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The President's Message

by Phillip A. Rutter

In the following lines I am going to try to share with you the tremendous experience it was for me to attend the opening of our new research farm in Meadowview Virginia. In every possible way, it was a dream come true for me, and for many of the others who were able to attend.

For me the activities started days before April 15th, the day decided on for the groundbreaking. I drove first, early in the morning, from my farm in southern Minnesota up to the greenhouses of the University of Minnesota in St. Paul; there I met Dr. Burnham who had selected about 30 of the 3/4 American chestnut trees he had been keeping there for transfer to the Virginia farm. These were carefully packed into my battered station wagon, and I drove as fast as the law allows down to Ames, Iowa, to meet our Vice President, Dr. Mark Widriechner, who gave me his entire crop of small potted trees from the past year's pollination 'work, also destined for Virginia. It was getting late by now, as you can imagine, and I soon stopped for the night. There was a good chance of a frost, so I had to (carefully) unpack all the trees from the car, and bring them (carefully) into the motel room with me-baby chestnut leaves don't appreciate frost.

In a few days I arrived in Meadowview.

with the little trees doing well. I transferred them to Dr. Fred Hebard's care (he is our Superintendent there), had a delightful dinner with the Wagners, and managed a few hours sleep before the preliminary festivities began the next day, the 14th.

At my request, Jennifer Wagner had arranged a fully packed day for me. In the short time I could be there, I wanted to reach as many of our new neighbors as I could, and I certainly had the chance. In the course of the day I gave talks to the entire elementary school, and the biology classes of nearby Emory and Henry University; talked with a representative of the Chamber of Commerce; had lunch as a guest of the Rotary Club; went to a delightful reception in our honor, and in the evening gave a slide show for the public: and loved every minute of it. Dr. Fred 'was with me much of the time and did a good share of the talking.

We are in good hands. The folks of Meadowview and Abingdon gave us a warm welcome, and as you will see below, they pitched right in to help.

Friday the 14th was a lovely day, although I will admit to being tired at the end of it. When I pulled my shoes off in the motel room, though, and watched the weather, it was not encouraging.

Great bands of rain were being predicted for the whole region-probability 70%. Just the right probability to allow some hope that the rain might stay away.

Peeking out the window the next morning, the hope started to fade: lots of gray clouds, wet pavement, and... rain. Never say die, though-maybe it would stop in time for us to meet, plant some trees, and have our ceremony.

Persistence pays, I find. While it never quite stopped raining, it did slow to just a slight misting drizzle. Not the weather that first comes to mind for a celebration, but in fact it turned out well. Perfect weather for planting trees, which is what counts. And we found out who our friends were. Basically, everybody came.

At the appointed time, we had 80 people there on the small section of the farm we were going to plant (besides the TV news crew). The folks came bringing their shovels in their hands, from 3 years old to 90. Dr. Fred had the spots for the holes marked; with trees laid out in the mist waiting to be planted. We said a few appropriate words, and our great crew dug in with gusto. Some of our help was a little untrained in tree planting technique (the kids, for example) so each hole had a "designated planter, someone with the experience to be sure the trees were set in right. Among our overseers were chestnut researchers from around the region: Dr. Scott Schlarbaum came from Tennessee; Drs. MacDonald and Elkins from West Virginia, Dr. Griffin from Virginia, and we had the help of a dozen or more of the local experts. The activity was fierce for a couple hours. Ford Cochrane, the writer from the *Geographic,* took a lot of notes for a while, but later I found him with a grin and dirty hands, standing by his newly planted tree. At least one gentleman came in a business suit, mostly to watch, I think, but he was unable to simply stand by, and wound up helping with a dozen trees. I hope he was able to get the mud out!

The trees we planted at this ceremony were very special. Almost all of them were gifts to the Foundation from nut nurserymen around the country, from Florida to Oregon. Our research hybrids were planted later, by Dr. Fred and his crew, in a more protected spot on the farm. We know that many of our hybrid trees, particularly in the early years, may not be much to look at. We know they will be somewhat susceptible to the blight. So to be sure visitors will have a chance to see *healthy* chestnuts, we have planted an avenue of grafted nut-type trees that are known to be resistant. They 'won't be very much like the American chestnut in their growth, but they will be healthy (and useful in the research). And when you visit, you will be able to see them, and some day walk in their shade, perhaps picking up the nuts. The

retail value of all the trees donated for our opening was very considerable, and I want to express here, again, how very much the generosity of the nurseries is appreciated. We couldn't have done it without them.

When the last tree was planted, two more things happened to show me what kind of folks live in Meadowview.

One of our volunteer planters turned out to also be a volunteer fireman. Even though it had been misting the whole time we planted, the ground was still somewhat dry; not the best for newly planted trees. We knew they should be watered in, and were figuring on a slow and painful process that 'would take several days, but the Fire Department came to our rescue. Our volunteer made the offer, and five minutes alter his phone call the Fire Department's huge tank truck, carrying thousands of gallons, was slowly making its way up our slippery, muddy, hill; deep-soaking each tree. No charge, just helping out.

Then, at last, lunch. The Wagners had planned a scrumptious picnic for the 'workers, to be eaten among the newly planted trees, out on the hill. Trouble was, the hillside was entirely mud, with no place at all to sit down. The Wagners solution was simple-they opened their lovely nearby home to a hundred uncleanably clay-muddy feet, with smiles. And the food was delicious, too.

After the lunch, those of us with the time and the energy went for a fascinating hike up in the mountains. We all saw a few new phenomena, learned something, and had a delightful time, mist and all.

Nightfall found me back at the farm, picking up my car. All the others were gone by now, and you will not be surprised that I took the time to walk in the dusk through the new orchard. Quiet. And hopeful. The American Chestnut Foundation has found a very fine, very caring, home. Thanks to the Wagners, and you all. Thanks.

CHESTNUT TREES AT MEADOWVIEW WHICH ARE GOOD FOR NUT PRODUCTION, AND THEIR SOURCES AND COST

by Fred Hebard

Sources of seed, seedlings and grafted cultivars

Empire Chestnut Company
3276 Empire Rd-. SW
Carrollton, Ohio 44615

65A	$10.00	Chinese chestnut- Good nut producer (This is a graft of the parent, they also sell its seed or pennies per seed).
72-211	$10.00	Chinese chestnut. Produces large nuts (this also is a graft of the parent).
Caha	$10.00	Scion from old tree in CAHA (pronounced "saw-haw") plantation in Nebraska. Appears to be European- American hybrid. may be 'Paragon'. Very vigorous and productive. May be relatively blight susceptible.

Waynesboro Nurseries, Inc.
P. 0. Box 987
Waynesboro, VA 22980

(These people primarily sell bare-root Chinese chestnut seedlings).

Chestnut Hill Nursery, Inc.
RR 1, Box 341
Alachua, FL 32615

(These people also sell grafted cultivars).

Dunstan Hybrid Seedling Chestnuts

These are primarily Chinese.

Sources of grafted cultivars

Nolin River Nut Tree Nursery
797 Port Wooden Rd.
Upton, KY 42784

Meiling	$17.95 & $11.99	One of the three Chinese chestnut cultivars originally released by the USDA. Highly blight resistant. About 32 nuts per pound.
Eaton	$17.95	Chinese chestnut.
Orrin	$17.95	Chinese chestnut.
Armstrong	$17.95	Chinese chestnut.
Ford's Tall	$17.95	A complex hybrid.

Ford's Sweet $17.95 A complex hybrid.

8ear Creek Nursery
P.O. Drawer 411
Northport, WA 99157

Douglass Select	A complex American-Chinese hybrid.
Layeroka	A complex hybrid.
Sleeping Giant	A Chinese-Japanese-American hybrid released by the Conn. Ag. Expt. Station.
Manoka	A Gellatly hybrid.
Wards	
Meader	A complex Korean-American hybrid.
Skookum	A Gellatly hybrid.

Whitman Fanns Nursery
1420 Beaumont N.W.
Salem, Oregon 97304

SeftlemeierA European chestnut cultivar. These are quite blight susceptible.

A FEW SERIOUS WORDS FOR THE FIRST TREE PLANTING AT OUR NEW MEADOWVIEW RESEARCH

FARM
April 15, 1989

Dearly Beloved,

We are gathered here today, on the sight of the rain and the wind, the mountains and the trees, to join with each other and with this land in a bond of holy determination.

We are determined that these trees we plant will survive, and grow, and flower. We are determined that this farm shall succeed in its goal, which is: to grow chestnut trees, in the face of wind and sun, flood and drought, lack of funds, and blight; until chestnut trees are found which can once again grow free on these hills.

Why are we determined? We could talk about economics, and ecological necessities, but the future of all life in the Earth in our hands. We must care for it, and nurture it. The chestnut especially deserving, because we caused its current plight. It is only fair for us to restore its health.

Today we place our feet firmly on the trail that will lead to the restoration of the American Chestnut Tree.

Let Us Plant!

Minneapolis, Minnesota

A SERVICE OF DEDICATION
of a Bicolor Oak on the Cathedral Grounds

The Day of Pentecost, 14 May 1989

When all have assembled, *the Officiant says;*

Through the ages, Almighty God has moved His people not only to build houses of prayer and praise, but also to surround those buildings with majestic trees that give beauty and remind us of the power and love of their Creator. With gratitude not only for our Cathedral but also for its natural adornments, we are now gathered to dedicate and consecrate this Bicolor Oak in God's Name. Let us pray.

O merciful Creator, whose hand is open wide to satisfy the needs of every living creature: Make us, we beseech thee, ever thankful for thy loving providence; and grant that we, remembering the account that we must one day give, may be faithful stewards of they bounty; through Jesus Christ our Lord, who with thee and the Holy Spirit liveth and reigneth, one God, for ever and ever.

Amen. BCP 208.

A Reading from the Book of Isaiah, Chapter 61, verses 1-3:

The Spirit of the Lord God is upon me; because the Lord hath anointed me to preach good tidings unto the meek. He bath sent me to bind up the broken-hearted, to proclaim liberty to the captives and the opening of the prison to them that are bound; to proclaim the acceptable year of the Lord and the day of vengeance of our God; to comfort all that mourn; to appoint unto them that mourn in Zion, to give unto them beauty for ashes, the oil of joy for mourning, the garment of praise for the spirit of heaviness; that they might be called the trees of righteousness, the planting of the Lord, that he might be glorified.

A Reading from the Book of Revelation, Chapter 22, verses 1-3 and 12-14:

And he showed me a pure river of water of life, clear as crystal, proceeding out of the throne of God and of the Lamb. in the midst of the street of it, and on either side of the river, there was the Tree of Life, which bare twelve manner of fruits, and yielded her fruit every month: and the leaves of the tree were for the healing of the nations. And there shall be no more curse: but the throne of God and of the Lamb shall be in it, and his servants shall serve him.

... [and the Lord said] "Behold, I come quickly, and my reward is with me, to give every man according to his work shall be. I am Alpha and Omega, the beginning and the end, the first and the last." Blessed are they that do his commandments, that they may have the right to the Tree of Life and may enter through the gates into the city."

A Reading from the Book of Isaiah, Chapter 55, verses 813:

For my thoughts are not your thoughts, neither are your ways my ways, saith the Lord. For as the heavens are higher than the earth, so are my ways higher than your ways, and my thoughts than your thoughts. For as the rain cometh down, and the snow front heaven, and returneth not thither, but watereth the earth, and maketh it bring forth and bud, that is may give seed to the sower and breath to the eater: So shall my word be that goeth forth out of my mouth. It shall not return unto me void but shall accomplish that which I please, and it shall prosper in the thing thereto I sent it. *For ye shall go out with joy* and be *led forth with peace; the mountains and the hills shall break forth before you into singing* and *all the trees of the* field *shall clap their hands.* Instead of the throne shall come up the fig tree, and instead of the briar shall come up the myrtle tree: and it shall be to the Lord for a memorial for an everlasting sign that shall not be cut off.

Dedicatory Remarks: Donald C. Willeke

Remarks on the Occasion of
The Dedication of a Bicolor Oak
On the Grounds of the Cathedral Church of St. Mark
Minneapolis, Minnesota, the Day of Pentecost, 14 May 1989.

by *Donald C.* Willeke,
A Director of the American Forestry Association
and a Member *of the Global Releaf Advisory Committee*

On the day when we commemorate the descent of The Holy Spirit as a guide and comforter to God's people, it is fining and proper to plant and dedicate a tree here on the grounds of our Cathedral.

It is especially fitting on this day because wherever we *turn* we see that the Spirit of the Creator has been disregarded by the Spirit of Man. who is fouling the very air we breathe with the pollution of his engines and his ever-burning fires.

Not a day goes by when we do not read about further havoc which unthinking humankind has wrecked upon the garden into which God set us. It is absolutely true that with respect to our environment "We offended against [God's) holy laws, we have left undone those things which we ought to have done, and we have done those things which we ought not to have done' and, I might add, in the words now dropped from the Confession, 'There is no health in us.

Today we are being called to account literally for the fouling of our own nest. We have dumped the sewage of our civilization into the very air we breath, and as a result we are in grave danger of turning the Earth into a hellishly hot place unfit for human habitation.

Yet all this frightening news we read every day is not without a hope of salvation. We will have to change the way we live.

And we will have to plant tree-millions of trees that will absorb the pollution we have released on the land and, in addition, will give us beauty and comfort and procure us many other benefits.

The Bicolor Oak we dedicate today is just a symbol of what we must all do. It is a tree born of adversity; Its original home was in the swamps where soils are low in oxygen and alternatively have too much water and then too little. It can take our tough city conditions, and can suffer the insults of polluted air, little water, compacted soils and winter salt far better than most other species of trees. It grows rapidly and it is beautiful to look at-

Having planted one symbolic tree here today, we should all now go out and plant many trees, to provide "releaf" for our troubled planet. The Collect that opened our service cautions us that we will be called on to account for our stewardship of God's earth. And the Prophet Isaiah reminds us that trees are not only a sign (fully as much as is the rainbow) of God's Grace towards us, but also a symbol of those who are truly righteous. Finally, St. John the Divine concludes the Bible with a reminder that a tree - the Tree of Life was not only God's first symbol for man, but also His final symbol for us.

With thanks to the One who alone can make a tree, I am happy to join my friends at St. Marks in dedicating this Bicolor Oak to the glory of God.

[Then the Officiant leads the people in:]

A Litany of Praise to the Creator Or Trees:

Let us thank God whom we worship here amongst the beauty and holiness of His noble creations

Eternal God, the walls of temples made with hands cannot contain You. Graciously receive our thanks for these pillars of the larger temple which You have created so that we may worship You wherever we may be in the land.

For trees which only You can make, but which You expect us to water, prune. nurture and tend,
We thank You, Lord.

For trees to give us the air we breathe and remove the pollution with which we have fouled Your land,
We thank You, Lord.

For trees which cool the air is summer and lessen the winter's stormy blast,
We thank You, Lord.

For trees which give us wood for our churches, homes and workplaces, and paper for our books,
We thank You, Lord.

For frees which give us places of solitude, vast and small, in forests and groves,
We thank You, Lord.

For the privilege of helping to add another pillar to your great green cathedral that surrounds us,
We thank You, Lord.

For frees: for <u>Your</u> trees,
We thank You, *Lord.*

[The Officiant then gives the Benediction:]

May you go out with joy;
May you be led forth with peace;
May the mountains and the hills break forth before you into singing;
And, for you, may all the frees of the fields clap their hands. *Amen.*

WHY SHOULD WE BOTHER

ABOUT THE AMERICAN CHESTNUT?

from the Prospectus of
The American Chestnut Foundation - *established in 1984*

This tree was unique.

An integral part of The eastern forests, the American chestnut grew from Maine to Georgia and west to southern Michigan, Indiana, and Mississippi, often constituting 25 percent of the forest. According to some estimates, the blight killed enough trees to cover the State of Ohio.

It was a big tree: mature individuals probably averaged three to four feet in diameter and 80 to 100 feet in height. Maximum size was much greater. In the Great Smoky Mountains region, specimens nine to ten feet in diameter were reported, maximum height may have reached 140 feet.

The wood was remarkable. Straight-grained, lighter in weight than oak and more easily worked, it was as rot-resistant as redwood. It was used for virtually everything - telegraph poles, railroad

ties. flea construction, shingles, paneling, fine furniture, musical instruments, even pulpwood. It split easily and was widely used for fend rails and firewood. The tanning from the heartwood and bark was the best available for tanning heavy leathers and was a mainstay of the industry. Many of those uses would still find a place in our present economy.

From the foresters point of view, the tree was exceptional for several reasons. In addition to its broad utility, the chestnut grew rapidly, often adding 1/2 inch in diameter each year, occasionally putting on as much as one inch of new wood annually. This is remarkable in a high quality hardwood. Also unusual was the strong sprouting ability of the chestnut. When a tree was harvested, the remaining stump sent up new sprouts from its base. With proper thinning, the mature, established root system grew a new crop very quickly, with no need for replanting.

THE AMERICAN CHESTNUT BREEDING PROGRAM

by Charles Burnham

The current American chestnut breeding program is using the Chinese chestnut as the best source of resistance. The blight fungus tails to grow at the point of infection. The hybrid between the two species is moderately resistant, more resistant than the American, but less resistant than the Chinese chestnut. Resistance is incompletely dominant. By crossing that hybrid back to the American chestnut and following with

successive backcrosses to the American chestnut, using blight-resistant Selections each time for the next backcross, the American chestnut is recovered automatically, and at the same time resistance to the blight is being added by selection. The third backcrosses are, on the average, 15/16 American chestnut and some will have the one(s) for resistance derived from the Chinese chestnut, but only from one parent and, consequently, are only moderately resistant. Progeny from crosses between those moderately-resistant selections will include some that have received the gene(s) for resistance from both parents. They are homozygous for those genes are expected to be as resistant as the Chinese chestnut. They will "breed true" for resistance.

Corn breeders find that third backcross progeny are indistinguishable from the recurrent parent. In the current chestnut breeding program second backcrosses are now growing, one backcross away from the final backcross and the final two step, i.e., one to produce the true-breeding, highly resistant hornozygotes, and one for increasing them.

The goal of the present program is not a single-tree cultivar, but breeding populations ultimately, ones that will be adapted to different plant growth regions.

AN AMERICAN CHESTNUT WITH SPINELESS BURRS?

by Charles Burnham

This note is directed to those who have plantings of American Chestnuts that came from irradiated nuts.

Nuts were irradiated in 1956 at the Brookhaven National Laboratory, and by 1974 more than 10,000 trees were growing from treated seeds in Virginia, Maryland, West Virginia, Ohio, and Pennsylvania. Second generation trees are being grown and nuts from some of them have been given a second irradiation. The basic goal is to produce blight-resistant mutants of the American Chestnut. Thus far none nave been identified.

The purpose of this note is to direct attention to the possibility that mutants with other useful or interesting traits may be found. For example, one with spineless burrs would he useful as a shade tree. This mutant has been found in the Japanese Chestnut. A list of hybrids produced in the early USDA breeding program includes hybrids from an American Chestnut crossed with a Japanese Chestnut with spineless burrs. There is not later report on those hybrids. Also there is a spineless burr strain of Castanopsis, a related genus.

Since the trait may be recessive, and the chestnut is self-incompatible, as many nuts should be gathered from the mutant tree. The progeny can then be crossed with each other to recover trees with the trait. Resistance to chestnut blight will then need to be added by breeding

SIMULTANEOUS BREEDING OF THE AMERICAN CHESTNUT FOR MANY TRAITS

Dr. Lawrence L. Inman

Strategies for producing populations of blight-resistant American chestnuts, each adapted to a particular growth zone were discussed in an earlier issue of this Journal (Vol. 2(1):6-9, 1987). The current breeding program based on successive backcrosses to the American chestnut will recover automatically its forest-type growth and great competitive ability. As stated by Jaynes (1979), who American chestnut was not perfect."

Improvements in yield and other useful traits can be made simultaneously during the breeding for blight resistance and adaptation. Strategies for accomplishing this will be discussed here.

Although chestnut blight eliminated the American chestnut as a commercially-important forest tree, it survives through-out much of its natural range as scattered large trees, or as living sprouts that develop from buds in the root collars of the dead trees or from pre-blight seedlings. The latter now have a shrub-like form as the result of repeated blight infections followed by sprouting.

The natural range of the American chestnut was extensive. Over the centuries nature did the job of breeding for adaptation to the conditions of the local environment including the infrequent extremes. Hence the American chestnut species is comprised of a series of geographic races of natural varieties. When geographic races are crossed, the adapted combinations of genes will be broken up. The progeny may not be adapted to the area of origin of either parental race or any other. This must be avoided.

Drawing the boundaries of the geographic zones is impossible at this time. It can be postponed by making separate point collections each within a relatively small area, e.g., a radius of about ten miles. It resources are limited the first step is to make the initial point collections far apart throughout the natural range.

Each collection point is to be established in a breeding orchard, either in a blight area in which various means of protecting the trees from blight can be used or in a blight-tree area with similar climatic conditions. lithe point collection area has flowering trees or sprouts, they can be crossed immediately either as male or female with Chinese xAmerican hybrids. The resulting backcross progeny will be developing at the same time as the trees in the breeding orchard for the point collections are developing. The trees in the breeding orchard will be used, not only for the next backcross,

but also as a source of nuts for a progeny test to identify superior parent trees in that orchard described as follows

Lacking any other information, the appearance of the individual is the best indication of its breeding value, but the only sure way to determine its breeding value is to grow and observe its progeny.

Since chestnut trees are self-incompatible, each tree in the breeding orchard will be pollinated by a mixture of pollen from the other trees. Superior mother trees will have a high frequency of superior progeny.

When flowering and fruiting in each breeding orchard begin, enough nuts from each marked and numbered tree can be collected to plant a single row plot with at least 18 seedlings in a field trial in a blight free area, where the performance of the progeny rows can be compared for rate of growth, form and other traits. The superior mother trees identified by those observations can be used for the next backcross. By the time those backcross progeny are flowering, continuing comparisons of the performance of the progeny rows can be used to give a better measure of the breeding value of each tree in the breeding orchard.

Plant breeding is largely a matter of probabilities, in this case, having large enough numbers in each point collection. In tree breeding, cost-next to time - is a major consideration. With a spacing of 22 feet each way, 90 trees can be planted per acre. Less than 20 acres would be needed for a point collection of 1,200 trees. With an 11-

foot spacing each way about GO acres would be needed for the field trials of 1,200 single row plots of 18 trees each for the field trial of the open pollinated progeny of the 1,200 trees in each point collection. These figures are given to provide the basis for decisions to be made in planning the program.

This plan will result in the improvement of the American chestnut not only for blight resistance and adaptation, but also for other important traits as well.

THE LEGEND OF THE CHESTNUTS

by Max Lemouzi (1913)
translated by Nicote Hetod

Long ago, the countries of Berry and Limousin were very, very poor. Their inhabitants raised their most ardent prayers to heaven in vain. Saint Peter, the high minister of God, barely granted them their daily bread. This bread was not kneaded of the finest flour, but was coarse, heavy, black and so hard that one had to be gifted with a great appetite and good teeth to devour one's meager portion. Any improvement in their humble lot would have been most welcome. After all, it is not forbidden, while waiting for the next life, to wish for success in this one.

What good does it do us, lamented our ancestors, to have such illustrious patrons as St. Ursinus and St. Martial, it we are to be deprived of the blessings of this world?

And they decided to complain about this oversight to the two apostles. They did not make a formal petition (the use of these being as yet undiscovered) but instead they most humbly presented their entreaty. St. Ursinus was the first to be moved by their lamentations and spoke of them to St. Martial in order to persuade him to visit St. Peter, because, as he said: "It is preferable that you go yourself. That regions borders on your own, and I myself have already obtained great favors from the Master for my people of Brenne. Their swamps, which were once infected and unhealthy are now pure, and the water, thanks to a fortuitous arrangement of the plains, forms magnificent pools which are a delight to the eyes and which, stocked with fish, are a great resource for their country, furnishing healthy and abundant nourishment. But what we have obtained with a great deal of work and the help of God would be impossible in the lands that border your Limousin. You see, my dear Martial, all this is quite properly under your jurisdiction. and it requires your intervention to good St. Peter."

The saint, realizing the truth of this reasoning. resolved to obtain satisfaction of the request of the poor folk, because they were, it was true, quite deprived of the charms of this world.

He put on his lace surplice, his feast-day cape, took his cross and his shiniest golden halo and set out to see his cousin Peter.

The keeper of the keys of Paradise, his long beard resting on his chest, seemed sad and wonted.

"Well! Martial, what can I do for you today?"

"Great St. Peter, I've come to ask you a favor, it I am not too bold."

"I wish to Heaven that I were busy, my good Martial, but the devil simplifies my task. The pages of my register remain blank. All too often the wretched souls are taking the road to Purgatory or to Hell. Go ahead, tell me quickly what you want; you know quite well I can refuse you nothing."

And St. Peter, puffing aside his sad thoughts, adjusted his glasses, took up his great register and started rapidly turning the pages.

"Good St. Peter, it's just that I've come on behalf of the people of my Limousin and also, in agreement with Ursinus, to ask you some favors for his folk from Berry, who are, as you know, our good neighbors."

"Ah! Yes," said St. Peter. "Let's talk about these folk from Berry and from Limousin; they are to be commended. As stubborn as their goats and even more capricious. Add to that ignorant and making short shrift of the commandments of the Master. The people of Brenne, now that they have their pools full of golden carp, succulent pike and eels a yard long, are much more worthy. And your Limousin folk are really quite disagreeable. I would pardon them if they had some spirit and some cheer. Their Gascon cousins, liars though they be, have a certain animation that makes me smile. But your Limousin folk are unbearable."

"Oh! illustrious saint, what does the roughness of the bark matter when the sap is generous and good? Many wild herbs grow on our hills, but they are so lively and so sweet-smelling that one would hardly think of censuring their untamed state."

'What an excellent lawyer you make, my dear Martial. I am no longer surprised at the great number of conversions performed in your land of Aquitaine. Well, tell me what these incomparable people of Limousin and your neighbors of Berry are complaining about."

And Great St. Peter, half-convinced, buried his nose even deeper in his great register.

"What they're complaining about? Well, they say that their soil is arid, rocky, sown with heath and difficult to cultivate; that they have floods, hail, too much rain and too many frosts. They do have their white wine from the slopes to cheer them up a bit, but you see even that goes bad, and they will soon be reduced to drinking water. It's certain that they'll turn wicked. All those who drink water are wicked."
"Look here, Martial, your dependents, as interesting as they are, can't demand a new creation."

'Oh! God save us from wishing such a thing! But the smallest gift, the tiniest favor would be received with gratitude. Don't you hear the pleas of these gallant folk?: 'Saint Fronto, they say, is the favorite of St. Peter. That is why our neighbors in Perigord received as their share the exquisite truffle; the Gascons have their vines, the ruby juice of which sharpens the spirit and gladdens the heart; the Normans have the apple, the Provencals the orange. We alone, we have nothing and are the disinherited of heaven.'"

The apostle was silent; and St. Peter was, for a time, quite pensive. Finally he closed his register.

And, when St. Martial lifted a comer of the blue sky to look down lovingly on his dear Limousin, he was surprised and charmed. Huge trees shaded the land, balancing in their toothed foliage large spiny balls. St.. Martial smiled, understanding the malicious intention of the minister of God. These rough shells concealed a savory fruit: the Chestnut.

ALONG THE RIDGE: CHESTNUTS

by Kurt Reinheimer

The blight was too far along for them to have been American chestnuts, but there's a boyhood memory of my parents pulling a cookie sheet of hot chestnuts out of the over and then trying to talk us children into tasting one. I remember the nuts as 'risualty appealing-maybe just because the sight of a cookie sheet in my mother's hand *aiways* meant something good. And the shapethe split brown casing around something steaming and soft-looking -must have added to the initial enticement.

But they just weren't a children's delicacy. The taste to me was as much like

warmed-up, waterlogged chalk as anything else, so we left the full sheet for our parents to enjoy. I guess in our youth and ignorance we d have had not problem if we never saw them again. And while those were Italian chestnuts from the grocery store, the disappearance of our own native trees has continued unabated since those days back in the 1950s.

The blight was first noted in New York state in 1904, entered the Blue Ridge region from the north by 1908, and by 1945 was present in all areas of the tree's natural range from Maine south along and to either side of the Appalachians south into Alabama and even eastern Mississippi. Before the march of the disease, about one in every four trees in that huge 17-state area was a chestnut. Now, there are just a few survivors per state, and a great American resource not just for food. but for lumber, fences, railroad ties, landscaping, shape and more has virtually disappeared. A parallel event in the animal kingdom might involve the rabbit or the deer. But the impact on our culture of the disappearance of the tree that has been called the most useful hardwood species in the eastern United States has not caused much of a diminished place in the fabric of society. Try to think of a town without a Chestnut Street. Or recall, albeit out of season, one of the most recognizable line in American music - the one about the open fire and the chestnuts roasting.

Maybe it's that kind of entrenchment of the chestnut in our psyche that has helped spawn a resurgence of efforts to develop a blight-resistant tree. Efforts in the 1920s, '30s, and '40s most part attempting to breed a more resistant tree - ended in general failure. But a new generation of researchers in the Southern Appalachians (along with a national organization based in Minnesota) is at work trying to return the chestnut to its proper place in the Appalachian forest and the home landscape. Biologists and arborists at Virginia Tech in Blacksburg, Virginia, at Concord College in Elkins, West Virginia, and at the universities of West Virginia, Kentucky and Tennessee are exploring several distinct tracks toward eradication of the blight:

Trees infected with a hypovirulent (tow level) of the blight fungus have in isolated instances recovered. Though the early optimism of this situation providing a "vaccination" for other trees has ebbed, researchers still see hypovirulence as an important step toward recovery.

"Promise has also been shown with a breeding technique using blight-resistant American chestnuts in initial hybridization with fully blight resistant Chinese chestnuts. Subsequent generations are then bred to be more and more American (there are some 16 million different gene combinations in the chestnut) and at the same time carry the resistance from the Chinese strain.

There is also an attempt to use genetic material from several large, surviving American chestnuts through grafting.

Irradiation of nuts is being carried out with the hope that resulting mutants will produce a resistant strain.

The people doing the work - people like Gary Griffin at Virginia Tech-carry a quiet, passionate patience about what they do. The work is slow. It takes years to grow a free to the point that it will produce nuts, but the overall attitudes

seems to be that the task will be accomplished.

If the job does get done - Griffin says the chestnut could be restored to economic significance within 50 years-then the great Appalachian forest would be re-transformed. Not to the extent that some might dream-the virgin forest that covered these hills for thousands of years, and as late as the 1700s still encompassed the whole of the Blue Ridge region, had already been almost fully destroyed by the 1940s - but perhaps at least to the point where it stood before the advent of the blight, when the proudest of the chestnuts reached 120 feet into the blue mountain sky, with trunks up to 13 feet in diameter: The

oldest of the trees had stood for 500 years on the Appalachian forest floor; well anchored in their preeminence before the blight brought them down

The battle will be long and slow, and probably won't be won in the lifetimes of most of us- But past of our ties to this beautiful land are to the forests that give the mountains their life and shape and majesty. Among the many things we must preserve and present to the generations that come after us, those things that grow around us are surely among the most important.

And besides, every little boy of 10 or so should have to taste a roasted chestnut. An American chestnut

HYPOVIRULENCE: A BIOLOGICAL CONTROL FOR CHESTNUT BLIGHT

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The introduction of *Endothia parasitica* (Mum) P.J. and H.W. And. into North America at the turn of this century created one of the first major challenges to the relatively young science of plant pathology. This brightly pigmented orange Ascomycete introduced into the New York City area on oriental chestnuts, did not remain a curiosity for long. Careful studies by early scientists quickly unraveled the details of a host-parasite interaction that would have unparalleled ecologic, economic and sociologic impact on the eastern United States. Sadly, within 10 years of the discovery of the causal fungus, most of the same researchers admitted that little could be done to slow the epidemic. The frustration they felt is evidenced in their early writings (29). To them, the only remaining control strategy was to initiate breeding programs to preserve the best traits of the American chestnut *(Castanea dentata* (Marsh.) Borkh) and incorporate resistant germplasm from Chinese *(C. mollissina* BI.) and Japanese *(C. crenata* Sieb and Zucc.) chestnut. These undertakings, which met with limited success, were never designed to control chestnut blight in our eastern forests. Undoubtedly, many students of plant pathology and forestry have entertained romantic thoughts of controlling this destructive pathogen.

Fortunately, the American chestnut was saved from extinction in its natural range by its propensity to sprout from the roots. Ironically, this perpetual sprouting also may have provided for the development or expression of a natural biological control phenomenon that we may be able to exploit to regulate *E. parasitica* in our forests.

Discovery and Description of Hypovirulence

In the 1950's an Italian plant pathologist, Antonio Biraghi, observed 'spontaneous healing' of cankers on European chestnut *(Castanea sativa* Mill.) in northern Italy (4). His observations aroused the curiosity of Jean Grente, a French mycologist, who described a variety of abnormal *E. parasitica* isolates associations prompted him to call them "hypovirulent". While this observation was significant, more importantly, Grente and his coworkers found that the factors responsible for hypovirulence were transmissible. Using in vitro and in vivo tests he demonstrated that normal isolates became hypovirulent after hyphal anastomosis with hypovirulent isolates. He therefore considered the determinants of hypovirulence potentially useful as biocontrol agents (19).

Less than 25 years passed from the time chestnut blight was discovered in Europe until recovery was first observed in Italian chestnut stands (4). By this time, the disease had been present in North America for over 50 years, with few if any signs of resistance to or recovery from infection. The Italian situation, however, refocused attention on chestnut blight in the United States and lead to experimentation by Van Alfen and his colleagues at The Connecticut Agriculture Experiment Station (33). In greenhouse tests, they confirmed Grente's findings by demonstrating that European hypovirulent isolates of E. *parasitica* could be used successfully to stop the expansion of individual cankers initiated by North American virulent isolates. The introduction of hypovirulent isolates into expanding cankers induced the formation of callus tissue at the edges of cankers on young stems. Descriptions of their research and of the resulting abnormal/nonlethal cankers

were widely publicized. As a result of the attention this work received, one observant naturalist sent bark samples to The Connecticut Agricultural Michigan. These trees had been planted by early settlers, and although severely damaged by blight, were still alive and had abnormal cankers, similar to those described in Italy. Elliston et al. (14) found that although the cultures maintained the normal orange pigmentation of virulent strains, they fit many of the criteria then used to define hypovirulence; they had abnormal culture morphology, were less virulent than normal isolates and transmitted these traits to normal isolates.

Brewer (5) later determined that surviving blighted American chestnut trees in Michigan were common, and Fulbright et al. (17) found hypovirulent isolates in several of these blighted chestnut stands. Today, over 30 American chestnut stands consisting of large mature trees, saplings and seedlings have been identified in Michigan that are surviving infection. In many of these stands, blight is still the dominant biological stress, but in a few, almost all signs of E. *parasitica* have disappeared. Even though the natural range of the American chestnut reached into southeast Michigan, the recovering stands are all located in western or central Michigan, outside of the natural range.

Jaynes and Elliston (25) and Griffin (22) also have recovered isolates from surviving American chestnut in other states and found that many of these surviving trees were infected with hypovirulent strains. Other hypovirulent isolates have since been recovered from trees in Maryland, New York, Virginia, Tennessee, and West Virginia. Infected, surviving trees with hypovirulent isolates of E. *parasitica* also are present in southern Ontario (Colin McKeen, personal communication). Therefore, it is now apparent that Europe does not have a monopoly on surviving chestnut trees or hypovirulent strains of the fungus, but the recovery of chestnut in Europe has been so successful that the reestablished European chestnut industry is now exporting chestnuts to this country.

What Is Hypovirulence?

Grente first coined the term hypovirulent to describe the *E. parasitica* isolates recovered from surviving European chestnuts in Italy. As these types of isolates were characterized during the next 20 years, many of the phenotypic traits of those particular hypovirulent isolates were used to define hypovirulence. Because hypovirulent isolates possess many variable traits, it has been difficult to define hypovirulence in relation to synonyms such as 'less virulent' or attenuated'. What criteria must a pathogen posses to be termed hypovirulent? The first and primary trail of a hypovirulent isolate is reduced virulence. Although there is no 'normal" virulence level, there is an 'expected' level, which can be determined after observing the disease in situ. Pathogens that produce smaller cankers or sporulate less in a given time period may be less virulent (11). Elliston demonstrated hypovirulent isolates of *E. parasitica* exhibit a wide range of virulence, and that any given hypovirulent isolate may cause large cankers while others are unable to grow when inoculated into living trees. By measuring canker size and/or reproductive capacity, the virulence of field isolates can be characterized and compared.

Hypovirulent isolates of E. *parasitica* also often exhibit abnormal culture morphology on agar media. The first hypovirulent isolates recovered from Italy and France were non-pigmented and posed problems for species identification. In Michigan, and other locations in North America, hypovirulent isolates maintain pigment production, but some may lack the expected zonations when grown on media under alternating light/ dark photoperiods. Culture morphology has been widely used for the identification of hypovirulent isolates. However, it has become apparent that the use of this feature should be limited to well characterized strains that have distinct, recognizable morphologies. Culture morphology should never be the sole criterion used screening unknown culture morphology may be overlooked or virulent strains with abnormal morphology may be improperly classified as hypovirulent.

An important characteristic of hypovi rulent *E. parasitica,* that makes the biological control of blight possible, is the transmissible nature of the cytoplasmic agents of hypovirulence. Hypovirulent isolates can convert ones to hypovirulent after hyphal anastomosis. When conversion occurs, the recipient strain demonstrates all or most of the hypovirulence characteristics associated with the donor strain, including virulence, pigmentation, sporulation and culture morphology (12,13). Therefore, when an orange, American virulent isolate is converted by a white, European hypovirulent one, the American isolate will become hypovirulent and white.

What genetic factor(s) codes for these abnormal phenotypic characteristics in *E. parasitica?* The transmissible nature of hypovirulence demonstrates these genetic factors are cytoplasmic. There fore, it was not surprising to find that almost all hypovirulent *E. parasitica,* as previously defined, contain molecules of double-stranded ribonucleic add (dsRNA) (6). Most fungal viruses, however, are composed of a protein coat and a single or multi-segmented dsRNA genome. The dsRNA molecules found in E. *parasitica* whether originating from European or North American isolates are similar in that they do not appear to be associated with **a** protein coat; rather they appear to be associated with vesicles of host origin (8,29). A virus-like replicase activity has been found in one hypovirulent strain (34). While evidence strongly supports dsRNA as the genetic factor involved in hypovirulence, it has not been proven to be the cytoplasmic determinant of hypovirulence because Koch's postulates have not been fulfilled. Three strong pieces of correlative evidence, however, suggest dsRNA is responsible for the hypovirulent phenotype in most E. *pamsitica* isolates. First, nearly all hypovirulent isolates contain dsRNA; virulent ones usually do not. Second, a virulent isolate converted to hypovirulent will usually obtain the characteristics of the hypovirulent dsRNA molecular (based on size and number of dsRNA segments) as the hypovirulent isolate involved in the conversion. And third, when the dsRNA is eliminated from an isolate either by single conidial isolation or treatment with cycloheximide (15), the resulting culture changes to a virulent phenotype.

Hypovirulent isolates of *E. parasitica* frequently harbor more than one segment of dsRNA and the number and sizes of these segments are usually different and vary from isolate to isolate. The molecules range in molecular weight from approximately 6.0 x 106 (9 kilobases) to less than 0.6 x 107 (0.8 kilo-bases). Most hypovirulent strains have a 'large' molecule of dsRNA ranging in size from 406 x 106 (7). There is relatively little dsRNA in the hypovirulent strains, ranging from 10-75 ug/g dry weight of mycelium. On the whole, there seems to be more dsRNA in European than in American strains (7).

Although dsRNA molecules examined from European and North American isolates of *E. parasitica* are associated with hypovirulence they do not share extensive genetic similarity (28,30), however, terminal sequence analysis showed these dsRNA molecules share some common terminal sequences (23,32). This indicates that although there are similarities among the dsRNA molecules that infect *E. parasitica* these virus-like agents are not necessarily genetically identical.

The role of dsRNA in hypovirulence remains unknown. Recently, Powell and Van Men reported that isolates of *E. parasitica* that contained either North American or European dsRNA, did not accumulate proteins found in normal dsRNA-free isolates (31). The functions of these proteins are being determined, but this is the first evidence of biochemical differences between virulent and hypovirulent isolates due to the presence of dsRNA. Such studies may determine the role of dsRNA in hypovirulence and help us understand the phenotypic differences found among hypovirulent isolates.

It must be emphasized that there are E. *parasitica* isolates with reduced virulence without detectable levels of dsRNA. However, little work has been done to determine the cause of the reduced virulence phenotype in these isolates. Fulbright (I 6) demonstrated the transmissible nature of hypovirulence in one dsRNA-less isolate and showed its usefulness in biocontrol of chestnut blight.

Exploiting Hypovirulence

For the first time since the realization that the American chestnut was doomed, excitement has been generated by the prospects of a unique biological control to this devastating disease. Because we have assumed the survival of trees in Michigan and Italy results from the natural buildup of hypovirulent strains of E. parasitica, one approach has been to investigate various ways to artificially introduce these strains into the forest. The initial efforts were to follow Grente's procedure of treating individual infections by exposing cankers to some form (inoculum plugs, conidial/mycelial slurries in sprays) of hypovirulent inoculum (24). The results of such tests at first were promising, because expansion of individual cankers often was arrested and callus tissues formed at their margins. However, the failure to control some infections or to influence the development of subsequent cankers on the same stem was a common result. In some instances, a majority of infections (15 or more) on a single tree were arrested but as new cankers developed over time trees died from the shear number of infections they supported. Sprouts that remained alive after successive years of canker treatment are the exception (24,35).

Information, however, was obtained from these initial experiments. The strains first used for canker treatment were highly curative but they grew and sporulated so poorly that their persistence was limited. Therefore, as infections ceased to expand and periderm tissue formed, the amount of infected bark containing the agents of hypovirulence deteriorated rapidly. Failure to observe the perpetuation of strains led to the examination of isolates with greater potential to grow in bark and provide a persistent source of hypovirulent inoculum. Virulent and hypovirulent strains was dramatically restricted if the two strains are vegetatively incompatible (hyphal anastomosis limits this restricting dsRNA transmission) (1,3). This finding helped explain why the treatment of some virulent infections was unsuccessful and lead to an appreciation of the complexity of the vegetative compatibility situation in our eastern forests. Well over 70 compatibility types have since been described and the existence of as many as 128 hypothesized (3,9). Even though vegetative incompatibility may be a significant barrier to the successful interaction of virulent and hypovirulent strains, subsequent research also has demonstrated that some hypovirulent isolates are able to successfully transmit the agents of hypovirulence to virulent isolates from a variety of vegetative compatibility groups (2,27). Furthermore, combinations of these highly interactive hypoviwlent strains have successfully converted virulent isolates from most known vegetative compatibility groups both in vivo and in vitro (26). Yet, even with this information, a variety of field treatment strategies have done little to dramatically influence the course of disease or significantly prolong individual tree life.

We have assumed that for hypovirulence to be successful, a reservoir or source of hypovirulent inoculum would have to be continuously present to spread and interact with the natural *E. parasitica* population. Furthermore, for this interaction to be successful in the native chestnut forest it would have to occur on the only host material available, young chestnut sprouts. One approach used to establish hypovirulence reservoirs in West Virginia has been to initiate hypovirulent infections on healthy stems prior to their natural infection by virulent strains. In one study, large scratched-wounded areas of the bark on the main stem were
inoculated at various heights with hypovirulent isolates that were able to grow and reproduce but that did not kill their hosts. The objective of this study was to isolate E. *parasitica* from new infections as they arose in nonwounded areas of the bark, to determine if natural dissemination from the hypovirulent the experiment and increased with subsequent sampling periods up to 4 years. Infections that initially yielded only virulent isolates, when resampled one year later, often yielded one or more hypovirulent isolates and frequently a complex variety of interaction products. While detection of natural dissemination was very encouraging, almost all trees in these study plots have died because of an overwhelming number of virulent infections from the natural *E. parasitica* population.

Another dissemination study initiated in Michigan utilized a genetically marked hypovirulent strain and specific dsRNA molecules (18). Rather than directly inoculating bark with the hypovirulent strain, culture dishes containing asexually sporulating cultures of the hypovirulent fungus were placed in trees for six months above a series of small wounds made on the trunk of the 20 trees. The hypovirulent strain was found not only on treated trees but also on those on which it was not placed and dsRNA molecules were found in isolates from natural infections demonstrating dsRNA transfer in situ. Three years later, more than half of the trees harbored dsRNA-containing isolates. Therefore, hypovirulent strains and dsRNA may be disseminated by conidia produced from small cankers initiated by hypovirulent strains. Whether or not a hypovirulent epidemic is established in this plot will be determined by annual monitoring of the cankers.

The premise that either canker treatment or various other methods of hypovirulent strain dissemination can control chestnut blight over a short time is probably unrealistic. In the eastern United Stales, we are dealing with small stems whose survival is directly related to their circumference and the rapidity with which the tree is girdled by a virulent strain. Often areas in the Appalachian Mountains where chestnut sprouts abound are openings that have been created by some forest harvesting practice. When released, existing sprouts thrive and many new sprouts develop and as host substrate increases so does the virulent population of *E. parasitica* This rapid build-up in host and pathogen population eventually results in a 5-10 year epidemic and the death of most sprouts. This interval of time may not be adequate for the agents of hypovirulence to either become established or for their effects to become evident by prolonging tree survival. As the epidemic ensues, chestnut is over topped by competing vegetation and is eliminated or once again assumes a minor position as an understory shrub (21).

In retrospect, many of the field studies that were originally designed to test the hypothesis that hypovirulent strains can serve as Biocontrol agents were premature. Yet, such studies can be rationalized by the very fact they provided insight into a variety of research needs. Most significant was our lack of appreciation for the phenomenon of hypovirulence in nature, particularly the origin of the dsRNA associated with this phenomenon. In addition, the mode by which dissemination and establishment has allowed hypovirulent strains to significantly influence the virulent populations of *E. parasitica* was unknown. We mistakenly assumed dsRNA is not a common component of the chestnut blight fungus in our eastern forests. Recent examination of *E. parasitica* isolates from West Virginia has shown dsRNA is present in over 50% of the isolates from some locations (10). If dsRNA is a common component of E. *parasitica* in the eastern US, then the question must be asked, 'Why has this (these) dsRNA(s) not played a role in the recovery of chestnut within the native range as it presumably has in Michigan and Italy'? Several explanations exist. First, the dsRNA's carried by *E. parasitica* in the native range of American chestnut may not be responsible for reduction in pathogenicity and/or may not be readily transmitted. Whereas many of the Michigan and Italian strains even though significantly debilitated, appear to have spread among the virulent population. Furthermore, if the dsRNA's are common components of the *E. parasitica* population in the east and if some are appropriate for biocontrol, then perhaps the opportunity for expression has not been achieved. One possible explanation for the lack of expression comes from the observation of a major difference in the ecosystems (20). In both Michigan and Italy where hypovirulence expression occurs, chestnut regeneration has continued with little or no competition from other plant species. This seldom is the case in eastern forests, which indicates our need to examine the dsRNA from various regions in an effort to understand the role of these molecules in hypovirulence and biological control.

Another shortcoming in our knowledge is the lack of understanding of how a 'hypovirulence epidemic" develops. One must question how such debilitated strains can become so well established among a highly fit virulent population. The answer may rest in our knowledge of the source of hypovirulence and/or the examination of its contagious nature. We simply know little of the epidemiological details of the interaction between virulent and hypovirulent strains. For example, exposure of a virulent canker to hypovirulent inoculum not only may cause conversion of the canker but also may result in reduction of virulent inoculum production and initiation of hypovirulent inoculum production. The contagious nature of hypovirulence thus may be its one advantage and given time, a slowing of the virulent epidemic and development of a hypovirulent one might result. Remarkably, a gradual transition from virulence to hypovirulence appears to be responsible for the survival of chestnut in a small woodlot at The Connecticut Agricultural Experiment Station, where hypovirulent strains were introduced more than a decade ago (J.E. Elliston-personal communication). Another unique situation is occurring in Maryland where blighted chestnut sprouts in 20-year-old chestnut plantings are be-ginning to show obvious signs of recovery without hypovirulence introduction after blight reduced the young trees to sprouts. dsRNA-containing isolates are commonly recovered from the cankers, in this planting, but its effect on virulence is not clear. Both the Connecticut and Maryland settings are stands of pure chestnut.

Outlook

For most of this century we have only been able to describe E. *parasitica,* its effects and attempt some breeding experiments. Now, however, nature has provided us insight to a novel approach for the control of this devastating disease. Clearly, if we are to utilize hypovirulence we must first understand hypovirulence. This will take a multidisciplinary approach where the disciplines of plant pathology, ecology, epidemiology, genetics, molecular biology and others must merge into a coordinated effort if we are to affect the remaining chestnut biomass. The naturally recovering stands in Europe and Michigan indicate it is possible. With coordinated research efforts it may soon be realistic to once again entertain thoughts of controlling this destructive pathogen.

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SCREENING FOR RESISTANCE TO CHESTNUT BLIGHT

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Reliable screens for chestnut blight resistance currently require 5-year-old trees. We explored the possibility. of screening for **chestnut** blight resistance in first and second year seedlings growing in pots in the greenhouse. A usable screen would speed up a breeding program by shortening the time between planting and screening, and reducing labor and land needs. It also could be used safely in blight-free areas.

Materials and Methods

We used blight-resistant Chinese chestnut, and scarlet oak, and blight-susceptible American chestnut. The Chinese chestnuts were obtained from trees growing in Kentucky and Virginia. The parents probably originated from seed released by the U.S. Dept. of Agriculture. Such seed were from parents tested for blight resistance. The scarlet oak acorns were obtained from trees growing in Kentucky which did and did not exhibit the swollen butt associated with *Endothia parasitica* cankers. American chestnuts were obtained from trees in Kentucky and Maine. The parent trees bore numerous cankers typical of blight-susceptible American chestnut: a flat **to** slightly sunken interior, extending to the vascular cambium, abundant stromata over most of the canker, with numerous mycelial fans at the canker margin.

Seed were planted in 15 cm pots in a soil-tree potting mix composed of equal parts of peat moss, vermiculite and perlite. The pH of the medium was adjusted to ca. 6.0 with lime. Peter's soluble trace element mix and 20-20-20 Osmocote(TM) were added at concentrations recommended by the manufacturers. Peter's Professional 10-10-10 fertilizer supplemented with 0.1 M Fe-EDTA was added bimonthly at one cup per-pot. Daylength was maintained in excess of 13 hours with low-intensity (ca 5 m2 s-1) cool-white fluorescent lighting. Terminal buds were removed when plants were about 1 m high. Lateral branches less than 50 cm above the soil were removed when they were small. During winter (Nov-Mar) plants were placed outside in a cold frame covered with burlap.

Plants were acclimated to **a** growth chamber with 23 C, 14 hour days, and 18 C nights for one week prior to inoculation, and maintained in the chamber for about 2 months. Light intensity was 27 m-2 s-1. Plants too tall to fit the growth chamber were bent over and secured with string.
We assayed blight resistance by direct inoculation and by measuring rates of ethylene production after exposing stem segments to mycelium of *E. parasitica*. The seedlings were 5 to 18 months old and were in their first or second season of growth. Inoculations were made with the miniature cork-borer, agar-disk technique described by Hebard (1982). Two inoculations were made in each plant, at heights of a ca 6 and 12cm, on opposite sides of the stem. The midpoint of the stem between the inoculations ranged from 0.7 to 1.1 cm in diameter on American chestnut, 1.0 to 1.4 cm on Chinese chestnut, and 0.3 to 0.6 cm on scarlet oak. We used strain Ep 155, Ep408, and Ep905 of *E. parasitica* (Elliston., 1986).

For ethylene determinations, segments ca 0.5 cm in length were cut from the ends of stems and placed on 0.6 cm diameter agar-mycelium disks in 6 ml Vacutainer tubes. Further details are in Hebard & Shain (1988).

Results

<u>Direct inoculation</u>. There were several qualitative differences between cankers on Chinese and American chestnut which consistently differentiated the two species (Table 1). Cankers on American chestnut almost always encircled the stem and extended to vascular cambium, resulting in blight of distal The cankers were sunken., showed abundant stromata ca 40 days after inoculation, and had a symmetric, elliptical shape. Cankers on Chinese chestnut, by contrast, frequently did not encircle the stem and only extended to the vascular cambium over their entire circumference in stressed plants. Thus distal leaves rarely blighted. Stromata were rare on cankers on Chinese chestnut, and the cankers were mostly flat, neither sunken nor swollen. The cankers generally were highly irregular

in shape, due to restriction of expansion to only portions of the canker margin.

Cankers on scarlet oak mostly did not expand beyond the initial lesion. Occasionally, cankers expanded and encircled the stem, which resulted in blight of distal leaves. This occurred most frequently when plants were stressed by lack of water. Stromata occurred on such cankers but not on initial lesions. These cankers frequently extended to the vascular cambium on scarlet oak, probably because the plants were so small. Cankers which expanded beyond the initial lesion but did not encircle the stem were swollen on scarlet oak.

Table 2 presents rates of canker- length expansion for six experiments conducted over the last year. Cankers on American chestnut consistently expanded taster than those on scarlet oak and Chinese chestnut, but the difference with Chinese chestnut was significant ($p<0.05$) for only two of the experiments. After the first two experiments the lack of significance was due to low numbers
of cankers. These were the result of inoculation failures. Inoculation failures were indicated by no necrosis around the inoculation point and by callus formation in the inoculation hole. in experiments 1-5, there were five inoculations of each fungal strain on each species; so, for instance, there was one inoculation failure with strain 155 on American chestnut in experiment 1. In experiment 6, there were ten inoculations per species with strain 155. Experiments are in progress to try to reduce the rate of inoculation failure.

After the American chestnut were blighted, the stems of all plants were cut off near the soil line. Table 3 presents the fraction of stems which subsequently resprouted in four experiments. Generally, about 75% of stems resprouted, **and** recovery was higher in the more blight-resistant hosts.

<u>Ethylene production</u>. After exposure to *E. parasitica*, ethylene production increased over controls more in American chestnut than in scarlet oak or Chinese chestnut (Table 4). The difference was statistically significant when five replicate stem sections for each of the five trees per species were used (experiment 4, Table 4).

Discussion

The high blight resistance of Chinese chestnut and scarlet oak can be identified by direct inoculation and by measuring ethylene evolution. The qualitative differences we observed between the susceptible and resistant hosts after first inoculation would be extremely useful in a resistance screen. This is because the data can be collected and analyzed rapidly and because the data are consistent from test to test. This would permit us to screen one population over several tests, eliminating problems with inoculation failures. On chestnut, the differences are all highly correlated, and except for regular canker-margin, depend on whether or not a canker extends to the vascular cambium. There would be some loss of plants with the direct inoculation test undercurrent conditions.

The ethylene assay is nondestructive, fast, and can be conducted in regions where escape of *E. parasitica* inoculum is of acute concern. Further testing will be needed to demonstrate its reliability as the coefficient of variation is higher than for direct inoculation. Also, it would be desirable to understand the biological basis of the differences we observed between species. Experiments to this end are in progress.

Both tests need to be performed on F1 hybrids and backcross populations. We do not know whether the tests will be able to distinguish hosts of moderate blight resistance from hosts of low or else high resistance. Plants for such tests currently are growing in our greenhouse.

Cankers on 1 and 2-year-old potted seedlings expand faster-on average than cankers on larger plants. On larger Chinese chestnut, there frequently is no expansion beyond the initial lesion. On larger American chestnut, cankers expanded about 0.7 to 1.1 mm/day in length (Hebard, 1982), compared to 0.9 to 1.4 mm/day in the present experiments. Cankers may expand taster on small seedlings because the cankers exert relatively more stress on the smaller plants. We plan to evaluate the effect of number of cankers per- seedling on expansion rates.

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Addendum

Callus cultures were developed from hypocotyls of germinated seed of American and Chinese chestnut. Conditions have not been found which permit identification of cultures with different levels of innate blight resistance. Further tests are in progress.

EVALUATION AND USE OF LARGE AMERICAN

CHESTNUT SURVIVORS IN BLIGHT AREAS

by Charles Burnham

There are two possible reasons for their survival. First, those trees are susceptible but either somehow escaped the blight, or were saved by hypovirulent strains that stopped the growth of the cankers.

Second, the survivors may have genetic resistance. If widely scattered, they may have different genes for blight resistance. Thus far, the level of resistance in each is too low for use in plantings. The ultimate goal is to combine the different genes and thus increase the level of resistance. When a mutation from susceptibility to resistance occurs in blight areas it most likely will be dominant (complete or incomplete) and heterozygous, since heterozygotes for a dominant mutation for resistance would have a selective advantage. Heterozygotes for a recessive mutation for resistance would have no selective advantage. If the mutation occurred before the blight came, a recessive mutation for resistance could be carried and spread in the population. Eventually two heterozygotes might cross to produce some homozygous recessive trees that would be blight resistant. The first question to be asked for each source of resistance is, is that resistance completely dominant, incompletely dominant, or recessive? The answer comes from growing progeny from crosses between

the resistant tree and susceptible American chestnuts. This is the well-known and widely-used genetic test cross. If resistance is dominant, a resistant tree may be heterozygous or homozygous for the gene(s) for resistance. If homozygous and completely dominant, all the progeny from the testcross with susceptible tress should be as resistant as the resistant parent, if completely dominant but heterozygous, part of the progeny will be as resistant as the resistant parent, part will be susceptible. The ratio will depend on the number of pairs of genes involved. If one pair, the ratio will be 1 resistant:1 susceptible; if two pairs, 3:1. If resistance is incompletely dominant, homozygotes will have a higher level of resistance than heterozygotes. The test cross progeny not only provide the above information, but they also increase the number of trees carrying the genes for resistance. Some of the resistant trees will be cross compatible. Crosses between them will increase that source of resistance and also produce some that are homozygous (true-breeding for resistance).

If the resistance is recessive, heterozygotes will be susceptible. When the original recessive mutation occurred, the tree was probably heterozygous for that mutation. Progeny from crosses with surrounding trees would have produced more heterozygotes, and finally progeny from crosses between heterozygotes would have included some homozygotes which would be resistant and would have survived when the blight arrived. Susceptible trees in the same area would include heterozygotes and ones not heterozygous. Progeny from crosses between the latter and resistant trees would all be susceptible. Progeny from crosses between heterozygotes would segregate 3 susceptible:1 resistant if one pair of genes is involved.

When the above information for each source becomes available, progeny from crosses between the different sources of resistance can be tested for blight reaction to determine which ones have different genes for resistance.

The improved techniques for identifying different levels of resistance used by Griffin and Elkins (1982) will be useful.

Crosses are being made between the different sources of resistance on the assumption that different genes for resistance are involved (Griffin 1988). For the plantings from those crosses, consideration should be given to the need for maintaining pedigrees, making controlled crosses in successive generations to ensure that the genes for resistance from the different sources have a chance to combine. Also to be considered is the possibility of growing the next generation from plantings in which susceptible seedlings have been removed. This should not be done if any of the genes for resistance are recessive.

If pedigrees are not maintained, large numbers should be grown in one location and with a planting and crossing plan that gives a chance for generating the combinations of genes from different sources.

If information on the genetics of each source of resistance, as described above, is not available, not only is there a possibility that some of the sources may be lost, but also the potential from homozygotes for the largest number of different genes for resistance and the highest levels of resistance may never be realized.

I am not suggesting that crosses between the different sources should not be made, but now that more institutions and people are becoming involved, it should be possible to include the additional studies discussed above.

Meanwhile it is important to transfer the genes for resistance from the other known resistant Japanese and other species to the American chestnut, each in a separate backcrossing program, as first suggested by Inman. In these programs, each transfer is between different species. This may account for some of the oddball seedlings that are appearing among the progenies in first and second backcrosses.
After the genes from each source are transferred, crosses can then be made between them to combine the genes that are different.

These programs should be completed as rapidly as possible so that the resistant American chestnuts can be used in a later stage of the program for introducing blight-resistance into the various American chestnut populations that are adapted to different localities (Inman 1987).

An RFLP map of the American chestnut might be used as an aid in combining the different genes for resistance, but that will involve an intensive, long-term re

search effort.

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CHESTNUT TODAY IN THOREAU COUNTRY

This is a composite from several field sketches illustrating the typical dimensions and shape of chestnut sprouts in abandoned farmland in New England. The location is north of Boston in the town of Andover, not far from the places where Thoreau describes the Massachusetts countryside in his Journal. A very interesting glimpse of chestnut is given by photographs of places described by Thoreau taken around 1900 (H. W. Gleason, *Thoreau Country*, Sierra Club Books, 1975). The photograph on page 105 shows a nearly pure stand of chestnut all multi-stemmed trees having originated as stump sprouts. My illustration shows woods that were just returning to forest when chestnut blight struck in 1922. We know most large chestnut trees died that year because we have dated the outermost growth rings of large dead chestnut trees, like those leaning on the stone wall in the background of my sketch. Most of the pitch pines and many of the birches that first populated the abandoned pastures of Andover before 1900 have given way to oaks and maples. The pine one now sees abundantly is the white pine, which seeds into open oak woodlands. The chestnut trees killed by blight around 1922 eventually toppled over as their roots rotted out. Maps of chestnut logs show that they fell in almost random patterns, in sharp contrast with many pine logs all pointing to the northwest. That is the direction in which the winds blew during the infamous 1938 hurricane. The old chestnut wood shown in my sketch is still largely intact, compared to the thoroughly rotted pine wood from the 1938 storm. One final point to note is that the chestnut sprout in my sketch is not attached to the roots of one of the old blight killed trees. This is typical. Chestnut only sprouts from the root collar, and chestnut trees which topple pull any surviving sprouts out of the ground when they fall over. It took a lot of head scratching before this simple explanation for the tendency for salvaged chestnut trees (those that were cut before they fell over) to survive. Most of the living sprouts we see today were seedlings just established in the years before 1920. The present population of chestnut sprouts in New England may very well represent a "snapshot" of chestnut reproduction in the decade before the appearance of blight.

AGING CHESTNUT SPROUTS IN CONNECTICUT

It is not uncommon to find rather large chestnut sprouts in the mixed hardwood forests of southern New England (see illustration on page 24). These small tress are typically six or more inches in diameter at the base, and more than forty feet tall. That is. tall enough to be reaching up into the subcanopy. Where do such trees come from, and how have they managed to escape the blight torso long? These are subjects of current research, but we can illustrate the kinds of answers one obtains by examining a typical example. My sketch shows the profile of a chestnut tree in Rocky Hill, Connecticut - tree no. 54 in the research area maintained by Dr. Sandra Anagnostakis of the Connecticut Agricultural Experiment Station. My sketch shows the "mature" looking bark of the trunk, providing a strong hint that this tree is relatively old. Also notice the adjacent group of root crown sprouts with the multiple-stemmed growth form one expects when chestnut resprouts after blight infection. The large stem now has a blight canker in the upper part of the crown, which has killed the top branches of the tree. How old is this veteran chestnut sprout? The answer comes from an increment boring at the base. The growth rings show that this tree is almost forty years old, the current stem having originated about 1950. Since blight appeared in this area about 1915, the root crown from which this large stem originated was already 35 years old when the tree I have drawn started growth. Quite a few other borings of chestnut sprouts indicate similar results - old stems typically produce about one inch of diameter increase per decade when held in suppression. The ancient" looking fissured bark appears after about twenty years of age. The same kind of bark ridges and fissures appear at about the same age on blight-free, naturalized chestnut trees in the midwest-but at a diameter of 18 inches after sustained diameter growth rates approaching an inch per year!