

THE AMERICAN CHESTNUT

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SPHEX, September 7, 2023

SLIDE intro

There are notable North American species that we know are extinct in the modern era
passenger pigeon
Carolina parakeet
Franklin tree and 4 other small trees, as well as 65 other plants
For most people, we would likely put the American Chestnut on that list.

I would have too, until a couple years ago. We have a Wednesday hiking group that is frequently up in the mountains, often on the Appalachian trail. Occasionally we'll stop to look at the flora around us. Tom Melton, one of our hikers turned to me at one spot and said, "you know, that's an American chestnut." I was shocked. It was like turning around and getting introduced to George Washington! So it is not actually gone. It is "functionally extinct".

Bringing back the Woolly Mammoth from extinction with old DNA may be a long shot and controversial, but bringing back the ACN is a story for our day.

SLIDE giants

It's hard to understate the significance of this species to the Eastern US and particularly to Appalachia. In areas of concentration, it comprised as much 1 in every 4 trees in the forest.

"Keystone" or "Foundational" species, as its presence dictated the character of the forest, what grew nearby, and which animal populations were favored.



SLIDE family tree

Similarly, it prescribed the lifestyle and diet of those who lived in its range as it provided food, for both people and livestock, shelter, tools and a cash crop.



SLIDE perfect tree

- Fast growing
- Straight grained
- Fairly strong
- Very rot resistant
- Abundant nut crop (the "mast"), reliable every year

Only thing it wasn't good for was burning. Low heat generation. Tended to spark and fire was always a danger. The Iroquois Confederation constitution banned its use during official assemblies

SLIDE green tree

They were beautiful, giant trees, frequently 7 ft in diameter. They had dense dark green foliage and vertical dominant trunks that would sometimes rise 40 feet before branching with total heights reaching over 100 ft.



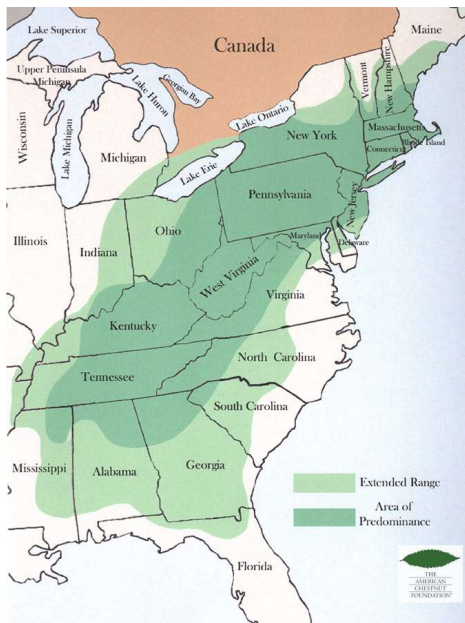
SLIDE blossom

In Spring the trees would cover the landscape with white to yellow blossoms and then fill the air with light clouds of pollen blowing across the treetops. Scented, but not always described as sweet or particularly pleasant.



SLIDE burrs

In Fall the chestnut burrs would form, eventually dropping to the ground providing a “banquet” of easily obtained nutrition as the nuts were released across the forest floor.



SLIDE range

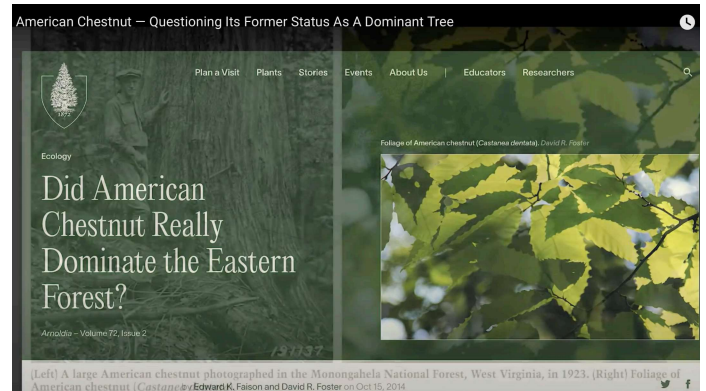
This was the approximate range when Europeans arrived in North America, although *Castanea dentata*, the American Chestnut, had already been on the continent approximately 24 million years. It established itself in the southern regions of what would be the United States after the last Ice Age, 20,000 years ago. As the earth warmed, it gradually moved North into its modern distribution, stretching from Mississippi up the East Coast and Ohio Valley all the way to coastal Maine.

The pollination occurs through the air, but the dispersal of seeds to extend its range was mostly by birds and squirrels, burying the nuts. Jays, in particular are known to carry nuts large distances and then to cache them into the ground. Crows and Passenger pigeons,

interestingly also contributed to their spread, especially up into the mountainous regions of the East. So it covered the lowland areas of the Deep South, mountain areas of the East as well as reaching the coastal forests of NE. It's range covered 200 million acres.

SLIDE not dominant

Accounts of how dominant ACN was can be exaggerated as a mythology has grown up around this tree. Studies of tree pollen in lake beds has shown it to have been common, but not dominant in many areas of its range. Where most concentrated, however, it could occupy 80% of the forest canopy. And the area where it was most dominant was Appalachia, particularly the southern Appalachians of Virginia, North Carolina, West Virginia and Kentucky.



SLIDE nuts

The “nut mast” of all species of trees, hickories, chestnuts, acorns was extremely important to early indigenous people as well as those that came after. The Lamoka, predecessors of the Iroquois, were a people of the Middle Archaic period, 5000 years ago who clearly used chestnuts. There is evidence they were able to store them as well as use them as a meal for bread.

They could be eaten raw or cooked and

provided calories comparable with other nuts, as well as all 9 essential amino acids.

And they were very abundant. It was a very reliable crop year to year, unlike acorns. The new Europeans arrived, with the hunters and trappers pushing first into the mountains. The chestnut was again crucial as it provided sustenance for the incredibly abundant game they found. Bison, elk, bear and deer all grazed on chestnuts. And they supported geese, turkeys and other small game. Where there were chestnuts, there was abundant game.

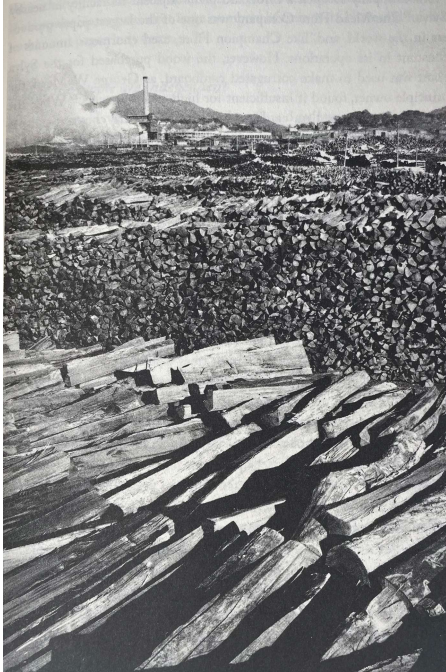
SLIDE farmers

Following on their heels were the subsistence farmers. The chestnuts provided crucial calories directly to their diets and could make the difference between survival and starvation over a winter. Perhaps even more importantly, the ACN provided abundant free forage for cattle and particularly pigs, which they could let out into the forest in the fall, in time to fatten them for market or personal use.



SLIDE for the farmer

- Food for pigs, cattle and family
- Cabins, shingles, furniture, tools, posts, barrels, boxes, fencing, coffins
- Cradle to grave



SLIDE woodlot

With more industrialization, the chestnut still played a crucial role. Even as the forests were disappearing enormous amounts of lumber were taken out for railroad ties and telephone poles and other uses. It was estimated that from 1875 to 1900 just the telephone and telegraph industry cut down 10 million trees.

Champion Paper and Fibre company, Canton, NC, 1937.
Cord wood for tannin production, another industrial use of chestnut.

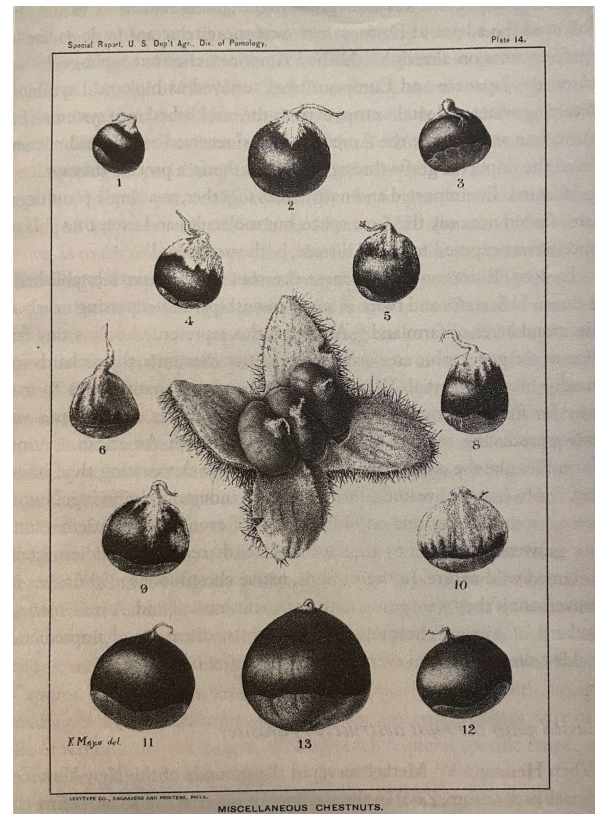
SLIDE chestnut assortment as of 1896

By the last decade of the 1800's the number of ACN's in the landscape was already declining due to unrestrained harvesting for its multitude of uses. At the same time, interest in other European, Asian and hybrid chestnuts was at a peak.

Although the ACN was admittedly the sweetest and best tasting variety, there was some bias toward these other species because they had long been cultivated as opposed to the "wild" American tree. And the ACN was smaller than many others so they were hybridized and bred for larger size.

1. ACN
2. Murrell, a hybrid named after George E. Murrell of Coleman Falls, VA.
11. European Numbo
12. Japanese Ridley
13. Japan Giant (up to 2" diameter)

As early as 1876, root stock from Japan and Europe began arriving. By 1900 there were at least 30 different cultivars available.



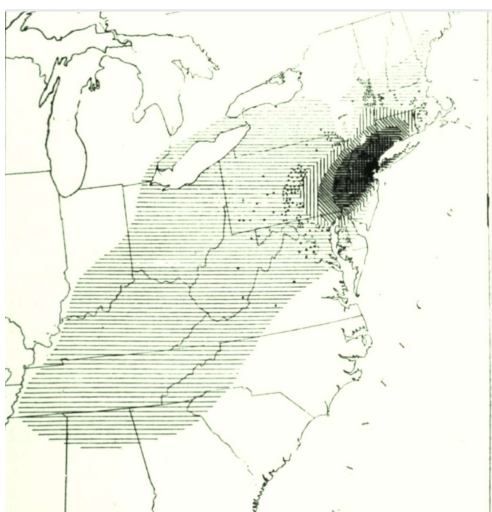
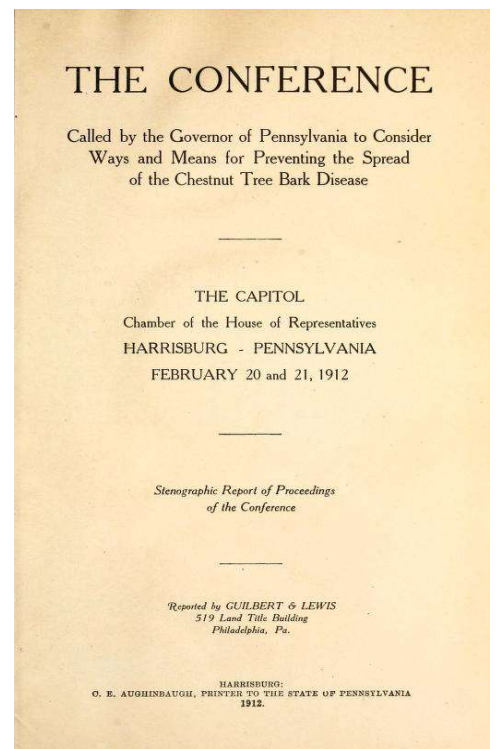


SLIDE zoo

It wasn't until 1904 that the forester at the Bronx Zoo in Prospect Park noted something was amiss. He saw cankers and evidence of disease on a few Chestnuts, which failed to respond to an anti-fungal spray. It was worse the next year and William Murrill, a mycologist and curator of the NY Botanical Garden in the park, became the first to describe what he called "*Diaporthe parasitica*", believing it to be a new fungus. In two years, all the trees in Prospect Park were dead or dying and the affliction spread out into NY, Connecticut, and New Jersey. Nothing seemed to stop it—no sprays, injections, pruning or even clear cutting in front of the advancing fungus was of any benefit.

SLIDE conference

Dr. Murrill, ironically, was one of the first to declare the ACN doomed. Others held out hope, and an academic conference was convened in Pennsylvania in 1912. No new remedies were revealed, but it did produce a graphic map of the devastating spread.



SLIDE map

NY was the epicenter and it was determined the Japanese chestnuts in the Botanical Garden were the likely source. There may have been other sites where the Chestnut Blight was introduced. A year earlier than NY, a very similar blight was reported in a grove in Bedford County, Va.

Even as the fungus advanced, there was no effort to reduce cutting which, in fact, accelerated because of the high demand. Because of the chestnuts resistance to

rot, the dead and even downed trees could continue to be harvested for decades.



SLIDE canker

The fungus would be renamed, with the current designation *Cryphonectria parasitica*. It kills by entering the tree through any minor external injury, then residing in and damaging the cambium layer beneath the bark. When it has encircled or girdled the tree, it essentially starves everything above the canker of water and nutrients and the branch or the entire tree dies. Trees typically can resprout and grow from the roots for several years, but are then attacked again. A young tree can succumb in 1-2 years, while a larger, established tree may hold on for years, depending on its general health.

SLIDE fungus damage

The appearance is typically the appearance of orange fungus on the bark, often around an area of injury. Progressive damage occurs as *cryphonectria* get down into the cambium layer.



SLIDE dead tree

As the 21st century progressed, the landscape became littered with these “ghost trees” as they were called. Estimated loss was 3-5 billion trees. Rot resistant, they often remained standing for many years. As a result, the animal, plant and human communities that depended on the chestnut gradually collapsed over the next 40 years with very few living trees documented by the 1940’s. Described in a PBS documentary as “the greatest ecological disaster since the last ice age.”

SLIDE insects etc.

Without the nutrition of the chestnut mast, the abundant wildlife dramatically declined and the very character of the forest changed. The dominant, tall chestnuts have been replaced with mostly chestnut oak, red oak and red maple as well as a variety of smaller trees.

All the species that regularly fed on the chestnuts, and their predators, began to disappear. James Hill, a biologist at Randolph Macon College, documented the decline of the goshawk, Coopers hawk, eastern cougar and bobcat.



SLIDE moths

There are 7 species of just moths, that are thought to be now extinct as a result of the

TABLE 1: Insects presumed to be extinct due to the loss of the American chestnut

<i>Argyresthia castanella</i>	Chestnut ermine moth
<i>Coleophora leucochrysell</i>	Chestnut casebearer moth
<i>Ectoedemia castaneae</i>	American chestnut moth
<i>Ectoedemia phleophaga</i>	Pleophagan chestnut moth
<i>Tischeria perplexa</i>	Chestnut clearwing moth
<i>Swammerdamia castaneae</i>	Chestnut Yponomeutid moth
<i>Synanthedon castaneae</i>	Chestnut clearwing moth

loss of the chestnut. Doug Tallamy has recorded that there were 125 species of butterflies that fed on the ACN. The human populations declined as well. Coupled with the economic disaster of the Great Depression, hardship abounded. The lack of chestnuts as animal feed, supplemental food for the family and as a cash crop, left subsistence farmers with few choices and many had to move off the land.

SLIDE root thicket

So what remains of the true American Chestnut? 100 years ago this is what you would have seen: “collar sprouts” from dead stumps. Fungus does not live in the soil, so root stock can resprout. Unfortunately, the fungus can be harbored by oaks where it causes no damage, but provides a reservoir of fungus to reinfect chestnuts when they sprout. A few individual large trees are occasionally found through its range. Mostly as a result of their isolation from the fungus and a degree of resistance. There may be 430 million root sprouts and individual trees across the chestnut’s range. If they can grow large enough to pollinate and produce



seeds, then they are very important source of the pure ACN genome for use in bringing back this tree.



SLIDE ACN leaf

If you are walking in the woods, here is what you would be looking for: boat shaped, sharply tapered at both ends, and sharp serrations along the edge (“dentata”).

SLIDE leaf from below

Underside light green; leaf thin and papery



SLIDE trail-side tree #1

Walking along the Appalachian Trail, or anywhere in our mountains, what are you going to see?

This is the White Rocks Falls Trail off the Blue Ridge Parkway: an inconspicuous, small trail-side tree is the most common finding.

SLIDE trailside tree #2

A far cry from the giants of the 18th and 19th centuries. Note the typical leaf shape. This young tree may have sprout from a nut. If there are a couple of somewhat larger individuals in the area surviving long enough to flower, the nut may have fallen or been carried here and germinated. More likely, it’s coming from surviving chestnut roots. It will, however, die of the blight almost surely before reaching the flowering stage.



SLIDE from root stock

This is a tree a little farther down the trail. You can see its dead predecessor centrally, surrounded by smaller sprouts. If you look, there is a surprising number of American chestnuts out there as our Blue Ridge in this part of Virginia was prime chestnut territory.



SLIDE ACN off the trail

Here's another tree just off the trail that you can find if you are



looking. In 2008, a joint project between The American Chestnut Foundation and the Appalachian Trail Club counted chestnuts along the trail, using volunteer hikers. Volunteers covered more than 400 miles of trail and counted 10,000 trees within 15' of the trail. More were counted in Virginia than anywhere else, but the density was highest in Georgia with over 70 trees per mile. Virginia had almost 22 trees counted per mile. They looked also for Large Surviving Americans, defined as more than 25 inches in circumference—only 5 were recorded.

THERE ARE PLENTY OF CHESTNUTS OUT THERE. WHAT CAN WE DO TO PROMOTE RESISTANCE TO *CRYPHONECTRIA PARASITICA* OR GIVE THEM RESISTANCE TO THE FUNGUS?

SLIDE species

There are species within the chestnut genus. These are 6 that are important to our part of the world, either as natives or hybrids: American, Chinese, the Chinquapin, European and Japanese. Two are native to America, *dentata* and *pumila*.

CASTANEA SPECIES	
CASTANEA DENTATA	AMERICAN CHESTNUT
CASTANEA MOLLISSIMA	CHINESE CHESTNUT
CASTANEA PUMILA	ALLEGHENY CHINQUAPIN
CASTANEA SALIVA	EUROPEAN CHESTNUT
CASTANEA CRENATA	JAPANESE CHESTNUT

SLIDE leaf species

Left to right: American, Chinese, Chinquapin, European and Japanese

Soon after the onset of the blight, recognized in 1904, the search was on for any kind of native resistance. None was found in the native trees, as the mortality seemed to be virtually 100%. The Chinese chestnut, however, did have a RELATIVE resistance to the blight, developing cankers but healthy trees would survive. Previous hybrid breeding efforts had been aimed at increasing nut size or production, but by about 1917, a USDA program under the direction of Dr. Van Fleet in Maryland was having some success with the Chinese hybrids. Subsequent researchers continued the work, both at the federal level and in state laboratories, but the early hybrids proved disappointing.



SLIDE dentata x mollissima

Problems with resistance, slow growth, wrong blooming time, conformation. Unlikely to compete and thrive in the wild and continue to hybridize with natives. By 1960 the federal chestnut breeding program was closed, although some state efforts continued.

Castanea dentata x mollissima

- Resistance attenuated
- Grew slower
- Bloomed at a different time
- Conformation smaller, multitrunk



THE
AMERICAN
CHESTNUT
FOUNDATION®

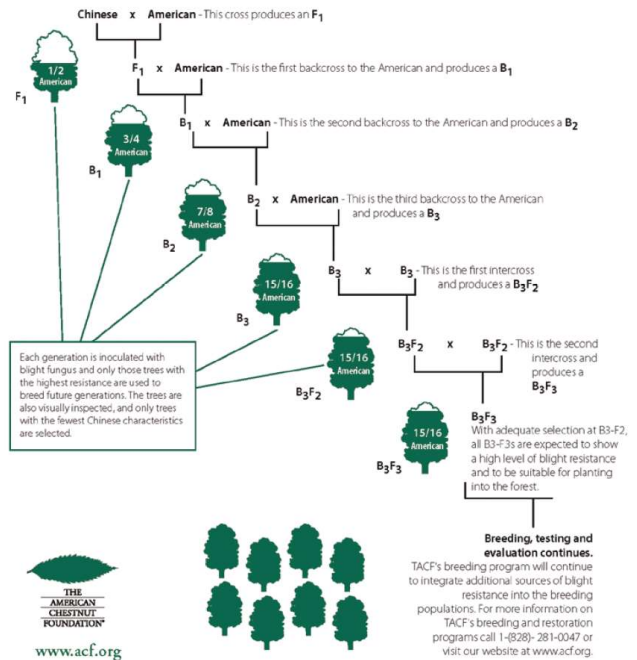
SLIDE TACF

In 1981, a noted plant cytologist, Charles Burnham, became convince that newer breeding techniques might make a resistant chestnut possible, still maintaining American traits. With several other plant scientists he began a breeding program and they founded a not-for-profit, The American Chestnut Foundation, in 1983. With the acquisition of a farm near Abingdon in 1989 and the establishment of a research station, they became the central resource for breeding programs.

SLIDE breeding chart (see page below)

The central theme of the breeding scheme was to introduce Chinese chestnut genes with an initial cross to the American, but then repeatedly back cross to the American, selecting only those individuals with the most resistance to the blight and with the most American traits for further breeding. Hoping to get more and more American genes and retain only the Chinese genes related to disease resistance.

Starting with the F1 cross and then back breeding X 3 to the American to produce a “B3”, which is 15/16 American. Then you intercross with other B3’s twice more, again selecting only those trees that are most American, but still with resistance. The result is a B3 F3 which TACF is now calling the “Restoration Chestnut 1.0” .



SLIDE chihuahua/great dane

Well, the genes don't always behave as planned. TACF has the best hybrid yet produced but they admit it is not sufficient for a large scale effort at reforestation. There are likely multiple problems, but primarily it may be because the trait for resistance is not on two or three genes, which would be ideal, but on 8 or 9 and perhaps many more. That makes it much less likely that you can get all those of genes into the Restoration Chestnut without retaining a significant amount of Chinese traits. However many hybrids have been planted both by TACF and a multitude of state and individual breeding programs.



SLIDE Large Surviving American (LSA)

Meanwhile, there is excitement, and some fear, about another approach to the problem using genetic engineering. In the 1980's, an engineer named Herbert Darling discovered a large surviving ACN on his land in Western NY. Despite its impressive size and height, he could see that it was dying from the blight. He turned his attention to trying to save the potential of this tree by pollinating it himself from another distant tree, hoping it bore nuts and potentially planting those as replacements. The result was about 100 nuts, some of which he planted, and nurseried as he watch the parent tree gradually die.



State University of New York College of Environmental Science and Forestry

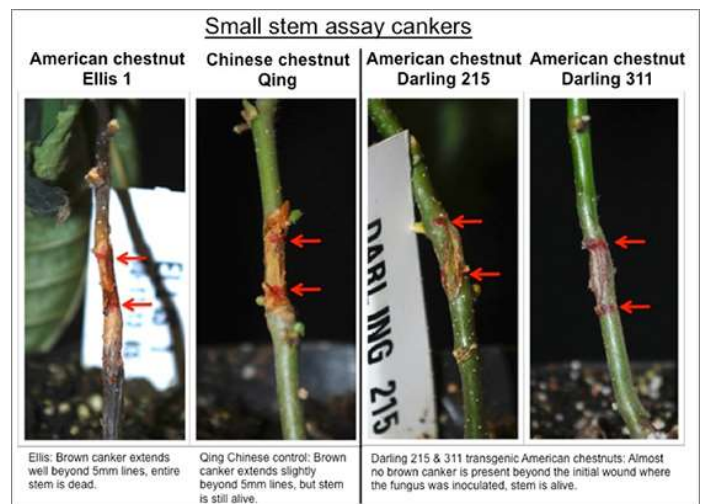
SLIDE State University of New York College of Environmental Science and Forestry

He also brought nuts to Dr. William Powell and Dr. Charles Maynard who were working in Syracuse at the State University of New York's College of Environmental Science and Forestry. They had an interest in the American Chestnut, but Darling asked them specifically if, as plant geneticists, they could bring back the chestnut, starting with the nuts from his tree. They took this on, and formed the American Chestnut Research and Restoration Center at SUNY. They first noted that the damage to the tree was caused by the excretion of oxalic acid from the fungus. The next step was finding a gene that conferred resistance to the fungus by coding for the production of oxalate oxidase to detoxify the acid. The gene they found, however, was in wheat. It's common, found in other plants as well and is throughout our environment and in our food.

Its transfer into chestnut embryos' DNA is accomplished by using *agrobacterium* as an agent to carry it in. This is a bacterium that normally enters a damaged tree, enters the cells and adds DNA that results in excretion of a sap beneficial to the *agrobacterium*. It can be hijacked, though, to carry in the 2-4 wheat genes necessary to impart protection from the fungus. Note that this is a defensive quality. The tree can't kill the fungus, it just protects itself from the toxic oxalic acid and continues to live with the fungus present. Its transfer to the chestnut, however, would create a transgenic plant, with significant regulatory and marketing implications.

SLIDE small stem cankers

They can test the susceptibility on very small stems, measuring the cankers that form. Standard ACN with large canker—dead Chinese—intermediate resistance Transgenic Darling (named after the gentleman who first brought them the nuts)—2 versions show even better resistance.



SLIDE small plants

These are somewhat larger plants, pictured just 8 days after inoculation, with the American AND the Chinese succumbing. The Darling versions with the wheat gene surviving. They found the transgenic tree even more resistant than the Chinese.



Figure 1

SLIDE larger tree with canker.

Trees tested at an older age, still showing resistance and healing canker with the wheat gene.

Environmental Interactions with Transgenic American Chestnuts

Andrew E. Newhouse and William A. Powell, with gracious acknowledgement to many research collaborators
SUNY College of Environmental Science & Forestry, Syracuse, NY

Background

During 38 transgenic American chestnuts have shown significantly enhanced tolerance to the chestnut blight fungus, and will soon be evaluated by federal regulatory agencies before potential public distribution. The oxalate oxidase enzyme expressed in Durling transgenic American chestnuts is naturally found in many food crops and wild plants, and it protects the tree by degrading a toxin rather than by killing the fungus, so it is unlikely to present novel risks when expressed in American chestnut. However, along with data from molecular, growth, and nutritional tests, it is valuable for regulators and prudent for environmental scientists to carefully evaluate potential environmental risks before deploying new restoration material. Here we summarize several comparative risk assessments of environmental interactions with transgenic American chestnuts and non-transgenic controls.

Leaf Herbivory by Insects

Aquatic mayfly larvae performed better on American chestnut leaves than oaks and other trees in a preliminary test, so chestnut re-introduction may be net fit stream ecosystems. Subsequent studies have shown that caddisfly larvae survival was not significantly different on transgenic vs. wild-type American chestnut leaves². Separate studies of terrestrial (cypress moth) caterpillars showed leaf consumption differences between Chinese and American leaves, but similar consumption between wild-type American and transgenic or B3F3 leaves.



Germination of Nearby Native Seeds

Seeds of several native plant types from American chestnut habitats (*Elymus* grass, *Cichorium* forb, *Galium* herb, *Acer* deciduous tree, *Pinus* conifer) were germinated in potting mix with different types of chestnut leaf litter³. *Pinus* seeds showed reduced germination in the presence of one wild-type control leaf type compared to two leaf types, and *Cichorium* showed reduced biomass in B3F3 leaves compared to Durling 38 leaves, but there were no significant differences in seedling germination rates or total biomass when grown in transgenic vs. wild-type American chestnut leaf litter.



Leaf Litter Decomposition

Leaf decomposition rates were compared by placing different chestnut leaf types (wild-type and transgenic American, and B3F1 hybrid) in mesh bags on the forest floor. All leaf types lost ~90% of their mass after 1 year, rates were not significantly different⁴. Transgenic chestnut leaves were also tested for persistence of oxalate oxidase enzyme activity after leaf drop in the fall. Enzyme activity essentially ceased as soon as leaves turned brown (approximately one week after leaf drop in outdoor conditions), though activity could be preserved for more than a month in artificial freezer conditions⁵.



Mycorrhizal Fungi

Several experiments have examined mycorrhizal associations with transgenic American chestnut roots. Most recently, Durling 38 roots were observed to be colonized at the same rate (~95% of root tips colonized) as wild-type American chestnut controls⁶. These results corroborate older studies on transgenic chestnut (including both greenhouse⁷ and field⁸ experiments) and studies on other transgenic trees (aspen⁹, elm¹⁰, *Eucalyptus*¹¹, poplar¹², and apple¹³), all of which indicate transgene presence does not inhibit mycorrhizal associations.



Bumble Bees and Pollen

Chestnuts are often considered to be wind-pollinated, but recent research has shown that insects (including native bees) contribute to successful pollination¹⁴⁻¹⁷. Bumble bees were reared in microcolonies (photo at right) and supplied with chestnut pollen containing the oxalate oxidase enzyme. There were no differences in survival, body size, pollen use, or reproduction when bees were exposed to a field-realistic concentration of oxalate oxidase in pollen¹⁴.



Transgene Inheritance



If or when transgenic American chestnuts are deployed for restoration purposes, their ecological significance ultimately will rely on inheritance of the transgene by offspring. Meaningful genetic diversity in a potential restoration population will be accomplished primarily by outcrossing transgenic trees with surviving wild chestnuts¹⁷. Since the transgene is only present on one half of the chromosome, about half the offspring from a cross with one transgenic parent are expected to be transgenic. This has been confirmed in analyses of two generations of Durling 38 offspring: 56 of 112 units tested to date show transgene activity. Growth rates and survival of these transgenic offspring are equivalent to their non-transgenic siblings (that didn't inherit the transgene), and *Ophiostoma parasitica* inoculations indicate that transgene offspring have blight tolerance similar to their transgenic parent.

Tadpoles in Vernal Pools

Wood frog tadpoles were raised in individual jars with different types of deciduous leaf litter to simulate an interaction that could take place in vernal pools¹⁸. Chestnut leaves (transgenic American, wild-type American, hybrid, or Chinese) were not detrimental to tadpole survival compared to sugar maple controls, only American beech leaves increased tadpole mortality. Development and growth rates were similar on most leaf types, but American chestnut leaves (both transgenic and wild type) allowed slightly increased growth rates compared to other leaf types when supplemental food was not present, suggesting American chestnut restoration could conceivably benefit amphibians.



Conclusions

Some environmental interaction experiments show differences between Chinese and American chestnuts, which is not surprising given the differences between these species. However, neither B3F3 nor transgenic Oxo-expressing American chestnuts have shown significant ecological differences compared to wild-type American chestnuts, apart from blight tolerance. Thus Durling 38 transgenic American chestnuts do not appear to present greater ecological risks than traditional breeding.

Background: 1. A. E. Newhouse, W. A. Powell, and J. M. Powell. 2014. "The Oxalate Oxidase Enzyme Expressed in Transgenic American Chestnuts is Naturally Found in Many Food Crops and Wild Plants, and it Protects the Tree by Degrading a Toxin Rather than by Killing the Fungus." *Journal of Agricultural and Food Chemistry* 62: 1111-1118. 2. A. E. Newhouse, W. A. Powell, and J. M. Powell. 2014. "The Oxalate Oxidase Enzyme Expressed in Transgenic American Chestnuts is Naturally Found in Many Food Crops and Wild Plants, and it Protects the Tree by Degrading a Toxin Rather than by Killing the Fungus." *Journal of Agricultural and Food Chemistry* 62: 1111-1118. 3. A. E. Newhouse, W. A. Powell, and J. M. Powell. 2014. "The Oxalate Oxidase Enzyme Expressed in Transgenic American Chestnuts is Naturally Found in Many Food Crops and Wild Plants, and it Protects the Tree by Degrading a Toxin Rather than by Killing the Fungus." *Journal of Agricultural and Food Chemistry* 62: 1111-1118. 4. A. E. 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SLIDE environmental interactions

The potential introduction of a transgenic plant into the environment has a multitude of implications. The researchers have tried to anticipate this and have done additional studies:

1. Multiple insects showed no ill effects, eating leaves from transgenic chestnuts.
2. Other plant species associated with chestnuts germinated without problem in the presence of transgenic leaf litter.
3. Leaf litter decomposed at the same rate and the oxalate oxidase was gone by the time the leaves turned brown.

4. Fungi colonized the root systems at the same rate as native trees.
5. Bumble bees fed pollen had no change in survival, body size or reproduction.
6. Tadpoles in vernal pools actually did better with transgenic leaf litter in the water.
7. Darling trees did pass on their full resistance to ½ of the next generation, as expected.

TACF was initially, in the 90's, not supportive of the transgenic program, preferring to just continue their breeding in the hope that a suitable tree would result. With considerable controversy in the organization, that policy finally changed and they now are partners and support the SUNY-ESF program of Dr. Powell. Prominent board members quit TACF in 2019 over this issue as transgenics are still controversial.

SLIDE 3BUR

Breeding resistance to *Cryphonectria* and *Phytophthora*
Biotechnology—transgenic
 Darling 58: stack genes from breeding program with Darling 58
Biocontrol—hypovirulence: introduction of a virus into the fungus to attenuate its ability to damage the tree.

TACF STRATEGY

“3 BUR”

BREEDING — Continued efforts to induce resistance to *Cryphonectria* as well as *Phytophthora* (ink rot disease)

BIOTECHNOLOGY — Transgenic tree approval with potential to “stack” genes for additional resistance from the breeding program

BIOCONTROL — Continued exploration of “hypovirulence” of the fungus by infecting it with a virus

United for Restoration

SLIDE agencies

The Darling 58 is the transgenic chestnut resulting from this long period of development. As a transgenic plant, it requires approval from the FDA, USDA, and the EPA. Submitted for approval in Jan 2020. Originally stated that approval could come “as early” as 2021”. It is still under consideration with no estimated date for release.



SLIDE GJEP

The anti-GMO forces, however, are lined up against it, most prominently the Global Justice Equality Project and the Center for Food Safety, organizations that oppose GMO products. They have a history of effectively mobilizing against projects with lobbying and “direct action”, meaning demonstrations, and physical interference. They have vowed to use “every means at their disposal to oppose” approval. They believe that Dr. Powell’s testing is too limited in scope and too short of duration. They are also afraid this is a “Trojan horse” for the industry to get genetically modified trees approved for massive tree farming projects in the future.

Others in the scientific community also are opposed, as a former TACF geneticist has advised that longer term testing is required, probably “over 50 years”.

SLIDE momentum

Momentum is on the side of saving the American chestnut now

Growing popular support:

- Enthusiasm for a nostalgic part of our culture
- Grass roots participation in breeding programs; TACF has 5000 members
- Prospects of a new commercial nut crop for people and animals
- Lumber source, with potential to replace chemically treated wood
- Renewed forests and the supported species
- Carbon sequestration

SLIDE Site visit

Meadowview Research Farms of the American Chestnut Foundation



SLIDE research laboratories, offices and greenhouses on a beautiful 150 acres

SLIDE farm

Seedlings that they propagate and pollinate are kept in the lab and greenhouses. You can see here the young trees that they test in the field.



SLIDE

Dr. Vasily Lakoba, the research director who gave a tour to Judy and me, with his ubiquitous posters that he used for us visual learners. In the background, a pure Chinese chestnut



SLIDE Chinese

You can see its conformation is much different, multitrunked, more spread out.

SLIDE F1

This is a grove of F1's, that is the first cross, 50/50 American and Chinese. You see first that there are empty spots in the rows as many have already died. The others have a little more vertical dominance, but not much, and there were a lot of cankers.



SLIDE B3F2

This tree is much farther down the breeding sequence, B3 F2. So it is an additional 3 backcrosses and one intercross. It's better, but still not there, with a thin canopy.



SLIDE

And at least one limb already dying of the blight.



SLIDE green house

Two open green houses and "one shade house", but no totally enclose green house (they are raising money now).



Ciera Wilbur, nursery manager who gave the tour.



SLIDE green house

Many of these young plants include the Restoration 1.0, which is being given out for planting.

SLIDE

These young plants also include the Darling 58 transgenics, from Dr. Powell's Syracuse lab, with which they are testing additional crosses now, even before the approval for release into the wild



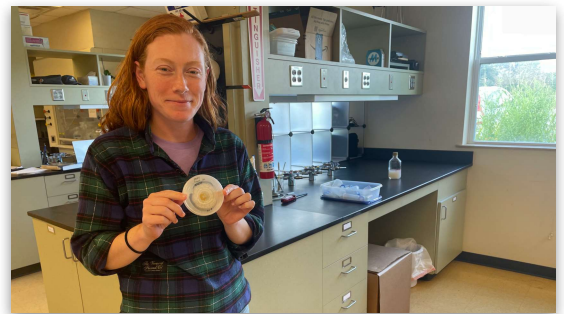
SLIDE

The lab and offices are in the building you initially saw in the first pictures.

Dr. Lakoba

SLIDE

Lauren Kerwein is the lab manager and showed us around the facility. She greeted us with a sample of *Cryphonectria*.



SLIDE

This was her demonstration of how they test the resistant of young seedlings.

SLIDE

The stem is purposely slit, then the fungus directly applied and sealed with a twist of plastic wrap



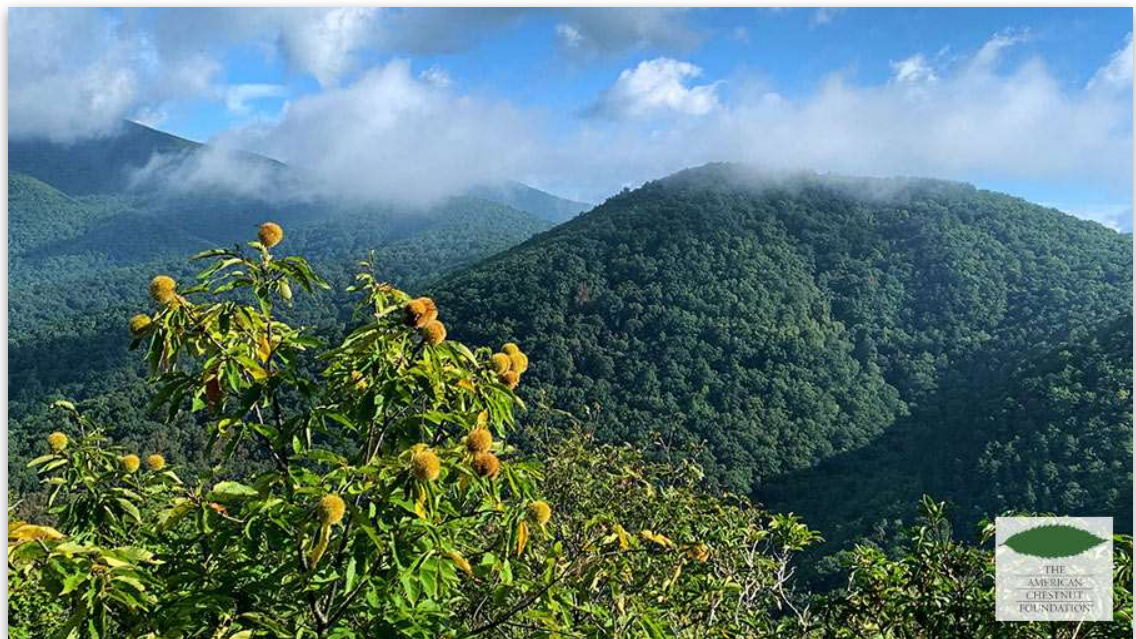


SLIDE

With additional intense light, they are able to push these small plants to blossom and produce pollen for the next generation experiments within 18 months, rather than waiting years for the trees to mature.

Conclusion

- After a century of work, we are on the verge of being able restore a foundational species.
- Even if successful, it will take 100 years to repopulate its range.
- It will face a very different world, with climate change and new pests, some yet unforeseen as well as new plant competitors.
- Yes, we should try anyway, despite some risk.
- Too important a species to let it go, especially since the germplasm is still out there.



- This genetic engineering is not unlike a lot of other GE we have already done successfully.
- Our forests are under assault, having lost the American Elm and now serious threats to ash and Eastern hemlock. We need this tool to help defend our forests.
- Darling 58 seems like the least of our threats, could restore a keystone species and offer hope for preserving other important species.

RECOMMENDED REFERENCES

Dr William Powell TedX talk:

<https://www.youtube.com/watch?v=WYHQDLCmgyg>

The American Chestnut: and environmental history, Donald Edward Davis, University of Georgia Press, 2021

The American Chestnut Foundation: <https://tacf.org>