Vol. XI, #1 Summer 1997

## Notes



## Memories



## Science and Natural History

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# EDITOR'S NOTES

*Summer 1997*

This issue of *The Journal* resembles the chestnut itself in terms of what it one time gave us — a little bit of just about everything. Where the species provided food and shelter for all kinds of living things above and in the earth that supported it, this *Journal* offers food for the mind in challenging articles and shelter for the spirit and imagination in poems and letters and photographs.

From Virginia come farm superintendent Fred Hebard's and research scientist Yan Shi's update on activities at the research farms, Yan Shi's latest on his work on male sterility in chestnut and its potential impact on The ACF's breeding program, and two poems by Ralph S. Coleman that will immediately transport you from today and here to then and the Appalachians. Halfway across the world, John W. Duffield's photographs of chestnut carvings in a Slovenian churchyard will usher you into an entirely different past.

Member Bill Lord worked hard to tell the story of the blight fungus itself, and closes his article with a message of hope. Hope was in the hearts of all who worked with the Clapper tree, a source of some of the genetic material now used at the foundation's research farms. This unusually vigorous tree grew more than six stories tall in a research plot on a refuge in southern Illinois before it finally succumbed to the blight. The story of the Clapper tree can be found here as excerpts from the refuge's files.

These and this issue's other stories and memories, drawings and photographs all celebrate an extraordinary tree which continues, despite its catastrophic decline, to provide us with any number of good things to strengthen and please heart and mind.

Shelly Stiles
Editor

# NOTES FROM MEADOWVIEW

BY F. V. HEBARD AND Y. SHI
MEADOWVIEW RESEARCH FARMS

In 1996, Meadowview was again blessed with abundant precipitation all year long, so the trees grew very well. After four seasons of growth, the trees average 12 feet in height, representing a range of three to 16 feet. After three seasons, the trees average eight feet, representing a range of two to 13 feet in height. After two seasons of growth the average height is five feet, and at one year of age the average is 22 inches tall.

Our trees continue to flower fairly early, usually first producing male flowers after two to four seasons of growth and female flowers after three to five seasons of growth. Most trees first produce male flowers at a younger age than they first produce female flowers, but sometimes both sexes are produced at the same age and, more rarely, female flowers are produced first.

## 1996 NUT HARVEST

In 1996, we harvested almost 6,000 nuts from controlled pollinations at the farms and in the surrounding mountains, about three times more than in any previous year. (Table 1 summarizes the year's harvest.) And we had little pollen contamination. As a result, it was by far and away our best crop ever!

The crop was larger than in years past because we placed about 2.5 times more bags than in previous years. (Each bag may contain several controlled pollinations.) We were able to place more bags because a large number of second backcross trees already selected for resistance flowered. Additionally, a large number of American chestnut trees at the farm produced heavily. Compared to previous years, we had much more material with which to work.

We were also better able to take advantage of this additional material. In 1996, Yan Shi joined our research team at Meadowview in time to help direct the pollination work. And the use of our new bucket truck also greatly sped up the bagging and pollinating process. The steady flow of volunteers also markedly increased our ability to take advantage of the additional material.

The yield per bag this year was good, more than one nut per bag. Yields may have been up compared to last year because we tried to pollinate ten to 13 days after bagging, instead of delaying some pollinations beyond 13 days after bagging.

We recorded the time of day at which pollinations were performed to assess whether that was a factor in the rate of nut set (as has been reported previously by Clapper). There were no significant differences between nut set for pollinations performed in the early morning, the afternoon and the evening, which yielded 0.52, 0.61 and 0.63 nuts per bur, respectively. (The pollinations performed in the late morning yielded only 0.37 nuts per bur, which supports Clapper's findings. Since we performed relatively few late morning crosses compared to those for each of the other times of day, however, we can reach no firm conclusions regarding late morning pollinations.) High humidity and moderate afternoon temperatures may have maintained morning nut yields into the afternoon and evening in our experiments. If afternoons had been hot and dry, we might have observed, as Clapper did, a decrease in nut set for afternoon pollinations compared to early morning pollinations.

In 1996 we harvested 3,517 third backcross nuts whose blight-resistant parents had been bred in 1990 using pollen from the 'Graves' and 'Clapper' second backcross trees. We also harvested 1,194 second backcross nuts most of whose blight-resistant first backcross parents had been bred in 1990. The harvest of first backcross nuts from first hybrids of the 'Nanking' Chinese chestnut, however, was a disappointing 147 nuts. (We had hoped this year to have finished advancing 'Nanking' trees to first backcross.)

If things go as expected, we will begin harvesting third backcross F3 nuts around 2006, only nine years from now! At the current pace, by 2011 at least twenty lines derived from the 'Graves' tree will be producing nuts, as will at least twenty lines from the 'Clapper' tree. By 2011, we also should begin releasing nuts derived from some of the other sources of resistance.

Several volunteers helped out with pollination in 1996. First time helpers that year were Ted Blaney, John Hoffman and his grandson Bruce Stocking, Carl Mayfield, Harry Norford, Robert Strasser, and Gene Whitmeyer. Bill Lord again put in several good days. Larry

Peters and Christine Bock came back for a second time. Barbara Cox, Chandis Klinger and Lou Silveri have returned so often we've lost count. Everyone helped generate a record number of nuts, and provided enjoyable company during the long days of pollination time.

## Plantings

Our total holdings are now close to 10,000 trees and planted nuts (Table 2). These include 2,869 second backcross (BC2) plants. Most of these are derived from the 'Graves' and 'Clapper' first backcrosses, but we also were able this year to add second backcrosses derived from other sources of blight resistance. In addition, there are now 2,216 third backcross (BC3) plants derived from the 'Graves' and 'Clapper' trees.

Table 3 presents the changes from 1996 to 1997 in the various types of trees at the farm. We were especially pleased to advance 12 additional sources of blight resistance to F1 last year and three to BC2.

Our Pennsylvania chapter has developed a very nice breeding program. Over the past three years they have planted 1,076 third backcross nuts, 145 first backcross nuts, and 104 F1 nuts. These numbers are in addition to those reported in Tables 2 and 3. We commend them for their fine efforts. Similar initiatives are underway in other states. Our breeding program is finally developing into a vigorous national effort!

## Planting Methods

We have been experimenting with planting methods for a number of years. In 1993, a local high school student, Randi Parker, found better nut emergence when styrofoam cups were placed over the aluminum cylinders with which we protect nuts from squirrels, in comparison to cylinders with no cups. The cups appeared to promote nut emergence by keeping rain out of the peat moss inside the cylinders.

In 1996, Fred's daughter Kyla Hebard tried a similar experiment indoors. In two flats she planted American chestnuts in ground, milled peat moss and in two other flats she planted nuts in a potting mix made from equal parts peat, perlite and vermiculite. One peat-containing flat and one mix-containing flat were watered to the saturation point (the "wet" condition) while the other two flats were kept at a moderate moisture level (the "moist" condition). After a month, 22 of

50 nuts in the wet mix had germinated and sent up shoots while 42 of 50 nuts in the moist mix had sprouted. In the peat flats, only five of 50 nuts had sent up shoots in the wet peat and 24 of 50 in the moist peat. It appears that wet conditions do in fact inhibit seedling emergence, and that a 1:1:1 peat-perlite-vermiculite potting mix gives better emergence than pure peat.

In the field, we have been planting nuts by punching a hole in the ground with a bulb planter, filling the hole with peat, planting a nut one-half inch deep, surrounding the nut with an aluminum cylinder and putting a styrofoam cup over the cylinder. In view of Kyla's results, we felt justified this year in switching from peat to the 1:1:1 peat-perlite-vermiculite potting mix.

## Blight Resistance Testing

The results of inoculations made this year, as in the past, continue to support the hypothesis that only two or three genes control blight resistance in Chinese chestnut.

We have been experimenting since 1990 to try to determine the best age at which to screen chestnut seedlings for blight resistance. Currently, we screen straight backcross trees for blight resistance when they are four years old. Since many of these trees produce male flowers when only two or three years old, we could speed up the breeding process and save plot space if we could screen trees for resistance at a younger age.

We have now found that although we can detect moderate levels of blight resistance in trees as young as one year old (we only expect to find moderate, not high, levels of blight resistance in straight backcross trees), one- and even two-year-old moderately blight-resistant trees are likely to succumb to blight before we can breed them to create the next generation of trees. Three years seems to be the minimum age at which straight backcross trees should be screened for blight resistance.

Our new orchards of first and second backcross trees will be screened for blight resistance when they are three years old. However, third backcross trees need to bear nuts for an intercross generation rather than merely provide pollen for a backcross generation. And since many seedlings do not bear nuts before they are five years old, we plan to continue screening third backcross trees for blight resistance when they are four years old.

*"The results of inoculations made this year, as in the past, continue to support the hypothesis that only two or three genes control blight resistance in Chinese chestnut."*

## TABLE 1

*American Chestnut Foundation 1996 Nut Harvest*
*from Controlled Pollinations and Selected Open Pollinations*

| Nut Type | Female Parent | Pollen Parent | Pollinated nuts | Pollinated bags | Pollinated burs | Unpollinated Checks nuts | Unpollinated Checks bags | Unpollinated Checks burs | Number of American Chestnut Lines* |
|---|---|---|---|---|---|---|---|---|---|
| $BC_1$ | Nanking $F_1$ | American | 147 | 360 | 711 | 6 | 23 | 42 | 3 |
| $BC_2$ | American | Douglas $BC_1$ | 55 | 91 | 191 | 0 | 14 | 17 | 3 |
| $BC_2$ | American | Mahogany $BC_1$ | 298 | 255 | 519 | 2 | 23 | 43 | 2 |
| $BC_2$ | American | S.Lot R1T10 $BC_1$ | 306 | 161 | 298 | 3 | 15 | 30 | 1 |
| $BC_2$ | PI#36666 $BC_1$ | American | 14 | 127 | 442 | 0 | 12 | 24 | 1 |
| $BC_2$ | Mahogany $BC_1$ | American | 373 | 250 | 819 | 4 | 15 | 58 | 5 |
| $BC_2$ | PI#70315 $BC_1$ | American | 73 | 38 | 45 | 0 | 3 | 4 | 1 |
| $BC_2$ | S.Lot R1T10 $BC_1$ | American | 75 | 140 | 215 | 1 | 16 | 21 | 2 |
| $BC_2$-$F_2$ | Clapper $BC_2$ | Clapper $BC_2$ | 9898 | open-pollinated | | | | | 1 |
| $BC_3$ | American | Clapper $BC_2$ | 1216 | 943 | 1794 | 11 | 97 | 169 | 9 |
| $BC_3$ | Clapper $BC_2$ | American | 570 | 326 | 1020 | 1 | 26 | 78 | 3 |
| $BC_3$ | American | Mahogany $BC_2$ | 329 | 368 | 509 | 10 | 32 | 49 | 7 |
| $BC_3$ | Mahogany $BC_2$ | American | 1192 | 602 | 2743 | 18 | 52 | 344 | 4 |
| $F_1$ | 65-18 Chinese | American | 3 | 5 | 7 | 0 | 0 | 0 | 1 |
| $F_1$ | 65-4 Chinese | American | 181 | 20 | 300 | 1 | 6 | 12 | 6 |
| $F_1$ | 72-211 Chinese | American | 148 | 101 | 148 | 1 | 6 | 12 | 6 |
| $F_1$ | Meiling Chinese | American | 356 | 628 | 436 | 2 | 20 | 51 | 6 |
| $F_1$ | Nanking Chinese | American | 185 | 236 | 550 | 3 | 11 | 21 | 12 |
| $F_1$ | Orrin Chinese | American | 175 | 83 | 191 | 1 | 6 | 12 | 6 |
| $F_1$ | opNanking Chinese | American | 54 | 16 | 27 | 0 | 2 | 2 | 1 |
| $F_1$ | Hubei Chinese | American | 66 | 72 | 112 | 9 | 10 | 16 | 1 |
| $F_1$ | FP 7284 Chinese | American | 27 | 25 | 41 | 3 | 2 | 2 | 1 |
| $LSF_1$ | American | Amherst | 136 | 203 | 313 | 0 | 16 | 49 | 1 |
| **Total Controlled Pollinations** | | | | **5,979** | **5,050** | **11,431** | **76** | **407** | **1,056** |

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

## TABLE 2

*Type and Number of Chestnut Trees and Planted Nuts at the ACF Meadowview Research Farms in April 1997, with the Number of Sources of Blight Resistance and the Number of American Chestnut Lines in the Breeding Stock*

| | Number of* | | |
|---|---|---|---|
| **Type of Tree** | **Nuts or Trees** | **Sources of Resistance** | **American Lines*** |
| American | 1041 | | 39 |
| Chinese | 392 | 42 | |
| Chinese x American: $F_1$ | 812 | 19 | 60 |
| American x (Chinese x American): $BC_1$ | 583 | 9 | 29 |
| American x [American x (Chinese x American)]: $BC_2$ | 2869 | 6 | 47 |
| American x {American x [American x (Chinese x American)]}: $BC_3$ | 2216 | 2 | 39 |
| (Chinese x American) x (Chinese x American): F2 | 284 | 3 | 4 |
| [Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:$F_3$ | 9 | 1 | 1 |
| [Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: $BC_1$-$F_2$ | 460 | 2 | 2 |
| {Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:$BC_2$-$F_2$ | 476 | 1 | 1 |
| Chinese x (Chinese x American): Chinese $BC_1$ | 145 | | |
| Chinese x [American x (Chinese x American)] | 44 | | |
| Japanese | 4 | 3 | |
| American x Japanese: $F_1$ | 1 | 1 | 1 |
| (American x Japanese) x American: $BC_1$ | 5 | 1 | 1 |
| Castanea sequinii | 48 | 3 | |
| Chinese x Castanea pumila: $F_1$ | 2 | | |
| Large, Surviving American | 1 | 1 | 1 |
| Large, Surviving American x American: $F_1$ | 331 | 9 | 10 |
| Large, Surviving American x Large, Surviving American: $I_1$ | 42 | 4 | 4 |
| Irradiated American | 48 | 3 | 3 |
| Other | 32 | | |
| **Total** | **9,845** | | |

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

## TABLE 3

*Changes between 1996 and 1997 in the Number of Chestnut Trees and Planted Nuts of Different Types at the ACF Meadowview Research Farms, Including Changes in the Number of Sources of Blight Resistance and the Number of American Chestnut Lines in the Breeding Stock*

| | Increase or Decrease in Number of* | | |
|---|---|---|---|
| Type of Tree | Nuts or Trees | Sources of Resistance | American Lines |
| American | 303 | | 18 |
| Chinese | 33 | 14 | |
| Chinese x American: $F_1$ | 628 | 12 | 34 |
| American x (Chinese x American): $BC_1$ | -45 | 0 | 3 |
| American x [American x (Chinese x American)]: $BC_2$ | 576 | 3 | 11 |
| American x {American x [American x (Chinese x American)]}: $BC_3$ | 1306 | 0 | 20 |
| (Chinese x American) x (Chinese x American): $F_2$ | 9 | 0 | 0 |
| [Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:$F_3$ | -9 | 0 | 0 |
| [Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: $BC_1$-$F_2$ | 38 | 0 | 1 |
| {Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:$BC_2$-$F_2$ | -114 | 0 | -5 |
| Chinese x (Chinese x American): Chinese $BC_1$ | 0 | | |
| Chinese x [American x (Chinese x American)] | 1 | | |
| Japanese | 0 | 0 | |
| American x Japanese: $F_1$ | 0 | 0 | 0 |
| (American x Japanese) x American: $BC_1$ | 0 | 0 | 0 |
| Castanea sequinii | 0 | 0 | |
| Chinese x Castanea pumila: $F_1$ | 0 | | |
| Large, Surviving American | -4 | -3 | -3 |
| Large, Surviving American x American: $F_1$ | 72 | 1 | 1 |
| Large, Surviving American x Large, Surviving American: $I_1$ | -6 | 1 | 1 |
| Irradiated American | 0 | 0 | 0 |
| Other | 10 | | |
| Total | 2,783 | | |

* Most of the decreases reflect lack of emergence of nuts planted in 1996, with the following exceptions: the decrease in Large, Surviving American chestnut trees is from mortality due to blight; the decrease in the number of American lines of BC2-F2 trees is due to a reclassification. A reclassification is also reflected in most of the increase in the number of pure Chinese chestnut sources of blight resistance. The number of American lines refers to unique combinations of all parents, grandparents and great grandparents.

# TWO POEMS

*by Ralph S. Coleman*

In a recent letter to The ACF, Mr. Coleman says "I feel a special closeness to the American chestnut because my father and mother lived during the times that it flourished in these southern Appalachian mountains. Most of my writing about the chestnut trees came from stories that they used to tell me."

Mr. Coleman's nature poems and essays have also appeared in *Appalachia*, *Snowy Egret* and elsewhere.

## On Granddad's Lap

When I sit
on Granddad's lap,
I see Model-T Fords
crawling down dirt roads,
old men playing
checkers
and sipping Nehi
in country stores,
neighbors talking
at white-washed fences,
and I see
passenger pigeons
resting in the shade
of American chestnut trees.

## The Passing

To write a poem about the American chestnut tree,
I talked with relics of these Appalachian mountains,
old people that might remember the chestnut trees.

Miss Ida, with eyes golden brown like chestnut
shells, pointed to the mountain with a bowed finger
and said, “I remember the chestnut trees. There were
thousands of them on the mountain. They were the
mountain! Then, one summer the mountain turned brown.”

Mrs. Annie Jones, black like the shadows
in these mountain hollows, told me of children
that picked up chestnuts. They put them in burlap
sacks and carried them on the backs of mules
to town and sold them.

Mr. Bent Arnold, wrinkled like a relief map,
said that autumns long ago, just before
the winter snow, chestnuts fell ankle deep
in the mountains around his farm. He turned
his hogs loose in the mountains and saved his corn.

To write a poem about the American chestnut tree,
I talked with relics of these Appalachian mountains,
old people; they remember the chestnut trees.

# DIARY OF THE CLAPPER TREE

*The U.S. Department of Agriculture spent decades breeding and testing chestnuts for blight resistance before canceling the program in defeat. Although failure was the norm, for a time USDA breeders and even the general public had high hopes. Those hopes rested on a single chestnut growing at one of six research sites established by USDA plant pathologist Jesse D. Diller and others.*

*The tree, a Chinese/American hybrid backcrossed to its American parent, was created in 1946 at the Department of Agriculture's Beltsville, Maryland laboratories by plant pathologist Russell B. Clapper. In 1949, the tree was planted on the U.S. Fish & Wildlife Service's Crab Orchard National Wildlife Refuge in southern Illinois. The 'Clapper' hybrid, as it was eventually called, was unusually vigorous and, despite the presence of the blight in the research plot, strangely safe from infection. As you will read in the following history of the tree drawn directly from the Crab Orchard files, it was not, however, in the end resistant to the blight fungus, although scions collected from the tree still live. In 1996, 'Clapper' clones were part of the parentage of more than 11,000 nuts harvested at The ACF's Meadowview research farms.*

*Thanks to refuge forester Tom Palmer for sharing the Crab Orchard chestnut files. And thanks to the editor's father, Harry E. Stiles, former Crab Orchard refuge manager since retired from the Fish & Wildlife Service, whose idea this story was.*

*Crab Orchard National Wildlife Refuge was established in 1947 and covers some 43,000 acres not far from the confluence of the Mississippi and the Ohio Rivers. The refuge is part of the Mississippi flyway, and hosts upwards of 120,000 Canada geese each winter. Like the Clapper tree, this editor found it a great place to grow up. Readers might find it a great place to someday visit.*

---

MARCH 1949

9

***The request for a research location went first to nearby Southern Illinois University. Notice how researchers defined the perfect chestnut planting site.***

*from Dr. Jesse D. Diller, plant pathologist, USDA to Dr. Lowell Tucker, Southern Illinois University (SIU), Carbondale, Illinois*

The Division of Forest Pathology is interested in establishing approximately 100 blight-resistant hybrid chestnuts in an experimental plot in southwestern Illinois.

... The hybrid chestnuts are trees ranging from 1- to 4-feet in height, and would be furnished by the Dept. of Agriculture and by Professor A. H. Graves.... At various and sundry times we would inspect these planted trees, take growth measurements and other pertinent data regarding them, and furnish the University with reports on our findings.

...The University would have to furnish a suitable planting site of approximately 0.4 acre, preferably a pole-sized, fully stocked stand of the following hardwood species: yellow poplar, northern red oak, white ash, dogwood, mulberry, American elm; lesser woody species such as papaw or spicewood; and the following herbaceous species: maidenhair fern, bloodroot, Solomon's seal, May apple. Such an association of plants is usually found in a protected site, as at the head of a draw, or slight ravine, generally on a north or northeast aspect, with a slope of 5 to 15%. The University would have to under-plant the pole-sized overstory trees with the chestnut planting stock (10 by 10 spacing), then girdle all woody growth five feet in height or taller. After furnishing the Division an establishment map, showing the location of every planted chestnut tree, the University's responsibility would be completed.

MARCH 1949

16

***When no University site would do, the refuge agrees to cooperate.***

*from the refuge manager to the Fish & Wildlife Service regional director, Minneapolis*

...Mr. Diller made an inspection of University-owned lands near the college and was unable to find the soil cover and planting type desired by the USDA for this rather specialized planting. Messrs. Tucker and Marberry of the University then brought Mr. Diller to the refuge.

... Mr. Diller was very much pleased with one site.... The site involved would occupy 0.4 of an acre in a 10-acre mixed hardwood stand.

...It is the recommendation of this office, in the encouragement of research, to go along on this experimental project.

MARCH 1949

# 18

*The first batch of seedlings is delivered.*

*From J. D. Diller's office to Dr. Lowell Tucker, SIU*

In accordance with the enclosed wire ... [we] are shipping you today 75 Chinese chestnut trees for an experimental planting in accordance with arrangements made by Dr. Jesse D. Diller.... Dr. Diller's instructions left with us were to the effect that you are to plant three of our chestnuts and then one of Dr. Graves', and continue on through the planting in this way...[*Arthur Graves of the Brooklyn Botanic Garden and, later, the Connecticut Agricultural Experiment Station, contributed a great deal of material to the USDA breeding program*.]

These two lots of trees include some of the more promising hybrids from Mr. Clapper's breeding work and from Dr. Arthur H. Graves' breeding work, and we hope that the planting will turn out well.

NOVEMBER 1952

# 16

*A report on the progress of the trees.*

*From J. D. Diller's field notes on the 1949 Crab Orchard plot*

15 Best Trees

| Tree # | [Tree code] |
|---|---|
| [B]26 | RBC-3146 |

MARCH 1956

# 21

*An update on the tree's progress four years later.*

*From a note to the refuge file*

Twenty Five Best Trees in Hybrid Chestnut Plots

| Tag no. | DBH | Height |
|---|---|---|
| 26 | 1.2" | 13' |

JANUARY 1959

12

*About three years later, another progress report on B26.*

*From the refuge manager to Albert G. Snow, Jr., US Forest Service*

The attached measurements were taken on January 9, 1959. Several of the larger trees produced a crop of nuts this year.

| Tree Tag No. | d.b.h. (inches) | Height (feet) |
|---|---|---|
| B 26 | 3 | 30 |

JULY 1962

1

*This next correspondent , who visits the tree for the first time thirteen years after it was planted, will eventually save the Clapper for posterity.*

*From Dr. Richard A. Jaynes, Connecticut Agricultural Experiment Station, to the refuge manager*

I am planning a trip out to the Northern Nut Growers Meeting in Evansville… and would like to visit the Hybrid Chestnut Plot established at Carterville in 1949. As you are probably aware, approximately fifty hybrids developed at the Connecticut Station were included in this plot.

FEBRUARY 1963

5

*Jaynes requests scions for the Connecticut Experiment Station.*

*From Richard A. Jaynes to Dr. Ernest Kurmes, Department of Forestry, SIU:*

... Could you send me scions from three or four of the best trees in the hybrid plot? My notes indicate that B26 and B59 are two of the best trees. Four seven-inch sticks from each tree would be fine.

FEBRUARY 1963

18

*The scions are safe.*

*From Ernest Kurmes to Richard A. Jaynes:*

I have checked with the refuge personnel, and they will be happy to have me collect scions and take measurements on the hybrid plot...To that end I have already collected scions from four trees on the plot, as you requested...

B26 is, of course, the outstanding tree on the plot. The other three are considerably smaller but of good form and not cankered.

*News of encouragingly vigorous B26 goes out to the world.*

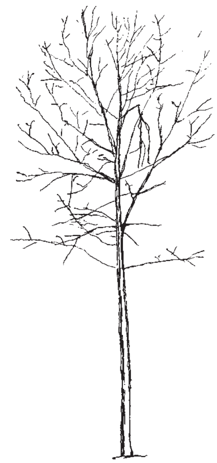
*from a Forest Service press release*

NOVEMBER 1963

29

During August and September 1963, R. B. Clapper and J. D. Diller inspected 12 of the 15 hybrid chestnut test plots established during the period 1947 to 1955...

The largest, blight-free, forest-type hybrid, B26:#3146 USDA, occurs in the test plot near Carterville, Illinois. It is an American x Chinese backcrossed on to an American; the cross was made by R. B. Clapper in 1946. After 17 growing seasons, this tree measured 7.3 inches d.b.h. and 45 feet in height — an increase of 0.43 d.b.h. per year, and a height of nearly 2 feet 8 inches per year. It apparently has a high degree of blight resistance, as chestnut blight is present in the plot.



◀ The November 1963 Forest Service press release included a drawing of the then 45-foot-tall hybrid chestnut.

*One of the first references to the "Clapper" tree rhymes, but Longfellow it isn't!*

*from an article on the Clapper tree entitled "Chestnut Coming Back Strong" in the Herrin, Illinois* Spokesman

AUGUST 1964

22

...While the village blacksmith may never return, hybridized or otherwise, the imminent revival of the tree undoubtedly will uncork a rash of parodies on a poetic classic that already has been parodied to death. So we offer the first:

Under the Clapper's chestnut tree
The refuge turkey stands
A strong and husky bird is he
With hybrids in his glands.

SUMMER 1968

***The last entirely good news.***
*from notes on a survey of B26 by a refuge employee*

1) good crop of burs
2) 11.3” diameter...
3) 63’ height...
4) some damage by leaf cutter or miners on lower leaves - not serious. No sign of blight that I could see.
5) overall appearance - very good

OCTOBER 1968 22

***The first bad news.***
*from the acting refuge project manager to J.D. Diller*

It is my sad duty to tell you that the Clapper chestnut, B-26, shows definite signs of blight infestation....

I can appreciate your disappointment as we all had high hopes for this tree. We will continue to observe the tree and report any changes to you.

MAY 1969 14

***There is still hope.***
*from the refuge project manager to J. D. Diller*

We are happy to report that the Clapper chestnut is in full leaf now and appears to have the vigorous health that it had last year at this time. The blight symptoms are still evident but show little, if any, increase in either area or vigor. We sincerely hope this favorable trend continues.

OCTOBER 1970 8

***Back East in Connecticut, ‘Clapper’ scions rescued in 1963 produce seed.***
*from Richard A. Jaynes to the refuge forester*

... I had a graft of Clapper produce about 20 burs this fall.

OCTOBER 1971

4

*An update on blighted B26.*

*from the refuge forester to Russell B. Clapper*

...Although the blight damage is becoming more noticeable, the Clapper (B-26) is still looking good. It now measures 13.0” DBH and 68’ tall, compared to 12.5” and 66’ a year ago.

MAY 1974

2

*Though blighted, B26 continues to put on girth and height.*

*from the refuge forester to Robert P. Clapper [Dr. Clapper’s son]*

I will be leaving shortly for a new assignment...One of the jobs I will miss most at Crab Orchard will be working with the chestnut plots and with #B-26, the Clapper Chestnut. It has been a pleasure and a privilege to have been a part of its history.

I checked the tree today. B-26 does not seem to be as extensively leafed out as in past years. There also seems to be a greater lower limb loss than I have noticed before. And for the first time, there is quite a lot of bole leafing taking place. I don’t know whether or not these conditions are significant or just normal responses to the severe weather fluctuations experienced here since February...

JANUARY 1977

27

*The main stem succumbs.*

*from refuge forestry technician to Robert P. Clapper*

Because of your past interest in the Crab Orchard Refuge B-26 Clapper chestnut, you may be interested to learn that the tree is dead.

In the spring and early summer of 1976 the foliage showed that the tree was dying, and by late fall it was dead. There are, however, live sprouts at the base of the tree, and we are thinking of removing the tree and later selecting the best sprout to see how long it will survive.

LAST ENTRY

*The last entry in the refuge file, thirty-two years after the first.*

Chestnuts alive as of 12-8-81 [in chronological order]

B1 (151B44), B22 (147B44), B23 (4546), B27 (145B44), B43 (4944)

# ROY J. OWEN'S LETTERS ON THE CLAPPER TREE

*After news of the Clapper tree appeared, the Crab Orchard chestnut file began to fill with letters from landowners interested in growing some of their own Clapper progeny. The most moving pieces of correspondence were those between Roy J. Owen of Terre Haute, Indiana and Richard Johnson, then refuge forester. We tried to track down Mr. Owen's descendants, with no success. We hope that if they do read his letters, which we print admittedly without permission, they'll see them as a tribute to an obviously wonderful man.*

FEBRUARY 1973

To the Postmaster (Carterville, Illinois)

I am writing you, as the only person whom I could think of for a little information.

I was looking through some old magazines yesterday, and in a 1965 magazine I came across a story I had read and was much interested in (but the magazine was misplaced and I had forgotten it).

The story was in regard to the activities of two scientists from the East, Dr. Jesse Diller and Russell B. Clapper, who had undertaken through cross-breeding, etc. to produce a strain of chestnuts which would be immune to the chestnut blight which had killed all the chestnut trees in the U.S.A.

… This story said these two fine men had planted one of their young trees close to the shore of Crab Orchard Lake near Carterville, and that the young tree, 18 years old, was 45 feet tall, and perfectly healthy, and was making a beautiful spreading top.

Now I would like to make a trip to your city to see this tree if it is still alive and healthy. Maybe I could make arrangement to get some seed and see some trees growing again on my premises before I leave this world. I am 84 years young.

Roy J. Owen

FEBRUARY 1973

Dear Mr. Owen:

Thank you for your interesting letter. Although it has a bad case of blight, the Clapper chestnut is still with us and doing remarkably well.

I'm enclosing a map of the refuge and some spare literature describing the hybrid chestnut planting program for you.

I also happen to have a few nuts from the Clapper that I will send to you within a few days. These nuts were gathered last fall and have been refrigerated all winter. They should be germinated in …

I hope you have good luck with the seedlings. If you should decide to visit us to see the Clapper, please let me know by mail a few days ahead. I will be happy to show it to you.

Richard J. Johnson
*Refuge Forester*

MARCH 1973

Dear Mr. Johnson:

I was amazed and overjoyed to receive today the precious chestnuts from the famous Clapper tree. A week ago I had thought if I could ever receive a few nuts from this famous tree it would not be before fall, and then not without a lot of red tape, etc. Imagine if you can how I felt this p.m. as I planted the little beauties, seven of them, in six-inch pots, and buried them in a cold frame. If they had been diamonds or gold nuggets I wouldn't have enjoyed it more.

… I expect to grow seven young trees, and have the location picked out for them, and hope to make a grove out of them. It is fertile ground and has plenty of sun. If they do well (and I expect them to), they will be named the "Richard Johnson" grove, for the fine man who made this possible.

Roy J. Owen

SEPTEMBER 1973

Dear Mr. Owen:

I managed to cheat the squirrels out of 25 nuts from the Clapper today and am sending them to you. Sincerely hope that you have good luck with these. Would be interested in your results.

Richard J. Johnson
*Refuge Forester*

OCTOBER 1973

Dear Friend:

This is to acknowledge receipt of the Clapper chestnuts, which arrived in good condition. I thank you for being so kind to me.

Do you know life is a strange thing? Here I am 85 years old, and I have met - no, not met, I should say have had dealings with a fine man like you, who if this had been years ago I am sure we would now be fast friends.

My friends are mostly all gone, and I feel sure that we will never meet in this life, but who knows, maybe there will be a place for people like you and me, who love to work with and see growing things.

Today I planted them and I hope it has been the best way. I thought if these nuts had been left for nature to care for them and they had not been eaten by squirrels, etc. they would have lain where they fell, and become covered with leaves, and the rains and snow of winter would keep them moist, and when spring came they would be ready to start to grow. Now why couldn't I try to simulate nature as far as possible?

So I enclosed them or rather wrapped some half-inch woven wire around them for protection, and buried them in a cold frame without the glass over them. Thanks again, good friend.

Roy J. Owen

p.s. I will write you in the spring about how they went through the winter. If anything should happen to me, my grandson, who is familiar with this, will take over.

memories

# OF SOUTHERN MARYLAND, CIRCA 1904

*Even before the arrival of the blight and when chestnuts were still very common, some thoughtful people worried about the future of the species. Reprinted here are cautionary excerpts from a U.S. Department of Agriculture bulletin originally published under the imprimatur of Gifford Pinchot, the founder and first director of the U.S. Forest Service. ACF members can learn more about Pinchot at the 1997 annual meeting, to be held in November at The Biltmore Estate in Asheville, North Carolina. The Estate contains several thousand acres of woods originally planted and managed under Pinchot's direction, the first "scientifically managed" forest land in the country.*

(To: Hon. James Wilson, Secretary of Agriculture)
US Department of Agriculture, Bureau of Forestry
Washington, DC
JUNE 28, 1904

Sir: I have the honor to transmit herewith a report entitled "Chestnut in Southern Maryland," by Raphael Zon, a forest assistant in the Bureau of Forestry, and to recommend its publication as Bulletin No. 53 of the Bureau of Forestry…

Very respectfully,
Gifford Pinchot, Forester

"Most of the data were obtained in the southeastern part of Prince George County and the northwestern part of Calvert County. They include analyses of 1,245 large chestnut trees and of 426 seedlings for the growth in height and diameter; of 338 trees for the taper [taper was important for telephone and telegraph pole brokers]; and of 1,690 for the relation between stump-high and breast-high diameters, together with measurements of 1,269 one-year-old chestnut sprouts for the purpose of determining the best time and way of cutting chestnut for coppice [or sprouting]….

The original stands of timber are mostly gone. The section studied was settled over two hundred years ago, and has remained principally an agricultural county ever since. Though the demand for chestnut timber in the early days was not great, it was used extensively even then for rails, fence posts, and vine props…. In more recent times chestnut has been cut on a larger scale and at a more rapid rate, on account of the increasing demand for chestnut ties and poles….

The silvicultural system to which chestnut is best suited is "pure coppice." It must not be forgotten, however, that a chestnut stump can not go on coppicing forever. With each new generation of sprouts the stump becomes more and more weakened…. The effects of repeated and bad coppicing manifest themselves in the increasing number of dying chestnuts all over Maryland….

The capacity of chestnut to produce sprouts from the stump in spite of the reckless and careless cutting now practiced may delay the entire disappearance of this most desirable of the trees possessed by the farmers of Maryland, but it will not save it from deterioration and eventually complete removal, unless efforts are made to provide also for its natural reproduction from the nut…. Although an abundance of seed is borne, the reproduction of chestnut from this source is exceedingly scant in Maryland. This is largely due to the fact that the nuts are a source of revenue. With chestnuts worth on an average $2.50 per bushel delivered in Baltimore,…the gathering of them is usually carried too far for the good of the woodlot… and the comparatively few which escape man are greedily devoured by the hogs which range freely in the woods, not to mention the squirrels and crows. If, after all, a chestnut seedling succeeds in coming up, the chances are that it will be destroyed by cattle…. [for] the use of the woodlot as a pasture is one of the chief enemies to the reproduction of the farmers' woods. To secure natural reproduction from the nut the woodlot must not be robbed wholly of its crops of chestnuts by turning them into money, the hogs must be kept entirely out of the woods during the season in which chestnuts fall and germinate,… and the young chestnut seedlings must be protected from the cattle until they reach the height at which no harm can be done to them."

# OF NORTH BRIDGEWATER, SUSQUEHANNA COUNTY, PENNSYLVANIA, CIRCA 1920

*Cecil Clink of Fort Myers, Florida got in touch with us last December, writing that "Three of us boys used to go with our mother with fifty pound flour sacks and gather chestnuts. Well, believe it or not, just this past summer I was back at the farm and along this old dirt highway there still are several chestnut trees still growing." When contacted by phone for more information on that old rural highway and the trees along it, he elaborated, and we paraphrase.*

"Almost all my life, until 1980 when I moved to Tunkhannock, Pennsylvania and then later to Fort Myers," he said, "I lived in North Bridgewater, between Forest Lake and Bridgewater, near Montrose, Pennsylvania. There were lots of chestnuts there then. From our farm over to the next farm, we could walk along a ridge where there were fifty or sixty chestnut trees. Every year we'd gather chestnuts there after the frost knocked them down. We'd fill old flour sacks half-way full with chestnuts. They must of weighed twenty pounds. My mother stored them in what we called the 'butry' [*and what we call "buttery"*], there with the smoked hams. We'd eat the nuts boiled, or put them on the cook stove and roast them."

But what about the dirt road? "Seventy years ago or so I used to walk along a dirt road, on the way to a little country school house where we used to go to school," Mr. Clink said. "The road was lined with all these chestnuts then, and last summer I found sprouts from the originals still there."

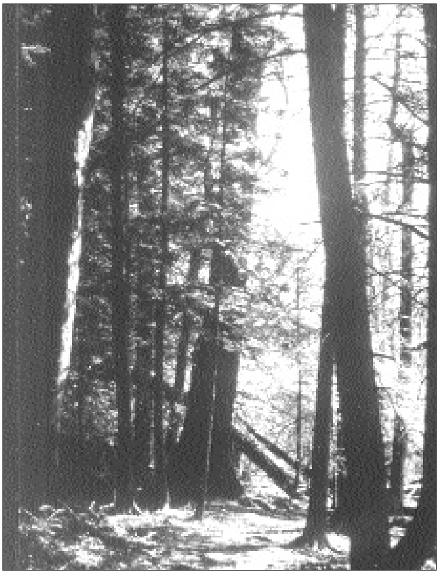


PHOTO BY ROGER LATHAM

Although they're no longer common, chestnuts were probably a big part of the hemlock forest at The Nature Conservancy's Woodbourne Preserve in Montrose, Pennsylvania, just four or five miles southeast of Cecil Clink's childhood home. ►

# OF KOSTANJEVICA, SOUTHERN SLOVENIA, AND PLACES LESS EXOTIC, CIRCA 19-TEENS AND LATER

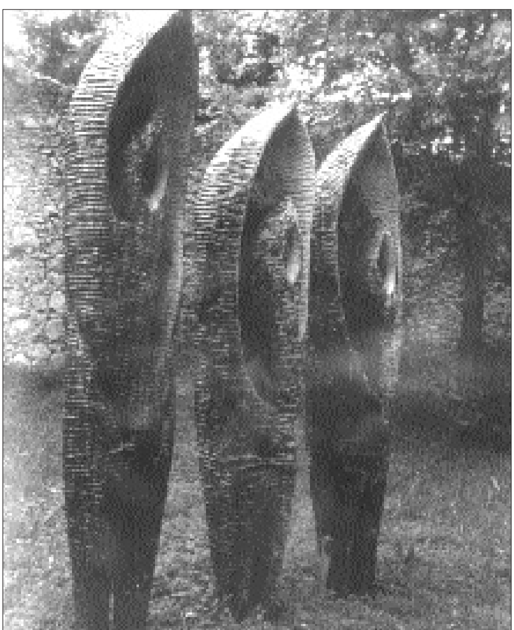
*The following note accompanied Mr. Duffield's photos, taken in 1963.*

Enclosed is a note that might interest readers of *The Journal*, despite the fact that it deals with an "un-American" species of chestnut, but one that shares the disease problem of our species.

I must admit that I stumbled onto wood sculpture. In 1963, I was a USAID "expert on the culture of white pine and Douglas fir," two North American species of interest for forestry in Yugoslavia. In my visits to the several republics of Yugoslavia, I was shepherded to various areas related to forestry and forest products. Kostanjevica was one of these.... On a second stay in Slovenia in 1968-69, I visited, in the little town of Kranj, an exhibit of linden sculpture, most pieces recently carved. The characteristic odor of freshly-carved linden permeated the exhibit room, and was most pleasant.

Durable chestnut wood is the medium for an outdoor collection of sculptures located at the restored monastery in Kostanjevica in southern Slovenia. Here, since the early 1960's, sculptors from as far as Japan have come together for an annual "Forma viva" wood sculpture seminar, carving massive chestnut logs from the nearby forests.

▼



PHOTOS BY JOHN W. DUFFIELD

My interest in chestnut far antedates that in wood sculpture. One of my earliest memories is of gathering American chestnuts in the woods of New York state with my father in the late 19-teens. Later, I attended a summer camp in western Connecticut, where we were housed in cabins built of rough lumber locally milled from blight-killed chestnut. In those pre-OSHA days, I was turned loose as a 12-year old, with an old farm horse, to skid dead chestnut poles out of the woods to erect backstops for our tennis courts.

# science and natural history

# *CRYPHONECTRIA PARASITICA*, STEALTH INVADER

by William Lord

*Dr. Bill Lord is a member of the Board of Directors of The ACF.*

Learn all you can about the enemy. This is essential to winning any war, and we are allied with the American chestnut in a war against the blight fungus, *Cryphonectria parasitica*.

Until recently, fungi were classified as primitive members of the plant kingdom but now are considered to be in a separate kingdom, neither plant nor animal. Plants and fungi have a similar cellular structure with rigid cell walls. And some fungi, like plants, contain the structural fiber, cellulose. Most, however, contain the structural fiber chitin, which is also found in the exoskeletons of insects and crustaceans. And fungi lack chlorophyll and therefore the ability to utilize the energy of the sun to produce nourishment. The evolutionary "objective" of fungi is to extract nourishment from organic matter, and to this purpose they are extremely successful.

Our enemy belongs to the Class Ascomycetes, or sac fungi, which includes the yeasts, molds, powdery mildews and the cup fungi. From the human standpoint, many of these fungi are beneficial. Yeasts help put bread on the table as well as nut brown ale. Molds are the source of penicillin. The cup fungi include the truffles and morels that delight the gourmet.

But our ascomycete is no friend. Its presence was first recognized at the New York Zoological Park in the Bronx in 1904, where it was underestimated by some as a transient phenomenon, perhaps the flare up of a native pathogen that would subside. Others suspected an extremely virulent foreign invader. And they were right. By 1908, airborne outbreaks had occurred throughout much of New York state and into Pennsylvania, Maryland, Virginia, New Jersey, Connecticut and Delaware. This invasion was to become the most extensive and destructive epidemic ever to strike a native tree in recorded history.

The fungus was first identified as an Ascomycete shortly after the outbreak captured the nation's attention by C. L. Murrill of the New York Botanical Garden. Murrill considered the fungus to be a hitherto unidentified species which he described and named *Diaporthe parasitica*. Shortly thereafter, it was recognized by C. L. Shear and others as belonging to the genus *Endothia*, and considered a new species, *Endothia parasitica*. In 1978 it was given its present classification, *Cryphonectria parasitica*.

The fungus may attack a chestnut tree from topmost twig to the base of the trunk, wherever it gains access into an open break or wound. On young stems and branches, the infection first appears as a discoloration of varying shades from yellow to deep red. This spreading sore or canker may become sunken as the living tissue of the tree is consumed by the fungus, or it may enlarge and develop lengthwise splits and fissures in the bark. On old trunks and large limbs, a canker is first noted by an area of splits and fissures. Within two or three growing seasons, cankers can girdle and kill a tree branch by branch, or entirely by girdling the trunk. So-called water sprouts grow from points below a canker. Soon they too succumb.

The fungus grows from a spore that has gained access through a wound in the bark. Given sufficient moisture, the spore swells and begins to germinate. A sharp-tipped, thread-like growth, the hypha, protrudes from the spore. Quickly, additional hyphae begin their outward growth from the spore. The hyphae branch repeatedly and form an expanding interwoven mat, the mycelium, which constitutes the body of the fungus.

Initially, the fungus nourishes itself from dead or damaged tree cells where it entered the wound in the tree. When it has gained a sufficient mass of mycelium, fan-shaped hyphal bundles begin to radiate through the living tissues of the tree. Soon, a dark, gelatinous band created by enzymes "digesting" the tissues of the tree forms along the leading edge of the fan and the vanguard of the invasion.

As the fungus feeds and prospers, sac-like fruiting bodies known as pycnidia, within which form the asexual spores, or conidia, begin to arise. As a pycnidium matures, it is imbedded in stroma, a dense layer of yellow-pigmented hyphae. On smooth bark, the upward-growing stroma causes the formation of numerous tiny mounds or blisters on the surface of the canker. As the stroma extends upward through the pores of smooth bark, it opens from each blister as a small, flat, yellow-orange pustule. As the season lengthens, the color of the pustules will trend into a deep red to brown. On older trees, the pustules often emerge in a line along a vertical fissure in the bark.

Given adequate moisture, the conidia are extruded in paste-like threads or "spore horns" through the surface of the pustule onto the bark. They coil on the bark like miniature horns and are generally an inch or less in length. Their emergence generally follows rain or periods of high humidity. The spores slide downward within their sticky matrix, which enables them to lodge in breaks or wounds in the bark. Or they may adhere to birds, insects, etc., and be transported to another tree.

In late summer or early fall, after several generations of conidia, the fungus begins to form sexual spores or ascospores. These result from the entry of conidia into the mycelium of another canker. In such instances, the conidia function as male sex cells or sperm. A conidium enters and joins its nucleus with that of the receptive hyphal cell. From these merged nuclei, a flask-shaped, spore-forming organ, the perithecium, develops, on the inside of which a crowded group of elongate, pod-like sacs or asci form. Each sac contains a single row of eight ascospores. As moisture swells the ascospores within each sac, they burst from the pustule through a tubular neck of the perithecium into the air. Each exit point is easily identified by the presence of a prominent black dot on the orange pustule.

Human attempts to combat the blight are remembered as heroic failures. One of the most ambitious was mounted by the Pennsylvania legislature, which, on June 14, 1911, by unanimous vote of both houses, "passed an act providing for the appointment of a five member commission to attack and destroy the Chestnut Bark disease by whatever method they may adopt." At this time the disease was present throughout the eastern half of the state, but was thought to be absent, or nearly so, in the western half. Control methods included the removal and destruction of all identified diseased trees with particular emphasis along the leading, westward edge of the pestilence. In 1913, Pennsylvania conceded defeat and deactivated the Commission.

Despite early failures to stop the spread of the blight, many workers persisted in efforts to learn more about the enemy. In 1913, the blight was recognized in American chestnut trees in a nut orchard in British Columbia. Because there were no other American chestnut trees within 500 miles, this infection was believed to have derived from nursery stock received from importers, wrapped in mats made in the Orient.

Conclusive proof of foreign origin was provided the same year by Frank N. Meyer, a botanist who traveled through many parts of China for the specific purpose of observing the Chinese chestnut, *Castanea mollissima*, and its possible association with the fungus blight. Specimens of infected bark were sent to C. L. Shear of the U. S. Department of Agriculture, as were specimens from the nut orchards in British Columbia. In each instance, the parasitic bark fungus was grown on culture media and proved to be identical with the fungus currently destroying the American chestnut. Final proof included transmitting the disease to the American chestnut and recovering the same organism in the laboratory.

In 1915 Meyer visited Japan, where he found the bark fungus on the Japanese chestnut, *Castanea crenata*. Then in 1938, as the blight was penetrating every ridge and hollow of the Appalachians, it was first reported in Europe on its native chestnut, *Castanea sativa*. Would the fungus yield the same destruction as in America? Fortunately, the European experience was less severe. Although many trees died, they were fewer in number than in America and a large number of trees withstood or recovered from the attacks. Two differences may account for this survival. First, the European chestnut is likely more resistant to the blight than the American. The second is hypovirulence, discovered by Antonio Biraghi of the Forestry Institute of Florence, Italy.

Biraghi cultured bark taken from a healing canker, and discovered that the decrease in the severity of the infection was due to the fungus itself being infected by a virus. The virus can enter the cytoplasm of fungus cells and disrupt the metabolism of the fungus, thus reducing the virulence or severity of the infection in the chestnut. Trees infected with this hypovirulent fungus are often able to wall off the fungus with healing callous tissue quickly enough to reduce or “heal” a canker.

When a piece of bark containing mycelium from a normal or virulent canker is cultured in the same petri dish with a similar specimen taken from a hypovirulent or abnormal canker, each produces its characteristic appearance until they meet along the surface of the nutrient media. Then an interesting change may occur. Sometimes the orange color of the normal, virulent mycelium changes to the white color of the mycelium infected with hypovirulence, indicating that, at the point of contact, the hyphae of the hypovirulent fungus have fused with the hyphae of the virulent fungus. When this occurs in nature in a virulent canker, it becomes much less destructive to the chestnut host. It becomes hypovirulent.

Hypovirulence has great potential as a biological control of the blight, and in Europe the appearance of hypovirulence is a success story. It has now spread throughout the range of the European chestnut. This and the native degree of resistance to the blight have brought about an increasingly stable chestnut population.

Native hypovirulence was first observed in America in 1976, but it is less prevalent and less effective than in Europe. A major reason is due to the existence of "vegetative compatibility" groups, a feature of the blight fungus that probably reflects its high rate of sexual recombination.

Field workers attempt to transmit hypoviruses by implanting "plugs" of hypovirulent mycelium in several small holes around the border of a virulent canker. In some instances the inoculations do not "take." The canker remains virulent and spreading. Why? A hypovirulent and a virulent fungus must be in the same vegetatively compatible or "VC" group for the hypovirulent fungus to infect the virulent fungus.

Unfortunately for the effectiveness of hypovirulence in America, there are many more VC groups identified in America than in Europe. The more VC groups present in an area, the more difficult it becomes to assure a successful conversion of a virulent canker to a benign hypovirulent canker wherein the tree can develop a callous around the canker and heal itself.

In nature, hypovirulence is spread mainly by conidial spores from a hypovirulent canker. Ample evidence exists in Europe of natural spread, but less so in America. But as the fungus mutates, or varies its genetic make up by sexual recombination, so can the hypovirus. Some hypoviruses have developed the ability to convert several rather than one VC group. The search is on for the perfect hypovirus, one with a high "conversion capacity," and that permits a high production of spores that produce hypovirulent colonies. When that time comes, hypovirulence will be much more effective.

It is interesting to conjecture how the fungus has changed since the epidemic years of *Cryphonectria parasitica*. Certainly it gave every evidence of being an extremely effective parasite during its early all-conquering decades. But a recent study by a graduate student at West Virginia University offers evidence that the fungus may be a less successful invader today. A survey of the 2-acre tract study tract revealed the generous presence of both conidial spores and ascospores amid a count of 6,000 artificial wounds in the bark of resident chestnut trees. Yet there are very few infected sites. Perhaps infection in nature is now the exception rather than the rule as *Cryphonectria parasitica* becomes a less effective parasite.

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# MALE STERILITY IN THE PROGENY DERIVED FROM HYBRIDIZATIONS BETWEEN *CASTANEA DENTATA* AND *C. MOLLISSIMA*

by Yan Shi and F. V. Hebard

*Dr. Shi is The ACF's research scientist;*
*Dr. Fred Hebard our farm superintendent.*

## Introduction

Male sterility refers to the failure of plants to produce functional anthers, pollen or male gametes (Kaul, 1988). Thus, male-sterile plants cannot pollinate mother trees. Chestnut is highly self-incompatible, which means that catkins can pollinate burs in another tree but cannot pollinate the burs in the same tree (Clapper, 1954). Therefore, in order to produce a large amount of nuts, two trees are needed and at least one of them must produce viable pollen.

Male sterility has been reported in several *Castanea* species and their offspring (Jaynes, 1964; Omura and Akihama, 1980; Soylu, 1992). Omura and Akihama (1980) found male-sterile chestnut trees in cultivars of Japanese (*C. crenata*), Chinese (*C. mollissima*), and European chestnuts (*C. sativa*). Soylu (1992) also reported that certain intraspecific crosses between plants of C. sativa produced male-sterile offspring.

Jaynes (1964) reported that male-sterile offspring (F1s) were produced from crosses between several *Castanea* species, including *C. sativa* x *C. sequinii*, *C. dentata* x *C. sequinii*, *C. mollissima* x *C. dentata*, *C. dentata* x *C. mollissima*, *C. mollissima* x *C. sativa*, and *C. crenata* x *C. sativa*. Furthermore, Jaynes (1964) noted that the catkins of the male-sterile progeny produced from crosses between American and Chinese chestnuts lacked anthers.

Generally, male sterility is induced by nuclear genetic factors, cytoplasmic genetic factors or an interaction of the two. With nuclear genetic factors, only those plants that carry homozygous recessive 'msms' genes are male-sterile, while male fertility occurs in those plants carrying 'Msms' or 'MsMs' genes. With cytoplasmic factors, a normal cytoplasm (designated as N cytoplasm) produces a male-fertile plant while a sterile cytoplasm (designated as S cytoplasm) produces a male-sterile plant; as long as the sterile plant is used as the female parent, all of its offspring would be male-sterile because these offspring usually inherit the sterile cytoplasm only from the female parent. With both genetic and cytoplasmic factors, a male-sterile plant is produced only when S cytoplasm is in combination with homozygous non-restorer 'frfr' genes (Kaul, 1988).

Based on progeny derived from crosses between plants within *C. sativa*, a European researcher, Soylu (1992), proposed that male sterility in C. sativa was genetically controlled. He indicated that there were two nuclear gene loci involved, and those two genes had a nearly equal and additive effect in contributing to male sterility. The mechanisms of male sterility in *C. dentata*, *C. mollissima*, and their hybrids are basically unknown.

Although the occurrence of male sterility has been reported in C. *dentata*, *C. mollissima* and their hybrids (Jaynes, 1964), the frequency of male sterility has not been addressed. Moreover, the mechanisms of male sterility in these two species are also unknown. In our backcross breeding program, male-sterile chestnut trees have been found in progeny derived from crosses between American and Chinese chestnut trees. An understanding of mechanisms of male sterility may help us prevent it from happening. The objectives of our study were to: (1) address the importance of male sterility in our breeding program, and (2) propose genetic mechanisms of male sterility.

## Materials and Methods

The study was conducted at the Wagner Research Farm in Meadowview, Virginia. Chestnut progeny seedlings were F1s, BC1s, and BC2s. Chestnut parents used in this study are listed in Table 1. The F1s were the first generation from the crosses between American and Chinese chestnuts. The BC1s were the progeny from the first backcross of F1s to American or Chinese chestnut as the recurrent parents. Those BC1s produced by using American or Chinese chestnut were called American or Chinese BC1s, respectively. The BC2s were the progeny from the second backcross of American BC1s to American chestnut.

The seedlings were observed for bloom and male sterility during

June and July of 1995 and 1996. Among flowering trees, only those trees with antherless catkins were classified as male-sterile trees. Those trees with anthers were regarded as male-fertile. However, some of these trees may have inviable pollen or may lack pollen in their anthers. Thus, frequency of male-sterile progeny might be even higher than reported here.

Frequency of male sterility was calculated based on either an individual cross or of an individual tree. If a cross produced even a single male-sterile progeny, then this cross was classified as a male-sterile cross. Percentages of male-sterile crosses were calculated separately for the F1, BC1, and BC2 populations as: (Number of male-sterile crosses / Total crosses) x 100. In each cross, we also calculated the percentage of male-sterile trees as: (Male-sterile trees / Total flowering trees) x 100.

## Results and Discussion

In our breeding program, male-sterile progeny were frequently detected in the F1, BC1, and BC2 populations produced by crossing American chestnut with Chinese chestnut (Tables 2, 3, and 4). In F1 populations, the occurrence of male-sterile trees depended on whether American or Chinese chestnut was used as the female parent (Table 2). When American chestnut was used as the female parent, all progeny produced were male-sterile. In contrast, when Chinese chestnut was used as the female parent, all progeny produced were male-fertile. This phenomenon indicates that the female cytoplasm likely plays a role in inducing male sterility. Because in most cases, only those trees used as the female parents would contribute their cytoplasm to their offspring, therefore the Chinese chestnut trees used in this study may carry normal cytoplasm (N cytoplasm) and the American chestnut may carry sterile cytoplasm (S cytoplasm).

However, in contrast to our results, Jaynes (1964) found that male-sterile F1s were produced when *C. mollissima* was used as the female parent and *C. dentata* was used as the male parent. This discrepancy may have occurred because the Chinese chestnut cultivars used in Jaynes' study differed from those used in our study; perhaps not all *C. mollissima* carry normal cytoplasm. In order to confirm the role of cytoplasm in inducing male sterility, reciprocal crosses need to be carried out using the same parent trees. In the current study, a different American chestnut tree was used as a male or female parent even when the Chinese parent was the same (Table 2).

In the BC1 and BC2 progeny populations, male-sterile progeny were found in some crosses but not in other crosses such as 'RW1 x C', 'RW3 x C', 'Mu x N', 'Meiling x F', and 'Mahogany x MF' (Table 3). Male sterility may have not been observed in some of those crosses because some of their seedlings had yet to flower.

In the BC2 population, the frequency of male-sterile progeny ranged from 0 to 67% (Table 3). When 'Clapper' and 'Graves' were used as the male parents to cross with different sets of American chestnut mothers, the frequency of male sterility was significantly higher (T-test, p=0.05) when 'Graves' was used (41%) than when 'Clapper' was used (12%). These data strongly suggest that male sterility was influenced by the male parent.

The frequency of male sterility also differed when different American chestnut trees were used as female parents to cross with the same male parent; when the 'Clapper' tree was the male parent, the frequency of male-sterile progeny ranged from 0 to 26% among on the female parents, while it ranged from 13 to 67% when the 'Graves' tree was the male parent (Table 3). This implies that the female parent is also involved in inducing male sterility. All the above evidence indicates that male sterility is probably also controlled by nuclear genetic factors, and there may be more than one nuclear gene locus involved. The exact number of gene loci can not be determined based on the current data.

Theoretically, the frequency of male-sterile progeny should decrease with each backcross generation as the Chinese genetic components are eliminated. However, our data indicate that percentages of male-sterile crosses (Table 4) were higher in the BC2 population than those in the American BC1 population. The percentage of male-sterile progeny (Fig. 1) was also higher but not significantly higher (T-test, p=0.05) in the BC2 population than that in the American BC1 population. These may have occurred because 1) there were insufficient samples of the American BC1 populations, 2) different Chinese chestnut trees were used to produce the American BC1 and BC2 progeny populations, and / or 3) interspecific hybrid breakdown results in male sterility. Hybrid breakdown generally occurs in F2 and later generations due to 1) break up of complementary alleles, and 2) if the gametes of F1 hybrid have chromosome deficiencies or duplications, those gametes will have deleterious effects on the F2 generation (Fehr, 1987). Further observations on male sterility are needed on additional backcross progeny populations.

We propose that the male sterility of *C. dentata*, *C. mollissima*, and their hybrids is controlled by both nuclear and cytoplasmic factors. *Castanea dentata* may carry sterile cytoplasm, and *C. mollissima* may carry normal cytoplasm. Determination of the type of cytoplasm can only be done when all nuclear gene loci carry dominant alleles. Additionally, there probably is more than one gene for male sterility involved in the nuclear factors. The genes for male sterility may be dominant and complementary; only those trees that carry S cytoplasm and dominant alleles at all loci will be male-sterile. These dominant alleles are inherited from both American and Chinese chestnuts.

We believe it will be helpful for us to select against male-sterile progeny during the breeding program, since this will eliminate the dominant gene(s) from Chinese chestnut associated with male sterility as well as eliminate an apparently undesirable trait. In general, during backcrossing, the sooner we can eliminate traits from the donor parent, the better.

To implement this strategy in our backcross breeding program, it may be better to use American chestnut (which apparently carries sterile cytoplasm) as the female parent. This will encourage the expression of male sterility and allow us to select against this trait.

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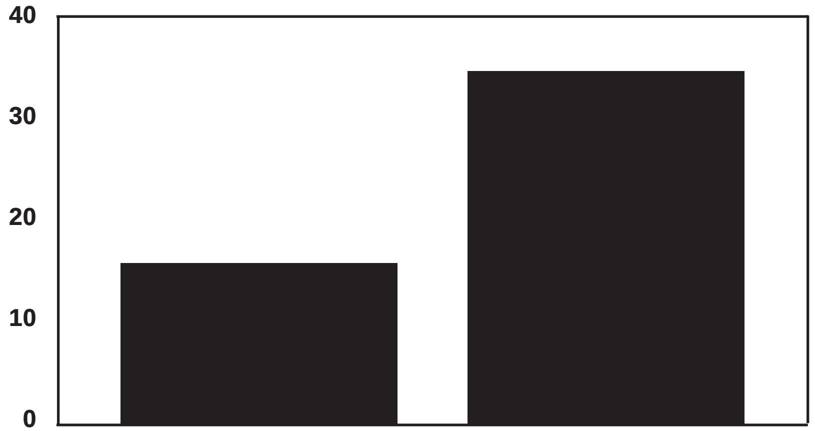
**Figure 1.**




Percentage of male-sterile progeny in $BC_1$ and $BC_2$ progeny populations from crosses between Chinese and American chestnut (Number of the $BC_1$ and $BC_2$ progeny were 100 and 418, respectively. Only those crosses with at least 10 progeny were used.

**TABLE 1.**

*Parents used to produce progeny for the study of male sterility.*

| Parent[y] | Species/Hybrid[z] | Parent[y] | Species/Hybrid[z] |
|---|---|---|---|
| Mahogany | C. mollissima | Musick | C. dentata |
| Meiling | C. mollissima | PG | C. dentata |
| Nanking | C. mollissima | QB | C. dentata |
| AC1 | C. dentata | RC1 | C. dentata |
| Am29 | C. dentata | RC2 | C. dentata |
| Am33 | C. dentata | RCLBig | C. dentata |
| Am59 | C. dentata | RD1 | C. dentata |
| B3 | C. dentata | RF1 | C. dentata |
| BurrOak | C. dentata | RF2 | C. dentata |
| CC1 | C. dentata | Rr1 | C. dentata |
| CC3 | C. dentata | RT4 | C. dentata |
| CR1 | C. dentata | RW1 | C. dentata |
| HW3 | C. dentata | RW2 | C. dentata |
| JK1 | C. dentata | RW3 | C. dentata |
| Lesesne | C. dentata | Test E #17 | C. dentata |
| MCBig | C. dentata | Clapper | BC1 |
| MCH | C. dentata | Graves | BC1 |
| Mu | C. dentata | | |

[z]BC1 was produced by crossing F1 to American chestnut.
[y]Lesesne was irradiated American chestnut.

**TABLE 2.**

*Percentage of male-sterile progeny in individual crosses of F1 progeny populations of Chinese and American chestnut.*

| Cross[x] | Total progeny | MF progeny[y] | MS progeny[z] | % MS |
|---|---|---|---|---|
| RC1 x Mahogany | 1 | 0 | 1 | 100 |
| BurrOak x Nanking | 6 | 0 | 6 | 100 |
| MCBig x Mahogany | 2 | 0 | 2 | 100 |
| RCLBig x Nanking | 5 | 0 | 5 | 100 |
| Meiling x Musick | 1 | 1 | 0 | 0 |
| Meiling x QB391 | 1 | 1 | 0 | 0 |
| Meiling x RC2 | 1 | 1 | 0 | 0 |
| Meiling x Am59 | 3 | 3 | 0 | 0 |
| Meiling x Am33 | 4 | 4 | 0 | 0 |
| Meiling x Am29 | 3 | 3 | 0 | 0 |
| Meiling x Test E#17 | 2 | 2 | 0 | 0 |
| Nanking x B3 | 1 | 1 | 0 | 0 |
| Nanking x Lesesne | 1 | 1 | 0 | 0 |
| Nanking x MCH | 3 | 3 | 0 | 0 |
| Nanking x Musick | 2 | 2 | 0 | 0 |
| Nanking x QB | 4 | 4 | 0 | 0 |

[x]First tree served as female parent and second tree as male parent. RC1, BurrOak, MCBig, RCLBig, Musick, QB391, RC2, Am59, Am33, Am29, Test E#17, B3, Lesesne, MCH, and QB were pure American chestnuts and Mahogany, Nanking, and Meiling were pure Chinese cultivars.
[y]MF = male fertile.
[x]MS = male sterile.

**TABLE 3.**

*Percentage of male-sterile progeny in individual crosses in the BC1 and BC2 populations.*

| Cross[v] | Population[w] | Total # of progeny | % Flower progeny | % MS[x] |
|---|---|---|---|---|
| RW1 x C | BC2 | 37 | 81 | 0 |
| RW3 x C | BC2 | 16 | 63 | 0 |
| HW3 x C | BC2 | 21 | 100 | 5 |
| RW2 x C | BC2 | 19 | 95 | 6 |
| JK1 x C | BC2 | 26 | 50 | 8 |
| RT4 x C | BC2 | 18 | 72 | 15 |
| RF1 x C | BC2 | 35 | 74 | 19 |
| CC1 x C | BC2 | 200 | 19 | 26 |
| Clapper[y] | BC2 | 372 | 69 | 12 |
| CR1 x G | BC2 | 40 | 60 | 13 |
| PG x G | BC2 | 31 | 84 | 23 |
| Rr1 x G | BC2 | 73 | 48 | 23 |
| CC3 x G | BC2 | 139 | 58 | 44 |
| RF2 x G | BC2 | 27 | 44 | 58 |
| AC1 x G | BC2 | 88 | 50 | 61 |
| RD1 x G | BC2 | 24 | 75 | 67 |
| Graves[z] | BC2 | 422 | 57 | 41 |
| Mu x N | BC1 | 70 | 66 | 0 |
| MCH x N | BC1 | 33 | 88 | 45 |

[v]First tree served as female parent and second tree as male parent. All female parents were pure American chestnut in the forest. C = The original Clapper (BC1) tree and G = The original Graves tree (BC1). N = (Nanking) x (Lesesne irradiated American chestnut). F = F1 derived from Meiling x American chestnut. MF = F1 derived from Mahogany x American chestnut.

[w]BC2 used American chestnut as recurrent parent and BC1 used American or Chinese chestnut as recurrent parent. 'Mu x N' and 'MCH x N' were American BC1s while 'Meiling x F' and 'Mahogany x MF' were Chinese BC1s.

[x]MS = male sterile.

[y]Totals for Clapper as the male parent.

[z]Totals Graves as the male parent.

**TABLE 4.**

*Comparison on the frequency of crosses that produced at least one male-sterile progeny in the F1, BC1, and BC2 populations.*

| Progeny population[w] | Female parent | No. of cross[x] | MS cross[y] | % MS cross[z] |
|---|---|---|---|---|
| F1 | Chinese | 12 | 0 | 0 |
| BC1 | Chinese | 2 | 0 | 0 |
| F1 | American | 4 | 4 | 100 |
| BC1 | American | 4 | 1 | 25 |
| BC2 | American | 29 | 27 | 93 |

wF1 = Progeny derived from crosses between Castanea dentata and C. mollissima. BC1 = Progeny derived from crosses between F1s and American or Chinese chestnut. The BC1s with American or Chinese as the female parent were American or Chinese BC1, respectively. BC2 = Progeny derived from crosses between BC1s and American chestnuts.
xThe crosses included: (1) those with at least one male-sterile progeny, and (2) those with no male-sterile progeny, but with at least 10 progeny.
yNumber of crosses that had one male-sterile progeny.
zPercentage of male-sterile crosses.