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Preserving Chestnut Memories

Are you one of the fortunate few who was around to witness the majesty of forests full of towering chestnut? Or perhaps you have a parent or grandparent who regaled you with stories that featured this mighty giant? Whatever your story, we want to hear it! Please send articles you would like to be considered for publication to:

Jeanne Coleman, Publications Director
The American Chestnut Foundation
469 Main St., P.O. Box 4044
Bennington, VT 05201
Or e-mail publications@acf.org.

Are you more the talkative type? Please let us call you to record your story. You can leave your name and telephone number with our main office, at 802-447-0110.

FROM THE EDITOR

Greetings TACF Members and Supporters. What a year it has been for TACF! From a record setting year-end campaign in 2006, to planting a chestnut sapling on the Department of the Interior's lawn in mid-July, to the publication of TACF's first book, "Mighty Giants: An American Chestnut Anthology", in late-October, to the fast approaching 24th Annual Meeting in Burlington, VT, it has been quite a year indeed!

Since taking over this position in early-March, I have been shown an organization that has accomplished more in six months than any other company where I have ever been employed. It is a remarkable feeling to be a part of a mission that actually matters, with people who actually care about more than just themselves.

Now that Fall has officially come to Vermont, as evident by the myriad of colored leaves atop the Vermont National Forest trees, I am reminded of a favorite quote from one of my best friends who often says, "Time is an arbitrary measure of existence." While the statement resonates sound advice, at 31 years of age I often wonder, is this a true statement? Do we, the human race, measure our failures and successes simply by the passage of time? Do we humans judge something to be a "success" only after it leads us somewhere else? These are rhetorical questions to be sure, but these are also questions each person must answer in their own lives.

Case in point, at 93 years-of-age, Dr. Norman Borlaug received the Congressional Gold Medal in July of 2007, for saving an estimated one billion lives. Dr Borlaug is famous, though he is not super-rich or super-powerful. He is a man who did his job to the best of his abilities and continues to provide a better world for all of us to live. Yet, when Dr. Borlaug won the Nobel Peace Prize, he speculated on his award:
"When the Nobel Peace Prize Committee designated me the recipient of the 1970 award for my contribution to the 'green revolution', they were in effect, I believe, selecting an individual to symbolize the vital role of agriculture and food production in a world that is hungry, both for bread and for peace".

To this day, almost 40 years later, Dr. Borlaug remains a relatively unknown hero to the rest of the world, yet he carries on, striving to make each day better for billions of people whom he has never met. The fact

Dr. Borlaug is over 90 years-old and continues his craft is astounding, but, it also makes me take a real hard look at my friend's statement that, "Time is an arbitrary measure of existence."

Within this issue of *The Journal of the American Chestnut Foundation*, it is my sincerest hope that the readers are left examining this statement for themselves. Read the powerful prose of *Tender Leaves of Hope* and feel the forlorn loss from the poem *Elegy* in the "Memories" section. Check out what is happening at the Meadowview Research Farm, with TACF's Annual Science report: *Meadowview Notes* located within the "From Then to Now" section. Finally, complete the reading by getting up-to-date information on a *Review of the Historic and Current Status of the Asian Chestnut Gall Wasp in North America*, the *History of Chestnut Survival in the Appalachians (Prehistory to Present)*, the *Decomposition of American Chestnut Leaves* and *Small Stem Chestnut Blight Resistance*.

In my opinion, 2007 marked a year of hope for TACF. If it is possible, step back and take a look at what TACF has accomplished in so little time. Look to the future and you can see the horizon brightening. As TACF moves into our 25th anniversary, every person associated with TACF can honestly state, "We got involved, we made something happen, imagine what we can do from here."

Good Day.

Louis Bedor III
Publications Director for TACF

memories

TENDER LEAVES OF HOPE

NORTH CAROLINA SENIOR GAMES 2004

Silver Arts

Literary Arts: Essay

Dr. Page Hudson, MD

Greenville/Pitt County

Addendum: This article was awarded the first place gold medal in the essay competition on North Carolina Senior Games Awards Day, October 2, 2004, at the McKimmon Center, North Carolina State University campus. Literary judges were: Diane Jones, Bonny Harrison, and Jerry Barrax. Competition was open to all NC seniors, male and female, 55 years and up. A companion piece, “A Little Poison In My Life,” by the same author placed third in the “Life Experiemces” category. The other two Literary Arts categories were Short Story and Poetry. All of North Carolina’s 100 counties participated in Senior Games in 2004 via 53 individual and groups of counties. Approximately 50,000 senior citizens participated in local Games, over 3000 at the 20th Annual NC Senior Games held primarily in Raleigh, partly in Winston-Salem and Greenville, in September and October.

This is the state of man; Today he puts forth
The tender leaves of hope, tomorrow blossoms
And bears his blushing honors thick upon him.
(W. Shakespeare: Henry VIII, Act III, Sc. 2)

Where there is no hope, there can be no endeavor.
Samuel Johnson

Indulge me a few minutes with your advice and aid. Help me design a tree!

Help me conjure up an ideal tree for the East, for particularly that great band of mountains, foothills and piedmont stretching gloriously from Alabama and Georgia into Canada — Appalachiana, I like to call it.

We want our tree to rapidly grow uncommonly tall, thick and shapely whether it stands alone or is crowded by its fellows in the forests. Long, dark, prettily veined and deeply serrated leaves would serve the tree well and look grand. A robust bark supplying significant quantities of tannic acid would be an asset. Wouldn't we want a tree as strong as the oak but with wood that is lighter and easier to work? As long as we're imagining, let's make the wood uncommonly resistant to weather and to decay. Utility poles, fence posts, even caskets could be made from this ideal material as well as lovely furniture. Since it is weather and rot resistant we could use it for super durable studding and siding for houses and barns. We'd like a straight and pretty grain lending itself to clear finishes. This tree must propagate easily and grow rapidly. Since it should be remarkably tall and straight we want the branches to take off high on the tree so there will be a very long trunk length for multiple 16 foot logs without troublesome knots. Stout, straight, graceful limbs should add to the efficiency of the tree. Newly cut planking would warp minimally as it cured, if any at all.

Our tree should flower but with dignity befitting its size. Don't you think blossoms could be too showy, a bit much? — Certainly nothing like the Magnolia grandiflora, a fine tree — in its place. Even flowers like those of the lovely Silver Bell that sequesters its floral glory in the forests at two or three thousand feet above sea level might be overdone. If you agree, let's have draping, six to eight inch, creamy white catkins that in bloom will light up the forest as the tree's floral and reproductive expressions. We'll have them flower in June after the frosts to ensure superior fertilization.

If this is not enough we can have the tree *do* something. It shall produce in great quantity large nuts that raw, roasted or boiled are tasty to humans and that provide abundant healthy nutrition additionally to deer, turkeys, bear, elk, swine — the large herbivorous and omnivorous creatures of the forests, hillsides, and fields. The myriad of smaller creatures, the chipmunks, skunks, raccoons, weasels, field mice and the like would feast as well.

We could continue in this vein but by now you are suspecting that I've come undone, that perhaps I suffer a raging fever or something more exotic like jimson weed hallucinations. Hold on; I'll make it even worse. Suppose I told you that this tree really *existed*! But it did! And it may live and flourish again!

At about age six years — not really terribly long ago I contend — I saw for the first time the forest remnants of the American chestnut, *Castanea dentata*, in the hills of Charlottesville, Winchester, and Waynesville, Virginia. I can faintly recall the giant ghostly remains reaching straight and starkly toward the heavens. Bared of bark and sun-bleached, the towering specters absent their leaves in death contrasted dramatically with the summer verdancy of adjacent forest. The image had little significance to me then as a small boy but it lives on in my mind's eye.

Only a century ago foresters estimated there were between three and a half and four *billion* chestnut trees in this country. Twenty five percent of all hardwoods, perhaps of all trees, in the area were chestnuts. Conifers were scarce. One wag claimed a Georgia squirrel could make his way to Maine bounding through only chestnut trees without putting a paw on the forest floor! One hundred years ago this year the fungus *Cryphonectria parasitica* was discovered blighting the chestnut trees that proudly lined the avenues of the Bronx Zoo, among other grand display vistas. Some authorities say that the infection came from imported contaminated Chinese chestnut trees. Others believe it was introduced in imports to New England in the late nineteenth century and was only discovered in 1904 in New York. The chestnut trees of the New York Botanical Garden exhibited the disease in 1907. The spread was steady and rapid until by the middle of the twentieth century there was scarcely a living chestnut in the country. It was all but extinct in its natural range in the eastern United States, including the western stands of the tree in Illinois and Indiana.

In still another fifty years, at the first blush of the 21st century, those who knew the tree still feel deeply lessened by the loss. Even to grow up on one's elders' stories of this great creation was to love it and to feel diminished by its absence. *Foxfire 6* voices some who witnessed the effect of the loss: The worst lick ever to hit the South and the United States, in the timber line, was when they lost the chestnut timber;   That was one of the greatest losses of natural resources that this country has ever suffered;   That blight was the awfulest lick the South ever got. It hurt everybody because so many people could get to work because of the trees; Economically, it was the worst blow this area ever had.

Millions of land acres once shaded by Longfellow's spreading chestnut tree became tree graveyards into which grew that giant weed called pine plus a relatively second-rate line of hardwoods, all in less than 50 years. As the bleak scene of death of the forest mainstay evolved, commercial interests leaped in to harvest from the remaining forest giants the valuable bark for tannic acid and the wood for timber before the great trees all died and decayed. It may well be that any chance of blight resistance was thus eliminated from the gene pool and doomed before it could develop. That development was a very reasonable biological possibility. We shall never know now.

Perhaps the lack of awareness of the perfection and bounty of the American chestnut among most people today is a monument to human resiliency and capacity to adapt. But maybe the apparent attitude of Oh, that's ancient history, let the dead bury the dead, or, Well, what can anyone do about it now? or, Say whaaat? is our real monument, one to superficiality in its worst contexts. Why has there not been always a powerful hope of bringing back — reconstituting if you will — this magnificent element of our resources?

There have been a stubborn, hopeful few souls who fought the disaster of the fungal blight and tree loss. In the first years of tree destruction a quarantine to minimize disease spread was employed. The movement of infected trees was proscribed. The torch was applied to newly affected areas but fire was not effective in slowing the epidemic. A gamut of chemical agents has been in vain. As tiny, light-as-air fungal spores transmitted the affliction, there was nothing effectively retarding the disease spread and the process continued rapidly. Huge groves of the arboreous giants of Appalachiana would die in a single season. Even after it seemed the disease would affect the entire population of chestnut trees there was a minute glimmer of hope to reverse this disaster. Fresh shoots arose from the roots in the somber forests of dead and bleaching trees and raised their little trunks and branches 10, even 25 feet in the air. But then, alas, the omnipresent spores would find the young trees which would be then girdled by the cankerous scarring via which the blight destroyed the vital cambium layer beneath the bark. There was hope when the shoots appeared from the roots of dead trees that the regrowth would be immune to the fungus. But no such luck! Teasing further those who yet had hope of resurrection of the chestnut forests is that almost none of the shoots bear fruit before succumbing to the blight. A very occasional mature chestnut tree, even a minute grove, that seems resistant or unaffected is encountered in diverse areas. Many of these are in regions to which the tree was exported years ago, away from the Appalachiana zone, remote from the persistant fungus. But those do provide enough nuts/seeds and germplasm to furnish the investigations of biological scientists who, in slowly increasing numbers, believe that they can recreate a near likeness of the classic *Castanea dentata*, if not create an immune exact replication.

Professor Sam Cox wrote, As the ultimate cause of the blight s effects on the American chestnut, man may also be the ultimate salvation of the American chestnut. Efforts to stop the blight waned and research shifted predominantly to breeding a blight-resistant chestnut. The Chinese chestnut *C. mollissima* was susceptible to the fungus but was little affected by it. But that tree is a very poor substitute for the American chestnut, having few of the attributes so loved in species *C. dentata*. The several species of the chestnut genus *Castanea* cross easily so various investigators produced a plethora of crosses or cultivars. Resistance to the blight was demonstrated but the desired characteristics of *C. dentata*, e.g. size and stature and quality of fruit, were hopelessly attenuated.

More recently two or more genes on different loci have been found by plant geneticists to be associated with resistance to infection by *C. parasitica fungus*. As these scientists express it, the resistance trait is incompletely dominant, meaning the need for the genome to be homozygous for the resistance allele at two loci for the tree to be immune. Trees that are three-quarters Chinese chestnut demonstrate insufficient resistance to be effective. Crosses of species must have the offspring getting the total complement of resistance genes from the Chinese chestnut parent. Of course significant genes would come from the American *C. dentata* parent. This led to crossing the two species and repeatedly backcrossing with the Chinese. Most of the products were resistant but without sufficient characteristics of *C. dentata*. Interest waned after many years of fruitless endeavor.

Reconstitution of the American chestnut has at times become almost a hopeless and forgotten mission in this country. A few persisted and kept the concept alive. Scientists in Europe added enormously to the energy of the mission. The blight had spread to Europe's extensive chestnut (*C. sativa*) forests and caused great destruction. The Italian pathologist Biraghi found chestnut trees living with the blight. From them the French mycologist Grente cultured out a strain of *Cryphonectria parasitica* that was virtually never poisonous to the tree. This strain is referred to as hypovirulent. Without the effect of repeated dosing of the American chestnut with hypovirulent fungus the American laboratory trees would not live long enough for meaningful research. The hypovirulent fungus, it is now believed, is made so by a virus that affects the fungal RNA cytoplasm (cell fluid or juice). Cytoplasmic content has essentially no genetic impact in reproduction, so mating (hyphal fusion) between the usual virulent and the hypovirulent fungi results in the off-spring all being virulent. A promising line of research now is trying to get the hypovirulence viral protein into the reproductive DNA of the genome inside the fungus nucleus. Then *C. parasitica* proliferation could result in effective hypovirulence proliferation.

Arguably the most exciting and promising research is a relatively long term endeavor that involves several research stations in various states, many investigators and scores of volunteers — encouraged by hundreds of chestnut nuts cheering on the sidelines and helping when they can. Hope, kept alive by Dr. Arthur Graves of Connecticut from about 1930, and endeavor led to a several decade project. Based largely on his efforts the American Chestnut Foundation was started in 1983 in Virginia to produce a blight resistant tree via the backcross method. This involves as a start crossing adult Chinese chestnut trees with adult American chestnuts. The products of the cross are screened for blight-resistance so that only the more resistant are used in continuing the progression. These survivors are backcrossed to the American parent. Assays for resistance are repeated. Yet again the survivors are backcrossed to the American parents. This is repeated to result in a generation that is 15/16ths American, 1/16th Chinese. Then the off-spring are crossed. This is part of revealing the parents that are not fully resistant. After yet another cross between surviving resistant progeny, the ineffectual American type resistance genes should have been eliminated from the gene pool. Thus, after six crosses a tree is 15/16ths *C. dentata* and 1/16th *C. mollissima* but still has resistance to the fungal blight like the Chinese chestnut. In the expressions of the biological scientists, it is homozygous for resistance, meaning all off-spring have resistance to the fungus *parasitica*.

You ask, Does it work? The project goes well but is not quite far along enough to answer yes or no. Each backcross takes over five years for nut production. With the number of crosses necessary more than 30 years must pass before there are definitive answers. The project s first crosses were made in 1977. So there just a few more years before this endeavor should permit planting of the first groves or forests of the new tree. The degree of success is yet to be seen. Work in progress with hypovirulence could lead to weakening of the extant fungus stock. Then the sprouts from the roots of diseased and dying tree might be able to survive and mature.

Thus you have a glimpse of a wonderful possibility — dare we think probability? There are other approaches and encouraging lines of research on American chestnut restoration. Because of the hope, endeavor, and patient work of a hard core few, and benefits of modern botany, we are close to seeing restoration of what has been, and can be again, a magnificent creation of nature. In addition, the benefits of lessons learned along the way, and the inspiration gained, can be beyond measure.

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ELEGY

Birthed in bursting soil,
the chestnut once feathered her top
in creamy flowers—
miniature puffs on every branch, waiving
farewell to frost ghosts,
budded into papery leaves,
nutted to brown goodness,
retreated in cold to her core.

So cycling for one hundred years—
rounding, heightening-straight and strong,
resisting rot like her granite companions,

until
a fungus of foreign name
stretched its spidery web around her heart,
and, she, leaning east,
signaled her finality—
a massive pounding to moss.

She lies beyond my kitchen window—
chipmunks burying into her softened crevices,
ferns bending to grace her grave-
and, I grieving her softening to eternity.

Gay R. Paluch

A retired teacher, Gay Paluch has found joy in observing nature and trying to capture its beauty and serenity in poetry. She is an editor of *Freshwater: a Poetry Magazine*. Two of her poems about nature, "Brown-eyed Susans" and "Dignity" have been recently published and she is currently working on her first chapbook.

from then to now

HISTORY OF CHESTNUT SURVIVAL IN THE APPALACHIANS (Prehistory to Present)

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The American chestnut (*Castanea Dentata*) is rich in history, having been important to people, animals, and forest ecosystems. Chestnut is central to Appalachian forest history, because it was a common tree and was an important source of food, lumber, and natural beauty from prehistoric times until the chestnut blight pandemic in the early 1900s. After the blight, the chestnut still plays an important role in the forest understory, competing with other tree species and providing food for insects, grazers, and microorganisms. Appalachian history, like all history, is full of change, and chestnuts have so far survived and changed along with the rest of the forest.

Prehistory

Chestnuts arrived in the southern Appalachians from their warm southern Pleistocene refuges sometime between 7000 and 5000 B.C (Russell, 1987). Native Americans, called the Mississippians, appeared in the Appalachians around 900 A.D (Davis, 2000). Chestnut was an important food source for them, and some believe Native Americans were key in chestnut's migration northward (Russell, 1987). These people also regularly burned areas to encourage wildlife, cleared out brushy forest undergrowth, and generally improved their hunting areas. This addition to the natural fire regime certainly impacted the development of forests during the time that Native Americans occupied this land and impacted the growth of chestnuts, which can re-sprout prolifically following fires and can grow quite rapidly when light is increased (Paillet, 2002). A historical relationship between fire and chestnut dominance has been suggested (Russell, 1987).

European Settlement

Europeans began fur trading with the Native Americans in the 1600s and 1700s. Around 1760, Euro-Americans began settling in the Appalachians, bringing livestock and clearing land. Land clearing impacted forests, but livestock had an even greater impact. Livestock were not fenced in, but instead were allowed to roam freely in the forests. Their grazing encouraged certain plants and nearly eradicated others (such as river cane) (Davis, 2000). Pigs had an especially large impact because of their habit of rooting, thus killing rather than cutting back plants. No doubt, grazing and rooting of tree seedlings began to change the forest. Livestock, especially pigs, ate chestnuts, and the new settlers did as well. Germination of chestnuts probably decreased after livestock introduction, though this is speculative. Chestnuts were also important in building log cabins and as fence posts because of their resistance to decay due to the high concentration of tannins in the wood.

Commercialization and Industrialization

Local water-driven sash sawmills were common in the 1800s, but were mostly used to provide lumber to locals. Cuts were normally selective; clearcutting was a rare practice (Sarvis, 1990). Chestnut was certainly a desirable species in selective timbering operations, but its ability to resprout and its rapid growth rate allowed it to recover faster than many other timber species that had to be started from seed. Forests of resprouted (coppiced) rather than seed-germinated chestnut became more common and were often encouraged. Copper and iron mining and large-scale commercial agriculture became more prominent in the Appalachians following the Civil War. The post-war depression caused many small farmers to abandon land and head West or to urban areas, selling their land to commercial landowners. Most areas, especially in the valleys, had been logged or cleared by the 1870s.

The early 1900s was a busy time in Appalachian forests. Not only was the chestnut blight introduced in 1904 in New York and encroaching on expanses of beautiful trees and valuable lumber, but the area's value was just beginning to be realized as the industrial revolution progressed. With the invention of the portable steam sawmill in the 1850s, more areas could be timbered at a faster rate, and timbering increased considerably but was still selective and rather local in scale.

However, with the construction of railroads in the 1880s through the early 1900s, commercial, large-scale logging operations became widespread in the Appalachians (Davis, 2000). Chestnut was highly preferred for railroad ties because of its resistance to decay. Most oak-chestnut forests that had not been logged before were logged during this time period. Railroads made remote forests on high ridge-tops (where chestnuts often grew) accessible to lumbering operations and also increased incidences of intense forest fires during this period. The deer population, which had been almost eradicated by 1900 due to over-harvesting during the fur trade, began to increase as open areas were created through clearcutting operations (Shrauder, 1984).

Figure 1. Floods promoted by logging on watershed slopes damaged a sawmill in May 1901 on the Nolichucky River, TN (above, left). Agriculture on steep slopes caused erosion, as you can see in this small mountain cornfield (above, right).

The Great Depression and the National Forests

Erosion and floods became a problem because of excessive clear-cutting and agriculture on steep slopes (Figure 1) (Ayres and Ashe, 1902). The Weeks Act was passed in 1911 to allow the federal government to purchase forests around watersheds to help prevent floods and topsoil loss (Davis, 2000). This legislation paved the way for the creation of the U. S. Forest Service, which made its first purchase, Pisgah National Forest in North Carolina, in 1912. Purchases of Forest Service land in the Appalachians peaked in the 1930s, when many small farmers sold their land and left for logging or coal camps during the Great Depression and when many timber companies sold off previously logged land. In addition to land sales and the droughts of the Great Depression, it was also during this period that the chestnut blight pandemic swept through the majority of the Appalachians. The Forest Service, often employing the Civilian Conservation Corps, conducted chestnut salvage operations and tree plantings (usually pine), and private landowners adopted these practices as well. The Forest Service also began its forest fire prevention campaign through Smoky the Bear. Fire suppression over the next few decades, combined with forest recovery following the large-scale loss of chestnut from the canopy, caused changes in forest composition, since Appalachian forests had endured periodic ground fires since prehistoric times. However, the conservation and expansion of national forestland probably promoted chestnut's ability to survive through this difficult time.

Recent Times: 1950-2007

In recent times, pollution from urbanized areas has caused acid rain on some high ridges and mountains. The loss of large predators and the decrease in hunting have caused deer over-population in some areas, and deer eat chestnut sprouts. And, to top it off, the climate has fluctuated, warming since the Pleistocene and especially following the industrial revolution, with periods of cold and drought as well.

Surviving Chestnuts

Chestnuts in the wild have lived through major changes in the environment throughout history. It's absolutely amazing that many still survive, though usually in the understory rather than in the canopy. The root stocks of most surviving chestnuts must be at least a half century old (and probably much older) and have endured disease, timbering, fires, grazing, acid rain, and weather extremes.

Research on chestnuts at Mountain Lake Biological Station in southwestern Virginia focuses on linking their past to their present state and also predicting their future. In 1932, the community ecologist E. Lucy Braun sampled old-growth forests on Salt Pond Mountain near Mountain Lake Biological Station finding 50-80% chestnut in the canopy (Braun, 1950). If you go to the same spot on Salt Pond Mountain today, you will find.six chestnuts (about 3%). But, if you travel less than a mile to another stand on the same mountain, you will find an abundance of chestnuts. One begins to wonder: What caused the majority of chestnuts to die on one site and thrive on the other? Chestnut survival varies with many environmental and historical factors, since a live chestnut today has had to keep surviving, or the rootstock has had to continue re-sprouting, for 50 to 100 years.

Today, chestnut as an understory shrub has different survival requirements than it did as a canopy dominant (Table 1). Sites that may have been unfavorable to the chestnut before the blight may be more favorable to chestnut today if they disfavor the blight. By comparing research on favorable chestnut characteristics before and after the blight, one can see how chestnut-preferred habitat has changed since the blight introduction. Chestnut abundance in western Virginia is greater at high elevation, dry sites (Stephenson et al., 1991). Chestnuts also grow better where canopy hardwood basal area is low, suggesting other hardwoods out-compete chestnuts for light (Griffin et al., 1991). Chestnut grows best where light availability is highest (Griffin, 1992). It has been shown that chestnut sprouts are more abundant on old agricultural fields adjacent to former woodlots in New England and Ohio (Schwadron, 1995; Paillet, 2002), although it is unknown if this phenomenon is site-specific or widespread. Chestnut grows best on moist, well-drained sites of intermediate slope when blight is controlled with a virus causing blight

TABLE 1

Comparison of site conditions for highest chestnut abundance before and after blight introduction.

Site characteristic	Most abundant before Blight	Most abundant after Blight	Source
Slope/Elevation	Mid-slope	Mid-slope	Zon, 1904; Stephenson et al., 1991.
Elevation	427-1372 m	1079-1372 m	Braun, 1950; Whittaker 1956; Stephenson et al., 1991.
Soil	Moist, well-drained soil Acid loam Low calcium	Dry soil Acid loam Low calcium	Zon, 1904; Whittaker, 1956; Griffin, 1989; Stephenson et al., 1991; Russell, 1987.
Light requirements	Moderate	High	Zon, 1904; Griffin, 1992.
Aspect	N - W	S	Zon, 1904; Stephenson et al., 1991.

Figure 2. Older chestnut stumps (above, left) produce fewer sprouts the year following cutting than young stumps (above, right). Thus, chestnut survival varies with many complicated factors. When you are walking in the woods and you come across a patch of chestnuts, stop a moment and ponder on what that little chestnut has endured over the years - fires, timbering, disease, drought, ice- and wind-storms, climate change, acid rain, shade competition, and grazing - in order for it to be there today.

hypovirulence (Griffin et al., 1991). However, blight epidemics erupt in the 10 years following a clear-cut on sites with many chestnuts (Hebard, 1982). Because these epidemics are most severe on moist, well-drained sites, chestnut survival was highest on dry sites (Griffin, 1989).

Furthermore, a chestnut growing from a seed and a chestnut sprouting from a rootstock (coppiced) have different qualities that affect their survival (Zon, 1904). Seed germination was not common by the turn of the 20th century, before the blight, due to increases in livestock and humans that ate the seeds and seedlings and also due to the economic value of the nuts and lumber (Zon, 1904). However, the existence of seedlings (rather than coppice sprouts) certainly was more common before the blight. Today, seed production and thus germination of chestnuts is a rare event (Paillet, 1988). Seedlings grow more slowly, have a less developed root system when young, reach a greater height and diameter, and re-sprout for a longer time period (Zon, 1904). Certainly, this faster growth, more developed root system, and shorter re-sprout period affects survival in sprouting chestnuts we find today in the wild. According to the forester Raphael Zon (1904), a root stock will continue to re-sprout, on average, 120 years (though his methods for determining this age are unknown), and older trees produce less sprouts than young trees (Figure 2). Thus, young trees recently germinated from seeds when the blight was introduced may have a greater chance of survival through re-sprouting than trees that were older when the blight epidemic arrived. The timbering and fire history of a site in the early 1900's may also affect chestnut survival by influencing the age of the trees when they were first infected with the blight.

The Future

Predictions of the chestnut's future have covered both extremes, from extinction to survival and preservation as an understory plant. For example, consider Donald Culross Peattie's dramatic words in his "A Natural History of Trees" in 1948: "All words about the American chestnut are but an elegy for it. This once mighty tree, one of the grandest features of our sylva, has gone down like a slaughtered army before a foreign fungus disease, the Chestnut blight." On the other hand, Frederick Paillet in 1988 countered that in a forest stand in Connecticut "the small proportion of total stand basal area composed of chestnut stems has been increasing since 1930," and "American chestnut will be an important component of upland deciduous forests for many years to come." Chestnuts are not yet extinct and do remain important in the forest understory even one hundred years after their extinction was first predicted. However, a decline in chestnuts has been monitored at Mountain Lake Biological Station over the past twenty years (Parker et al., 1993; Wilbur, unpublished data). With so few seed-bearing trees and even fewer successful germinations in the wild, the number of chestnuts that die in a year far exceeds the number of recent seedlings. From this perspective, wild chestnuts must be declining. The American Chestnut Foundation shows great promise for introducing a blight-resistant backcross. When considering American chestnuts growing in the wild, two questions arise: What factors promote their survival? And how much longer can they hang on?

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REVIEW OF THE HISTORIC AND CURRENT STATUS OF THE ASIAN CHESTNUT GALL WASP IN NORTH AMERICA

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The Asian Chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus*) is an exotic insect that infests all *Castanea* species. As with all gall formers, the ACGW hijacks the physiology of its host plant to form abnormal plant growths (galls') in which the immature wasps develop. These galls provide the developing gall wasp larvae with protection and nutrition.

The ACGW produces one generation per year. Adults emerge from the galls in late June or July and immediately lay eggs in dormant buds, where the larvae over-winter inconspicuously. The following spring, concurrent with bud-break, the gall wasp larvae begin feeding and induce the production of leaf or stem galls (Fig. 1A and 1B). Galls are greenish-red and eventually reach 1-1.5 cm (0.4-0.6 inches) in diameter, but can be as large as 3 cm (1.2 inches). Galling prevents normal shoot growth and flowering and eliminates nut production. Severe infestations can kill smaller trees. The ACGW poses a significant threat to chestnut cultivation and American chestnut restoration (Schlarbaum et al., 2001). This article reviews the status of the ACGW in the eastern US, with an overview of our research on biological control.

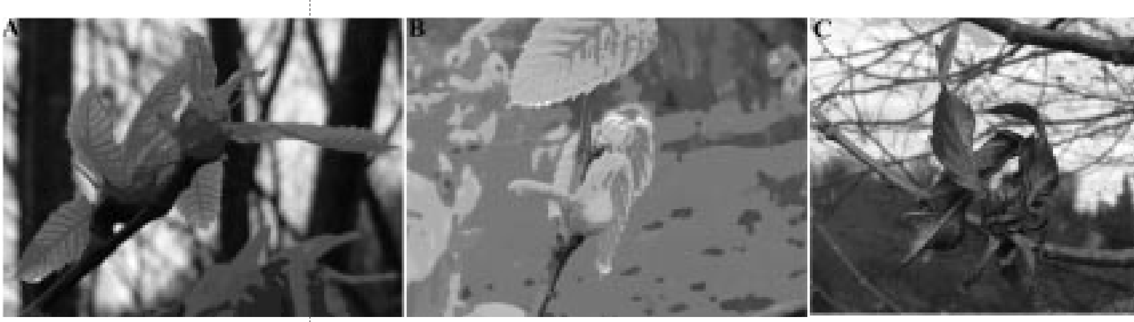

Fig. 1. Leaf (A) and stem (B) galls produced by the ACGW.
After adult emergence, the galls become brown and desiccated (C), but still may harbor parasites.

Asian Chestnut Gall Wasp: A Historic Perspective

A native of China, the ACGW was accidentally introduced into Japan and Korea, where it became a major pest of cultivated chestnut (Yasumatsu, 1951; Murakami et al., 1995). Asian chestnut varieties resistant to galling were initially used for management, but virulent gall wasp isolates soon became dominant, eliminating the efficacy of host plant resistance (Shimura, 1972). In the 1970s, a parasitic wasp (*Torymus sinensis*) (Hymenoptera: Torymidae), was collected from China and released in Japanese chestnut orchards, leading to a rapid decline in ACGW populations (Moriya et al., 2003).

The ACGW was introduced into the United States on infested plant material near Byron, GA (Peach Co.) in 1974 (Payne et al., 1975) (Fig. 2). Within a decade, ACGW had nearly eliminated the chestnut industry in Georgia (Anagnostakis and Payne, 1993), and was also infesting native American chestnut in the southern Appalachian mountains (Anagnostakis, 1999; Schlarbaum et al., 2001). Twenty five years after its introduction, ACGW was found in chestnut orchards throughout the southeastern US (Anagnostakis, 1999). It reached southwestern Virginia and southern Kentucky by 2003 (Fig. 2) (Cooper and Rieske, 2007). An isolated infestation also occurred in northern Ohio (Fig. 2), which likely resulted from movement of infested plant stock (Stehli, 2003; Cooper and Rieske, 2007). ACGW now reportedly occurs in Maryland and Pennsylvania and may be unknowingly shipped throughout the eastern US (Rieske, 2007). More recently, the ACGW was accidentally introduced into European chestnut orchards and now threatens the chestnut industry in Europe (Melika et al., 2003).

Fig. 2. The ACGW was accidentally introduced near Byron, GA and has dispersed throughout the southeastern U.S. (Cooper and Rieske 2007). The natural range of American chestnut is shaded grey.

Advances in Biological Control of the Asian Chestnut Gall Wasp

Throughout the late 1970's several parasitic wasps, including *Torymus* spp., were introduced to Georgia from Japan with the hope of providing biological control against ACGW (Payne, 1978), but the efficacy of these introductions was not tracked. In addition, several native parasitoids were observed emerging from chestnut galls in Georgia (Payne, 1978), but again, their prevalence and effectiveness are not known.

We investigated parasitism in several locations throughout the expanding ACGW distribution in the eastern U.S. We collected galled chestnut from three distinct geographic locations (Fig. 2). The first collection site is on The American Chestnut Foundation's research breeding farm in Meadowview, VA. This infestation was initially noticed in 2001. The second collection site, in Bowling Green, KY, is located in a privately owned deciduous forest mosaic where several tall (5-15 m) American chestnut trees occur naturally, despite infection with the blight fungus. This infestation was first observed by the landowner in the winter of 2003-04. The third collection site is in a suburban setting in Broadview Heights, OH. The infestation was first recorded in 2003 (Stehli, 2003), and occurs on three ornamental Chinese chestnuts.

Galled chestnut shoots were collected from each site at 3 week intervals, from before bud break in April, to after adult emergence in early July (Cooper and Rieske, 2007). Infested shoots were separated into current and previous generations' galls. Current generation galls developed during the spring of 2005 and are green, pliable, and contain within them gall wasp larvae and/or parasites (Fig. 1A and 1B). Previous generation galls are withered, woody galls that do not contain gall wasp larvae, but may still contain parasites (Fig. 1C). Collected shoots were monitored daily for insect emergence.

We collected six parasitic insects from chestnut galls (Cooper and Rieske, 2007). Two of the six occurred in relatively high frequency and may have a significant impact on gall wasp populations. The most prevalent parasite collected, *T. sinensis* (Fig. 3A) (Cooper and Rieske, 2007), had been introduced into the US for ACGW control in the late 1970's (Payne, 1981). It is also a highly successful biological control agent in Japan (Moriya et al., 2003), and its use against the ACGW in Europe is under investigation (Aebi et al., 2006). Larvae of the parasitic *T. sinensis* consume the gall wasp, and remain in the galls until the following spring. *T. sinensis* was collected from previous generation's galls on both American and Chinese chestnuts collected in Virginia and Ohio. Given the success of *T. sinensis* as a sustainable biological control agent in Japan, and the high emergence rate in our collections from Virginia and Ohio, *T. sinensis* may also prove useful for control of *D. kuriphilus* in the U.S.

A second parasitoid, *Ormyrus labotus* (Fig. 3B) (Hymenoptera: Ormyridae), also occurred in high numbers and may play an important role in population suppression of ACGW (Cooper and Rieske, 2007). *O. labotus* is a common generalist native parasitoid of oak gall wasps. It occurred most commonly in the forested Bowling Green, KY site, and emerged from both current and previous generation galls. It was absent from the suburban Broadview Heights, OH site. This has promising implications for restoration of American chestnut, since *O. labotus* could potentially provide natural control of ACGW in mosaic forests containing oak and chestnut.

The remaining four parasitoids we collected are of unknown origin, and are likely associated with native oak galling insects. Nevertheless, they may be able to exploit galls of the ACGW, thereby contributing to the developing natural enemy complex of this pest.

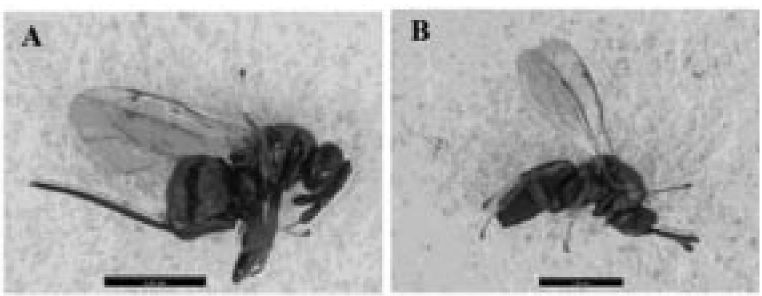

Fig. 3. The introduced parasitoid *T. sinensis* (A) and the native parasitoid *O. labotus* (B) emerged from chestnut galls in high numbers and likely play an important role in population regulation of the ACGW (Cooper and Rieske, 2007).

Current Research

Our initial study evaluated adult parasitoid emergence from galls but it did not provide information on the actual rates of parasitism by *T. sinensis*, and *O. labotus*. In 2006 we added a fourth collection site in a commercial chestnut orchard near Hiram, OH located ~50 km east of Broadview Heights (Stehli, 2006). Woody vegetation at each site was assessed to more fully characterize habitats. Galls were collected monthly from May through August, and a winter (January) collection date was included. Each gall was weighed, measured for volume, assessed for signs of herbivory or pathogen attack, and dissected to reveal the chamber contents. ACGW larvae are easily distinguished from parasitoids based on morphological characteristics. However, *T. sinensis* and *O. labotus* are morphologically indistinguishable. Therefore, we are currently using molecular techniques to evaluate parasitism rates by these two parasitoid species (Fig. 4).

Preliminary results corroborate our earlier findings that *T. sinensis* is the dominant parasitoid in Broadview Heights. *O. labotus* and *T. sinensis* both parasitize ACGW in Meadowview, VA, and *O. labotus* is the dominant parasitoid in Bowling Green, KY. Parasitism is the leading cause of mortality for ACGW, but the rates of parasitism vary between sites. The ultimate goal of this study is to characterize the relationships between the occurrence of the ACGW, its expanding natural enemy complex, and the habitats in which they occur.

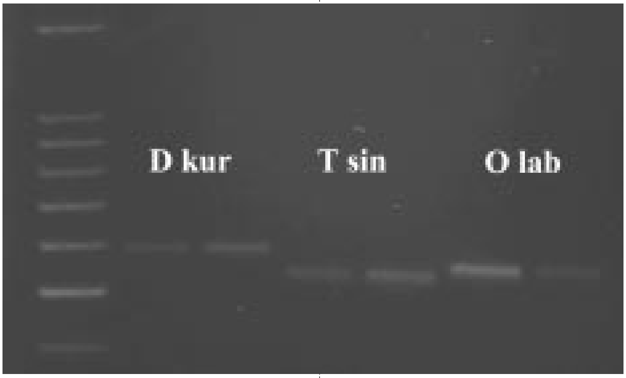

Fig. 4. *T. sinensis* (T sin) and *O. labotus* (O lab) are distinguished based on differentially sized ITS2 DNA products determined by the distance they travel through an electrically charged agar gel. ACGW (D kur) ITS2 is larger than both parasitoids, allowing easy detection of host DNA contamination.

Implications

Our findings have implications for ACGW management in orchards, horticultural settings, and chestnut breeding farms. Chemical suppression of ACGW is impractical because the wasp is protected within the gall for most of its life cycle and no chemical insecticides are presently registered. Cultural control recommendations involve pruning out and burning visible galls (Payne, 1978). However, we now know that many of these galls harbor parasites of the gall wasp, and pruning out galls could be counterproductive in that it also negatively impacts natural enemies. In spite of their unsightly appearance and initial damage, leaving galls on the tree could help promote parasitism and the establishment of *T. sinensis*, and potentially offer sustainable, long-term control of ACGW (Cooper and Rieske, 2007).

O. labotus and other generalist parasitoids commonly associated with oak galls may also suppress the ACGW. The high incidence of *O. labotus* parasitism in our forested site has promising implications for the restoration of American chestnut in close proximity to oaks.

Our work also highlights the importance of careful inspections when transporting chestnut stock (Rieske, 2007). Although finding *T. sinensis* in Broadview Heights is encouraging due to its biological control potential, *T. sinensis* is only found in visible galls, implying that ACGW-infested plant material was shipped to uninfested areas.

The ACGW is spreading throughout eastern North America, and new infestations are problematic. However, over time native and introduced biological control agents become established, potentially reducing ACGW populations. As we learn more about the biology and ecology of the ACGW and its parasitoids in North America, we may develop techniques to propagate and enhance biological control and lessen the impact of this exotic gall wasp.

Acknowledgements

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science and natural history

SMALL STEM CHESTNUT BLIGHT RESISTANCE ASSAY

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The current standard method for testing blight resistance in chestnut (Griffin et al., 1983) typically requires trees that are a minimum of 4 cm (~1.57 in) in diameter (Anagnostakis, 1992). Even though chestnut is known for its rapid juvenile growth, it will often require three or more years for a seedling to reach that size. Although the current methods are accurate and can detect intermediate or partial resistance, it would be useful to have a method to determine resistance at a much earlier age. Hebard and Shain (1989) were the first to develop a method to inoculate small stems 7-14mm in diameter (5-18 month old seedlings) that could differentiate resistant Chinese from susceptible American chestnut. Independently, we have developed a similar assay that can distinguish between susceptible American chestnut and resistant Chinese chestnut on stems as small as 3 mm (~1/8 in) in diameter. Some differences in the two techniques include the wounding method in which we use a thin vertical slit as opposed to a circular hole created with a miniature cork-borer, wrapping the inoculation site with Parafilm instead of tape, and removing the inoculum after 5-7 days as compared to leaving the mycelial plug attached. Both methods are useful, but the method described here has the advantage that it can be used on younger seedlings with smaller stems, thereby saving some time. Both methods have the disadvantage that they appear to distinguish only between trees with high levels of blight resistance and those with essentially no resistance and therefore neither method as described is designed to detect intermediate levels of resistance. We currently use highly virulent isolates of *Cryphonectria parasitica*, strains EP155 (ATCC#38755) and EP42 (ATCC#38751), which can be obtained from the American Type Culture Collection (http://www.atcc.org/). A lesser virulent strain SG1 2-3 (Hebard, 2005) might be able to detect intermediate resistance, but haven't tested this yet. Even though this small-stem assay only detects high levels of resistance, it should prove very useful for screening homozygous F_2 and F_3 trees from the backcross-breeding program (Hebard, 2005) and for screening trees produced from the transgenic program (Polin et al., 2006). Below is the step-by-step method. If you have any questions, please contact Dr. Powell.

Method:

Note: Pure American chestnut seedlings should be used as a susceptible control and pure Chinese chestnut seedlings should be used as a resistant control and included along side of the trees you are testing.

1. Grow test seedlings and controls in pots until the stem diameter about 10cm (~4 in) above the soil line is greater than 3 mm (1/8 in). Depending on how long the nuts have been stratified and the temperature and light intensity of the growth room or greenhouse, it usually takes 12 or more weeks to grow seedlings to the minimum 3 mm diameter. (We routinely grow seedlings in pots in a greenhouse, but the assay might also work with seedlings growing in nursery beds, but this has not been tested.)

2. Once the trees have reached the appropriate size, grow *Cryphonectria parasitica* (EP155 and EP42 were used to develop assay) for 3-5 days on Potato Dextrose Agar (PDA) medium.

3. Using a very fine-line pen, place a mark 1.5 mm from the tip of a sharp scalpel. We use a Fisherbrand #11 scalpel blade. The mark will be used to ensure your cuts do not go too deep and are consistent from seedling to seedling. (Note: you should also be able to use a razor blade, Exacto knife, or other very sharp, thin cutting tool.)

4. With a marker pen, place two marks 5 mm apart (one above the other) on the stems about 10 cm above the soil line.

5. Sterilize the tip of the scalpel by dipping in 95% ethanol and then passing the tip though a flame. With the sterile scalpel, cut a thin, 5 mm long wound (from mark to mark) parallel to the stem. This might take a few passes through the same cut to get down to the 1.5 mm depth. (Note: the idea is to make a thin vertical slit in the stem, not to carve out a piece of stem. When done correctly, no wood or bark is removed. This step is critical to a successful assay.)

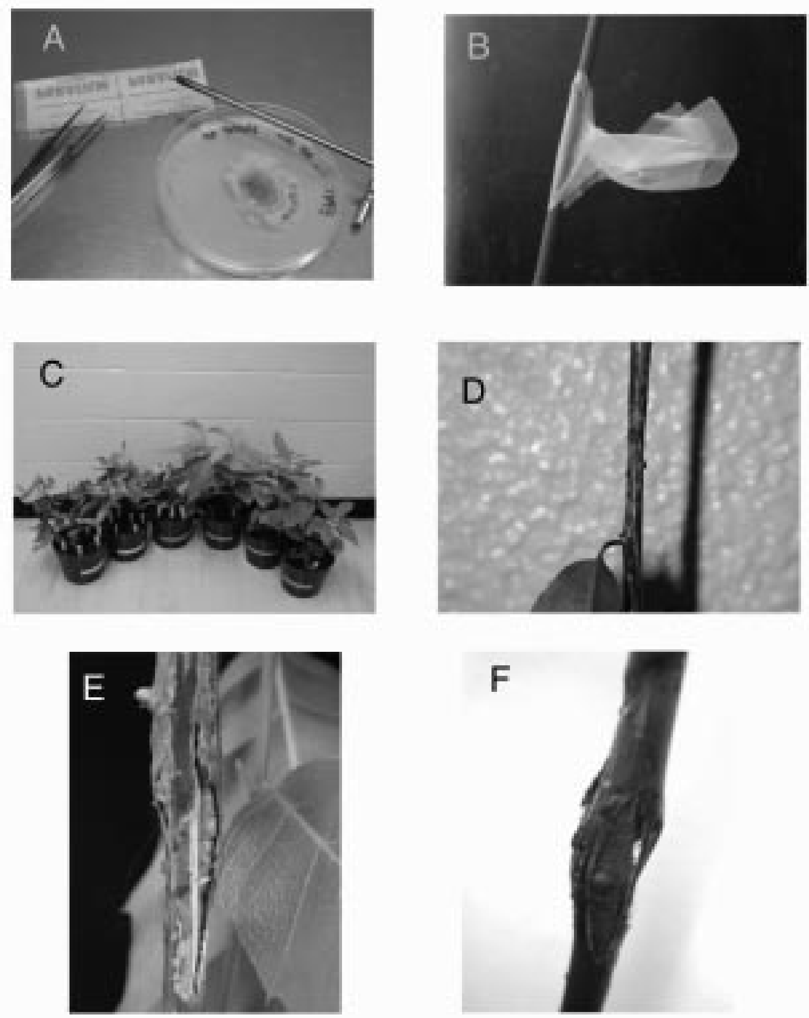

Figure 1. Examples of selected stages of the small stem Chestnut blight resistance assay.

A. *Cryphonectria parasitica* prepared for inoculation.
B. Fungal plug placed on wound and sealed with Parafilm.
C. American chestnut (left) and Chinese chestnut (right) after a few weeks.
D. Killing canker on American chestnut seedling stem
E & F. Healing cankers on Chinese chestnut seedling stems

6. Aseptically cut many plugs of mycelium from the front edge of the *C. parasitica* colony using a #1 cork borer (~3 mm diameter or 1/8in.) (Figure 1A). (Note: you can also cut the plugs in other ways, such as using the scalpel to cut 3mm size squares.) Remove some of the excess agar from the bottom of the plug to make it easier to handle.

7. Take a strip of Parafilm cut to approximately 2.5 cm (1 in) by 10 cm (4 in) and stretch it slightly to make it pliable. Place a plug, mycelium side up, on a strip of Parafilm (Figure 1A). Then carefully place the mycelium against the wound while pulling the parafilm against the stem to make a seal (Figure 1B). Leave enough Parafilm hanging off the opposite side of the stem for easy removal later. This is an important step. Make sure the mycelium is in firm contact with the wound and seal the parafilm around the stem so that the plug does not dry out. (Note: if the mycelium plug slips out of the parafilm, start over with a new plug and a new strip of parafilm.)

8. Leave the mycelial plug parafilmed to the wound for 5-7 days. (Note: 3 days was too short in our tests.)

9. Remove the parafilm and plug and allow the canker to grow.

10. You should see a difference between the controls in about 3-4 weeks (Figure 1C-F). The resistant plants will form callus at the inoculation site and retain their leaves. The susceptible plants will have a sunken canker that completely encircles the stem, the leaves above the inoculation site will wilt, and sometimes new shoots will form below the inoculation site. (Note: We have been able to rescue and reuse many of the susceptible American chestnut seedlings after the assay by cutting off the stem several cm below the canker and allowing a new shoot to form either from an axillary bud or from the root collar.)

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DECOMPOSITION OF AMERICAN CHESTNUT LEAVES

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Introduction

The elimination of an important species, such as American chestnut (*Castanea dentata*), from forests may cause significant changes in the rate at which nutrients are recycled in the ecosystem. Nutrient cycling in a forest ecosystem is affected by a number of factors-geology and topography, climate, disturbance history-but one of the most important factors is the species of plants that comprise the forest. Tree and shrub species vary in the chemical components of their tissues, which affects the rate at which those tissues eventually decompose and release nutrients for reuse. For instance, alders (*Alnus* spp.) have nitrogen-fixing bacteria associated with their roots allowing their leaves and fine roots to contain more nitrogen than most other species. Oaks (*Quercus* spp.) defend their leaves and seeds with high quantities of condensed tannins, which bind proteins and make these tissues less digestible. Structural compounds, such as lignin and lignified cellulose, and defensive compounds, such as tannins, terpenoids, and alkaloids, make leaves and twigs less palatable to herbivores. These compounds also slow their decomposition and release of nutrients. Swift et al. (1979) suggested that the rate at which plant residues decompose is determined by the interaction among the chemical makeup of the residue, the physical and chemical environment, and the activities of decomposer organisms. Trees, through leaf and root senescence, contribute the organic matter that serves as both environment and nutrient source for soil organisms. Soil organisms, in turn, break down plant residues and release nutrients for plant and microbial uptake. This process of decomposition is largely conducted by soil bacteria and fungi, but their activities are mediated by soil invertebrate animals.

The studies described here begin to examine the ecological impact of the loss of American chestnut from Connecticut forests. The data presented in this paper were collected as part of undergraduate research projects by two students, Kristen Zepko and Breamond Ostrander, at Western Connecticut State University.

Methods

This research was conducted in the Westside Nature Preserve on the campus of Western Connecticut State University, Danbury, CT. The 13 ha site, a second growth forest on hilly terrain, was part of a dairy farm for at least 200 years prior to 1930. Current tree species on the site include Northern red oak (*Quercus rubra*), American beech (*Fragus grandifolia*), and pignut hickory (*Carya glabra*) in well-drained soils and red maple (*Acer rubrum*), witch-hazel (*Hamamelis virginiana*), and tulip poplar (*Liriodendron tulipifera*) in moist soils. Sugar maples (*Acer saccharum*) are found throughout. There are also a few American chestnuts in the understory.

In the autumn of 1999, red oak, sugar maple, and American chestnut leaf litter was collected from the study site. Oak and maple leaves were raked from the ground promptly after senescence. Marcescent American chestnut leaves were picked from the trees. The air-dried leaves were sorted by species and put into packets. Each packet consisted of approximately five grams of leaves tied together at the petioles by monofilament line. The packets were tethered to a flag and placed on the forest floor on 15 November, 1999. Twenty packets of each species were placed in a well-drained site high on a hill slope and a moist-soil site near a small stream. There were 120 packets in all. Each month through May 2000, five packets of each species were retrieved from each site. Retrieved packets were air dried and weighed.

In autumn 2002, a similar study was done at the same location with the following differences. A fourth species, American beech (*Fragus grandifolia*), was added to red oak, sugar maple, and American chestnut. For this study, the American chestnut leaves were collected from the Connecticut Agricultural Experimental Station in Hamden, CT. The station maintains an orchard of American chestnut trees infected with a hypovirulent strain of the blight fungus. Each leaf packet contained five leaves and was placed on the forest floor on 2 December, 2002. Packets were retrieved monthly through July, 2003. Data from both studies were analyzed by Analysis of Variance (ANOVA).

Results

In the 1999-2000 experiment, litter mass loss was observed through the winter months. There were significant differences in mass lost among the three species ($P < 0.001$) and between the two sites ($P = 0.008$). Bonferroni post-hoc tests showed that American chestnut litter lost mass fastest, and red oak the slowest (Figures 1 and 2).

The 2002-2003 data covered winter, spring, and early summer. Over this period, American chestnut and sugar maple lost similar mass, but both lost more than American beech and red oak (Figure 3; $P < 0.001$). There was no difference between sites.

Discussion

The environment for decomposition varies over time as seasonal and climatic conditions vary. The sites-one high on the hill slope and well-drained, the other low on the slope in moist soil near a small stream-were less than 50 m apart and both shaded by canopy trees. We expect them to be similar in temperature and soil nutrient availability.

The 1999-2000 study reflects decomposition in the winter months. Winter processes are understudied, but decomposition and microbial activity in the soil at this time can be significant (Taylor and Jones, 1989). Soil fauna are mostly inactive during the winter months in New England and similar climates. Therefore, this first study reflects the activities of soil microbes only. The 2002-2003 study extended through spring and early summer, a period when the soil invertebrates that eat leaf litter are active. This, along with differences in weather between the years, may explain differences in decomposition we observed.

Both of the experiments confirm that American chestnut litter decomposes quickly in comparison to other common species. Sugar maple litter has previously been shown to lose mass rapidly (Gosz et al., 1973). This is due to lower quantities of some plant secondary metabolites, as well as to the thinness of the leaves. Oak species produce thick, leathery leaves that contain a high quantity of tannins.

American beech appears to decompose similarly to red oak. There are in the same family of plants (Fagaceae) and may share a similar chemistry. American chestnut is also in this family, but decomposes quicker than American beech or red oak. We do not currently have data on the tannin content of American chestnut leaves, although the bark and wood were previously important as a source of tannic acid for the leather tanning industry. American chestnut decomposes quickly at least in part due to its nitrogen content, which is significantly higher that the other three species considered here. The winter activity of decomposer microbes on American chestnut litter appears to be high. Other data shows that not only does chestnut litter start with high N, but it accumulates N at a high rate throughout the winter (Slemmer and Wagener, unpublished data). The rate at which leaf litter decomposes determines the rate at which the nutrients contained in that litter are made available for uptake by plants and microbes. If a tree with leaves that contain a high N content and decompose fast, such as the American chestnut, is replaced by species with less N and slower decomposition, overall rates of nutrient cycling may decrease.

Conclusion

The rate at which nutrients cycle in a forest ecosystem is influenced by the plant species present. When an abundant tree, such as the American chestnut, is virtually eliminated, the effects could be significant. Our study found that American chestnut leaf litter decomposes rapidly as compared to other common species of New England forests. This may be due to the high nitrogen content of American chestnut leaves.

When trying to understand the ecological impact of the chestnut blight, we must consider the contribution of the American chestnut to nutrient cycling in the forest ecosystems to which they previously played such a large role. This study is a first step in addressing this question.

Acknowledgements

We wish to acknowledge the contribution of Dr. Sandra Anagnostakis, Agricultural Scientist at the Connecticut Agricultural Experimental Station in Hamden, CT, who allowed us to rake up her chestnut leaves.

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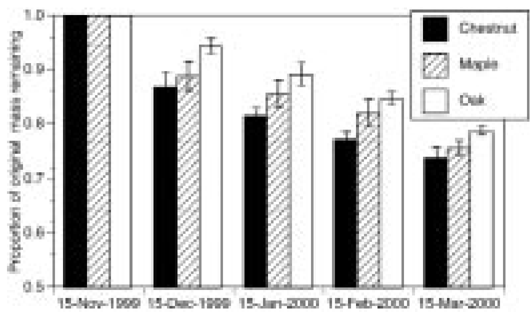

Figure 1. Mean mass loss of American chestnut, sugar maple, and northern red oak litter at a well-drained site in 1999-2000. Error bars represent standard errors.

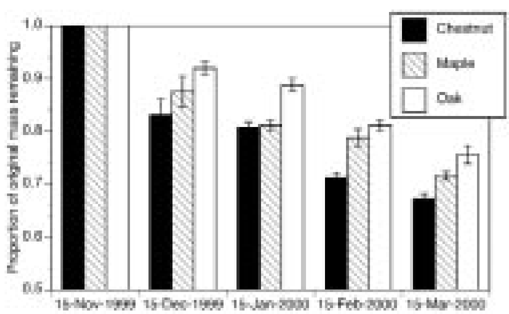

Figure 2. Mean mass loss of American chestnut, sugar maple, and northern red oak litter at a moist-soil site in 1999-2000. Error bars represent standard errors.

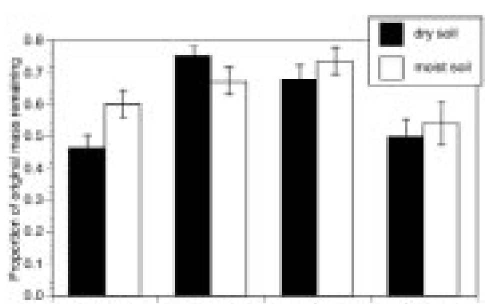

Figure 3. Mean mass loss of American chestnut, American beech, northern red oak, and sugar maple litter from December, 2002 - July, 2003. Error bars represent standard errors.

MEADOWVIEW NOTES 2006-2007

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American Chestnut Foundation Research Farms, Meadowview, VA

Meadowview experienced fairly normal rainfall in the summer of 2006, and adequate rain in the fall, but it was fairly dry in January thru March of 2007, and extremely dry in May and early June. There was enough rainfall in April to delay spring plowing for local farmers, but our plowing in February and March was not hampered by wet weather beyond late February.

Inventory.

Our current holdings are presented in Table 1, and changes from 2006 to 2007 are indicated in Table 2. We now have more than 33,000 trees and planted nuts, an increase of approximately 7,400 over last year (Table 2). The addition of B_3-F_2 trees has been offset by the removal of straight backcross trees as we have made selections and rogued the rejects. We now have screened all of our `Clapper' $B_3$ trees for blight resistance and completed rouging of rejects in those orchards. We also are largely finished screening and roguing our `Graves' B_3 trees. Many of the B_3 trees listed in Table 1 are from sources of blight resistance other than `Clapper' or `Graves.' There also is now significant roguing of rejected B_3-F_2 trees, so their numbers may be close to peaking, with new plantings offset by removals. We have some hope that we will finish planting `Clapper' B_3-F_2 seed in the next two to three years.

Our 14 state chapters have numerous additional `Clapper' and `Graves' B3 trees to those reported in Table 1. The numbers of trees in the chapters is now reported separately in the Spring edition of the Journal.

Harvest.

The most noteworthy event of the 2006 harvest (Table 3) was that it was our largest crop ever, including seed from open pollination. The crop from controlled pollinations was our second largest ever, and we placed the largest number of bags ever this year.

Part of the reason for the large number of bags was that Bob Paris headed up an effort to make a number of crosses among pure Chinese chestnut trees, to see whether we can determine if multiple sources of resistance to chestnut blight exist within the Chinese chestnut population. We also made crosses on a more limited scale with European, Japanese, and large surviving American chestnut. In January of 2007, we made grafts of the Chinese parents in the greenhouse, and in the spring we planted the grafted parents along with the progeny from the crosses in three plots. Based on emergence data, we had to redo some cross combinations in the summer of 2007. We hope that examination of the progeny under blight screening will reveal additional genes for resistance not already in use in TACF's breeding program. Additional sources, if found, could prove valuable to the breeding program by possibly providing increased levels of resistance. However, if no new sources of resistance are found, we will know that the current breeding efforts are being maximized with all of the available resistance from Chinese chestnut.

The yield from controlled pollinations was average, about one nut per pollination bag, which was a welcome respite from last year, when yield was very poor.

We used a large number of dried pollens this year, but the yield from those pollinations was much worse than some of the chapters report, around 0.7 nuts per bur. This was significantly ($p<0.05$) better than the yield of pollinations with fresh catkins, however, which was about 0.4 nuts per bur. The yield from open pollinations was about 1.0 nuts per bur.

The open-pollination yield from older, larger trees may have been higher (about 1.3 nuts per bur) than the yield from smaller younger trees (about 0.8 nuts per bur). This difference in tree size also may be associated with the better yield seen in some chapter pollinations than in pollinations at Meadowview. After accounting for tree size, there were no apparent differences between the yield from open-pollinated backcross trees and the yield from open-pollinated pure species. This question will need to be revisited when it is not confounded with tree size.

Last year, William White measured the germination of all pollens sent out to the state chapters and compared that to nut yield. As we found in three previous years of this comparison, there was no correlation between the percentage of pollen grains that germinated and nut yield. This past winter, William worked long hours to optimize the pollen germination assay. We found that pollen and sugar concentration were critical factors in germination, with optimal germination occurring at concentrations of about 4 mg of pollen per 1.5 ml of 0.5% (w/v) sucrose solution.

More complete instructions are posted on our website at http://www. acffarms.org/papers/Pollen%20germination%20assay.pdf.

With the improved assay, we measured the germination of all pollens sent out this year. Several B_3 pollens germinated at over 70%, which is much higher than reported previously for chestnut. Most B_2 and B_3 pollens germinated at levels comparable to those seen in pure species, although pollens from more pure species will need to be examined to confirm this. It will be interesting to see whether we can establish a correlation with nut yield using germination data from the improved assay.

One point that emerged concerning the pollen assay was that pollens that had been taken to the field appeared to have poorer germination than those that had remained in the refrigerator. It may be helpful to store your pollen in a cooler on ice except when in use. To avoid freeze injury, you may want to ensure that the pollen is insulated from the ice itself by crumpled up paper or a similar item. However, if you remove pollen from the refrigerator, be sure to let it warm before removing the vial cap, to avoid condensation of water on the cold pollen.

Improving chestnut establishment on croplands.
Bob has also begun a multi-year experiment (2006-2010) to address some of the challenges in establishing chestnut on lands previously used for cropping. We suspect that chestnut establishment and growth on land formerly used for cropping may be affected by the type of crop previously grown. As TACF approaches the time when chestnut plantings will be on the increase by land owners, we think it will prove valuable to know how to most efficiently and successfully grow chestnut. We designed this experiment to examine the effects of chestnut growth following corn, tobacco, and grass crops on land that has been tilled for crop production, and on pasture land that has remained relatively undisturbed. We hope that, based on this experiment, we will be able to make recommendations for chestnut establishment on various types of land.

Blight resistance screening in B_3-F_2 seedlings.

The year 2006 was the third in which we screened 'Clapper' B_3-F_2 seedlings for blight resistance and the second for 'Graves' B_3-F_2 seedlings. The results of the 'Clapper' tests are presented in Table 4 and those for the 'Graves' test in Table 5. This year we inoculated many more 'Clapper' and 'Graves' B_3-F_2 seedlings than in 2004 and 2005. However, as discussed in last year's Meadowview Notes, we have moved to staged screening for blight resistance in B_3-F_2 seedling seed orchards, which means this year we only inoculated with a relatively nonaggressive, but virulent, strain of the blight fungus. This will separate out the weakest trees, those in resistance classes 4 and 5. Next year, in 2008, we will inoculate the stronger trees from the 2006 test with an aggressive strain of the blight, to separate out the strongest trees.

Interestingly, this year, for the first time, we saw statistically significant family differences between American backgrounds, for both the 'Graves' and 'Clapper' sources of blight resistance. Further testing will reveal whether the difference has biological significance.

Overall, Fred Hebard has cautious optimism that we will be able to recover highly blight-resistant progeny among the B_3-F_2 seedlings from the 'Clapper' and 'Graves' sources of resistance, but we certainly do not have firm evidence for this yet. Stay tuned!

We would like to thank Lou Silveri, Dave Lazor, Dick Olsen, Steve Barilovits, and Sam Fisher for helping with pollinations and inoculations. Special thanks to Dave Slack for volunteering two days a week all year round for the past two years(!), and to Ignazio Graziosi for interning this summer. Also, we need to acknowledge the role of George Sykes and Danny Honaker in keeping the farms running from day to day. Thanks to all — this wouldn't get done without their help. If you are interested in helping to pollinate next year, plan on any time in June (call 276 944-4631). If you are interested in learning more about the Elder Hostel program, call 617 426-8055 or write 75 Federal St., Boston MA 02110.

We would like to remind all TACF members that you are welcome to visit the farms at any time. We are in a white house on the northeast side of Virginia Route 80, one-third of a mile southeast of Exit 24 on Interstate 81, the Meadowview Exit. We generally are there during normal work hours, but it might be good to call ahead (276 944-4631).

TABLE 1

Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2007, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

	Number of		
Type of Tree	**Nuts or Trees**	**Sources of Resistance**	**American Lines***
American	2162		222
Chinese	1149	53	
Chinese x American: F_1	511	20	83
American x (Chinese x American): B_1	582	16	40
American x [American x (Chinese x American)]: B_2	1683	11	95
American x {American x [American x (Chinese x American)]}: B_3	1683	9	78
Am x (Am x {Am x [Am x (Chin x Am)]}):B_4	30	3	3
(Chinese x American) x (Chinese x American): F_2	253	5	9
[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	471	4	6
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	223	5	7
(A x {A x [A x (C x A)]}) x (A x {A x [A x (C x A)]}):B_3-F_2	18169	2	35
B_3-F_3	217	1	5
Chinese x (Chinese x American): Chinese B_1	191	3	3
Chinese x [American x (Chinese x American)]	41	1	1
Chinese x Chinese	2255		
Chinese x Japanese	109		
Chinese x European	140		
Chinese x Large, Surviving American	28		
European	1	1	1
European F_1	2	1	1
Japanese$_1$	3	3	3
Japanese F_1	11	2	2
Japanese B_1	10	2	2
Japanese B_2	133	2	2
Japanese x European	157		
Japanese x Large, Surviving American	42		
Castanea seguinii	48	3	3
Large Surviving American F_1	548	15	32
Large Surviving American B_1	531	8	27
Large Surviving American B_2	9426		
Large Surviving American I_1	1411	19	21
Large Surviving American F_2	374	5	10
Large Surviving American other	146	10	13
Other33			
Total	**33456**		

* 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 parent would be counted as two lines rather than one line (this only occurs rarely).

TABLE 2

Changes between 2006 and 2007 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.

	Increase or Decrease* in Number of		
Type of Tree	Nuts or Trees	Sources of Resistance	American Lines
American	-1		-13
Chinese	457	2	
Chinese x American: F_1	-12	-2	-7
American x (Chinese x American): B_1	157	1	7
American x [American x (Chinese x American)]: B_2	124	1	4
American x {American x [American x (Chinese x American)]}: B_3	-2135	0	1
Am x (Am x {Am x [Am x (Chin x Am)]}):B4	21	2	2
(Chinese x American) x (Chinese x American): F_2	457	-1	3
[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	-298	0	-2
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	-118	0	-2
(A x {A x [A x (C x A)]}) x (A x {A x [A x (C x A)]}):B_3-F_2	5793	0	6
B_3-F_3	96	0	3
Chinese x (Chinese x American): Chinese B_1	0	-1	-1
Chinese x [American x (Chinese x American)]	0	0	0
Chinese x Chinese	2255		
Chinese x Japanese	109		
Chinese x European	140		
Chinese x Large, Surviving American	28		
European	1		
European F_1	-1	0	0
Japanese	10	1	1
Japanese F_1	0	0	0
Japanese B_1	0	0	0
Japanese B_2	110	1	1
Japanese x European	157		
Japanese x Large, Surviving American	42		
Castanea seguinii	0	2	2
Large Surviving American F_1	220	2	3
Large Surviving American B_1	128	1	16
Large Surviving American B_2	0	0	3
Large Surviving American I_1	722	9	3
Large Surviving American F_2	-74	-1	2
Large Surviving American other	-29	1	3
Other	-2		
Total	**7443**		

* The decreases in B_1, B_2, B_3, and large, surviving American B_1 & F_2 trees reflect roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting.

TABLE 3

The American Chestnut Foundation Meadowview Research Farms 2006 nut harvest from controlled pollinations and selected open pollinations.

Nut Type	Female Parent	Pollen Parent	Pollinated nuts	Pollinated bags	Pollinated burs	Unpollinated Checks nuts	Unpollinated Checks bags	Unpollinated Checks burs	Number of American Chestnut Lines*
AxA	American	American	225	83	131	0	9	16	2
B_1	European F_1	American	95						1
B_1	mollissima11 F_1	American	102	171	332	1	12	28	3
B_1	mollissima12 F_1	American	286	284	812	0	23	90	2
B_1-F_3	Clapper;Graves B_1-F_2		2657		1446	open pollinated			10
B_2	72-211 B_1	American	6	107	174	0	11	17	2
B_2	American	Nanking B_1	152	211	449	6	25	61	19
B_2	Nanking B1	American	301	706	2071	0	87	235	18
B_2	PI#104016 Japn B1	American	193	126	405	1	13	33	3
B_2-F_2	Clapper B_2		182		161	open pollinated			3
B_2-F_3	Clapper B_2-F_2		5872		4342	open pollinated			5
B_2-F_3	Graves B_2-F_2		729		698	open pollinated			3
B_3	American	Meiling B_2	5	44	268	0	8	14	1
B_3	American	Nanking B_2	68	108	277	3	13	30	7
B_3	Meiling B2	American	10	15	48	0	2	8	1
B_3-F_2	Clapper B3		9365		11215	open pollinated			47
B_3-F_2	Graves B3		7609		10092	open pollinated			27
B_3-F_2	Graves B3	Graves B_3	194	324	1037	0	32	145	3
B_3-F_3	Clapper B3-F2		97		134	open pollinated			7
B_4	American	R11T14 B_3	9	20	30	0	5	8	2
B_4	Douglas B3	American	8	22	38	0	2	3	1
B_4	R11T14 B3	American	6	122	196	0	10	15	1
F_1	Kuling Chinese	American	7	77	132	0	8	17	1
F_1	Nanking Chinese	American	212	364	610	0	40	40	1
F_1	Richwood Chinese	American	11	19	34	0	2	4	1
F_1	opMacBoyd Chinese	American	15	32	55	0	2	3	1
LSA F_1	American	DaresBeach LSA F_2	141	58	198	0	5	11	2
LSA B_1	Amherst LSA F1	American	9	15	30	0	2	6	1
LSA B_1	DaresBeach LSA F2	American	1	39	68	0	4	8	1

(Continued on next page)

TABLE 3 *(continued)*

Nut Type	Female Parent	Pollen Parent	Pollinated nuts	Pollinated bags	Pollinated burs	Unpollinated Checks nuts	Unpollinated Checks bags	Unpollinated Checks burs	Number of American Chestnut Lines*
LSA B_1	NCChamp LSA F_1	American	20	171	257	0	14	25	2
LSA B_2	NCF179 LSA B_1	American	4	28	52	0	2	4	1
LSA F_1	American	CareyMac2 LSA op	144	108	241	0	61	22	6
LSA F_1	American	WayahBig LSA op	54	76	213	0	10	37	4
LSA F_1	CareyMac2 LSA op	American	6	14	47	0	1	3	1
LSA F_1	GaultSciCliffs LSA I_1	American	15	19	42	0	1	1	1
LSA F_1	WayahBig LSA op	American	7	21	53	0	1	1	1
LSA I_1	Ort LSA B_1	Amherst LSA F1	172	71	219				2
LSA I_1	Ort LSA B_1	Gault LSA F2	60	75	229	0	8	40	1
LSA I_1	Ort LSA F_1	Weekly LSA F1	129	100	179	0	3	6	1
LSA I_1	SciCliffs LSA B_1	Amherst LSA F1	164	122	297	0	13	34	2
LSA I_1	SciCliffs LSA B_1	Weekly LSA F1	200	76	292	0	8	27	1
LSA I_1	SciCliffs LSA F_1	Weekly LSA F1	14	17	31	0	1	1	1
CxC	Mahogany Chinese	Nanking Chinese	319	289	494	2	30	39	5
CxC	Nanking Chinese	Mahogany Chinese	43	209	416	0	22	47	2
	Parent 1	**Parent 2**							
CxC	Eighteen Chinese	Mahogany Chinese	773	344	546	0	31	46	
CxC	Eighteen Chinese	Meiling Chinese	227	431	657	2	31	48	
CxC	Eighteen Chinese	Nanking Chinese	558	373	593	1	28	39	
LSAxJ	Five LSA American	Japanese	45	87	197	0	8	11	
LSAxC	Five LSA American	Nanking Chinese	27	66	122	0	9	15	
ExJ	European	Japanese	147	25	61	0	4	8	
ExC	European	Chinese	143	29	62	0	2	5	
JxC	Japanese	Chinese	119	84	143	0	6	17	
Total Controlled Pollinations			**5446**	**5782**	**12838**	**16**	**609**	**1271**	

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

TABLE 4

Number of 'Clapper' B_3-F_2 seedlings ranked in various blight resistance classes in 2006.

Susceptible Great Grandparent	LS Mean Resistance Rating**	Standard Deviation of Resistance Rating	Number of Progeny Tested		Blight Resistance Class* 3	4	5
QBF3CL	4.0	BC	0.7	295	75	146	74
LFR4T14	4.0	C	0.7	308	98	103	107
QBA1CL	4.1	BC	0.7	65	16	31	18
LFR4T10	4.1	BC	0.7	132	39	40	53
Bu3C1C	4.2	BC	0.6	72	13	38	21
LFR4T9	4.2	BC	0.7	628	142	226	260
LFR4T12	4.3	BC	0.6	23	2	10	11
LFR4T1	4.3	B	0.7	142	29	49	64
QBF2CL	4.4	ABC	0.6	96	17	34	45
HBF2C	4.6	A	0.5	83	5	17	61
QBF3CL	4.0	BC	0.7	295	75	146	74

* Trees were only inoculated with a weak, but virulent strain of the blight fungus in early June. A rating of 3 indicates that the cankers were small, about 1-cm long, 5 months after inoculation. A rating of 4 indicates the cankers were slightly larger, 2-4 cm long, and a rating of 5 indicates the cankers were over 5 cm long.
** Means followed by the same number are not significantly different at $p<.0.05$ by a Tukey-Kramer HSD test.

TABLE 5

Number of 'Graves' B_3-F_2 seedlings ranked in various blight resistance classes in 2006.

Susceptible Great Grandparent	LS Mean Resistance Rating**	Standard Deviation of Resistance Rating	Number of Progeny Tested		Blight Resistance Class* 3	4	5
HBF1G	3.1	C	0.6	10	8	2	0
QBF3G	3.7	B	0.7	137	57	51	29
HBW1G	3.8	ABC	0.8	31	12	10	9
Bu3C3C	3.9	AB	0.8	263	89	88	86
PaulGalloway	4.0	A	0.7	415	125	170	120

* & ** See footnotes to Table 4.

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, or B-three
B_3 x American	=	B_4, fourth backcross, or B-four
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-one 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

Castanea Guide: A Quick Comparison of Chestnut Species

	CHINKAPIN	JAPANESE	EUROPEAN	CHINESE	AMERICAN
Leaf Taper to Stem	straight	curved	curved	curved	straight
Taper to tip	straight	curved	curved	curved	straight
Teeth	1-3 mm, small, sharp, no hook	Tiny, often only bristles, no hook	Big, sharp or rounded, no hook	Large or small, no hook	6mm, big, sharp, and often curved (hooked)
Leaf Underside	*Sun leaves noticeably hairy	Sun leaves noticeably hairy	Sun leaves noticeably hairy on some specimens but not others	Sun leaves obviously hairy	Sun leaves not hairy, long sparse hairs only on midrib
Twig (those that have overwintered at least one year)	hairy tips, purple or brownish grey	Pink to light red, large white **lenticels	Stout, dark, brown, small white lenticels	Hairy tips, Tan to pea green Large elliptical yellow lenticels	Slender, smooth, hairless reddish brown, small white lenticels
Bud	Up to 3 mm, downy dark red, pointed, longer than wide, sticks out from stem	Glossy brown, As long as it is wide (rounded)	Dark red, fat and globular	Hairy, tan, dull brown to black, rounded and flat against stem	Up to 6mm, smooth, reddish brown to yellow, pointed, or longer than it is wide, sticks out from stem.
Nut* **	1 nut, ½" tip pointed with a round cross section	2-3 nuts, 1-2" no sunburst pattern at base, moderate brown	2-3 nuts, 1-2 " no sunburst pattern, dark brown, black stripes	2-3 nuts, ¾- 2", rounded hairy tip, no sunburst pattern, often light brown	2-3 nuts, ½ -1", pointed tip, top ⅓ to ⅔ downy, sunburst at base
Taste** **	sweet	not sweet	starchy	sweet	sweet
Resistance to blight:	None	Moderate	Slight	High	None

*Sun leaves arethose leabes that are most exposed to the sunlight on a tree.
** A lenticel is an aerating organ on the surface of a twig or branch. They may appear as bumps on the surface of twigs.
*** Nut size may vary a lot within each species. Sizes provided are maximum possible.
****Taste refers to those commonly found in the U.S. and may not reflect that of all members of a species.
Be aware that all chestnuts can cross-pollinate, so that a tree that seems clearly of one species or another, may actually be a mix of two or more different types of chestnuts, known as hybrids. Please refer to TACF's website www.acf.org for more information on identifying American chestnuts.

The American Chestnut Foundation is incorporated in the State of Virginia. It is exempt from tax under §501(c)(3) of the Internal Revenue Code, and is qualified as a public supported organization under §509(a)(1) and §170(b)(1)(a)(vi) of the Code. Donors to The American Chestnut Foundation may deduct contributions as provided in §170 of the Code.

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