination with *cms-Amer* cytoplasm.

s: The American allele of the same nuclear gene. This allele confers fertility in any cytoplasm.

S is dominant to *s*.

DEGREE OF MALE-FERTILITY OF VARIOUS COMBINATIONS OF CYTOPLASM AND NUCLEAR GENES

(*cms-Amer*) *SS* or *Ss* — the only combinations of cytoplasm and nuclear genes that result in a male-sterile tree. All the other combinations of cytoplasm and nuclear genes result in a male-fertile tree, namely: (*cms-Amer*) *ss*, (*Amer^F*) *SS*, *Ss*, or *ss*; and (*Chin^F*) *SS*, *Ss*, or *ss*

MF = male fertile and MS = male sterile. P's = parental trees

ASSUMPTIONS

CMS occurs in interspecific crosses of chestnut and occurs in Amer x Chin F₁ hybrids but not in the reciprocal Chin x Amer F₁ hybrids.

Chinese chestnut trees vary in resistance to chestnut blight. Thus not all loci for resistance to chestnut blight are in any one Chinese tree, and there may be a number of alleles for resistance at a specific locus within a population of Chinese chestnut trees.

Blight resistance will be treated as a quantitatively inherited character. The identity of Amer x Chin F₁ hybrid trees and all subsequent generation trees are identified by maternal tree, allowing maternal line selection within and among maternal lines. Amer x Chin F₁ hybrid trees and all Amer trees utilized are verified as to identity to eliminate possible contaminants.

PROCEDURES

Plant orchards in Randomized Complete Block (RCB) design with at least 5 replications, to reduce sibbing.

CMS orchards must be adequately isolated from all chestnuts except Amer chestnuts when CMS orchards include Amer chestnuts.



Despite a complex, “tricky” aspect of CMS to some, the practitioner conducting a CMS program needs only to know how to:

- (1) produce and confirm Amer x Chin F_1 hybrids;
- (2) confirm that the recurrent parents are pure Amer chestnut trees;
- (3) differentiate between MS and MF trees;
- (4) inoculate and select for blight resistance and for Amer chestnut characters (e.g., tree form, leaf hairs, twig color, bud shape); and
- (5) grow chestnut trees optimally!!

Leffel (2004) summarized progress through Year 2003 with CMS orchards established in Pennsylvania and Maryland in the Year 2001. A basic CMS plan for three generations of backcrossing is relatively simple, as outlined in the Fig. 1.

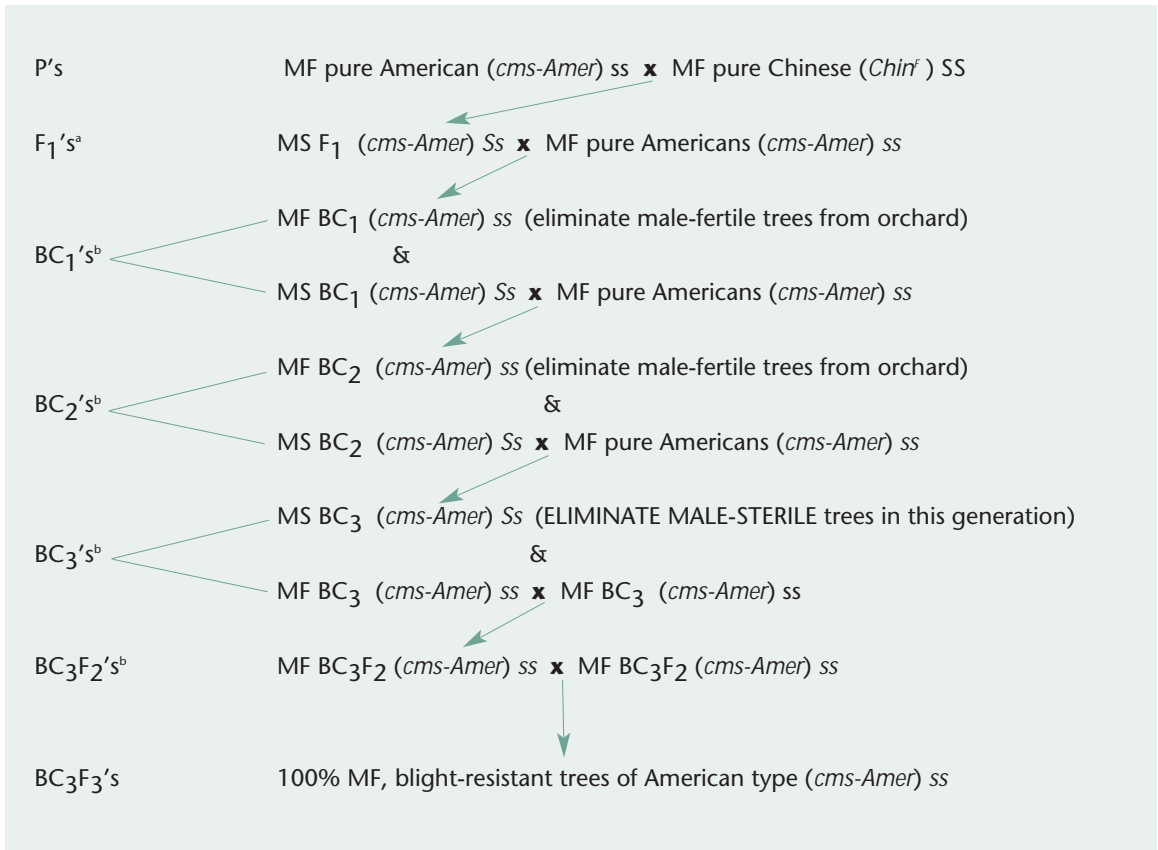
Thus selection may be practiced for MS or MF each BC generation. As many generations of backcrossing can be conducted as desired utilizing a large and diverse population of locally-adapted pure American chestnut trees each cycle of backcrossing as recurrent parents. In the BC generation in which MF is selected, MS trees are eliminated from the program along with any possible undesired donor parent genes closely linked with its *S* gene of Chinese origin. As few as 10 F_1 hybrid seed per Amer x Chin cross are required. Thus the number of long-lived, blight-resistant, and locally-adapted Chinese and Japanese donor parents can be maximized, formulating a germplasm pool with multiple sources of resistance. Seed harvested from American trees, established within Amer x Chin F_1 hybrid or BC orchards, perpetuate the germplasm pools for American chestnuts. Seed produced by open-pollinated American trees within F_1 and BC orchards should be pure American in genotype, because all F_1 and selected MS BC trees will be male-sterile and thus cannot cause contamination.

Easiest system involves only one Chinese gene controlling sterility

CMS has been reported in chestnut as controlled by one or more Chinese genes (Shi and Hebard, 1997). CMS will be most efficient when governed by interaction of American cytoplasm with only one dominant Chinese locus from the donor parent. It may be possible to select for a single-gene system only, if more than one gene are involved in some crosses.

FIG. 1

Crossing scheme using CMS and open-pollination



^a Controlled F₁ hybrids can be avoided by utilizing a single self-incompatible American tree surrounded by Chinese and/or Japanese chestnut trees. If there is more than one A tree in large C and/or J orchards, A x A crosses can be eliminated by examination, since many C and J characters are at least partially dominant in the F₁.

^b Inoculate and select for blight resistance and American chestnut characteristics.

Environment can cause variation in the expression of male-sterility

CMS in other species has been subject to environmental variation, such as temperature, moisture, and day length. An individual plant or tree can vary from MS to partially MS to MF, depending on environmental variation. Obviously, CMS will be most successful when not subject to such environmental variation. Will the breeder be able to select for CMS that is stable enough over environments to meet the requirements of this program?

The use of CMS in chestnut breeding is still experimental

Only experimentation can determine the future of CMS as breeding methodology in chestnuts. The crosses of American x Chinese and American x Japanese chestnut trees will create germplasm pools with multiple sources of resistance and regional adaptation regardless of the fate of CMS as a breeding methodology.

Advantages of the use of CMS in breeding blight-resistant chestnut trees

CMS, if it works, offers many advantages in breeding blight-resistant chestnut trees and deserves immediate and thorough investigation. Advantages of CMS in backcross breeding chestnuts include:

1. Genetic emasculation can be obtained when desired, eliminating the requirements of thousands of controlled crosses for BC and second filial (F_2) generations.
2. The gene(s) from the Chinese parent that causes male sterility is dominant. Thus MS is easily eliminated by selecting for fertile trees in American cytoplasm.
3. Male-sterility is an easy trait to score.
4. There is at least a 10-fold increase in seed production of seed via open-pollination vs. controlled crosses at far less expense and risk of injuries. More seed can be produced than can be utilized in a single program, but surpluses can be distributed for new programs.
5. Seeds produced on pure American trees within F_1 and BC orchards can perpetuate American germplasm pools, because the hybrid trees selected for MS produce no pollen.
6. MS Amer x Chin F_1 hybrids or MS BC's may be established in clear cuts and shelter cuts known to contain a goodly population of sprouts of American trees. In this case, no seeding of Americans will be required.
7. It is easier to include multiple sources of resistance in the breeding pro-

gram as recommended by 1999 TACF Science Review (Mehlenbacher et al., 2000).

REFERENCES

Leffel, R.C. 2001. Cytoplasmic male sterility - potential methodology for breeding blight-resistant, timber-type chestnuts? *The Chestnut Tree** 7(2): 7.

Leffel, R.C. 2004. Selection and experimentation within CMS F₁ hybrid/American chestnut orchards. *The Chestnut Tree** 9(1): 5.

Mehlenbacher, S.A., Phillips, R.L., and J.P. van Buijtenen. 2000. The TACF Breeding Program Review. *J. Amer. Chestnut Found.* 14(1): 16-24.

Shi, Yan and F.V. Hebard. 1997. Male sterility in the progeny derived from hybridization between *Castanea dentata* and *C. mollissima*. *J. Amer. Chestnut Found.* 11(1): 38-47.

* *The Chestnut Tree* is the newsletter of the Pennsylvania Chapter of The American Chestnut Foundation. Issues can be obtained by contacting the chapter <http://www.patacf.org>

The author acknowledges the advice of Dr. D.N. Duvick on CMS in plants and the assignment of CMS nomenclature in chestnut and the editing of this paper by Dr. Paul Sisco.



