

Contents

Volume IX, Number 2

Editor's Notes	1
Letters to the Editor	2

MEMORIES

<i>ACF Board members and friends</i>	Tributes to Charles Burnham and Angus McDonald	3
<i>Sandra L. Anagnostakis</i>	An Historical Reference for Chestnut Introductions into North America	4
<i>Timothy Van Vliet</i>	Letters from a Teenage Naturalist	5

SCIENCE

<i>T. L. Kubisiak</i>	Molecular Markers Linked to Resistance to <i>Cryphonectria parasitica</i> in Chestnut	6
<i>Sandra L. Anagnostakis</i>	Hypovirulence in Orchard Management	7
<i>Fred Paillet</i>	One of the World's Last Truly Wild Chestnut Forests	8

In Memory of Angus McDonald
1941-1995

EDITOR'S NOTES - JOURNAL FALL/WINTER 1995-96

Inevitably it seems those things that capture and hold our imagination are complex, with many sides and considerations, like a diamond. It takes a great force of nature to capture and hold the attention of an entire culture.

The American chestnut had that profound of an impact on Americans. Perhaps that is why so many people have sought to understand, appreciate, and use the American chestnut and its sister species, Chinese, Japanese, and European chestnuts.

Over the decades and centuries, as you will see, people have interacted with chestnut trees in a variety of ways for many reasons. The result is a myriad of perspectives; each person, each writer, explorer, geneticist, ecologist, naturalist, cook, artist, entrepreneur, and neighbor tells one small part of the whole story.

With this issue of *The Journal* we are indeed fortunate to be able to sample some of the varied perspectives of those working with chestnut both today and in the past.

Fred Paillet on tour in the Caucasus Mountains south of the Ukraine gives us a picture of wild chestnut forests and what our Appalachian Mountains likely looked like before the blight. As always, Paillet gives us a picture in words and images - this time with both photographs and his excellent pen and ink drawings.

Tom Kubisiak takes genetic mapping to new levels, confirms assumptions developed from hands-on breeding practices, and draws interesting parallels between the genetics of blight susceptibility and resistance.

Sandy Anagnostakis offers two very different articles. One describes the success of using hypovirulence in orchard management and offers thoughts on how environmental factors may affect different research efforts with hypovirulence - certainly a topic which those who toured the West Salem stand during the Annual Meeting will find interesting.

In the other, the first of a two-part series, Anagnostakis takes us through the history of chestnut importation in which we travel back more than 100 years before the appearance of blight in this country. In a series of letters written over two years, young Timothy Van Vliet keenly observes a grove of chestnuts that stand in the way of "progress" and eloquently pens his frustrations.

Also in this issue, ACF Board Directors and friends say good-bye and thank you to Charles Burnham and Angus McDonald, two men who have led, inspired, and educated every ACF member in one way or another.

An anonymous voice from the past brings chestnuts home, to the kitchen, and lets the fears of childhood out of the closet. A new illustrator for ACF Byrne Murphy Sleeper (herself a mother of 5!) brings the vision to life.

Thanks to Bon Appétit, Storey Communications, Inc., and my own kitchen fiddling we have a few new recipes for your holiday pleasure.

As you read, consider the voice of the writer and the vision of the artist - where is this person in relation to his or her subject? Is the writer looking at his neighborhood, in a foreign land, or focusing through a microscope? Is she reviewing history and seeing patterns that have a different meaning for us today than what contemporary society saw? Is this work a life's ambition?

Is he writing with his memory, his heart, his ideals? Is she drawing with her imagination? And how do you the reader, the person living with this culture, this history, how do you feel having read this material - enlightened, saddened, optimistic, sentimental, cynical?

All these perspectives are part of the chestnut story, as people have lived the story and as we dream and hope for the return of the American chestnut.

Rachel Kelly

LETTERS TO THE EDITOR

Dear John,

I had real good luck starting chestnuts this year. I put them in planter pails, the kind you get small trees in. I mixed them with compost and put them in my outside cellar steps with a cover over it. On the 10th of March they had sprouts on them.

I took 2-liter pop bottles, cut the tops off, and put topsoil and compost mix in. Then I put the nut in with the sprout down, adding another 2 to 3 inches of soil. I made a place for them, and put sawdust around them. About 190 of 207 came up. They are looking good.

Hope to see you at the meeting.
Chestnut Downs (Virgil Downs)
Mansfield, Ohio

Dear Sir:

As a forester, I am eternally interested and constantly alarmed at the hosts of pests, pathogens, fungi, blights, and harvest practices that affect, to near dissolution, the health and wealth of this country's forest lands.

Several years ago, perhaps the mid-1970s, my father, Dr. Donald Anderson, a veterinarian, and his brother were cleaning the grave of my great-great-grandfather in White County, Tenn., when my father noticed adventitious sprouting from a stump near the grave site. Recognizing the growth as chestnut, he broke several sprouts from the stump and brought them back to his home in Salem, Ore., where he placed them in a root solution. Later, he planted the sprouts in his backyard.

The sprouts flourished. Before the trees reached 25 feet in height, they were producing viable seed which were germinating seedlings in the shade of their crowns. I have since identified this species as *Castanea dentata*.

I gathered several nuts, but was unsuccessful in germinating them here on the Oregon coast. However, I transplanted seedlings and they are doing well. They are in the sapling stage, about 5 to 7 feet in height after two years of growth. Deer browse has been the biggest setback.

It is unfortunate that, just prior to my father's death, he had his chestnut trees removed from the backyard due to leaf problems on the lawn and guttering, a problem that I was quite unaware of. When I learned of this removal and its reasons, I, naturally, felt a loss far greater than space here allows one to verbally express.

However, my trees (saplings) remain as muted sentinels. It is my fondest hope . that the first filial generation of seedlings will have, or will have begun to develop, a resistance to the chestnut blight that hit this country in the early 1900's.

Sincerely,
Richard Anderson
Pacific City, Oregon

**TRIBUTES TO CHARLES BURNHAM
AND ANGUS MCDONALD**

ACF Board Directors and friends

My first interactions with Dr. Burnham 40 years ago got off to a rather shaky start. As a beginning graduate student I enrolled in his course in cytogenetics without having taken his prerequisite course in advanced genetics. Though I had no difficulty with the course, Dr. Burnham was obviously not at ease with a nongenetics major (a plant pathologist at that) taking his courses out of sequence, and having no difficulties.

Dr. Burnham was a very good teacher. I liked the way he laid out the logic of a cytogenetics experiment and the types of experiments described to investigate chromosome behavior and inheritance with chromosome rearrangements. I took his advanced genetics and then three special seminar courses with him. One of these special seminars is easy to remember.

About five of us graduate students were interested in learning why the X-ray crystallographer's data supported the Watson and Crick model of DNA. We went to Dr. Burnham to see if this could be covered in a special seminar. His reply was simply, "I don't understand it either, but if you are interested, I will help to assemble the appropriate faculty resource people, and maybe we can learn this together." His attitude and openness to learning astonished me in an unforgettable way. Dr. Burnham was also on my guidance committee. He was most helpful and certainly the most critical of my research of anyone on this committee.

Approximately 10 years ago Dr. Burnham got me involved in the activities related to restoration of the American chestnut. It began with a request to review a proposal, then a request to serve on the science committee, then to chair the science committee, then to serve on the board, etc. When he moved to a nursing home, I would stop to visit with him several times a year. He always had many questions about what was transpiring, and many suggestions of what must be done next. My last visit with him was just one month before he died. As he talked about the status of the Chestnut Breeding Program, he seemed to have a premonition that he wouldn't be available to help much longer. Dr. Burnham was a very inspiring mentor. He expected students to develop a burning, driving interest in their research. He was critical in a very constructive manner.

I have considered Dr. Burnham to be a prime example of an optimist. Who would start a tree breeding program at the age of 75? Only an optimist who looked forward to the future with enthusiasm.

- Dr. Albert H. Ellingboe
Vice President Science Cabinet

first met Charles Burnham in 1991. I had only been on board as The American Chestnut Foundation's executive director a few months, and felt a desire to introduce myself to the foundation's "other" cofounder. Philip Rutter, ACF's President and Burnham's founding partner, had hired me in 1990. Dr. Burnham was my missing link to the origin of The ACF.

At the age of 87, he still had more to do. He fought off a bout of poor health, and was transferred to a care center. When I saw him in 1992, he was feisty, all business, and did not engage in social talk. His mind was constantly thinking about plant breeding and letters flowed from his pen to all those interested in his genetic ideas.

My last visit with Charles was in January 1994 when friends and colleagues gathered to celebrate his 90th birthday. He was nervous about seeing so many people and wanted to be dressed in a suit, a drastic change from his day-to-day bathrobe. I will never forget the image of that day-an intellectual giant in his field, being wheeled down the hallway to meet admirers. He later returned to his shared room after the party to talk genetics.

For the last years of his life he worked under adversity in a cramped double room with only a bed, night stand and stacks of papers and books, thinking, writing and reading with a thin curtain of privacy separating him from his roommate.

I didn't really know you, Charles, but thank you from all of us for giving us hope and opening our eyes to the scientific techniques that will bring back the American chestnut for future generations to enjoy.

- John Herrington
ACF Executive Director

I had the good fortune to get to know Dr. Burnham three different ways. Because of his expertise in cytogenetics, I first made his acquaintance while I was working on my dissertation on azalea breeding and genetics at the University of Minnesota. I came to him many times for advice as I studied the pollen abnormalities of azalea

hybrids. At the time of my dissertation defense, my committee member representing the Agronomy Department was unable to attend, and although Dr. Burnham had been officially retired for some years, he kindly agreed to replace the absent member. After a fair amount of bureaucratic shuffling, the university finally approved this arrangement, which made for a more interesting and challenging defense.

During my M.S. program at the University of Illinois, I had worked on corn. When I came to Minnesota, I wanted to continue researching corn, so my wife and I began a project to breed corn for decorative purposes. In 1982, we were able to share a corner of Dr. Burnham's corn field. That summer we were amazed by Dr. Burnham and his stamina and dedication to research. He spent an unbelievable amount of time in the field making crosses and studying corn genetics throughout the heat of the summer for a man of his age - at that time he was in his late 70s. The year I finished my studies at Minnesota, I organized a seminar series on the breeding of perennial plants. This is how I became aware of Dr. Burnham's other devotion, the one you all know so well, the restoration of the American chestnut. Dr. Burnham was eager to present an overview of past efforts to breed disease-resistant chestnuts and to outline his initial hypothesis on the genetic control of blight resistance, which has since been refined and is being tested by the ACF. His seminar was well received and began the longest lasting phase of our relationship. In so many ways, I will always be grateful for his clear thinking, his powerful dedication, and his kind help.

- Mark Widriechner
ACF Publication Committee Chair

worked on my American chestnut plantings and tramped the hills looking for seed for some time before I heard of The

American Chestnut Foundation. When I did I, resolved to go to the next meeting.

Angus was one who could easily recognize a new member. When he saw one he went out of his way to say hello. He was a tall, imposing man with a manner that was empathetic and to the point. He made me comfortable and he listened. I have been dedicated to the ACF, as he was, ever since. Somehow Angus epitomized what the ACF is and can be.

We are a better organization because he has been here. We will become better as we remember this strong empathetic man who was a true "Servant Leader."

- Bud Coulter
President ACF

I am thrilled that Angus' active mind was able to receive the I news last year that the efforts he helped to promote are already resulting in some blight-resistant American chestnut-type trees. I know his soul leaped for joy, even though his body could not.

King David is believed by many to be the author of Psalm One, which defines the righteous life:

Blessed is the man that walketh not in the counsel of the unholy, nor standeth in the way of sinners, nor sitteth in the seat of the scornful. But his delight is in the law of the Lord; and in His law doth he meditate day and night. He shall be like a tree planted by the rivers of water, that bringeth forth his fruit in his season; his leaf also shall not wither, and whatsoever he doeth shall prosper.

And Winston Leonard Spencer Churchill defined the purpose of such a life: "What be the use of living," Churchill said, "if it be not to strive for noble causes, and to make the World a better place for our having lived in it?"

Angus McDonald's wonderfully full, but sadly brief life amply meets the tests of both King David and Winston Churchill. Angus has made the World a better place.

Angus, for you, we invoke not only the ancient Latin salutation, Ave Atque Vale - Hail and Farewell - but also the Psalmist's premier tribute: In our hearts you are like a tree planted by the rivers of water; your leaf shall not wither.

-Donald Willeke
ACF Board Director

I met Angus McDonald five years ago at his home in Minneapolis to discuss my contract as ACF's newly hired executive director. My first reaction was one of slight intimidation-Angus was taller than me, had a commanding voice, and was ready to delve into business. As I soon learned, though, behind his serious appearance was a soft caring man with a wonderful sense of humor and infectious laughter.

Angus' contribution to ACF was felt in many ways. His knowledge of Macintosh computers helped our Mac-based office; his business acumen moved us toward regular, workable financial reports for the Board; as editor of The Journal, his skills kept us on track with annual issues of our publication; and he was a board member who was always available for consultation.

I feel honored to have known Angus. At board meetings, no matter where they were held around the country, Angus would ask to go for a walk with me to check in on how I was doing personally and how I felt the organization was evolving. He was not timid if an issue needed to be addressed, he would approach it squarely, which helped cut through layers of chaff.

In the spring of 1993 he called to say that he might not be able to attend the board meeting in Airlie, Va., because he had just been diagnosed with Lou Gehrig's disease. Fortunately, he did come and it gave me the opportunity to visit his beloved family home at the 'Bower' in the eastern panhandle of West Virginia. He was beginning to feel the neurological effects of the disease, and it turned out to be the last ACF board meeting he was able to attend. In the Fall we held a development committee meeting at Don Willeke's in Minneapolis and Angus drove himself to the meeting, but relied heavily on a cane.

Four months later I saw Angus again at Charles Burnham's 90th birthday party. By now Angus was in a wheelchair and unable to hold his head up by himself. His core support group, known affectionately as "Angus' chestnuts" was already at work transporting and helping to care for him. The disease did not affect his intellectual prowess; his computer was modified and words continued to flow, although with painstaking slowness.

Through sheer interest in wanting to see the return of the American chestnut, Angus committed time and energy to a project and an organization he believed in and loved. We miss you Angus.

- John Herrington
ACF Executive Director

I never met Dr. Burnham personally. But, as a graduate student, I became acquainted with him in both cytogenetics and advanced corn breeding classes at Iowa State in 1950. These courses required an understanding of Dr. Burnham's proposed "Oenothera Translocation Stock" method of producing homozygous diploids in corn, the theory of a great scholar. Yes, Dr. Burnham helped us to understand chromosome behavior and meiosis - the basis for genetics!

I pursued a career in soybean and forage crop improvement while Dr. Burnham worked mostly on corn and barley. Dr. Burnham retired in 1972. During a visit to the National Agricultural Library in 1992, I encountered Dr. Burnham, P.A. Rutter, and D.W. French, *Planting Breeding Reviews* 4:347-397. 1986. WHAT A SHOCK!!

"I knew vaguely that the USDA had a chestnut breeding program, but not until 1959 did I write asking about the program. I read the references given. I could not believe what I was reading. Others reacted similarly. In the USDA chestnut breeding program, the backcross method had not been used in the manner required to produce American chestnut with blight resistance."

WOW!! This is "REVELATION" - By C.R. Burnham!! Never in the annals of plant breeding has anyone critiqued a major, long-term plant improvement program that failed - and corrected the approach -as has Dr. Burnham in blight-resistant American chestnut. This is why I support The American Chestnut Foundation, founded by Dr. Burnham and colleagues.

WAKE UP AMERICA!! Dr. Burnham's REVELATION should be our CRUSADE!!

- R.C. Leffel

ACF PA Chapter

AN HISTORICAL REFERENCE FOR
CHESTNUT INTRODUCTIONS INTO
NORTH AMERICA

Part one of a two-part series

Sandra L. Anagnostakis
The Connecticut Agricultural Experiment Station
New Haven, CT

When people find chestnut trees of any size growing in the New England woods they frequently call The Experiment Station, sure that they have found an American chestnut tree resistant to chestnut blight. It usually turns out that this tree is Asian or an Asian hybrid.

In previous centuries, chestnut trees were very important to the people on this continent. They took advantage of "new and different" material much more than is generally realized and were planting Asian species long before chestnut blight was discovered in New York City in 1904. The species are listed in Table 1. Since I am often faced with the problem of telling an enthusiast that some nice tree is not *Castanea dentata*, I have started compiling some information about the history of chestnut importations into North America.

EUROPEAN CHESTNUT TREES

The first recorded importations were those of Thomas Jefferson, who brought cuttings to his home, Monticello, and grafted them onto native American chestnut trees. Eleuthere Irenee DuPont de Nemours, who in 1799 moved from France to Bergen Point, NJ, and then to Brandywine, Del., brought many European chestnuts (*Castanea sativa*) with him, imported more later, hybridized lots, and planted them all over the area. By 1889, some of the popular varieties of *C. sativa* and *sativa* x *dentata* hybrids were: 'Anderson,' 'Bartram,' 'Comfort,' 'Cooper,' 'Corson,' 'Dager,' 'Darlington,' 'DuPont,' 'Miller,' 'Moncur,' 'Numbo,' 'Paragon,' 'Ridgely,' 'Scott,' 'Spanish,' and 'Styer.'

JAPANESE CHESTNUT TREES

In 1876, S.B. Parsons of Flushing, NY, imported a few trees of *Castanea crenata* and sold them as 'Parson's Japan.' Two of these are still growing very well in Connecticut; one in Old Lyme on the grounds of the Bee and Thistle Inn, and one in Cheshire behind the Congregational Church. Major importation of Asian chestnuts trees began in 1882 when William Parry, of Parry, NJ, imported 1,000 grafted *C. crenata* trees. Parry selected 'Parry' as his best, but sold several other varieties as well.

CHESTNUT ORCHARD SUPPLIERS IN BUSINESS BY 1900

The Albion Chestnut Company, Clementon, NJ
150 acres of stump land grafted with *sativa* ('Numbo') and *crenata*

J. W. Beecher, Pottsville, Penn.
80 acres with 18,800 grafted trees

Arthur J. Collins, Moorestown, NJ
30 acres, mostly with grafted 'Alpha' *crenata*, and 'Paragon' *sativa*

Henry W. Comfort, Fallsington, Penn.
56 trees on one acre, mostly 'Numbo'

J.T. Lovett, Emilie, Penn. (near Trenton, NJ)
about 22 acres with 1,200 grafted 'Paragon' and 25,000 seedlings

The Mammoth Chestnut Company, Clementon, NJ
about 150 acres, mostly grafted 'Numbo'

Samuel C. Moon, Morrisville, Penn.
originator of 'Numbo' = magnum Bonum *sativa*

Parry Brothers Nursery, Cinnaminson, NJ
many crenata seedlings and selections
Coleman K. Sober, Lewisburg, Penn.
300 acres, sprouts grafted with 'Paragon'

Joseph Williams, Riverton, NJ
7,500 dentata seedlings planted, many grafted with crenata and sativa scions

In 1886 Luther Burbank imported 10,000 nuts from Japan for selecting and hybridizing. In 1893 his "New Creations" catalog advertised his 'New Japan Mammoth' chestnut and he sold three seedlings to Judge Andrew J. Coe of Connecticut. These were sold in 1897 to J. H. Hale of South Glastonbury, Conn., who named them 'Coe,' 'Hale,' and 'McFarland' and sold them from his nursery' and through his catalogs starting in 1898. There were 21 varieties of Japanese chestnuts listed in T.H. Powell's 1898 Bulletin (#42, Delaware Agricultural Experiment Station). These were discussed in gardening magazines such as *The Rural New Yorker*, and advertised in plant and seed catalogs. Mail order spread these Asian trees all over the country. By' the turn of the century', Asian and European chestnut trees were available by' mail from many nurseries such as Burbank (California), Parry Bros. (New Jersey), Hale (Connecticut), Kerr (Maryland), Biltmore (North Carolina), Boehmer (Japan), and the Yokohama Co. (New York and Tokyo).

CHINESE CHESTNUT TREES

Chinese chestnuts are not mentioned in the early' catalogs that I have seen, but plant explorers were sending seed to the United States. In 1903, Dr. Charles Sprague Sargent sent *C. mollissima* seed to The Arnold Arboretum near Boston, Mass., for their collection. No trees from this seed lot have survived. In 1908, E. H. Wilson sent them seeds of his collection #55 1, *Castanea henryi* from Western Hupeh, China. This was planted in their collection as tree #6849, which survived better than most imports of this species, but finally died in 1934. Cuttings were sent to the U.S. Plant Introduction Department in the Bureau of Plant Industry.

Around the turn of the century', several plant explorers were traveling around the world collecting things not found in North America. These people were often careful observers of plant ecology, and their notes make fascinating reading. When the Boxer Rebellion opened up China to exploration, several expeditions were made. The most famous explorers were probably Ernest H. "Chinese" Wilson who collected for an English Nursery' and later for The Arnold Arboretum, and Frank N. Meyer, who was hired by' David Fairchild to explore for the U.S. Plant Introduction Division of the U.S. Department of Agriculture. The two had very different personal styles, and their travels resulted in vast numbers of importations. I have found only two

CHESTNUT TREES BY MAIL-ORDER

Catalog	Date	Species	Cost each, \$
---------	------	---------	---------------

Reading Nursery.1900 Jacob W. Manning, MA	American	0.50 to \$1.00	
J.T. Lovett Co. 1888 Little Silver, NJ	'Japan Giant' Spanish	0.75 0.30	
	American	0.1 0-0.25	
	'Numbo'	0.75	
Storrs and Harrison Painesville, OH	1888 American	0.50	
	'Japan Giant'	0.50-0.75	
	Spanish	0.50	
Shady Hill Nursery Cambridge (Somerville), MA, F.L. Temple	1888/1889	American	0.10-0.35
Highlands Nursery Boston, MA, H.P. Kelsey	1899/1901	American	0.25
Biltmore Nursery1900/1901 Biltmore, NC	American	0.15-0.50	
Mt. Hope Nursery Ellwanger and Barry Rochester, NY	1897	C. Americana C. Japonica C. vesca	0.50 1.00 0.50
Elm City Nursery1901 New Haven, a	American	0.50-1.00	
	Spanish	0.25-1.00	
	'Numbo'	1.50	
	Japanese	0.50-1.00	
Fmitland Nurseries Augusta, CA, P.J. Berckmans	1900	American	0.25-1.00
		Spanish	0.25
Hale's Fruits South Glastonbury,CT	Japanese hybrids (from Luther Burbank)		
	'Coe,' 'Hale,' 'McFarland,' J.H. Hale		
C.B. Homor and Son Mt. Holly, NJ	1897	American	0.25-0.35
	'Numbo'	0.75	
	'Paragon'	1.00-2.50	

certain survivors of Frank Meyer's chestnut imports. The Rochester (New York) Parks Department has a specimen of PI 36666 growing in their Durand-Eastman Park as #G25, and there is one at the Bartlett Arboretum in Stamford, Conn., that was named as the cultivar 'Bartlett.'

CHESTNUT BLIGHT

Chestnut blight or chestnut bark disease is caused by the fungus *Cryphonectria parasitica*, formerly called *Endothia parasitica*. Cankers were found on American chestnut trees lining the avenues of the Bronx Zoo in New York City

in 1904. In 1907 and 1908, the fungus attacked other species of chestnut in the New York Botanical Garden. Rapid spread of the disease followed, and within 50 years the fungus was found throughout the native range of *C. dentata*, from Maine to Georgia, and west to the edge of Michigan.

In 1913, David Fairchild asked Frank Meyer to look for the disease in Asia, and Meyer reported that he had found it in early June. He wrote:

This blight does not by far do as much damage to the Chinese chestnut trees as to the American ones. Not a single tree could be found which had been lulled entirely by this disease, although there might have been such trees which had been removed by the ever active and economic Chinese farmers.

Shear and Stevens grew cultures from Meyer's samples, and in July they inoculated the Chinese fungus into American trees near Washington, D.C. Rapid death of the sprouts confirmed that this similar-appearing fungus caused chestnut blight.

Meyer went to Japan in 1915 and was again first in finding chestnut blight. He wrote that the Japanese chestnut trees were generally more resistant to the blight disease than the Chinese chestnut trees that he had seen, and suggested:

This Japanese chestnut, *Castanea japonica* might be used as a factor in hybridization experiments together with American, European, and Chinese species to create immune or nearly immune strains of chestnuts.

HYBRIDIZATION

Many people took up Meyer's suggestion, and hybrids made earlier to improve the orchard qualities of chestnut trees were examined for their resistance to chestnut blight.

Arthur H. Graves, of the Brooklyn Botanical Garden, started planting chestnut trees and making hybrids in the early 1930s. Trees were planted on his property in Hamden, Conn., and on land owned by The Connecticut Agricultural Experiment Station. His work was aided by Hans Nienstaedt and Richard Jaynes, who both did their doctoral research on chestnut at Yale University and The Connecticut Agricultural Experiment Station. Now that we can keep American chestnut trees alive with biological control by hypovirulence, breeding can continue. Species and hybrids of chestnut were distributed by The Experiment Station to homeowners all over the northeastern United States. Today often records of origin have been lost, tags are unreadable, or row lines are confused by the planting efforts or squirrels I try to identify the trees found by citizens, using leaf and twig characteristics. The pure species are easy, but the complicated hybrids must sometimes be a case of "best guess."

My file on chestnut history gets larger every year, as I find yet another catalog or letter from the early days of this century. Many fine Asian trees have withstood 50 to 120 years of New England winters bugs, and blight. We can use these in present and future breeding programs, as long as we remember to write it down for the people trying to puzzle this out 100 years from now.

USEFUL REFERENCES

Trees:

Buckhout, W.A. 1896. Chestnut Culture for Fruit. Bulletin #36, The Pennsylvania State College Agricultural Experiment Station, State College, Pennsylvania.

Frothingham, E.H. 1912. Second-growth Hardwoods in Connecticut. U.S. Department of Agriculture, Forest Service Bulletin #96, Washington, D.C.

Fuller, A.S. 1896. The Nut Culturist: A Treatise on the Propagation, Planting, and Cultivation of Nut-bearing Trees and Shrubs Adapted to the Climate of the United States. Orange Judd Co., New York.

Meyer, F.N. 1911. Agricultural Explorations in the Fruit and Nut Orchards of China. U.S. Department of Agriculture, Bureau of Plant Industry Bulletin #204, Washington, D.C.

Powell, G.H. 1898. The European and Japanese Chestnuts in the Eastern United States. Delaware College Agricultural Experiment Station Bulletin #42, Newark, Delaware.

Zon, R. 1904. Chestnut in Southern Maryland. U.S. Department of Agriculture, Bureau of Forestry Bulletin #53,

Washington, D.C.

Blight:

Anderson, P. and Rankin, W.H. 1914. *Endothia* canker of chestnut. Cornell University Agric. Exp. Station Bulletin #347, Ithaca, New York.

Fairchild, D. 1913. The Discovery of the Chestnut Bark Disease in China. *Science* 38:279-299.

Merkel, H. W. 1905. A Deadly Fungus on the American Chestnut. *New York Zool. Soc., 10th Ann. Rep.* New York.

Murrill, W.A. 1908. The Spread of the Chestnut Disease. *J. NY Botanical Garden* 9:23-30.

Shear, C.L. and Stevens, N.E. 1913. The Chestnut-blight Parasite (*Endothia parasitica*) from China. *Science* 38:295-297.

Shear, C. and Stevens, N.E. 1916. The Discovery of the Chestnut-blight Parasite (*Endothia parasitica* and Other Chestnut Fungi in Japan. *Science* 43:173-176.

People:

Cunningham, I.S. 1984. Frank N. Meyer: Plant Hunter in Asia. Iowa State Univ. Press, Ames, Iowa.

Fairchild, D. 1938. *The World Was My Garden*. C. Scribner's Sons, NY.

Sutton, S.B. 1970. Charles S. Sargent and the Arnold Arboretum. Harvard Univ. Press, Cambridge, Massachusetts.

Sutton, S.B. 1974. *In China's Border Provinces: The Turbulent Career of Joseph Rock, Botanist-Explorer*. Hastings Co., New York.

LETTERS FROM A TEENAGE

NATURALIST

July 29, 1994-August 18, 1995

Timothy Van Vliet
Somerville, NJ

July 29, 1994

Dear ACF,

I examined the 10 seedlings that sprouted last year and managed to survive the harsh winter. (*Editor's Note. Winter 93-94 was a record setting season for snow and deep cold for the Northeast.*) The ice storms take their toll, particularly on birches, weeping willows, silver maples, any sort of coniferous tree, aspens, and cottonwoods. The ice bent our neighbor's birch tree so that its top touched the ground and glazed it, giving it a chandelier appearance in the sun.

All of the trees grew new leaves, but on nine of them the leaves are only four inches long and a pale yellow-green, which they have always been. The same nine seedlings, which I planted in June of last year, are still less than a foot tall. In contrast, one seedling is 3 feet tall with greener leaves up to 8 inches in length. They still pale in comparison to a stand near Freehold, NJ, the trees of which average 10 inches with many being 13 or more inches. Its trunk is 3/8 of an inch in diameter.

I had to build a new cage to put around it because it grew branch-Cs. I used the original cage to protect an oak that grew a new terminal bud after being bitten by deer, which number about 80 or more per square mile. If gray wolves were reintroduced, perhaps I would not have to keep cages around the trees to keep them safe from squirrels, rabbits, and groundhogs.

I am rather disappointed in the slow growth of the nine nuts. However, if they were oaks, their growth would be exceptional.

If you have any intention of using chestnuts in New Jersey or eastern Pennsylvania in your breeding program, I

better write to the state government to protect the few remaining trees left. The reason is that rank after rank, regiment after regiment, brigade after brigade of development houses, stark as packaging crates, are taking over the land.

The endless rows, some in blue, most in gray, are making an assault on the wheat fields and surrounding forests. Clones of over-priced piles of rubbish, the very hallmark of unimaginative architects, stand against piles of sod and mosquito breeding mud puddles in the tracks of the heavy artillery.

Another 200-acre farm has the appearance of ground zero as the farm house, apparently spared as well as 1/4 of an acre of greener' on which the house was built, stands like an island of the past. Farming in New Jersey pays about \$25,000.00 annually for working more than 12 hours every day of the year like a horse in the blazing sun. An acre of land sells for about \$50,000.00 to a land developer. Selling a farm, most of which are 250 acres, means getting 500 years pay plus 4% annual interest in an account.

I see the last generation in the fields with their hats and crimson faces, thickened like leather, wrinkled like raisins, but held high. You may ask, why do today's farmers of New Jersey still work their land? That, I can answer you in one word: Tradition!

May 1, 1995

Dear ACF,

I examined the 10 seedlings that sprouted from the nuts that I had planted in June of 1993. All of the trees grew new leaves in 1994, and nine seedlings today average about 1 foot tall each. The 10th tree, by contrast, is now over 3 feet tall on two different shoots, each 3/8 of an inch in diameter with deeper green leaves reaching 8 inches during the past two summers. The original shoot already shows ominous scars of the blight. The leaves are still modest compared to those that I have seen on stump sprouts in Freehold in 1993, some of which were nearly 30 feet tall and several inches across.

I found the stump sprouts while looking for the American chestnut listed in the 1991 register of the largest trees of each species in the state of New Jersey, printed by the Department of Environmental Protection. After traveling more than 40 miles to the corner of Five Points Road and Asbury Avenue in Monmouth County, I found a housing development where there was reportedly an American chestnut standing 3 feet, 2 inches in circumference. I investigated the fragmented remnants of forest on land marked Lot 16, adjacent to piles of bulldozed soil, new roads, holes in the ground for foundations, and recently planted hedgerows of our state tree, the telephone pole. I have seen many fine specimens of chestnuts, some with rich green leaves over 12 inches long. The abundance of chestnuts of considerable size on the well-drained sand and loam almost suggests that clues about the future of American chestnuts is (or more accurately was) on the property surrounding the street corner.

Current land use is poor. If land is not built on, used for agriculture, left in its natural state, or put to use other than as a landfill, it is being wasted. Sprawling corporate and residential lawns produce no timber, food or useful products. The forests, except for a few scattered patches are of spindly trees with little wildlife. The disappearance of farming makes little difference in the supermarket as New Jersey is far too densely populated to be self-sufficient. So uneconomical is agriculture that many landowners leave their fields fallow, leading to exponential deer populations that eat tree seedlings and girdle the bark, destroying the remaining woodland. The rural way of life is gone with the wind, but as tomorrow Is another day, from the ruins may one day grow American chestnuts. Then the developers might actually build out of real wood again, wood that has not been transported 2,500 miles, and the farmers would have shade, lumber, and a crop without chemicals that only needs harvesting.

August 11, 1995

Dear ACF,

Yesterday afternoon, August 10, 1995, I had the opportunity to reinvestigate the chestnuts near the intersection of Five Points Road and Asbury Avenue. None of the trees described still stand; several 25-foot chestnut trees have been leveled.

Several small groves have been spared, which I did not previously inspect as wild blueberries 1/2-inch across were there by the thousand. I saw four stump sprouts. The blight-killed poles, stripped of their bark, were

about 6 feet high. Shoots grew to varying heights shorter than the blight-killed stems.

I dug around the stump sprouts and other areas and found much forest litter decaying into black soil. Beneath that, there was sand. The soil contained a white fungus throughout the grove. The larger of the living stump sprouts had cankers.

One chestnut tree near the edge of the grove is currently as tall as its neighboring two-story development house, about 25 feet tall. It was not a stump sprout, but a tree of considerable size. Its bark was well-furrowed into the broad, flat ridges. Its diameter was about 6 inches. The first branch was 5 feet from the ground. Another branch was dead and broken, but was still attached to the tree at a splintered point, apparently damaged from something falling on it. There were no flowers on it, nor maturing nuts.

(Editor's Note: Timothy Van Vliet, now 16 years old, lives with his family in New Jersey. In addition to his literacy abilities, Van Vliet writes about chestnut genetics, genetic mapping, and biotechnology. He is also a top student and active in his school's choir.)

MOLECULAR MARKERS LINKED TO RESISTANCE TO CRYPHONECTRIA PARASITICA IN CHESTNUT

T.L. Kubisiak

*Plant Research Geneticist
USDA Forest Service
Southern Research Station
Southern Institute of Forest Genetics
Saucier, MS*

In recent issues of *The Journal* several articles have appeared which describe The American Chestnut Foundation's breeding program for backcrossing the blight resistance of Chinese chestnut into American chestnut (Hebard 1994a). The breeding program involves repeated crossing of selected *C. dentata* x *C. mollissima* hybrids and progeny to *C. dentata* (American chestnut), hence reconstituting the *C. dentata* genome with the addition of genes that condition resistance from *C. mollissima* (Chinese chestnut). Recent issues of *The journal* have also discussed the application of molecular markers in the backcross breeding program and advantages that such markers offer (Mulcahy and Bernatzky 1993; Ellingboe 1994; Hebard 1994a; Hebard 1994b). Ellingboe (1994) pointed out two things that The American Chestnut Foundation hoped to gain by applying molecular markers in the backcross breeding program. First, molecular markers and a corresponding genetic linkage map would help ACF identify the markers that bracket genes (or gene families) that condition blight resistance. Second, markers and such a map would give ACF the information to select against unwanted Chinese chestnut DNA in advanced backcross generations, greatly reducing the number of backcrosses required to reach a relatively "pure" American chestnut.

In the following paragraphs I describe how I came to work on the chestnut blight problem. I touch on the underlying theory behind recombinational linkage mapping, mention some current results in work with chestnut, and how these results compare to prior knowledge regarding the suspected pattern of inheritance of blight resistance. Finally, I look ahead and suggest where our efforts might best be focused next.

THEORY OF RECOMBINATIONAL LINKING

I met Fred Hebard of The American Chestnut Foundation in January 1995 when he visited the Southern Institute of Forest Genetics in Saucier, Miss., to discuss strategies for a joint molecular-marker mapping project in chestnut between the USDA Forest Service and The American Chestnut Foundation. Fred's study particularly interested me at the time because I had just finished my Ph.D. at Louisiana State University; my dissertation had focused on molecular-marker mapping in the southern pines. Since this visit we have spent numerous hours in the laboratory, on the computer, and on the phone analyzing and discussing the results of our efforts.

To understand how a recombinational linkage map is constructed, it is first necessary to recognize that genes are arranged on chromosomes. Chromosomes are microscopic structures that can be observed in actively dividing cells. Chestnut species have 24 chromosomes (Jaynes 1962) which are arranged in pairs during meiosis.

Meiosis is a special form of cell division leading to the formation of pollen in male flowers and eggs in female flowers. During meiosis, chromosome pairs separate, and at fertilization, when the pollen grain and egg unite, the "normal" or diploid number of 24 chromosomes is restored. For example, in normal first hybrids between Chinese and American chestnut there will be one Chinese chromosome and one American chromosome in each of the 12 pairs of chromosomes after fertilization (see Figure 1).

Usually, every gene on a chromosome will also be found on its counterpart, or homologous chromosome, at the same location, although frequently a gene will differ slightly between the homologous chromosomes. Those genes that are slightly different but are located at the same place on homologous chromosomes are called alleles. Allelic differences between or among various individuals are often referred to as polymorphisms. In American chestnut, likely there are genes that match those conditioning blight resistance in Chinese chestnut. The difference is they are alleles for susceptibility to blight, instead of alleles for resistance. This difference between alleles of the same gene is crucial to plant breeding.

The idea that chromosomes behave as units for segregation of alleles on chromosome pairs during meiosis led to the expectation that all genes located on the same chromosome should be transmitted as a unit, and therefore show linkage (Sturtevant 1913). Likewise, genes on separate chromosomes are never linked. However, linkage between two separate genes on the same chromosome is never complete, because each pair of chromosomes usually exchanges segments of DNA during meiosis. The occurrence of such an exchange event, called a cross-over, results in recombination between genes located on the same chromosome pair. For instance, one would expect that most chromosomes in pollen or eggs formed by a first hybrid between Chinese and American chestnut would be part Chinese and part American (see Figure 2), unlike the chromosomes in the first hybrid, which are either pure Chinese or pure American. The alleles on the chromosomes in the pollen or eggs of the first hybrid would have recombined during meiosis.

The frequency of crossing over between any two genes serves as a measure of the genetic distance between them. This has been discussed in a recent issue of *The Journal* (Hebard, 1994b), although that discussion only briefly described the process by which the genetic distances between separate genes on a chromosome are transformed so they can be related in a linear fashion. For instance, for three linked genes the distances from the middle gene to each flanking gene should add up to the distance between the flanking genes. The percent recombination values are mathematically transformed by an appropriate mapping function (Haldane 1919; Kosambi 1994) into a measure of genetic distance known as a centiMorgan (cM). The cM distances between genes are linearly related and can be used to construct a genetic map of each chromosome.

CURRENT RECOMBINATIONAL MAPPING EFFORTS

Using various molecular markers, I constructed a genetic linkage map for the Chinese x American hybrid chestnut genome using an F₂ population developed by Hebard. The map is based on segregation data for a maximum of 102 offspring. In our F₂ population, 241 polymorphisms were scored; 45 were found to deviate significantly from their expected Mendelian inheritance ratio based on chi-square analyses ($p < 0.01$). The 45 distorted polymorphisms were excluded from any further analyses as I could not confidently conclude that each represented a single genetic locus, or location on a chromosome. Additionally, the use of distorted polymorphisms can often lead to the declaration of false linkages. The remaining 196 loci (genetic locations) were employed for linkage mapping and in the search for genomic regions conditioning a blight-resistant response in the tree. Six isozyme, 14 RFLP, and 176 RAPD loci were entered into the computer program JoinMap (version 1.1) (Stam 1992).

Of the 196 loci analyzed, 185 were mapped. In total, 2 isozyme loci, 14 RFLP, and 169 RAPD loci mapped to 12 linkage groups spanning a total genetic distance of 660.9 cM. Using the partial genetic linkage data and the method-of-moments estimator (Hulbert et al. 1988), I obtained several estimates of genome size. My current estimates suggest a genome size ranging from approximately 780 cM to 900 cM. Based on these results, the map spans at least 73% of the Chinese x American hybrid genome.

Once I had the map, the next step was to search for genes that condition a blight-resistant response. Many of the statistical tests employed for mapping traits such as blight resistance are based on specific underlying assumptions (Ott 1991). A major assumption for many of these statistical models requires that the phenotypic trait data be normally distributed. Indeed, the resistance data collected for the central trees used in this experiment, American and Chinese chestnuts and their first hybrid, were approximately normally distributed, with similar variances.

In order to determine whether any of the markers might be closely linked to genes conditioning resistance in the

chestnut hybrids, a standard analysis of variance was carried out using marker genotype groups as class variables (Young et al. 1993). An association between a marker and a blight-resistant response was considered significant if the probability of observing an F-value as extreme or more extreme than the observed value was less than or equal to 0.001. This significance level was chosen to ensure that a minimum number of false positives was declared throughout the experiment (Lander and Botstein 1989). Using single-marker analyses, four unlinked regions were suggested to be conditioning blight resistance.

To confirm the results of the single-marker analyses, the data were also analyzed using the program MAPMAKER/QTL (version 1.1, Lincoln et al. 1992). Upon further investigation with MAPMAKER/QTL and multiple locus models, only two of these regions were found to be significantly associated with resistance to chestnut blight. Unfortunately, I cannot yet publish the map, but I can state the approximate location of the genes conditioning a blight-resistant response.

TABLE 1

Markers bracketing regions conditioning a blight resistant response. Primers beginning with a C were obtained from J.E Carlson (University of British Columbia, Canada) and primer X03 was obtained from Operon Technologies Inc. (Alameda, Calif. USA).

Primer	Sequence (5' to 3')	Molecular Weight of Marker Band (bp)	Linkage Group
C258	CAGGATTACCA	450	B
X03	TGGCGCAGTG 1050	B	
C153	GAGTCACGAG1900	B	
C202	GAGCACTTAC 1025	G	

The first region was on linkage group B; most likely it is located between RAPD markers amplified by the primers C258 and X03 (see Table 1 for primer information). The second region was located on linkage group G between RAPD markers amplified by primers C202 and C153. These two marker-intervals were responsible for explaining as much as 56.8% of the phenotypic variation observed.

RESULTS IN HISTORICAL CONTEXT

Those results are extremely promising in light of the long-standing hypothesis that blight resistance is conferred by two incompletely dominant genes (Clapper 1952). If blight resistance were controlled by only two genes, then it should be a relatively straightforward task to introduce those genetic regions into American chestnut using the backcross method. Of course, mapping results suggest that the situation is somewhat more complex, with over 40% of the resistance response unexplained.

WORK AHEAD

In spite of these promising results, a tremendous amount of research still needs to be conducted. Integrating the morphological markers discussed in a recent issue of *The Journal* (Hebard 1994b) into the molecular map must still be completed.

It would also be helpful to confirm the influence of the regions conditioning a blight-resistant response in both the first and second backcross generations. If the influence of these regions is confirmed, the breeding program will serve to introduce these regions into many different selections of American chestnut. One unique approach might be to identify shrubs and sprouts of American chestnut still persisting in the wild throughout the natural range, bring them into flower by exposing them to full sunlight, hybridize these to select hybrid individuals, and repeatedly backcross to restore native genotypes.

In addition, molecular studies of other pedigrees involving two or more species should be undertaken as these may help determine whether genes for blight resistance from different sources (such as Japanese chestnut) map to the same or different locations in the genome. Such information would help identify other possible sources of blight resistance, avoid the use of redundant sources of resistance, and greatly increase breeding efficiency in terms of the number of off-spring needed for selection efforts.

Hopefully the development and re-introduction into the wild of blight-resistant, primarily American, chestnut trees will restore the chestnut throughout much of the eastern United States.

To conclude, I want to inform the readers of *The Journal* that I would be happy to supply as much information as possible about the markers used in this study.

ACKNOWLEDGMENTS

I would like to thank Fred Hebard for all his help on this project, and commend him for the important work he is pursuing for The American Chestnut Foundation. I would also like to thank R. Bernatsky for supplying RFLP data and H. Huang for supplying isozyme data.

REFERENCES

- Anagnostakis, S.L. 1995. Field tests of transgenic strains of the chestnut blight fungus for biological control. Abstract Published In: Proceedings of the Mycological Society of America Annual Meeting, Aug 6-10, San Diego, CA. p1 .
- Clapper, R.B. 1952. Relative blight resistance of some chestnut species and hybrids. *J. Forestry* 50:453-455.
- Ellingboe, A.H. 1994. Breeding blight-resistant American chestnut. *J. American Chestnut Foundation* 8(1): 15-20.
- Haldane, J.B.S. 1919. The combination of linkage values, and the calculation of distances between the loci of linked factors. *J. Genet.* 8:299-309.
- Hebard, F.V. 1994a. The American Chestnut Foundation breeding plan: beginning and intermediate steps. *J. American Chestnut Foundation* 8(1):21-28.
- Hebard, F.V. 1994b. Genetic mapping of chestnut. *J. American Chestnut Foundation* 8(2):31-38.
- Hulbert, T.W., Legg, E.J., Lincoln, S.E., Lander, E.S., and R.W. Michelmore, 1988. Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. *Genetics* 120:947-958.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen. (London)* 12:172-175.
- Lander, E.S., and Di Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
- Lincoln, S., Daly, M.J. and E.S. Lander. 1992. Constructing genetic linkage maps with MAPMAKER/EXP (version 3.0): A tutorial and reference manual. Whitehead Institute for Biomedical Research.
- Michelmore, R.W., Paran, I., and Ri V. Kelleli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. *Proc. Natl. Acad. Sci. USA* 88:9828- 9832.
- Mulcahy, D.L., and R. Bernatzky. 1993. Speeding restoration of the American chestnut by using genetic markers in a backcrossing program: an homage to Dr. Charles Burnham J. *American Chestnut Foundation* 7(1):33-36.
- Ott, J. 1991. Analysis of human genetic linkage. The John Hopkins Press Ltd., London. 302 p.
- Stam, P. 1992. JoinMap Version 1.1-A computer program to generate genetic linkage maps.
- Sturtevant, A.H. 1913. The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *J. Exp. Zool.* 14:43-59.
- Young, N.D., Danesh, D., Menancio-Hautea, D., and L. Kumar. 1993. Mapping oligogenic resistance to powdery mildew in mung-bean with RFLP's *Theor. Appl. Genet.* 87:243-249.

HYPOVIRULENCE IN ORCHARD MANAGEMENT

Sandra Anagnostakis

The Connecticut Agricultural Experiment Station

New Haven, CT

Previously printed in the Proceedings of the World Chestnut Industry Conference, July 1992

The blight fungus has maintained its destructive vigor for at least 80 years in New England (1). Chestnuts have sprouted, become wounded become infected by *Cryphonectria parasitica* and died, to sprout again. Abnormal strains of the fungus were isolated in Italy in the early 1960s by Jene Grente of France, who showed that these "hypovirulent" strains lacked the orange pigment of virulent strains, and produced fewer spores. As these strains spread through the chestnut orchards of Italy and France, the trees began to survive longer, "healing" over the blight cankers with lumpy bark tissue. This spurred searches in this country for hypovirulent strains, and similar. "healing" cankers in Michigan, Tennessee, Virginia, and West Virginia yielded orange strains of the blight fungus that were also less able to kill chestnut trees (5). Virologists have now confirmed that these American strains of *C. parasitica* are infected with viruses (4). These viruses represent three very different types (three "families"), with wide variation in their effects on the fungus, and with varied effectiveness for biological control of chestnut blight. When scientists put bits of a hypovirulent blight fungus into holes in the bark around killing cankers, viruses move into the virulent stains that caused the cankers. The cankers then stop expanding, and the tree's natural defenses of walling off invaders succeed in protecting the tree's living cambium, the zone responsible for growth and active transport of nutrients. Once hypovirulence has been established in a chestnut blight population, hypovirulent spores are moved around in test orchards and in the forest by creatures that move up and down the trees (8,9,11). The European hypovirulent strains lack pigment, and do not grow quite like the virulent strains in culture in the laboratory. They have effected a biological control of chestnut blight in the orchards of *C. sativa* in France and Italy (3,7,10). Grente sent me hypovirulent cultures in 1972, which we tested in the laboratory and the greenhouse (2). When we had the permission of the U.S. Plant Quarantine Division (in 1978), tests were begun on trees growing outside at The Experiment Station Farm in Southern Connecticut. An orchard of 71 3-year-old American chestnut trees was planted at The Experiment Station farm in Hamden, Conn., in 1976. The seed for these came from Michigan and Wisconsin, and the seedlings were raised by E. Thor of the University of Tennessee. Trees became infected with the chestnut blight fungus, and were treated for four years in a row (from 1978 through 1981) with a mixture of hypovirulent strains of the blight fungus. No cankers on these trees have been treated for the last 11 years. In 1971, 43 of the original, main stems were still alive. In 1992, 28 of the original stems survived, and there were 28 sprout-clumps with stems that reached the canopy. New cankers form each season, but usually become superficial (and non-lethal) before they kill any cambium. When they kill twigs or branches, they are pruned off (late July is the best time to prune chestnuts).

In 1980, J. E. Ellison recorded 40 cankers on 59 trees; in 1992 they were uncountable. Many stems have cankered and calloused bark from their base up to 15 feet above the ground. Killed stems are removed each spring. The cankers in this orchard have the appearance of hypovirulent cankers, and we assume that birds, insects, and arthropods are moving hypovirulent strains of the fungus from cured cankers to newly formed killing cankers. Some trees seem to be surviving better than others. Since we have no records of the exact source of each of these trees, it is not possible to draw conclusions about population fitness. Perhaps some trees are simply better able to thrive with extensive hypovirulent cankers. Nut production is heavy, and since 1989, these trees have been used in a backcross breeding program.

It is clear from the report given at this meeting by William MacDonald that hypovirulence is not succeeding as well at controlling chestnut blight in West Virginia as it is in Connecticut. I have thought a lot about the possible differences between our results, and several possibilities occur to me. First, the trees may be different in each population (provenance) in the United States. Second, there are clearly many different kinds of viruses in the blight fungus population in West Virginia. We have never found "native" viruses in our *C. parasitica* cultures from Connecticut. If these interfere with the infection of the fungus by hypovirulence viruses, it could certainly reduce the possibility of a biological control. The third "southern factor" is the presence, in states from Pennsylvania south, of two fungal species that are closely related to the blight fungus and have never been found in Connecticut. One is *Endothia gyrosa*, found on oaks and chestnuts, and the other is *Cryphonectria radicalis*. The latter has not been found in the United States since blight reached its native range. These fungi might be interacting (or might have interacted) in some way that we have not yet discovered.

It is a challenge for us to find reasons for the differences in usefulness of hypovirulence. Fortunately, the scientists who work on chestnuts and chestnut blight are committed to working together.

Clearly hypovirulence can be used to maintain orchards of American or hybrid chestnut trees in some parts

of the country. This will be an advantage if partially resistant trees are chosen as good nut producers and planted in commercial orchards. I hope that the science will keep pace with the needs of the industry.

REFERENCES

1. Anagnostakis, S.L. 1987. Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* 79:23-37.
2. Anagnostakis, S.L. and R.A. Jaynes. 1973. Chestnut blight control: use of hypovirulent cultures. *Plant Dis. Rep.* 57:225-226.
3. Bisiach, M., A. DeMartino, E. Gobbi, M. Intropido, and G. Vegetti. Studies on chestnut blight: activity report. *Rivista di Patologia Vegetale* 24:3-13.
4. Choi, G.H. and D.L. Nuss. 1992. A viral gene confers hypovirulence-associated traits to the chestnut blight fungus. *EMBO J.* 11:473-477.
5. Day, P.R., J.A. Dodds, J.E. Elliston, R.A. Jaynes, and S.L. Anagnostakis 1977. Double-stranded RNA in *Endothia parasitica* *Phytopathology* 67:1393-1396.
6. Grente, J. 1965. Les formes H⁻ovirulentes d'*Endothiaparasitica* et les espoirs de lutte contre le chancre du chataigner. *C.R. Hebd. Seances Acad. Agr. France* 51:1033-1037.
7. Grente, J. and S. Berthelay-Sauret. 1978. Biological control of chestnut blight in France. pp 30-34 IN: *Proceedings of the American Chestnut Symposium*. Eds. W.L. MacDonald, F.C. Cech, J. Luchock, and C. Smith. West Virginia University, Morgantown 122p.
8. Sharf, S.S. and N.K. DePalma. 1981. Birds and mammals as vectors of the chestnut blight fungus (*Endothia parasitica*). *Can. J. Zool.* 59:1647-1650.
9. Turchetti, T. and G. Chelazzi. 1984. Possible role of slugs as vectors of the chestnut blight fungus. *Eur. J. For. Path.* 14:125-127.
10. Turchetti, T. and G. Chelazzi. 1984. Mixed inoculum for the biological control of chestnut blight. *Bulletin OEPP* 18:67-72.
11. Wendt, R., J. Weidhaas, G. J. Griffin, and J.R. Elkins. 1983. Association of *Endothia parasitica* with mites isolated from cankers on American chestnut trees. *Plant Disease* 67:757-758.

ONE OF THE WORLD'S LAST TRULY WILD CHESTNUT FORESTS - THE ACHIPSEE RIVER VALLEY OF RUSSIA'S CAUCASUS STATE BIOSPHERE PRESERVE

Fred Paillet
United States Department of the Interior
Geological Survey
Denver, CO

About 10 million years ago, geologic records show that most of the northern temperate regions were covered by a diverse forest containing many familiar kinds of broad-leaved trees such as oak, beech, maple, magnolia, sassafras - and chestnut. Later trends toward a cooler and drier climate fragmented those forests into local populations and replaced the deciduous trees at higher latitudes with the spruce, pine, and other conifers typical of the modern taiga. At the end of the latest glacial period about 12,000 years ago, chestnut forests were restricted to three geographic locations: eastern North America, south-central China, and the Caucasus Mountains of south-eastern Europe.

The chestnut we know today from other parts of Europe is believed to have been introduced by the early Romans. Chestnut blight and ink disease have eliminated chestnut from the otherwise undisturbed fragments of forest preserved in the Appalachians; the intense agricultural activity in China has probably disturbed most natural chestnut forests in Asia. However, a combination of extremely rugged topography and chronic political instability

has served to limit the impact of human activity on the forests of the western Caucasus region.

Russia's Caucasus State Biosphere Preserve (Figure 1) contains many hectares of essentially virgin deciduous forest known to contain chestnut. In this article I describe my impressions of Caucasian chestnut forests after a week-long visit to the preserve.

The visit was arranged through my colleagues Dr. Michael Pridyna and Dr. Leonid Plotkin, Russian scientists currently conducting research in the preserve. Pridyna and Plotkin arranged for me to be greeted in the resort city of Sochi and transported to a remote preserve station on the southern flank of the preserve. I then had the pleasure of spending a full week exploring the Achipsee River Valley for a first-hand experience of mostly undisturbed forests containing European chestnut (*Castanea sativa*) trees of all sizes ranging from seedlings with the nut still attached to forest giants more than a meter in diameter.

FORESTS OF THE ACHIPSEE RIVER BASIN

The Achipsee River upstream from the Laura Station drains about 50 square kilometers (20 square miles) of forest and alpine meadow (Figure 2). Elevations in the watershed range from 600 to over 3,000 meters (2,000 to 10,000 feet), with the tree line at about 1,800 meters (600 feet). Yearly rainfall is about 1.5 meters (60 inches). The valley has never been logged and contains an assortment of wildlife, including wild boar, wolves, deer roughly equivalent to North American elk, and bears roughly equivalent to North American grizzly bears. All soils in the watershed seem to be relatively acidic brown forest soils developed over metamorphic rocks such as slate or schist. Although I have no actual measurements of soil fertility, the uniform abundance of such characteristic plants as high-bush blueberry and azalea testify to the acidic quality of the soil. The habitat of these forests is characterized by a combination steep slopes, acidic metamorphic substrate, and humid temperate climate - a combination of elements which is similar in many ways to the habitat of the former oak-chestnut forests of the southern Appalachians. The most common tree and shrub species in the watershed are listed in Table 1. The forests in the basin are of three general types: 1) a river bottom forest dominated by European black alder and containing maple and walnut; 2) a lower slope forest of beech, chestnut, oak, and hornbeam; and 3) an upper slope coniferous forest dominated by fir and planetree maple.

The transition between deciduous and coniferous forest occurs at about 1,200 meters (4,000 feet) in elevation - about the same elevation as the transition to boreal forest in the Great Smoky Mountains of North Carolina. My inspection of the Achipsee Valley showed that chestnut is a major component of the forest everywhere except in the uppermost coniferous forest and in the limited alder woodlands immediately adjacent to the river. Preliminary forest cover maps being prepared by preserve foresters divide deciduous slope forests into three classes: beech, chestnut, and oak. My assessment of the forests in the Achipsee basin suggests that these are all phases of a single forest association spread over the lower slopes of the watershed. Beech, chestnut, and oak each locally assume dominance, with chestnut seeming to show a preference for lower slopes and eastern exposures. Very approximate local sampling of the forest gives a rough estimate of the average deciduous

TABLE 1
FOREST TREES AND SHRUBS OF
THE ACHIPSEE VALLEY

Major Forest Trees	
Oriental Beech	<i>Fagus orientalis</i>
European Hornbeam	<i>Carpinus betulus</i>
Italian Chestnut	<i>Castanea sativa</i>
Durmast Oak	<i>Quercus petraea</i>
Caucasian Fir	<i>Abies nordmanniana</i>
European Black Alder	<i>Alnus glutinosa</i>
Field Maple	<i>Acer campestre</i>

Minor Forest Trees

Planetree Maple	Acer pseudoplatanus
Norway Maple	Acer platanoides
Caucasian Linden	Tilia caucasica
Bird Cherry	Prunus avium
Aspen	Populus tremula
European Ash	Fraxinus excelsior
English Walnut	Juglans regia
Common Pear (introduced)	Pirus communis

Major Shrubs

Common Hazel	Corylus avellana
High-bush blueberry	Vaccinium spp
Azalea	Rhododendron spp
Rhododendron	Rhododendron spp
Blackberry	Rubus spp
Elderberry	Sambucus spp

forest composition: 40 percent hornbeam, 25 percent beech, 20 percent chestnut, 12 percent oak, and 3 percent other species such as maple, aspen, and cherry (Figure 3A). These estimates are based on stem counts in small areas, and by viewing hillsides where the distinctive colors of tree foliage and the presence of ripening chestnuts in tree crowns made it possible to distinguish canopy coverage by species. A typical example of the size and shape of chestnut, beech, and hornbeam in a representative sample of forest is shown in Figure 4.

My sampling of a small site chosen pretty much at random can be compared to sampling on a site chosen by Michael Pridyna to represent a chestnut-dominated forest (Figure 3B). Michael's data can probably be taken as an example of the upper limit of chestnut stem density in the Achipsee River Valley. Data from some of his other sampling sites in other drainages indicate chestnut density can locally exceed 90 percent. In the Achipsee Valley, chestnut and beech stems are usually larger than those of other trees, so that the relative percentage of chestnut and beech would be increased to perhaps 25 percent and 30 percent at the expense of hornbeam if the analysis were based on basal area rather than stem counts. Either way, chestnut is clearly one of the dominant forest trees in the Achipsee River Valley.

SPECIFIC ISSUES RELATED TO CHESTNUT ECOLOGY

After establishing that chestnut is one of the leading tree species in the Achipsee River Valley, I began to consider specific issues involved in chestnut ecology in general, and comparison between Caucasian and American chestnut in particular. Here are a few comments on some of the important issues. The reader is once again reminded that these are very preliminary conclusions based on a limited sampling from a single local population of Caucasian chestnut.

CHESTNUT GROWTH RATE AND TYPICAL TREE AGES.

Trail clearing, road construction near the Laura Station, and a few cores removed from trees deeper within the forest gave me a rough idea of chestnut tree ages and growth rates (Figure 5). Most stem sections showed ages from 115 to 150 years. I found no examples of stems showing initial extended periods of suppression followed by release, and only one example where a stem had been released for a second spurt of growth late in life. Most stems showed a gradual decline in growth from an initial rate of about half a centimeter (about a quarter of an inch) per year of diameter increase. Some trees were still growing at a good pace after more than 100 years (Figure 5A), while others had slowed so that rings were too closely spaced to count by visual inspection (Figure 5B). From these measurements it seems that Caucasian chestnut, like its American relative, grows faster than most of its competitors.

CHESTNUT SEED CROPS AND SEED PREDATION.

All of the chestnut trees in the Achipsee Valley were bearing heavy crops of new fruit at the end of July, and the large number of new seedlings on the forest floor clearly demonstrates that these trees are now producing a large nut crop. Chestnut crowns on hillsides could be distinguished from other trees by the glint of sunshine on the spines of

developing burs. At this point in time the burs were still relatively small. In fact, chestnut was still in flower at the highest elevations (above 1,500 meters; 5,000 feet), in the Achipsee Basin.

It appears that Caucasian chestnut flowers and fruits on the same schedule as American chestnut in the Appalachians. No damaged immature chestnuts were present on the ground, although there were large numbers of freshly opened immature beech burs on the ground. Each of the latter had been opened on one side and the green seeds extracted. I saw few squirrels or other rodents moving about, but there were many European jays active in the forest. Circumstantial evidence (they had both the motive and the opportunity) suggests that jays are the culprit responsible for the destruction of immature beech seed. There was some evidence of boars churning the soil, and I did see one wild boar sow with piglets in the forest. However, boar foraging for mast did not seem extensive here. By contrast, Wayne Martin of the U.S. Forest Service reports that he saw extensive signs of boar disturbance to the soil in the same area in November, 1993. Conversations with local foresters indicated that rodents (especially field mice) were the main predators on chestnuts, and they made no mention of boar damage to the seed crop.

REPRODUCTIVE STATUS OF CHESTNUT.

Although Russian authors have repeatedly cited a lack of chestnut reproduction, the numbers of chestnut seedlings on the ground were impressive. Many of these were new seedlings, but many others showed several seasons of growth nodes (Figure 6). Chestnut seedlings have undoubtedly been generated for at least the past few years in the Achipsee Valley. In contrast, there was a significant shortage of advanced chestnut reproduction (Figures 3c and 3d). I saw relatively few chestnut saplings greater than 1.5 meters tall.

Subcanopy chestnut stems were outnumbered 10 to 1 by beech saplings, even though these two trees comprise equal proportions of the overstory, and even though beech and chestnut seedlings seemed to be present in nearly equal numbers.

I also attempted to verify that oriental beech was not propagating by root sprouting. All seedlings and small saplings I investigated showed no indication that they were attached to the roots of mature trees. On the basis of these observations, I conclude that chestnut is now producing viable seedlings, but that seedling establishment was either reduced in the past, or events conspire to prevent the development of chestnut seedlings into subcanopy saplings.

UPPER ALTITUDINAL LIMIT OF CHESTNUT.

Although Russian foresters map the upper limit of deciduous forest at about 1,200 meters (4,000 feet), I found chestnut and most other deciduous species present up to about 1,500 meters (5,000 feet). In fact, all deciduous species continued as part of the fir-dominated forest right up to the limit of tree growth except for oak and chestnut. Oak seemed to fall out at about 1,200 meters (4,000 feet) whereas chestnut disappeared higher up. Beech and hornbeam continued to the upper tree line, with linden, planetree maple, and aspen becoming more prominent near the upper forest limit. This distribution of chestnut seems to correspond roughly with the distribution of American chestnut, extending beyond the range of the oak-chestnut forest itself, but not as far as northern hardwoods such as beech and maple.

CHESTNUT AND THE DISTURBANCE REGIMEN.

The natural disturbance regimen of the Achipsee River Valley appears completely dominated by slope instability and windstorm. My limited sampling of disturbed sites indicated that most chestnut reproduction is arising in the form of sprouts from the base of former large trees. Often these sprouts "emerge" through the tangle of soil and roots of rootplates of overturned trees. The lack of suppressed chestnut saplings being released by disturbance seems directly related to the small number of such stems. As with American chestnut, these suppressed "old seedlings" or subcanopy saplings appear capable of strong response to release, and probably could compete very well with hornbeam and beech if they were available to respond when gaps were created.

CHESTNUT PATHOGENS.

I found absolutely no indication of chestnut blight in the Achipsee River Valley. Furthermore, my queries about chestnut blight or "desiccation" as it is called in the Russian botanical literature were uniformly negative. These questions were asked to local foresters through an interpreter, so I cannot be certain whether the questions were

understood, or that I understood the answers. The one pathogen that was widespread was some kind of bark and wood fungus causing chronic canopy die back and sprouting from the main stem. This fungal pathogen did not form cankers, and no distinct slowdown in diameter growth was observed in cores taken from infected trees.

CHESTNUT DISTRIBUTION AND SOIL PARENT MATERIAL.

The substrate of the Achipsee Valley is composed entirely of schist, thus I have little information about how these chestnuts grow in other soil types. The Russian literature indicates that Caucasian chestnut is not found on calcareous soils, just as American chestnut avoids such soils. All I can say is that on my travels chestnut was a consistent component of the mountain forest all the way from Sochi to the Laura station, and that I did not see chestnut anywhere in the forests on the rugged limestone ridges in the immediate vicinity of Sochi.

SUMMARY

All of my observations of European chestnut in the forests of the western Caucasus support the conclusion that the ecological character of chestnut in the humid forests of the Caucasus Mountains is analogous to the former ecological character of American chestnut in the Appalachian forests of America. In particular, I believe the forests of the Achipsee River Valley could provide a useful natural laboratory in which to study the factors that influence the natural reproduction of chestnut.

Chestnut blight now makes it impossible to study the ecological factors that determine the rate and distribution of chestnut reproduction in American forests. The information derived from study of chestnut in the Caucasus State Biosphere Preserve could be very helpful in understanding how climate, seed predation, microsite conditions, and the disturbance regimen influence the quality and quantity of chestnut reproduction. Such information will become very important when ongoing research efforts provide blight-resistant American chestnut for reintroduction into North American forests.

On a more personal note, it was something approaching a mystical experience to visit this wonderful forest. My Russian colleagues were amazed that I stood silent for 10 minutes in front of a few of the largest chestnut trees, just contemplating these forest giants -chestnut, beech, oak, and linden. My photographs go only a short way toward conveying the sense of delight and wonder these trees create. It was interesting to note the appearance of a forest floor that is largely determined by the texture of chestnut leaves and the carpet of recently fallen male chestnut catkins. I found that it is somewhat painful to rummage around the forest floor on hands and knees when there are chestnut burs mixed with the leaf litter. The number of chestnuts maturing in their burs was also something to contemplate, when the color of whole hillsides is governed by the young burs filling chestnut crowns. Perhaps there will come a day when similar experiences will be part of an excursion along the Blue Ridge Parkway or a hike on the trail to Thunderhead Peak in the Smokies.