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## FROM THE EDITOR

In the second half Ralph Lutts' adaptation of his history of the American chestnut trade in southwestern Virginia, we are reminded of the chestnut commons of the Blue Ridge mountains. He describes why the loss of the chestnut was a double blow to farmers in an area where animals were free-ranged on vast tracts of unfenced pasture and forest.

Dr. Fred Hebard provides us with extensive detail about the work being performed by the dedicated Meadowview staff. Inventory and harvest tables reflect considerable growth in the breeding program, combined with the effects of unusual spring weather conditions at the Research Farm. In 2004, blight resistance screening was started on 'Clapper' B<sub>3</sub>-F<sub>2</sub> trees, and the results are in, as well as some very exciting news regarding the first crop of B<sub>3</sub>-F<sub>3</sub> nuts from selected Clapper B<sub>3</sub>-F<sub>2</sub> trees! Further information is provided for state chapter programs

In his article, *The Potential Use of American Chestnut for Reclaiming Mine Lands*, Dr. Douglas Jacobs discusses the many challenges faced by foresters in reclaiming lands that have been stripped and/or chemically altered due to mining. Dr. Jacobs highlights traits of the American chestnut, such as tolerance to harsh environmental conditions and rapid initial growth, as advantageous for this project, and introduces a new collaboration between TACF and Peabody Energy of St. Louis, MO, for a 5-year, \$100,000 study that will test the adaptability of American chestnut on reclamation sites in Kentucky.

*Curbing the U.S. carbon deficit* addresses the need for carbon sequestration, in order to reduce the gases currently emitted into the atmosphere as harmful CO<sub>2</sub>. Various approaches, including use of forest plantations as a means of biological sequestration are examined. Studies on the viability of using American chestnut in these sequestration programs are currently underway, and the results will be shared in a future edition of this journal.

In *Seed Dispersal, Seed Predation, and the American Chestnut?*, Michael A. Steele, Brian C. McCarthy, and Carolyn H. Keiffer provide a glimpse into how the loss of American chestnut may have altered the makeup of today's forests through the modification of seed dispersal. With the loss of a primary short-term food source, animals such as the gray squirrel adapted their habits of dispersing various seeds and, in turn, regenerating the forests.



Finally, Dr. Hebard describes the history, procedures, and expected future results of the backcross breeding program of TACF. In his introduction, he states that “...the most unusual aspect of this breeding program in comparison to similar programs for crop plants is the large acreages over which trees are grown, and the fact that the objective is recovery of a genetically diverse species rather than an improved cultivar.”

Exuberance and optimism abound as we enter our 24th year of research and development. Much progress lies behind us, and there may be unforeseen pitfalls ahead. As always, we are indebted to our members, to our chapters, and to our committed staff as we plow ahead towards the restoration of the King of the Forest.

A handwritten signature in black ink, appearing to read "Joanne", is positioned in the upper right quadrant of the page.



f r o m   t h e n   t o   n o w

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## THE CHESTNUT COMMONS IN THE BLUE RIDGE OF SOUTHWESTERN VIRGINIA

*Excerpted and slightly modified from Ralph H. Lutts. 2004. Manna from God: the American chestnut trade in southwestern Virginia. Environmental History 9(3):497-525. This is the second in a series reprinted in The Journal of The American Chestnut Foundation.*



*Memories of the chestnut loom large for many elderly residents of southern Appalachia. It is a memory of abundance—of manna that dropped from the forest canopy.*

### THE CHESTNUT COMMONS

For mountain folk, chestnuts were more than a source of food for themselves; the nuts were an abundant communal resource. Animals, such as hogs, turkeys, and cattle, were allowed to range freely in these Blue Ridge counties of Virginia without regard to property lines until well into the twentieth century. They grazed, foraged, and watered wherever they wandered. The free-range agricultural tradition was widely practiced in the South from colonial times and was strongly supported in the mountains, where farmers often owned large tracts of unimproved land. This practice was particularly beneficial to slaves, small landholders, renters, sharecroppers, and to the poor who did not own enough land to support their animals. (1) This open-range tradition was upheld in 1900 by the Virginia Supreme Court of Appeals, which declared that “the rule of the common law which requires the owner of animals to keep them on his own land or within enclosures is not in force in [Virginia] . . . and the owner of animals, being under no obligation to restrain them, is not liable for damage done in consequence of their straying on the unenclosed lands of another, unless he drives them there.” (2)

### FENCE-OUT VS. FENCE-IN LAWS

If an animal damaged a neighbor’s crops, there were few grounds for legal action unless that animal had broken through a fence. People were required to fence their neighbors’ animals out, rather than fence their own animals in. A county board of supervisors had the local option to pass an ordinance requiring owners to keep their animals on their own land. However, at the beginning of the twentieth century, only one Virginia county, Accomac, required residents to fence in their animals. The Blue Ridge counties of southwestern Virginia did not begin to require this until long after the blight

wiped out the American chestnut. Thus, much of the unfenced rural landscape of this region was a grazing and foraging commons. The ubiquitous rail fences that surrounded household gardens and farm crops bore witness to this. Although individuals owned the land, their unimproved acreage was a communal resource open to everyone's animals.

Cattle, hogs, and domesticated turkeys foraged through unfenced pasture and forest. In the autumn, hogs and turkeys fattened on the bounty of acorns and chestnuts. Hogs were important because their meat could be salted and stored through the winter by people who lacked refrigeration. Although the animals foraged across property lines, there was great respect for ownership of the animals. Owners of hogs and cattle could be identified by unique patterns of notches and holes cut in the animals' ears. The marks were sometimes registered at the local courthouse. An unmarked young hog born in the woods, however, could be claimed by the first person to find it. Farmers who lived at a lower elevation or had few chestnuts nearby sometimes fattened their hogs by hauling or driving them to more desirable locations to forage on chestnuts. Abraham Helms recalled, for example, that his father would take his hogs to Patrick County's Jones Mountain to fatten them. (3)



"Drovers" from Harper's,  
October 1857

**Impact of *Phytophthora* root rot disease and the chestnut blight.** The American chestnut was in trouble in its southern range long before the blight arrived. The U. S. Commissioner of Agriculture's 1878 Report Upon Forestry noted that the American chestnut was dying out in the Piedmont region of North Carolina. "The chestnut was formerly abundant in the Piedmont region down to the country between the Catawba and Yadkin Rivers," the North Carolina state geologist reported, "but within the last thirty years they have mostly perished." Chestnuts in the Piedmont of Virginia experienced a similar fate. "Throughout the Piedmont section of Virginia, especially in the lower portions," reported one observer in 1914, "there has been for thirty years or more a gradual

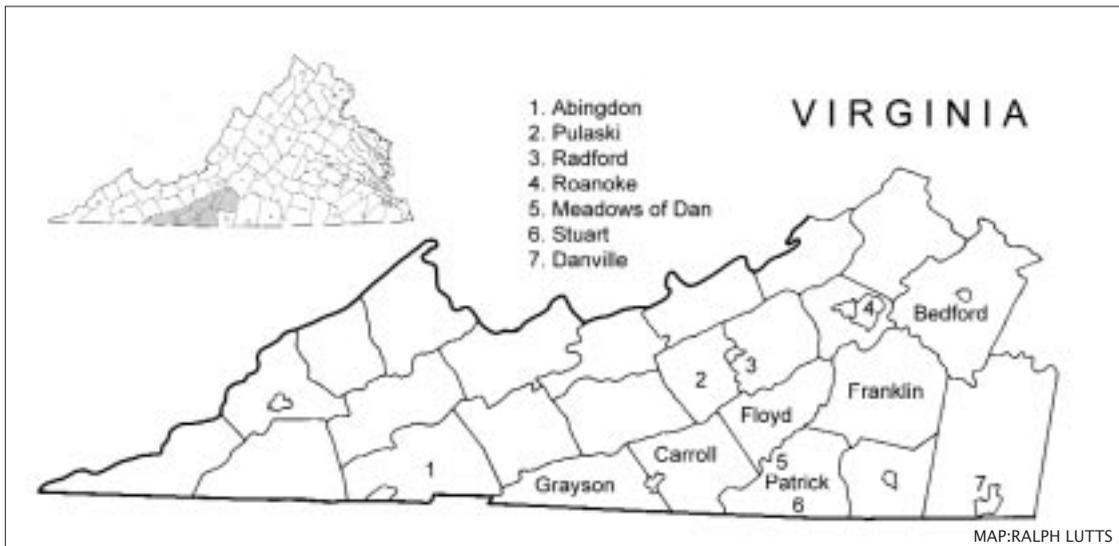
dying or recession of the chestnut toward the mountains.” It turned out that the problem was caused by a fungus in the soil, *Phytophthora cinnamomi*. (4) The southern and southeastern range of the tree was already shrinking when the chestnut blight arrived. (5) However, [root rot] had little or no impact upon the chestnut . . . in the southern Appalachian [mountains]. The slow death of the chestnuts in the lower Piedmont regions [caused by root rot] was quickly surmounted by the rapid destruction of the tree throughout its range by the chestnut blight.

Virginia experienced an outbreak of cicadas in 1911 and millions of these insects opened avenues for blight infection by piercing tree bark with their mouthparts. This hastened the spread of the disease. The blight reached Virginia around 1912 and by 1914 it was found in eighteen counties. It spread at breakneck speed, with the infection rate increasing at a reported rate of 600% per year in areas that had experienced the cicada outbreak. By 1914, 100 infected trees were found in Bedford County, located just northeast of Franklin County. This was an isolated outbreak ahead of the main infestation. The Bedford trees were destroyed and the advance of the infection halted in the county, but this was a temporary reprieve. Nearly all American chestnut trees throughout Virginia were infected by 1920. (6)

For many mountain people, . . . the loss of the tree brought economic hardship or devastation. The tree and its nuts failed just as the Great Depression arrived, which compounded their economic problems. The loss of the nuts was a double blow. The best sources of cash for the poorest in the southwestern Blue Ridge counties were chestnuts, hogs, moonshine, and, perhaps, dried apples. The blight ended the chestnut trade and the loss of the nuts brought an end to the hogs. Allowing hogs to forage for chestnuts was both the best and cheapest way to fatten them for slaughter. Acorns were not sufficient and most farmers could not afford to raise or purchase hog feed.

**The closure of the commons** in southwestern Virginia Blue Ridge counties, and perhaps in other Appalachian mountain counties, followed a very different path from that of much of the South. Following the Civil War, many interests worked to close the commons. People who wanted to constrain the liberties of formerly enslaved African Americans, large landowners who wanted to protect their property rights, mercantile interests, and even railroad companies concerned about liability when trains killed live-





stock tried to pass laws requiring farmers to fence in their livestock. They encountered considerable resistance on the part of small farmers and others, particularly in mountain communities. However, through persistence, political skill, and skullduggery, they often succeeded. (7)

They did not succeed in southwestern Virginia. Fence-in ordinances were not passed until late in the 20th century, if at all. Floyd County, Virginia, did not pass an ordinance requiring owners to fence their animals until 1975. Henry County did not pass a similar ordinance until 1977. Franklin County passed an ordinance requiring people to fence in their livestock in 1997. (8) According to local folklore, Grayson County had two of its four districts governed by fence-in laws and two by fence-out laws, but there is not documentation to confirm this. The county established a committee to examine the matter, and, in 2004, it recommended adopting a countywide fence-out ordinance, which would preserve the open range. Carroll County has yet to pass a fence ordinance, so it has been and continues to be a fence-out county. (9) But these are now only issues of legal liability, since the practice of free-range grazing was abandoned long ago. Although the commons was not legally closed, farmers stopped free-ranging their animals and it informally passed out of use. After the loss of the chestnuts there was not enough forage to fatten hogs, and free-ranging other animals ended as new agricultural practices were adopted.



**The death of the chestnuts** helped to destroy a semi-subsistence economy and forced many mountain residents to find wage labor. In the first decades of the 20th century, many Blue Ridge residents left to find jobs in Piedmont mills in nearby Fieldale and Danville (Virginia), Spray and Draper (North Carolina), and elsewhere. The timber and coal industries drew others. Jobs in cities in Ohio and other urban areas also attracted Appalachian residents. The population decline in Patrick, Floyd, and Franklin counties accelerated during the blight years of the 1920s. It is likely that out-migration in response to the loss of the chestnut was a significant contributing factor.

Memories of the chestnut loom large for many elderly residents of southern Appalachia. They remember it fondly and mourn its loss. These feelings of loss also may encompass the loss of a way of life that the chestnut has come to symbolize. It is a memory of abundance—of manna that dropped from the forest canopy.

#### REFERENCES

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7. Hahn, *The Roots of Southern Populism*, 239-68; Steinberg, *Down to Earth*, 106-10.

8. Judge Gino Williams, Floyd County, personal communication, 19 May 2004; Floyd County, Virginia, Board of Supervisors minutes, 11 August 1975; Floyd County Code Sec. 10-5; Patrick County, Virginia, Supervisors Order Book No. 9, 348; Franklin County, Virginia, Code 4.1 (ordinance approved 21 October 1997). The Floyd and Patrick ordinances closed the commons by defining property lines and streams as fences, regardless of whether a physical fence was present. The Franklin County code simply requires owners to prevent strays and to fence in their animals. This part of the ordinance may replace an earlier one. However, the code does not cite it (earlier ordinances are cited for other parts of this "Animal and Fowl" section of the code) and no one in the county supervisor's office or town clerk's office recalls an earlier ordinance of this sort. The hunting commons also are closing in response to cultural change as newcomers (called "foreigners" by local people), who do not share a family tradition of hunting, post their land against hunting.

9. Donald G. Young, county administrator, Grayson County, Virginia, personal communication, 14 May 2004, 21 June 2004; Ronald L. Newman, acting county administrator, Carroll County, Virginia, personal communications, 29 January 2004, 21 May 2004.







science and natural history

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## MEADOWVIEW NOTES 2004-2005

Frederick V. Hebard  
Staff Pathologist

In the year 2004, Meadowview was blessed with above average rainfall, but not the excessive amounts that occurred in 2003. The temperatures also were fairly normal during the growing season, leading to relatively “normal” patterns of canker expansion, making it fairly easy to assess blight resistance.

It has been quite wet in the winter and spring of 2005, delaying plowing until March, as in 2004. But, once again, we were able to finish planting by early April, as we now have sufficient equipment to prepare orchards quickly once the weather breaks. We have sufficient equipment because of the generous support of TACF members, and we thank you once again!

**Inventory.** Our current holdings are presented in Table 1, and changes from 2004 to 2005 are indicated in Table 2. We now have more than 22,000 trees and planted nuts, about 1,000 more than last year (Table 2). The addition of B<sub>3</sub>-F<sub>2</sub> trees has been offset by the removal of straight backcross trees as we have made selections and rogued the rejects.

Table 3 presents the current holdings of ‘Graves’ and ‘Clapper’ third backcrosses in the various state chapters. The number of trees, lines, and chapters continues to grow. This year, we count 18,771 third and fourth backcross trees and planted nuts in the various chapters. The count is up this year by about 5,000 nuts, reflecting the vigorous breeding occurring

in more and more chapters. Hopefully, before long, some chapters will be able to breed with additional sources of resistance to ‘Clapper’ and ‘Graves.’ We have been trying hard to produce backcross F<sub>2</sub>s that I feel will serve as good new sources of blight resistance for chapters.

**Harvest.** In 2004, 2,211 nuts were harvested from controlled pollinations in

Meadowview, which is a respectable number overall. Our yield of nuts per bag was much improved compared to 2003, which was fortunate



because we pollinated 726 fewer bags in 2004 than in 2003. The higher yield may have occurred because we used dried pollen for a number of the pollinations, rather than fresh catkins. Although controlled experiments have never shown a statistically significant increase in nut yield with dried pollen compared to fresh catkins, the predominance of the evidence generated in Meadowview and at state chapters indicates this is the case. This higher yield from dried pollen is most evident if one examines the yield from pollinations at Meadowview in comparison to the yield at state chapters. Most Meadowview pollinations have been done with fresh catkins, while most state chapter pollinations have been done with dried pollen. The state chapter yields have consistently been much higher, and we plan in the future to use dried pollen for most of our pollinations here in Meadowview.

We placed such a low number of bags in 2004 due to a hard frost in the first week of May, which killed most of the flowers at the Price Research Farm. Although our average first frost-free date is around the 15th of May, warm weather in April caused almost all the tree species in the Meadowview region to be leafed out by the first week in May, approximately two weeks earlier than usual. Hopefully, this will not happen again in the near future.

Flower death from the spring frost also reduced considerably our harvest of second generation nuts from the third backcross ( $B_3-F_2$ ). This was a disappointment, as many Clapper lines are coming into production, and I had hoped to finish advancing several of these.

**Blight resistance screening in  $B_3-F_2$  seedlings.** The year 2004 was also the first in which we screened ‘Clapper’  $B_3-F_2$  seedlings for blight resistance. The results of that test are presented in Table 5. I was very pleased to be able to rank a few trees in most families as highly resistant. However, not shown in the table are the results of the supplemental rankings of the most resistant trees performed during the spring of 2005. I was not as pleased with the spring results, as only one tree retained the high ranking. I am still fairly satisfied as of October 2005, with the appearance of the one tree that retained a high ranking.

At this point, I do not consider this a setback because the trees were fairly small when inoculated, only two years old and entering their third growing season. Previously in 1993, when we inoculated  $F_2$ s and  $B_1-F_2$ s,



not many of those survived through the 1994 growing season. In fact, all the grafted ‘Nanking’ Chinese chestnut trees included in that test as check trees succumbed by the end of 1995, and ‘Nanking’ is the most blight-resistant cultivar known in Chinese chestnut. After the 2004 test, five of the highest ranking ‘Clapper’ B<sub>3</sub>-F<sub>2</sub> seedlings survived and bore nuts in 2005 (see below)!

In view of these results, we have decided to start using a staged screening for blight resistance in B<sub>3</sub>-F<sub>2</sub> seedlings. We will first test resistance with a weakly virulent strain of the blight fungus, to weed out the most susceptible trees. The remaining trees will be tested with a strongly virulent strain a few years later, when they have become large enough to survive the test.

There are biological reasons for the lack of survival of small chestnut trees being screened for blight resistance using the cork-borer, agar-disk method with inoculation to the vascular cambium. Even highly blight-resistant trees are likely to die because the blight fungus can grow through the center of their small stems. In older, larger trees, the blight fungus has to grow around the stem to kill the tree. Thus, the larger trees have more time to marshal their defenses. Larger trees also are able to withstand the onslaught of blight better than smaller trees because they are considerably larger relative to the size of a canker. Thus, the canker is much less of a systemic stress, enabling the tree to mount a stronger defense.

In 2005, we have inoculated quite a few additional B<sub>3</sub>-F<sub>2</sub> seedlings, using both strong and weak strains of the blight fungus, as the inoculations were made prior to the decision to move to a staged resistance screening. The inoculations were done on trees located both at Meadowview by our staff, and at Penn State University by Sara Fitzsimmons, Tim Phelps, Kim Steiner and their crew. The results of these tests should give us a clearer picture of the levels of blight resistance we can expect to see in the future. Patience is indicated!

**Effective population size.** In 2002 and 2003 (J. of The American Chestnut Foundation, Vol. 16:1, 7-18 and Vol. 17:1, 7-14), I presented the results of computer simulations of the inbreeding levels expected in the products of our breeding program, based on assumed pedigrees of B<sub>3</sub>-F<sub>4</sub> trees. This year, I was able to calculate the inbreeding effective population size of our breeding stock from the results of those simulations,

and include the contributions of our chapter breeding programs to the effective population size.

Effective population size is an important parameter in population and quantitative genetics, as well as conservation genetics. There is a famous rule in conservation genetics known as the 50/500 rule (Franklin, I.R., & Frankham, R. 1998. How large must populations be to retain evolutionary potential. *Anim. Conserv.* Vol. 1, 69-71.), which states that, for obligate outcrossers like chestnut trees, the effective population size must be at least 50 to avoid immediate collapse of a population from inbreeding depression and at least 500 for mutation to offset the slow loss of alleles from genetic drift.

The basic building block of our breeding program is a set of 20 lines from one source of blight resistance. The 20 lines were chosen in order to capture alleles occurring at frequencies of greater than 5%. One source of resistance is used in order to ensure a fairly high level of homozygosity for blight resistance in later generations. The effective population size depends not only on these 20 lines, but also on how rapidly the real population size is increased from straight  $B_3$  to  $B_3-F_4$ . It is approximately the harmonic mean of the size of each generation.

Table 6 presents the results of these calculations and indicates that our basic building blocks of 20 lines will have an inbreeding effective population size of 72. Adding contributions from five chapters builds it to 248, and adding another source of blight resistance for five chapters would double it. Thus, our effective population sizes are comfortably above the thresholds of the 50/500 rule. It should be noted that these calculations assume there will be no variation in family sizes. The inevitable variation in family size, especially at  $B_3-F_3$  and  $B_3-F_4$ , will reduce the effective population size. But it does appear we will be OK in this regard. [As a side note, the effective population size of the West Salem, WI, stand of American chestnut trees would be about 30 or 35 if all nine founders contributed equally to the bulk of the second generation, perhaps explaining why that population does not appear to be foundering from inbreeding depression].

I would like to thank Lou Silveri, Dave Lazor, Chandis Klinger, Gene Whitmeyer, and Harry Norford for helping out with pollination and inoculation in 2004. They came down on their own and stayed at Emory and Henry College. We also had a group come down under an Elder



Hostel program. Sam Fisher, Neil Rich & Chrystle Gates of the Southwest Virginia 4-H Center have been very helpful managing the Elder Hostel program, which would not occur without their initiative. Thank you — this wouldn't get done without your help. If you are interested in helping to pollinate next year, plan on any time in June (call 276 944-4631). If you are interested in learning more about the Elder Hostel program, call 617 426-8055 or write 75 Federal St., Boston MA 02110.

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



**TABLE 1**

Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2005, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

| Type of Tree   | Number of     |                       |                 |
|--|---------------|-----------------------|-----------------|
|  | Nuts or Trees | Sources of Resistance | American Lines* |
| American   | 2082          |                       | 206             |
| Chinese  | 814           | 55                    |                 |
| Chinese x American: F <sub>1</sub>   | 858           | 22                    | 95              |
| American x (Chinese x American): B <sub>1</sub>  | 386           | 13                    | 38              |
| American x [American x (Chinese x American)]: B <sub>2</sub>                                     | 1512          | 10                    | 81              |
| American x {American x [American x (Chinese x American)]}: B <sub>3</sub>                        | 4198          | 8                     | 75              |
| Am x (Am x {Am x [Am x (Chin x Am)]}):B <sub>4</sub>   | 86            | 1                     | 1               |
| (Chinese x American) x (Chinese x American): F <sub>2</sub>                                      | 710           | 6                     | 6               |
| [Ch x Am] x (Ch x Am) x [Ch x Am] x (Ch x Am):F <sub>3</sub>                                     | 6             | 1                     | 1               |
| [Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B <sub>1</sub> -F <sub>2</sub>                  | 688           | 3                     | 3               |
| {Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B <sub>2</sub> -F <sub>2</sub>                 | 365           | 5                     | 5               |
| [A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B <sub>3</sub> -F <sub>2</sub> | 7295          | 2                     | 23              |
| Chinese x (Chinese x American): Chinese B <sub>1</sub>   | 191           | 3                     | 4               |
| Chinese x [American x (Chinese x American)]  | 41            | 1                     | 1               |
| Japanese   | 3             | 2                     | 2               |
| American x Japanese: F <sub>1</sub>  | 11            | 2                     | 2               |
| (American x Japanese) x American: B <sub>1</sub>   | 10            | 2                     | 2               |
| Castanea seguinii  | 48            | 1                     | 1               |
| Chinese x Castanea pumila: F <sub>1</sub>  | 9             |                       |                 |
| Large, Surviving American x American: F <sub>1</sub>   | 272           | 11                    | 29              |
| (Large, Surviving American x American) x American: B <sub>1</sub>                                | 582           | 6                     | 9               |
| [(Large, Surviving American x American) x American] x American: B <sub>2</sub>                   | 126           | 1                     | 2               |
| Large, Surviving American x Large, Surviving American: I <sub>1</sub>                            | 474           | 14                    | 14              |
| Large, Surviving American: F <sub>2</sub> = F <sub>1</sub> xF <sub>1</sub> , same LS parent      | 467           | 5                     | 5               |
| Large, Surviving American Other  | 59            | 2                     | 7               |
| Irradiated American x American: F <sub>1</sub>   | 1             | 1                     | 1               |
| Other  | 24            |                       |                 |
| <b>Total</b>   | <b>22,038</b> |                       |                 |

\* The number of lines varied depending on the source of resistance. We will have to make additional crosses in some lines to achieve the desired number of 75 progeny per generation within a line. In keeping with past practice, the number of lines for each source of resistance are added separately; thus, progeny from two sources of resistance that share an American parent would be counted as two lines rather than one line (this only occurs rarely).

**TABLE 2**

Changes between 2004 and 2005 in the number of chestnut trees and planted nuts of different types at TACF Meadowview Research Farms, including changes in the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

| Type of Tree   | Increase or Decrease* in Number of |                       |                |
|--|------------------------------------|-----------------------|----------------|
|  | Nuts or Trees                      | Sources of Resistance | American Lines |
| American   | -34                                |                       | -4             |
| Chinese  | 145                                | 5                     |                |
| Chinese x American: F <sub>1</sub>   | 241                                | -1                    | -3             |
| American x (Chinese x American): B <sub>1</sub>  | -392                               | -3                    | -5             |
| American x [American x (Chinese x American)]: B <sub>2</sub>                                     | -20                                | 0                     | -6             |
| American x {American x [American x (Chinese x American)]}: B <sub>3</sub>                        | -357                               | 0                     | 2              |
| Am x (Am x {Am x [Am x (Chin x Am)]}):B <sub>4</sub>   | 0                                  | 0                     | 0              |
| (Chinese x American) x (Chinese x American): F <sub>2</sub>                                      | 0                                  | 1                     | 1              |
| [Ch x Am] x (Ch x Am) x [Ch x Am] x (Ch x Am):F <sub>3</sub>                                     | 0                                  | 0                     | 0              |
| [Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B <sub>1</sub> -F <sub>2</sub>                  | 0                                  | 0                     | 0              |
| {Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B <sub>2</sub> -F <sub>2</sub>                 | 22                                 | 1                     | 1              |
| [A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B <sub>3</sub> -F <sub>2</sub> | 1459                               | 0                     | 6              |
| Chinese x (Chinese x American): Chinese B <sub>1</sub>   | 49                                 | 0                     | 1              |
| Chinese x [American x (Chinese x American)]  | 0                                  | 0                     | 0              |
| Japanese   | 0                                  | 0                     | 0              |
| American x Japanese: F <sub>1</sub>  | 0                                  | 0                     | 0              |
| (American x Japanese) x American: B <sub>1</sub>   | -69                                | 0                     | 0              |
| Castanea seguinii  | 0                                  | 0                     | 0              |
| Chinese x Castanea pumila: F <sub>1</sub>  | 0                                  |                       |                |
| Large, Surviving American x American: F <sub>1</sub>   | 10                                 | 2                     | 20             |
| (Large, Surviving American x American) x American: B <sub>1</sub>                                | -49                                | 0                     | 0              |
| [(Large, Surviving American x American) x American] x American: B <sub>2</sub>                   | 89                                 | 0                     | 0              |
| Large, Surviving American x Large, Surviving American: I <sub>1</sub>                            | 89                                 | 2                     | 2              |
| Large, Surviving American: F <sub>2</sub> = F <sub>1</sub> x F <sub>1</sub> , same LS parent     | 0                                  | 0                     | 0              |
| Large, Surviving American: Other   | 0                                  | 0                     | 5              |
| Irradiated American x American: F <sub>1</sub>   | -2                                 | 0                     | 0              |
| Other  | 0                                  |                       |                |
| <b>Total</b>   | <b>1181</b>                        |                       |                |

\* The decreases in B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, and large, surviving American B<sub>1</sub> & F<sub>2</sub> trees reflect roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting.

**TABLE 3**

Number of third-backcross chestnut at TACF Chapters in 2005, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

| Chapter       | Number of     |                       |                 |
|---------------|---------------|-----------------------|-----------------|
|               | Nuts or Trees | Sources of Resistance | American Lines* |
| Maine         | 1879          | 2                     | 29              |
| Massachusetts | 4345          | 2                     | 34              |
| Pennsylvania  | 5371*         | 2                     | 39              |
| Maryland      | 418           | 2                     | 7               |
| Indiana       | 2970          | 1                     | 19              |
| Kentucky      | 802           | 2                     | 7               |
| Carolinas     | 1093          | 2                     | 17              |
| Tennessee     | 2011          | 3                     | 19              |
| Alabama       | 267           | 1                     | 8               |
| <b>Total</b>  | <b>19,156</b> |                       |                 |

\*Numerous B<sub>3</sub>-F<sub>2</sub>s also have been planted but these are not included in this table.



**TABLE 4**

The American Chestnut Foundation Meadowview Research Farms 2004 nut harvest from controlled pollinations and selected open pollinations.

| Nut Type                       | Female Parent                          | Pollen Parent                   | Pollinated |                 |      | Unpollinated Checks |      |      | Number of American Chestnut Lines* |
|--------------------------------|--|---------------------------------|------------|-----------------|------|---------------------|------|------|------------------------------------|
|                                |  |                                 | nuts       | bags            | burs | nuts                | bags | burs |                                    |
| Am x Am                        | American                               | American                        | 277        | 267             | 581  |                     | 21   | 60   | 1                                  |
| B <sub>1</sub>                 | F <sub>1</sub> Mahogany                | American                        | 194        | 256             | 472  | 1                   | 24   | 39   | 1                                  |
| B <sub>2</sub>                 | American                               | B <sub>1</sub> MusickChinese    | 234        | 43              | 141  | 0                   | 5    | 5    | 1                                  |
| B <sub>2</sub>                 | B <sub>1</sub> MusickChinese           | American                        | 5          | 19              | 31   | 1                   | 3    | 3    | 1                                  |
| B <sub>2</sub>                 | American                               | B <sub>1</sub> Nanking          | 106        | 242             | 247  | 5                   | 26   | 26   | 11                                 |
| B <sub>2</sub>                 | B <sub>1</sub> Nanking                 | American                        | 30         | 50              | 108  | 0                   | 3    | 4    | 3                                  |
| B <sub>2</sub> -F <sub>2</sub> | B <sub>2</sub> Clapper                 |                                 | 865        | open pollinated |      |                     |      |      | 3                                  |
| B <sub>2</sub> -F <sub>2</sub> | B <sub>2</sub> R1T7                    | B <sub>2</sub> R1T7             | 180        | 60              | 138  | 0                   | 5    | 8    | 1                                  |
| B <sub>2</sub> -F <sub>3</sub> | B <sub>2</sub> -F <sub>2</sub> Clapper |                                 | 191        | open pollinated |      |                     |      |      | 3                                  |
| B <sub>2</sub> -F <sub>3</sub> | B <sub>2</sub> -F <sub>2</sub> Graves  |                                 | 19         | open pollinated |      |                     |      |      | 1                                  |
| B <sub>3</sub>                 | B <sub>2</sub> Gr                      | American                        | 119        | 141             | 447  | 3                   | 15   | 30   | 5                                  |
| B <sub>4</sub>                 | B <sub>3</sub> Gr                      | American                        | 1          | 97              | 316  | 0                   | 10   | 30   | 1                                  |
| B <sub>3</sub>                 | American                               | B <sub>2</sub> Nanking          | 155        | 128             | 162  | 1                   | 14   | 21   | 3                                  |
| B <sub>3</sub>                 | B <sub>2</sub> Nanking                 | American                        | 0          | 3               | 0    | 1                   | 1    | 1    | 1                                  |
| B <sub>3</sub>                 | American                               | B <sub>2</sub> R1T7             | 86         | 82              | 141  | 1                   | 9    | 17   | 6                                  |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Clapper                 |                                 | 1106       | open pollinated |      |                     |      |      | 17                                 |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Graves                  | B <sub>3</sub> Graves           | 26         | 14              | 27   | 0                   | 2    | 2    | 1                                  |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Graves                  |                                 | 555        | open pollinated |      |                     |      |      | 8                                  |
| Chin B <sub>1</sub>            | FB <sub>1</sub> Mahogany               | Chin Mahogany                   | 50         | 98              | 224  | 0                   | 10   | 17   | 1                                  |
| F <sub>1</sub>                 | Chinese Kuling                         | American                        | 13         | 66              | 121  | 3                   | 5    | 7    | 1                                  |
| F <sub>1</sub>                 | Chinese Nanking                        | American                        | 101        | 261             | 499  | 2                   | 28   | 51   | 3                                  |
| Isa B <sub>2</sub>             | American                               | B <sub>1</sub> ScientistsCliffs | 431        | 70              | 228  | 1                   | 4    | 4    | 2                                  |
| Isa I <sub>1</sub>             | American                               | opDaresBeach                    | 65         | 63              | 50   | 0                   | 6    | 5    | 4                                  |
| Isa I <sub>1</sub>             | F <sup>1</sup> DaresBeach              | B <sub>1</sub> ScientistsCliffs | 8          | 18              | 21   | 0                   | 2    | 2    | 1                                  |

(Continued on next page)

**TABLE 4 (continued)**

| Nut Type                             | Female Parent      | Pollen Parent                    | Pollinated  |             |             | Unpollinated Checks |            |            | Number of American Chestnut Lines* |
|--------------------------------------|--------------------|----------------------------------|-------------|-------------|-------------|---------------------|------------|------------|------------------------------------|
|                                      |                    |                                  | nuts        | bags        | burs        | nuts                | bags       | burs       |                                    |
| Isa I <sub>1</sub>                   | B <sub>1</sub> Ort | BB <sub>1</sub> ScientistsCliffs | 61          | 61          | 114         | 0                   | 7          | 13         | 1                                  |
| Isa I <sub>1</sub>                   | F <sub>1</sub> Ort | BB <sub>1</sub> ScientistsCliffs | 69          | 53          | 143         | 0                   | 4          | 14         | 1                                  |
| <b>Total Controlled Pollinations</b> |                    |                                  | <b>2211</b> | <b>2092</b> | <b>4211</b> | <b>19</b>           | <b>204</b> | <b>359</b> |                                    |

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

**TABLE 5**

Number of 'Clapper' B3-F2 seedlings ranked in various blight resistance classes in 2004.

| Code of Mother Tree | Code of Resistant Grandparent | Blight Resistance Class* |   |    |    |    |
|---------------------|-------------------------------|--------------------------|---|----|----|----|
|                     |                               | 1                        | 2 | 3  | 4  | 5  |
| CH271               | CL285                         | 2                        | 4 | 11 | 11 | 8  |
| CH199               | CL112                         | 1                        | 6 | 14 | 10 | 5  |
| CH34                | CL198                         | 0                        | 1 | 2  | 4  | 4  |
| CH726               | CL130                         | 0                        | 4 | 17 | 40 | 31 |
| CH283               | CL98                          | 3                        | 3 | 12 | 14 | 9  |
| CH526               | CL287                         | 0                        | 1 | 12 | 14 | 9  |

\* 1 is the most resistant class and 5 the least. A rating of 1 indicates that cankers caused by both strongly and weakly virulent strains of the blight fungus were small (2-3 cm long) after one season of canker expansion. A rating of 2 indicates that cankers incited by the strong strain were intermediate in size (3-6 cm long) while the weakly virulent strain yielded small cankers. A rating of 3 indicates that the strong strain yielded large cankers (>6 cm long) and the weak strain small cankers. A rating of 4 indicates that the strong strain yielded large cankers and the weak strain intermediate cankers, and a rating of 5 indicates that both strains yielded large cankers. Typically, Chinese chestnut trees achieve a rating of 1 or 2 and American chestnut trees a rating of 4 or 5.

**TABLE 6**

Effect of adding sets of 20 B3-F2 progeny from TACF's chapter breeding program on inbreeding and effective population size, assuming that the base population of B3-F1 trees are not inbred.

| Number of Chapters | Inbreeding Coefficient at B <sub>3</sub> -F <sub>4</sub> | Inbreeding Effective Population Size |
|--------------------|--|--------------------------------------|
| 1                  | 0.02066  | 72                                   |
| 2                  | 0.01153  | 130                                  |
| 3                  | 0.00850  | 176                                  |
| 4                  | 0.00698  | 214                                  |
| 5                  | 0.00603  | 248                                  |

**A Quick Guide to Chestnut Breeding Terminology**

| PARENT  | = | OFFSPRING  |
|---|---|--|
| American x Chinese  | = | F <sub>1</sub> , "F-one"                         |
| F <sub>1</sub> x F <sub>1</sub>                                 | = | F <sub>2</sub> , F-two                           |
| F <sub>2</sub> x F <sub>2</sub>                                 | = | F <sub>3</sub> , F-three                         |
| F <sub>1</sub> x American                                       | = | B <sub>1</sub> , first backcross, or B-one       |
| B <sub>1</sub> x American                                       | = | B <sub>2</sub> , second backcross, or B-two      |
| B <sub>2</sub> x American                                       | = | B <sub>3</sub> , third backcross                 |
| B <sub>3</sub> x American                                       | = | B <sub>4</sub> , fourth backcross                |
| B <sub>1</sub> x B <sub>1</sub>                                 | = | B <sub>1</sub> -F <sub>2</sub> , B-one F-two     |
| B <sub>1</sub> -F <sub>2</sub> x B <sub>1</sub> -F <sub>2</sub> | = | B <sub>1</sub> -F <sub>3</sub> , B-two F-three   |
| B <sub>2</sub> x B <sub>2</sub>                                 | = | B <sub>2</sub> -F <sub>2</sub> , B-two F-two     |
| B <sub>2</sub> -F <sub>2</sub> x B <sub>2</sub> -F <sub>2</sub> | = | B <sub>2</sub> -F <sub>3</sub> , B-two F-three   |
| B <sub>3</sub> x B <sub>3</sub>                                 | = | B <sub>3</sub> -F <sub>2</sub> , B-three F-two   |
| B <sub>3</sub> -F <sub>2</sub> x B <sub>3</sub> -F <sub>2</sub> | = | B <sub>3</sub> -F <sub>3</sub> , B-three F-three |

**Addendum: 2005 Harvest.** These notes include the results of the 2005 harvest in Meadowview, which are detailed in Table 6.

The most noteworthy event of the 2005 harvest was our first crop of  $B_3$ - $F_3$  nuts from selected Clapper  $B_3$ - $F_2$  trees! We expect this number to increase sharply in future years. We also harvested a large crop of  $B_3$ - $F_2$  nuts; the harvest of nuts from the Clapper source of resistance should go a long way toward completing that generation of crosses.

Our harvest from controlled pollinations was a disappointment: we harvested less than two nuts for every third pollination bag. However, because we managed to place so many bags this year, the total size of the harvest from controlled pollinations was a respectable 1976 nuts.

The low yield may have been associated with cool temperatures in early June after a warm May, which delayed flowering, along with very dry conditions in June. Trees were ready to bloom for several weeks, but didn't, which may have affected pollen viability. This hypothesis is supported by preliminary observation that some pollens shipped to chapters gave very poor yields. This will have to be confirmed by further analysis of the results of chapter pollinations, and further analysis of the results from Meadowview. The analysis is not finished yet because harvest was very late this year, delayed 2 to 3 weeks beyond normal. Another possibility for 2005's low yield was the hot, dry weather during June; this may have adversely affected pollen germination and growth into the style.



**TABLE 7**

The American Chestnut Foundation Meadowview Farms 2005 nut harvest from controlled pollinations and selected open pollinations.

| Nut Type                       | Female Parent                                 | Pollen Parent                 | Pollinated |                 |      | Unpollinated Checks |      |      | Number of American Chestnut Lines* |
|--------------------------------|---|-------------------------------|------------|-----------------|------|---------------------|------|------|------------------------------------|
|                                |   |                               | nuts       | bags            | burs | nuts                | bags | burs |                                    |
| B <sub>1</sub>                 | F <sub>1</sub> 72-211                         | American                      | 41         | 100             | 168  | 0                   | 15   | 17   | 2                                  |
| B <sub>1</sub>                 | American                                      | F <sub>1</sub> mollissima10   | 1          | 38              | 86   | 0                   | 3    | 17   | 3                                  |
| B <sub>1</sub>                 | F <sub>1</sub> mollissima10                   | American                      | 7          | 70              | 226  | 0                   | 7    | 19   | 1                                  |
| B <sub>1</sub>                 | American                                      | F <sub>1</sub> mollissima11   | 1          | 43              | 137  | 1                   | 3    | 15   | 2                                  |
| B <sub>1</sub>                 | F <sub>1</sub> mollissima11                   | American                      | 70         | 90              | 198  | 0                   | 11   | 35   | 3                                  |
| B <sub>1</sub>                 | F <sub>1</sub> mollissima12                   | American                      | 463        | 253             | 613  | 8                   | 25   | 71   | 3                                  |
| B <sub>1</sub>                 | F <sub>1</sub> mollissima13                   | American                      | 18         | 9               | 12   | 0                   | 1    | 2    | 1                                  |
| B <sub>1</sub>                 | F <sub>1</sub> mollissima7                    | American                      | 0          | 23              | 34   | 0                   | 2    | 3    | 1                                  |
| B <sub>1</sub> -F <sub>2</sub> | B <sub>1</sub> MusickChinese                  | B <sub>1</sub> MusickChinese  | 120        | 75              | 192  | 0                   | 7    | 22   | 2                                  |
| B <sub>1</sub> -F <sub>3</sub> | B <sub>1</sub> -F <sub>2</sub> Clapper-Graves |                               | 3255       | open pollinated |      |                     |      |      | 10                                 |
| B <sub>2</sub>                 | B <sub>1</sub> Mahogany                       | American                      | 46         | 51              | 78   | 0                   | 5    | 9    | 3                                  |
| B <sub>2</sub>                 | B <sub>1</sub> Meiling                        | American                      | 16         | 66              | 124  | 0                   | 8    | 27   | 1                                  |
| B <sub>2</sub>                 | American                                      | B <sub>1</sub> Nanking        | 30         | 305             | 601  | 0                   | 35   | 95   | 19                                 |
| B <sub>2</sub>                 | B <sub>1</sub> Nanking                        | American                      | 127        | 360             | 966  | 2                   | 43   | 128  | 12                                 |
| B <sub>2</sub>                 | American                                      | Japn B <sub>1</sub> Pl#104016 | 14         | 49              | 68   | 0                   | 5    | 4    | 1                                  |
| B <sub>2</sub>                 | Japn B <sub>1</sub> Pl#104016                 | American                      | 16         | 19              | 39   | 0                   | 3    | 7    | 1                                  |
| B <sub>2</sub> -F <sub>2</sub> | B <sub>2</sub> Clapper                        |                               | 417        | open pollinated |      |                     |      |      | 3                                  |
| B <sub>2</sub> -F <sub>3</sub> | B <sub>2</sub> -F <sub>2</sub> Clapper        |                               | 1990       | open pollinated |      |                     |      |      | 2                                  |
| B <sub>3</sub>                 | American                                      | B <sub>2</sub> Graves         | 9          | 83              | 156  | 0                   | 7    | 17   | 4                                  |
| B <sub>3</sub>                 | B <sub>2</sub> Graves                         | American                      | 50         | 175             | 383  | 2                   | 14   | 30   | 5                                  |
| B <sub>3</sub>                 | American                                      | B <sub>2</sub> Nanking        | 14         | 67              | 173  | 2                   | 9    | 25   | 7                                  |
| B <sub>3</sub>                 | American                                      | B <sub>2</sub> R1T7           | 30         | 17              | 40   | 0                   | 1    | 2    | 1                                  |
| B <sub>3</sub>                 | B <sub>2</sub> R1T7                           | American                      | 4          | 49              | 81   | 0                   | 4    | 15   | 1                                  |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Clapper                        |                               | 7399       | open pollinated |      |                     |      |      | 49                                 |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Graves                         |                               | 1451       | open pollinated |      |                     |      |      | 10                                 |
| B <sub>3</sub> -F <sub>2</sub> | B <sub>3</sub> Graves                         | B <sub>3</sub> Graves         | 54         | 27              | 79   | 1                   | 3    | 4    | 1                                  |
| B <sub>3</sub> -F <sub>3</sub> | B <sub>3</sub> -F <sub>2</sub> Clapper        |                               | 118        | open pollinated |      |                     |      |      | 5                                  |
| B <sub>4</sub>                 | American                                      | B <sub>3</sub> Clapper        | 21         | 81              | 96   | 2                   | 8    | 15   | 3                                  |
| B <sub>4</sub>                 | B <sub>3</sub> Graves                         | American                      | 1          | 4               | 2    | 0                   | 1    | 0    | 1                                  |

(Continued on next page)

**TABLE 7 (continued)**

| Nut Type            | Female Parent                     | Pollen Parent                | Pollinated |      |      | Unpollinated Checks |      |      | Number of American Chestnut Lines* |
|---------------------|-----------------------------------|------------------------------|------------|------|------|---------------------|------|------|------------------------------------|
|                     |                                   |                              | nuts       | bags | burs | nuts                | bags | burs |                                    |
| F <sub>2</sub> Chin | Chinese m12xm13                   | Chinese m12xm13              | 10         | 11   | 18   | 0                   | 1    | 1    | 1                                  |
| F <sub>1</sub>      | Chinese Kuling                    | American                     | 29         | 85   | 207  | 0                   | 3    | 17   | 1                                  |
| F <sub>1</sub>      | Chinese Mahogany                  | American                     | 55         | 131  | 203  | 2                   | 15   | 18   | 4                                  |
| F <sub>1</sub>      | Chinese Nanking                   | American                     | 27         | 129  | 256  | 2                   | 14   | 30   | 1                                  |
| F <sub>1</sub>      | Chinese Richwood                  | American                     | 30         | 50   | 98   | 0                   | 5    | 12   | 1                                  |
| LSA B <sub>1</sub>  | American                          | LSA F <sub>1</sub> Amherst   | 10         | 51   | 74   | 0                   | 8    | 10   | 7                                  |
| LSA B <sub>1</sub>  | LSA F <sub>1</sub> Amherst        | American                     | 10         | 12   | 26   | 0                   | 2    | 2    | 1                                  |
| LSA B <sub>1</sub>  | LSA F <sub>1</sub> Corrigan       | American                     | 0          | 13   | 28   | 0                   | 2    | 6    | 1                                  |
| LSA B <sub>1</sub>  | American                          | LSA F <sub>1</sub> NCChamp   | 0          | 19   | 51   | 0                   | 3    | 7    | 4                                  |
| LSA B <sub>1</sub>  | LSA F <sub>1</sub> NCChamp        | American                     | 9          | 82   | 142  | 0                   | 6    | 18   | 3                                  |
| LSA B <sub>2</sub>  | American                          | LSA B <sub>1</sub> Corrigan  | 6          | 78   | 154  | 0                   | 7    | 12   | 1                                  |
| LSA B <sub>2</sub>  | LSA B <sub>1</sub> SciCliffs      | American                     | 7          | 24   | 43   | 0                   | 2    | 1    | 1                                  |
| LSA F <sub>1</sub>  | American                          | LSA Green1                   | 145        | 95   | 172  | 0                   | 9    | 12   | 1                                  |
| LSA F <sub>1</sub>  | American                          | LSA opWeekly                 | 56         | 120  | 276  | 0                   | 11   | 32   | 1                                  |
| LSA I <sub>1</sub>  | LSA B <sub>1</sub> Ort            | LSA F <sub>1</sub> Amherst   | 102        | 65   | 250  | 0                   | 7    | 15   | 1                                  |
| LSA I <sub>1</sub>  | LSA B <sub>1</sub> Ort            | LSA F <sub>1</sub> NCChamp   | 52         | 63   | 110  | 0                   | 6    | 18   | 2                                  |
| LSA I <sub>1</sub>  | LSA B <sub>1</sub> Ort            | LSA B <sub>1</sub> SciCliffs | 20         | 24   | 60   | 0                   | 3    | 6    | 1                                  |
| LSA I <sub>1</sub>  | LSA B <sub>1</sub> SciCliffs      | LSA F <sub>1</sub> NCChamp   | 51         | 27   | 44   | 0                   | 3    | 8    | 1                                  |
| LSA I <sub>1</sub>  | LSA F <sub>1</sub> Weekly         | LSA B <sub>1</sub> SciCliffs | 21         | 70   | 97   | 0                   | 7    | 15   | 1                                  |
| LSA I <sub>2</sub>  | LSA F <sub>1</sub> DaresBeach     | LSA opWeekly                 | 36         | 37   | 69   | 0                   | 1    | 4    | 1                                  |
| LSA I <sub>2</sub>  | LSA F <sub>1</sub> Weekly         | LSA opDaresBeach             | 40         | 17   | 44   | 0                   | 2    | 6    | 1                                  |
| LSA I <sub>2</sub>  | LSA opDaresBeach                  | LSA F <sub>1</sub> Amherst   | 56         | 39   | 93   | 0                   | 4    | 9    | 1                                  |
| LSA I <sub>2</sub>  | LSA I <sub>1</sub> GaultSciCliffs | LSA F <sub>1</sub> NCChamp   | 51         | 49   | 88   | 0                   | 5    | 16   | 1                                  |

**Total Controlled Pollinations**

**1976 3345 7155 22 346 854**

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

## THE POTENTIAL USE OF AMERICAN CHESTNUT FOR RECLAIMING MINE LANDS

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### INTRODUCTION

Surface mining in the United States is a vast industry that has been affecting forested landscapes for centuries. The mining process substantially alters physical, chemical, and biological site characteristics (Singh et al., 2002), greatly influencing and delaying natural forest regeneration (Wali, 1999). Following completion of mining operations, efforts are made to quickly reclaim these sites. Reclamation often involves the planting of forest tree seedlings, and in many states reclamation plantings represent a significant portion of total tree plantings. In Indiana, for example, seedling orders for mine land reclamation account for nearly 20% ( $\approx 1$  million seedlings) of the State's annual plantings (Conrad, 1999). Long-term survivorship of seedlings planted onto mine reclamation sites is generally low and plantings often consist of species which are not deemed highly desirable by landowners (Rathfon et al., 2004).

With future mining activities expected to increase, there is a need to identify practices which improve the success of mine reclamation projects. Converting reclaimed sites into quality forestlands will offer many benefits to landowners and the public. Properly managed forests provide clean water, wildlife habitat, timber, aesthetically pleasing landscapes, and sequester atmospheric carbon dioxide. Long-term forest productivity on reclaimed sites can be ensured by identifying new species options that are easily established on reclamation sites, yet possess desirable timber, wildlife, and aesthetic characteristics.

### THE RECLAMATION PROCESS

Restoring forests on surface-mined lands is challenging because of adverse soil conditions and plant competition (Bussler et al., 1984; Andersen et



al., 1989). The post-mining site must be graded prior to reclamation, which may dramatically alter the soil's physical properties. For example, soils of mined sites have higher bulk density, coarse fragments, and clay content and, consequentially, lower porosity, permeability, and moisture-holding capacity than unmined sites (Bussler et al., 1984). Topsoil is generally replaced to an average depth of about 30 cm (12 inches), below which a hardpan layer may create a perched water table (Bussler et al., 1984; Andersen et al., 1989), restricting seedling root system penetration, subsequently reducing seedling establishment success.

Despite these obstacles, studies have shown that forests can be successfully restored on abandoned mine sites with equal or more productive roles than the native forests removed by mining (Burger and Torbert, 1992; Torbert et al., 1996; Andrews et al., 1998; Rodrigue et al., 2002). Successful restoration of these sites can result in many benefits, including improvements to hydrological processes resulting from decreased erosion and sediment flow, and more stable pH in runoff (Olyphant and Harper, 1995), as well as an increase in forest land area and provision of productive timber supplies (Torbert et al., 1996).

**TABLE 1**

Percentage abundance of established tree and shrub species on former Indiana mine sites that were reclaimed from 1988 to 1995, as determined by a survey (adapted from Rathfon et al., 2004).

| Species                                      | Proportion of total species surveyed (%) |
|--|--|
| Black locust ( <i>Robinia pseudoacacia</i> ) | 47                                       |
| Green ash ( <i>Fraxinus pennsylvanica</i> )  | 14                                       |
| Autumn olive ( <i>Eleagnus umbellata</i> )   | 7  |
| Northern red oak ( <i>Quercus rubra</i> )    | 3  |
| White oak ( <i>Quercus alba</i> )            | 2  |
| Other oaks                                   | 4  |
| Other species (desirable for timber)         | 12                                       |
| Other species (non-timber)                   | 11                                       |



Use of species with desirable characteristics (i.e., timber production, wildlife value, and aesthetics) may help to maintain reclaimed sites as forestland for the long-term. At present, species that are tolerant to degraded conditions, yet relatively undesirable to landowners, such as black locust (*Robinia pseudoacacia*) and green ash (*Fraxinus pennsylvanica*), are often used in reclamation projects (Rathfon et al., 2004) (Table 1). Thus, the resulting species composition on reclaimed sites typically reduces the prospective future value of the land. A potential new species option for reclamation projects, which has not been considered in the past, is American chestnut (*Castanea dentata*).



Figure 1. The historical range of American chestnut prior to introduction of chestnut blight (adapted from Saucier, 1973) and the location of the Rockland, WI tree plantation test site.

## POTENTIAL FOR INTEGRATION

American chestnut was once one of North America's most important trees, with a native range extending from Maine to Mississippi, encompassing over 800,000 km<sup>2</sup> (497,120 square miles) (Latham, 1992) (Figure 1). In portions of its range in Appalachia, American chestnut was thought to have represented 40-50% of trees in the forest canopy (Braun, 1950; Keever, 1953). American chestnut was critically important to the economic prosperity of the Appalachian region (Youngs, 2000), providing a major source of high quality timber, tannic acid, and nuts (Frothingham, 1912; Steer, 1948).

The original natural range of American chestnut (Figure 1) also represents a primary portion of the area of active mining in the eastern United States. However, American chestnut is almost never used in current reclamation or any other reforestation plantings because it is assumed that trees will inevitably succumb to blight. Thus, relatively little modern information is available regarding American chestnut silvicultural characteristics, such as environmental requirements or juvenile growth performance. Increasing optimism toward the release of a blight-resistant variety of American chestnut in the near future has stimulated some recent research to examine early growth and development of American chestnut. Analysis of these results, combined with examination of historical literature, provides a means to speculate as to the potential feasibility of incorporating American chestnut into future mine reclamation plantings.

Tolerance to harsh environmental conditions is a major consideration in selecting suitable species for mine reclamation programs. For instance, soil pH may be drastically altered on mine reclamation sites compared to unmined counterparts, often resulting in acidic soil conditions that may restrict growth of some species. American chestnut was adapted to a wide range of environmental conditions in areas of the southern Appalachians, where the species once dominated (Ashe, 1912). Many of these sites are characterized by moderately acidic soils (5.0-5.5), suggesting that the species may tolerate relatively acidic conditions. Evidence for this tendency is further supported by results from a test plantation of BC<sub>3</sub> hybrids and pure American chestnut on a site near Brevard, NC. Despite a pH of 4.4, the plantation is growing well after three seasons (Dr. Paul Sisco, The American Chestnut Foundation (TACF), pers. comm.). Additional indications that American chestnut may tolerate a wide range



Figure 2. Example of American chestnut dominance in a mixed interplanting with black walnut and northern red oak eight years following direct seeding on a study site in southwestern Wisconsin. Data published in Jacobs and Severeid (2004).



of environmental conditions was presented by Latham (1992), who evaluated seedling competitiveness of American chestnut relative to six co-occurring species by altering resources (e.g., light and mineral nutrient availability) experimentally. American chestnut ranked highest in traits associated with competitive ability over the broadest range of resource level combinations tested.

Rapid initial growth is another desirable quality of species for mine reclamation. Fast growth helps to ensure plantation success by facilitating prompt attainment of free-to-grow status above the height of competing vegetation and the level of deer browse. Reports from early in the last century indicate that American chestnut is highly competitive and fast growing initially (Zon, 1904; Graves, 1905), reaching 50% of ultimate height growth by age 20 (Ashe, 1912). A recent study of a rare stand of blight-free American chestnut in southwestern Wisconsin (Figure 1) helped affirm these historical observations (Jacobs and Severeid, 2004). Early plantation development of American chestnut interplanted with black walnut (*Juglans nigra*) and northern red oak was evaluated. American chestnut growth was exceptional (Figure 2), and trees averaged much greater height (47 or 77%) and diameter (50 or 140%) growth than

northern red oak and black walnut, respectively. Mean annual growth of American chestnut was nearly one m for height and one cm for diameter. Individual chestnut trees reached a height of 9.1 m (30 feet) and diameter of 10.2 cm (4 inches) within seven to eight growing seasons. These preliminary observations regarding early growth and development of American chestnut suggest the potential suitability of this species for mine reclamation programs and that trials should be established to further evaluate this potential.

### PROGRESS TO DATE

In 1998, The American Chestnut Foundation funded a study conducted by Dr. Greg Miller (Empire Chestnut Co., Carrollton, OH) to examine American chestnut performance on a mine reclamation site in east-central Ohio. Prior to planting, this site was graded as per standard reclamation procedures, limed, fertilized, seeded with a standard mixture of grasses and legumes, and topsoil was added (sandy loam mixed with sandstone and shale). Survival of chestnut seedlings after year-1 was 80-90% and was approximately 70% after year-3, with most mortality after the second year attributable to deer browsing (Dr. Carolyn Keiffer, Miami Univ., pers. comm.). Despite harsh site conditions and prolonged periods of drought, most of the planted American chestnuts were above the level of deer browse and had successfully established on the site following the third growing season.

A new collaboration established between The American Chestnut Foundation and Peabody Energy (St. Louis, MO) will test the adaptability of American chestnut on reclamation sites in Kentucky through a 5-year, \$100,000 study funded by Peabody. Peabody is the world's largest private-sector coal company and reclaimed nearly 2,400 ha of land and planted more than 500,000 trees in 2002. For this current project, six reclamation test sites were selected, representing a range of soil and topographic conditions. The sites will be planted with several varieties of BC<sub>2</sub>-F<sub>2</sub> chestnut material. Because this material is still being tested for degree of blight resistance and American chestnut character, it is likely that the trees planted on these sites will exhibit blight resistance ranging from very high to poor. The sites will be monitored for long-term plantation performance to help quantify the feasibility of integrating American chestnut into reclamation plantings in Kentucky.



### CONCLUSIONS

Following the release of blight-resistant material in the near future, American chestnut is likely to provide a valuable new species option for integration into mine reclamation projects. Fast growth, combined with high tolerance to a range of environmental conditions may allow American chestnut to rapidly establish within the degraded environmental conditions characteristic of mine reclamation sites. Additionally, excellent timber, wildlife, and aesthetic properties characteristic of the species may help motivate landowners to maintain reclaimed property as forestland for the long term. Incorporation of blight-resistant American chestnut into mine reclamation programs will also help facilitate the successful restoration of perhaps the single most important tree species in eastern North America back to its original range.

Though optimism for successful restoration in the near future is justified, several challenges must still be addressed. Chestnut breeding programs are largely supported by the National and State Chapters of TACF and establishment of future test plantations and seed orchards is likely to be limited by availability of funding and personnel. Additionally, plantings of American chestnut seem to be particularly susceptible to *Phytophthora cinnamomi*, a root rot common in the southern Appalachians, which suggests that site selection for restoration plantings may need to be limited to areas of low disease incidence (Rhoades et al., 2003). Despite these potential barriers, it is inevitable that a program to restore American chestnut to its original range will commence in the near future. Future research should continue to be directed toward examining the silvicultural requirements of American chestnut during early plantation development, which will help improve our understanding of the potential to integrate this species into mine reclamation programs.

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## CURBING THE U.S. CARBON DEFICIT

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*The U.S. emitted  $\approx 1.58$  petagrams (Pg) of fossil fuel carbon in 2001, approximately one-quarter of global CO<sub>2</sub> production. With climate change increasingly likely, strategies to reduce carbon emissions and stabilize climate are needed, including greater energy efficiency, renewable energy sources, geo-engineering, decarbonization, and geological and biological sequestration. Two of the most commonly proposed biological strategies are restoring organic carbon in agricultural soils and using plantations to sequester carbon in soils and wood. Here, we compare scenarios of land-based sequestration to emissions reductions arising from increased fuel efficiency in transportation, targeting ways to reduce net U.S. emissions by 10% ( $\approx 0.16$  Pg of carbon per year). Based on mean sequestration rates, converting all U.S. croplands to no-till agriculture or retiring them completely could sequester  $\approx 0.059$  Pg of carbon per year for several decades. Summary data across a range of plantations reveal an average rate of carbon storage an order of magnitude larger than in agricultural soils; in consequence, one-third of U.S. croplands or 44 million hectares would be needed for plantations to reach the target of  $\approx 0.16$  Pg of carbon per year. For fossil fuel reductions, cars and light trucks generated  $\approx 0.31$  Pg of carbon in U.S. emissions in 2001. To reduce net emissions by 0.16 Pg of carbon per year, a doubling of fuel efficiency for cars and light trucks is needed, a change feasible with current technology. Issues of permanence, leakage, and economic potentials are discussed briefly, as is the recognition that such scenarios are only a first step in addressing total U.S. emissions.*

As a nation, the U.S. emitted  $\approx 1.58$  petagrams (Pg) of fossil fuel carbon in 2001 (1), approximately one-quarter of the global production of  $\text{CO}_2$ . With climate change increasingly likely (2), strategies to reduce carbon emissions and stabilize climate are needed (3, 4). Such strategies include increased energy efficiency, renewable energy sources, geoengineering, decarbonization, and geological and biological sequestration (3, 4). Two of the most commonly proposed biological strategies are restoring organic carbon in agricultural soils and using plantations to sequester carbon in soils and wood (3, 5–10). Here, we compare scenarios of land-based sequestration in agricultural soils and forest plantations to emissions reductions that could arise from increased fuel efficiency in transportation. As an initial target, we examine ways to reduce net emissions in the U.S. by 10% or  $\approx 0.16$  Pg of carbon per year.

### TO SWARDS FROM PLOWSHARES

Land-based sequestration in agricultural soils restores all or part of the soil organic carbon (SOC) lost with plowing and intensive agriculture (6–10). Methods for restoring SOC in agricultural soils include no-till management and cropland retirement programs such as the Conservation Reserve Program (CRP) of the U.S. Department of Agriculture (USDA) Natural Resources Conservation Service. Established in 1985 as a tool to reduce erosion from agricultural lands, the CRP pays farmers to replace row crops with grasses and other perennial plants. As of January 2003, landowners had enrolled  $\approx 14$  million hectares (ha) of agricultural lands in the CRP (11).

Potential carbon storage in U.S. agricultural soils can be estimated by combining observed sequestration rates through the CRP and no-till agriculture with the extent of agricultural lands in the U.S. Recent reviews of  $> 100$  observations concluded that SOC increased  $\approx 450$  kg of carbon per ha per year after croplands converted to pastures or no-till management (9, 10).§ Maximum rates of storage peaked 5–10 years after conversion and slowed considerably within two decades (10). The U.S. also had an estimated 132 million ha of cropland in production in 2001 (11).¶ In consequence, if the U.S. converted its croplands entirely to no-till agriculture or, less likely, retired them all through the CRP, potential sequestration rates of 0.059 Pg of carbon per year might be possible for several decades (ref. 9 and Fig. 1).§ This upper limit for sequestration is

slightly more than one-third of the target of 0.16 Pg of carbon per year chosen here but still < 4% of total U.S. fossil fuel emissions.

Forest plantations grown on former agricultural lands have greater sequestration potentials because carbon can be stored both in the soil and as wood. Summary data across a range of plantations reveal an average rate of carbon storage of 3,600 kg of carbon per ha per year (12),<sup>2</sup> an order of magnitude larger than that in agricultural soils (9, 10).<sup>§</sup> Based on this rate, 44 million ha or one-third of all U.S. croplands would be needed for growing trees to reach the target of ≈0.16 Pg of carbon per year.

Although plantations provide greater rates of carbon storage than soils alone, the uncertainties may be larger. Sequestration rates somewhat higher than 3,600 kg of carbon per ha per year are likely possible in some locations and in the short term (13). However, none of these estimates takes into account the carbon costs of site preparation and planting, potential carbon losses from disturbance [e.g., storms, pests, and fires (14)], post-harvest carbon losses in timber use [e.g., sawmills or landfills (15)], and additional biogeochemical changes that might occur [e.g., decreased water yields (16)]. Because increases in plantation area do not automatically increase demand for wood products, some of the plantation carbon will likely return to the atmosphere after harvesting, if long-term uses for the wood are not found. Thus, the net storage will be lower than the technical potential and will reflect the proportion of harvested carbon that returns to the atmosphere and the regional chronology of planting and harvesting. A national policy promoting afforestation can store considerable carbon for decades, but the amount stored, the economic subsidies needed, and the environmental changes that would result require careful evaluation.

Issues of permanence and leakage (17, 18), activities shifted to locations outside of a sequestration program that counteract some of its benefits, are important for all analyses of carbon sequestration and management. Carbon stored as soil organic matter or wood must be protected from plowing, fire, storm damage, and/or decomposition to keep the carbon from returning to the atmosphere. An alternative approach that acknowledges these uncertainties is carbon “rental” payments (18), whereby farmers contract to store carbon for set periods of time only. Such payments explicitly acknowledge the uncertain permanence of biologically sequestered carbon.

**CAR TALK**

The transportation sector provides another opportunity to reduce carbon emissions to the atmosphere. Gasoline and related fuels comprise 28% of total energy use in the U.S. (19),\*\* mostly in passenger cars and light trucks. The category of “other two-axle four-tire vehicles” in the Bureau of Transportation Statistics includes light trucks, vans, and sport utility vehicles (SUVs) but does not include the heaviest SUVs. Cars and light trucks used 73 and 53 billion U.S. gallons of fuel, respectively, in 2001 (19). After converting fuel totals to carbon equivalents (the conversion factor used here is 2.42 kg of carbon per U.S. gallon of gasoline) ([http://bioenergy.ornl.gov/papers/misc/energy\\_conv.html](http://bioenergy.ornl.gov/papers/misc/energy_conv.html)), these vehicle groups generated ≈0.31 Pg of carbon in U.S. fossil fuel emissions that year.

To reduce net emissions by 0.16 Pg of carbon per year, a doubling of fuel efficiency for cars and light trucks is therefore needed (Fig. 1), a change feasible with current technology (3). Fleet mileages in the U.S. for the two groups in 2001 were 22.1 and 17.6 miles per gallon (mpg), respec-

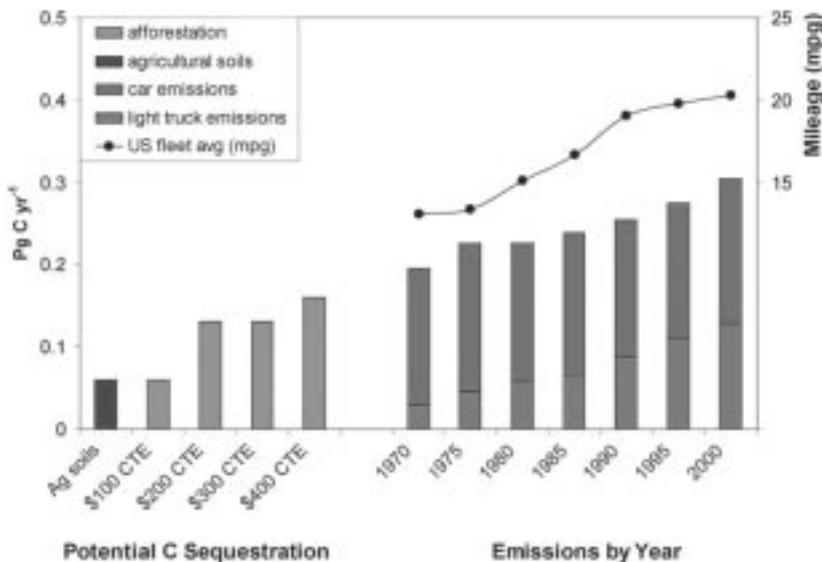


Fig. 1. Carbon emissions in Pg of carbon per year from cars and light trucks (blue and red bars) in the U.S. from 1970 to 2000 (ref. 19, and [http://bioenergy.ornl.gov/papers/misc/energy\\_conv.html](http://bioenergy.ornl.gov/papers/misc/energy_conv.html)), maximum potential carbon storage estimated for agricultural soils in the U.S. (brown bars; refs. 9–11),§ potential carbon sequestration for afforestation at carbon prices of \$100–400 per metric ton carbon equivalents (green bars; ref. 17), and average U.S. fleet mileage for cars and light trucks combined (black line; calculated from data in ref. 19).§

tively (19). Newer vehicles in 2001 were substantially better: 28.6 mpg for cars and 20.9 mpg for light trucks (19). Although improvements in mileage will likely occur as newer vehicles comprise a greater proportion of the U.S. fleet, some of these gains are being offset by the increasing proportion of less-efficient light trucks in the U.S. (Fig. 1).

Far greater efficiencies are already available from hybrid electric vehicles (HEVs) and additionally from advanced diesel engines and lightweight construction materials. More than 100,000 HEVs with mileage  $\approx$ 50 mpg (3) have been sold in North America to date. A policy to promote hybrid technology in new cars and light trucks would go a long way to doubling fuel efficiency to  $>40$  mpg (Fig. 1). Providing economic incentives for high-mileage vehicles could reduce oil imports and would not require cultural changes such as driving fewer miles or pursuing mass transit, two other useful options.

Just as with biological sequestration, permanence and leakage need to be acknowledged in improved fuel efficiency. Doubling the fuel efficiency of passenger cars and trucks will only cut vehicle emissions in half if the number of miles driven does not increase. Also, there is no guarantee that improvements in fuel efficiency would be permanent. However, unlike biological sequestration, where a fire or plantation harvest could liberate carbon stored over many years, the carbon emissions saved by improved fuel efficiency would not return to the atmosphere if mileage rates increased at a later date.

### HYBRID SOLUTIONS

Reducing net carbon emissions can best be accomplished with multiple strategies (3). Land-based sequestration has an important role to play in this effort, but large land areas are needed to have a sustained effect. Peak rates of carbon storage in agricultural soils are typically maintained for a decade or two (10). Farmlands enrolled in the CRP currently store  $\approx 0.005$  Pg of carbon per year compared with U.S. fossil fuel emissions of 1.58 Pg of carbon per year. The cropland area managed for carbon storage will need to increase by an order of magnitude to approach the technical potential of  $\approx 0.059$  Pg of carbon per year estimated here.

Policy changes promoting carbon storage on land will have additional environmental costs and benefits (17), some predictable and some unforeseen. Potential benefits include reduced erosion and pollution

from phosphorus and nitrogen runoff and improved wildlife habitat; potential costs include decreased food production in the U.S., increased food prices, and decreased agricultural exports, if large areas of farmland are taken out of production (17). In addition to evaluating the full benefits and costs of these policies, economic potentials also should be considered in making realistic projections of carbon storage. Recent economic models for the U.S. agriculture and forestry sectors suggest that carbon prices would need to be  $\approx$ \$125–400 per metric ton of carbon equivalents for potential sequestration rates in plantations to approach 0.16 Pg of carbon per year (17, 18).

All of the approaches analyzed here, combined with renewable energy sources, decarbonization, geological sequestration, and other technologies (3), will be needed to balance the U.S. carbon deficit. Scenarios for offsetting 1/10th of U.S. fossil fuel emissions as described above show the scale and scope of changes that are needed; they also highlight how far the U.S. is from addressing its total emissions of 1.6 Pg of carbon per year. Reducing fossil fuel emissions directly will be needed to approach that goal. As one of many opportunities, hybrid gas-electric cars are already widely available. A doubling in fuel efficiency through hybrid technology, advanced diesel engines, and lightweight materials could precede a transition to hydrogen vehicles, which themselves require fossil fuels or other sources of energy to generate the hydrogen (20). Coupled with changes in the way that agricultural lands are managed, doubling the fuel efficiency of our nation's vehicles seems a logical first step in balancing the carbon budget.

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\*\*Table 4-2 in ref. 19 provides data for U.S. energy consumption from primary sources and the proportion attributable to transportation (28.1%). Table 4-5 provides data for total fuel consumption; passenger cars consumed 73.452 billion U.S. gallons and "other two-axle, four-tire vehi-



cles” consumed 53.294 billion U.S. gallons in 2001. Table 4-23 gives the average fuel efficiency for the current fleet of cars and light trucks (22.1 and 17.6 mpg, respectively) and for new vehicles (28.6 and 20.9 mpg, respectively).

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Abbreviations: Pg, petagram; SOC, soil organic carbon; ha, hectare; CRP, Conservation Reserve Program; USDA, U.S. Department of Agriculture; mpg, miles per gallon.

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§The average estimate for soil carbon storage after a shift from agriculture to pasture is 332 kg of carbon per ha per year (based on 39 observations in ref. 9). The estimate for the change from conventional tillage to no-till agriculture in ref. 10 is based on 67 long-term agricultural experiments and was slightly higher, 570 kg of carbon per ha per year, but excludes wheat fallow systems where no significant increase in soil carbon was observed. Additional net savings of  $\approx 30$  kg of carbon per ha per year in no-till versus conventional tillage may be attributable to reduced emissions from tillage itself. For our analysis, we use the mean of the above estimates, 450 kg of carbon per ha per year. This estimate of carbon storage is then combined with the estimated cropland area in the U.S. (132 million ha; ref. 11) to place an upper limit on SOC storage in agricultural lands (0.059 Pg of carbon per year). The estimate is consistent with the lower range in potential sequestration presented by Lal et al. (7) for U.S. croplands:  $\approx 3,000$  million metric tons of carbon over a 25- to 50-year period (0.059 Pg of carbon year  $\times$  50 years = 2.95 Pg of carbon or 2,950 million metric tons of carbon).

¶The USDA estimate of U.S. croplands (132 million ha) is approximately one-third of the “farmland” estimate of 380 million ha from National Agricultural Statistics Service estimates. However, the latter also includes acreage for pasture lands, grazing lands, and woodlands and wastelands that are part of farmers’ total operations.

‡The article in ref. 12 presented data from a range of pine plantations in



their table 5. We calculated the average carbon gains based on age of the stands and the carbon gains above and below ground (3,640 kg of carbon per ha per year). Estimates of the amount of soil carbon alone stored after forestation of agricultural lands are similar to summary values for shifts from agriculture to pasture (338 and 332 kg of carbon per ha per year, respectively; ref 9).§

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## SEED DISPERSAL, SEED PREDATION, AND THE AMERICAN CHESTNUT

Michael A. Steele, Brian C. McCarthy, & Carolyn H. Keiffer

Although it is well known that the nuts of American chestnut (*Castanea dentata*) were a critical food resource for many wildlife species, as well as humans, few studies have explored the interrelationship between these nuts and the birds and mammals that consumed them. In this brief review, we attempt to evaluate this interrelationship based on the limited literature on *C. dentata*, and on recent studies on the behavior of nut consumers in today's forests. We specifically seek to evaluate how the loss of American chestnut (hereafter chestnut) may have altered nutritional resources for animals and affected the dynamics of seed dispersal in chestnut and other species.

### NUTRITIONAL VALUE TO WILDLIFE

It is clear, from a comparison with other nut-producing species common within the historic range of the chestnut (Table 1), that chestnuts provided a critical resource for a variety of wildlife in three important ways. First and most importantly, chestnuts added considerably to the overall food supply for mast consuming species. Although replacement of chestnuts by oaks and/or hickories may have occurred in many forests, there is good evidence to suggest that such succession did not fully compensate for the loss of chestnut mast. Based on comparisons of production of hard mast before and after (35 yrs.) the chestnut blight in a southern Appalachian forest, Diamond et al. (2000) estimated a 28% loss in basal area of nut producing trees and a 34% reduction (by mass) in nut production. In five of the 10 years of the study after the blight, nut production was only 5 to 27% of pre-blight production, leading to the inevitable conclusion that loss of chestnut resulted in a significant reduction in the carrying capacity of many wildlife species.

Second, it is also evident that in comparison to other nut-producing species, chestnuts represent a complementary food resource in several key ways. Once released from the outer husk, the soft shell of chestnuts makes them a highly suitable food for any species that also consumes acorns (which includes dozens of species in eastern deciduous forests; Van Dersel, 1949), hickories, or walnuts. Acorns, hickories, and walnuts all contain

**TABLE 1** Comparison of nutritional value of *Castanea dentata* in relation to the nuts of other selected species common within the historic range of *C. dentata*. Values are taken from Vander Wall (2001); original sources of data are indicated below.

| Species   | Dry mass of edible nut (g) | Caloric value (kJ/g) | Protein (% dry mass) | Lipids (% dry mass) | Carbohydrates (% dry mass) | Crude fiber (% dry mass) | Ash     |
|---|----------------------------|----------------------|----------------------|---------------------|----------------------------|--------------------------|---------|
| 1. American chestnut<br><i>Castanea dentata</i> |                            |                      | 8.6                  | 2.3                 | 82.9                       | 3.4                      | 2.8     |
| 2. Northern red oak<br><i>Quercus rubra</i>     | 2.12                       | 20.4                 | 5.3-7.0              | 18.9-20.8           | 67.1-69.1                  | 2.8-4.2                  | 2.4-3.1 |
| 3. Black oak<br><i>Quercus velutina</i>         |                            |                      | 6.9-7.0              | 23.0-24.1           | 64.6-65.1                  | 3.0-3.1                  | 1.7-2.0 |
| 4. White oak<br><i>Quercus alba</i>             | 0.4-0.83                   | 17.4-17.8            | 6.3-7.8              | 4.8-6.3             | 82.3-83.3                  | 2.5-2.7                  | 2.6-2.7 |
| 5. Chestnut oak<br><i>Quercus prinus</i>        | 1.21                       | 18.1                 | 5.8-6.9              | 5.1-10.1            | 78.9-83.2                  | 2.5-2.6                  | 2.2     |
| 6. Hickory                                      | 1.01                       | 27.5                 | 13.3                 | 74.4                | 8.8                        | 1.5                      | 2       |
| 7. Black walnut<br><i>Juglans nigra</i>         | 2.04                       | 26.1                 | 29.3-32.6            | 36.9-60.2           | 6.7-25                     | 1.0-2.1                  | 2.8-3.4 |

Original sources

1. McCarthy and Meredith, 1988
2. Wainio and Forbes, 1941; Baumgrass, 1944; Gysel, 1957; Lewis, 1982
3. Baumgrass, 1944; Gysel, 1957
4. Wainio and Forbes, 1941; Baumgrass, 1944; Gysel, 1957; Smith and Follmer, 1972; Lewis, 1982
5. Wainio and Forbes, 1941; Lewis, 1982; Smallwood and Peters, 1996
6. Wainio and Forbes, 1941; Smith and Follmer, 1972
7. Wainio and Forbes, 1941; Baumgrass, 1944; Smith and Follmer, 1972

higher levels of lipids than chestnuts. However, in contrast, chestnuts have higher levels of carbohydrates (sugars) than all three of these nut types, and lower levels of tannin (secondary compounds known to reduce palatability and digestibility; Robbins et al., 1987) than acorns. Chestnuts also possess higher levels of protein than is typically found in most acorns (Table 1).

These differences likely made chestnuts sweeter and more palatable than acorns and a better source of protein (Table 1). Acorns are low in protein and may have even less protein available for digestion because of their tendency to bind with tannins. The soft shell, and the ease with which most animals can open chestnuts, would have easily compensated for their lower lipid levels (and thus lower energy compared to other nut

species). This is especially noteworthy when chestnuts are compared to hickories and walnuts, which are consumed by relatively few species (e.g., squirrels, turkeys) because of their harder husks.

Third, the chestnut crop would have also provided an important alternative food when mast failure occurred in other nut-producing species. Many tree species, including most nut-producing species, undergo the process of masting—the widespread episodic and synchronized production of seeds in some years, followed by years of complete crop failure (Silvertown, 1980). Masting is particularly evident in oaks and is now considered an evolved strategy that allows the trees to satiate seed predators in years of high mast and cull these same predators during mast failures (the predator satiation hypothesis; Koenig and Knops, 2002). Because chestnuts rely on a mixed pollination syndrome (i.e., pollination by both wind and insects) and flower later in the season, they are far less likely to experience flower (and mast) failures due to late spring frosts as compared with hickories and oaks.

From the animal's perspective, alternative resources such as chestnut mast during mast failure of other species are critical for survival. Chestnuts likely buffered against such periods of extreme food shortage as suggested by Diamond et al. (2000) who found that the post-blight variation in hard mast was significantly higher than prior to the blight. They attributed this increased variation entirely to the loss of mature chestnut trees. Regular production of chestnuts also was likely to accentuate the effects of satiation during mast production of hickories and nuts. Numerous observations suggest that seed predators are rarely satiated in today's forests and that there is often insufficient seed of oaks and hickories for regeneration (McCarthy, 1994; McCarthy and Keiffer, 2004). Although such effects may be due in part to increasing fragmentation (McCarthy, 1994) and changing land use practices (McCarthy and Keiffer, 2004), the loss of chestnut may also contribute significantly to the problem.

### DISPERSAL OF AMERICAN CHESTNUT

A critical process in the regeneration of forests involves the dispersal of seeds to sites some distance away from parent trees where the probability of germination, establishment, and survival are higher (i.e., the probability of density dependent predation, seedling competition, and subsequent interbreeding are all reduced; van der Pijl, 1972; Vander

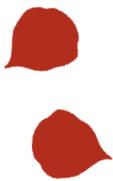


**TABLE 2**  
Nut characteristics

| Nut type          | Dormancy | Nut perishability | Tannin levels | Physical protection                                     |
|-------------------|----------|-------------------|---------------|---|
| American Chestnut | Yes      | Mod               | Low           | High during pre-dispersal;<br>Low during post-dispersal |
| Red oaks          | Yes      | Low               | High          | Low   |
| White oaks        | No       | High              | Mod           | Low   |
| Hickories         | Yes      | Low               | Low           | High  |
| Walnuts           | Yes      | Low               | Low           | High  |

Wall, 1990). For many of the oaks, for example, establishment is far more likely in open sites, away from the parent trees (Harrison & Werner, 1984; Crow, 1992). Similar conditions were likely required for chestnut, and our best estimates of the manner in which chestnuts were dispersed follow from the physical and chemical characteristics of the nuts.

Chestnut dispersal can best be inferred from recent studies on the oaks (Steele et al., 2001; Steele and Smallwood, 2002; Steele et al., 2005). Although consumed by numerous wildlife species, oaks are dispersed by relatively few species, mostly the mammals (e.g., mice, chipmunks, and squirrels) and birds (e.g., jays) that frequently scatterhoard nuts in widely dispersed cache sites and then, on occasion, fail to recover a portion of these stores (Vander Wall, 1990; Steele and Smallwood, 2002). Noted for their long-distance dispersal (> 1 km, .6 miles) of primarily small-seeded nuts, jays are credited with the rapid, northward migration of oaks, beech, and chestnut at the end of the Pleistocene (Johnson and Webb, 1989). Scatterhoarding mammals, in contrast, disperse oaks relatively short distances (< 150 m, 164 yards); Steele and Smallwood, 2002) and may be critical for the dispersal and maintenance of genetic diversity of nut-producing trees within forest patches (Smallwood et al. 1998; Steele and Smallwood, 2002). Because of the many similarities between the oaks and chestnuts, many of these same mammals and birds were certainly critical for chestnut dispersal. However, a number of specific characteristics of the chestnuts further suggest how their dispersal patterns were both similar and yet different from those of the oaks.



Compared to dispersal of other nut species, which begins while nuts are still attached to the tree, the process in chestnuts is likely limited to secondary dispersal (i.e., after nut drop). The heavy, well-protected husk of chestnuts is clearly an adaptation for preventing pre-dispersal predation. The spiny husk likely ensures that nuts are not harvested from the tree while the cotyledons and embryos are still developing (Vander Wall, 2001). When mature though, the husks dehisce and the nuts are dropped to the ground where they are readily available for consumption or storage. At this point, both scatterhoarding mammals and jays likely scrambled to cache as many nuts as possible. And from here the fate of chestnuts likely followed from several other characteristics, including the duration of dormancy, perishability, nutrient content, and protective chemistry of the nut (Table 2).

For the oaks, such characteristics directly influence the fate of acorns. The two major groups of oaks in North America, the red oaks (RO; subgenus *Erythrobalanus*) and white oaks (WO; *Leucobalanus*, now *Quercus*) show very different nut characteristics that directly influence their dispersal patterns (Steele et al., 2001; Steele & Smallwood, 2002). The ROs are high in both tannins and lipids (the primary energy source for nut consumers). ROs also exhibit a long period of dormancy before germinating in the spring. WOs, in contrast, typically have lower levels of tannins and lipids and germinate immediately in the fall, soon after nut drop (Steele et al., 2005). Some WO species even begin to germinate prior to nut drop while still attached to the tree (Steele, pers. obs.).

Seed dispersing mammals exhibit a high sensitivity to these characteristics that, in turn, markedly affect the manner in which these two acorn types are dispersed. Where RO and WO acorns occur together, several species of small mammals selectively consume the WO acorns and disperse and cache those of RO (Steele et al., 2001). Moreover, behavioral experiments with gray squirrels indicate that this response is due entirely to the reduced perishability of RO acorns (i.e., greater storability due to delayed germination) rather than other physical or chemical characteristic of the seed (Hadj-Chikh et al., 1996). More recent studies further show that the specific proximate cue that controls this behavior is located in the shell of the acorn (Steele et al., 2002). Tree squirrels quickly determine a seed's potential dormancy by rolling the acorn and licking it.

In comparison with RO acorns, those of WO are selectively eaten in the autumn, (Barnett, 1977; Fox, 1982; Smallwood and Peters, 1986),



especially if long-term stores of RO acorns are available for storage. Although gray squirrels will also disperse and cache WO acorns, when they do so they usually first excise the embryo by notching the apical end of the seed and killing the acorn (Fox, 1982; Pigott et al., 1991; Steele et al., in press). This behavior, now shown to be an innate trait in tree squirrels (Steele et al., in press), allows them to store these unviable WO acorns for up to six months without spoilage (Steele et al., 2001). Although this embryo excision appears limited to the tree squirrels, numerous small mammals under a range of conditions (different forests, mast abundance) show this propensity to selectively disperse and cache RO acorns and consume WO. Such a response may ultimately affect the structure of oak forests (Steele and Smallwood, 2002; Steele et al., 2005).

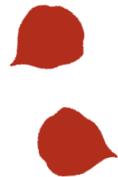
What can we learn about chestnut dispersal from these studies on oaks? Chestnuts share a number of characteristics common to the acorns of both RO and WO (Table 2). Their limited physical protection following nut drop, coupled with their high palatability (lower tannin and sweet taste), make them similar to WO acorns, especially in the context of a storable RO, hickory, or walnut crop (Table 2). We would also predict that this should result in strong selection for a rapid period of nut drop in chestnut, allowing quick satiation and possible storage of some nuts, at least for shorter periods. Their short dormancy and shorter shelf life indicate that they were of limited long-term value to scatterhoarding mammals and birds, and further suggest that they would have represented a prized food for consumption in the fall, especially when other seeds and nuts were available for storage.

Some scatterhoarding mammals likely stored chestnuts for shorter periods and this may be how they were dispersed. However, just as WO acorns influence patterns of RO dispersal, chestnuts were likely critical in promoting the dispersal and establishment of many other non-perishable nut species (RO, hickory and walnut). Hence, it can be inferred that the demise of the American chestnut dramatically modified the dynamics of forest regeneration in several tree species in Eastern deciduous forests.

As we learn more about today's forests, it is becoming clear that the chestnut blight meant far more than just the loss of an important resource for wildlife. The disappearance of this species changed numerous species' interactions, as well as the dynamics of forest regeneration in ways we may never fully appreciate.

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## THE BACKCROSS BREEDING PROGRAM OF THE AMERICAN CHESTNUT FOUNDATION

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### ABSTRACT

The blight resistance of oriental chestnut trees is being backcrossed into American chestnut using traditional plant breeding techniques. Progeny are screened for blight resistance by direct inoculation with the blight fungus, when they are old enough to survive inoculation, which is 3 or 4 years for trees with intermediate levels of blight resistance, and 1 or 2 years for trees with high levels of blight resistance. Trees are grown using intensive horticultural techniques. Probably the most unusual aspect of this breeding program in comparison to similar programs for crop plants is the large acreages over which trees are grown, and the fact that the objective is recovery of a genetically diverse species rather than an improved cultivar. Highly blight resistant progeny have been recovered from intercrosses of straight F<sub>1</sub>s, B<sub>1</sub>s and B<sub>2</sub>s, suggesting strongly that it should be possible to backcross blight resistance into American chestnut. Currently, two sources of blight resistance are being advanced to B<sub>3</sub>-F<sub>2</sub>. These are expected to begin producing progeny suitable for outplanting within 2 to 3 years.

### INTRODUCTION

The American chestnut tree, *Castanea dentata* (Marsh.) Borkh., has been destroyed as a dominant forest tree by a canker disease, chestnut blight, incited by *Cryphonectria parasitica* (Murr.) Barr. The blight fungus was introduced into eastern North America around the turn of the 20th Century, probably in blight cankers on imported Japanese chestnut, *C. crenata* Sieb & Zucc., nursery stock (Metcalf and Collins, 1909). By 1950, the disease had killed almost all of the large American chestnut trees throughout their range.

By 1930, when the American chestnut was thought to be doomed, attempts had begun to breed blight-resistant replacements. These attempts



were abandoned, for the most part, around 1960, when no trees had been developed that combined the blight resistance of oriental chestnut trees with the large size of American chestnut trees (Jaynes, 1994).

In 1961, what later proved to be viruses (Hillman *et al.*, 2000) were found infecting *C. parastica* (Grente, 1961). The infected strains had been isolated from blight cankers on European chestnut trees, *Castanea sativa* Mill., growing in Italy. The viruses reduced the virulence of the blight fungus enough that infected strains could no longer kill European chestnut trees. Additionally, the viruses spread from one canker to another, resulting, apparently, in the protection of entire stands of European chestnut. When viruses were introduced into blight cankers on European chestnut in France, the disease there was ameliorated. This discovery led to efforts to control blight on American chestnut with these viruses, which continue today. To date, the results of this effort have not been entirely satisfactory (Anagnostakis, 1990).

In 1981, Charles Burnham proposed that the blight resistance of oriental chestnut trees, primarily Chinese chestnut, *Castanea mollissima* Blume, could be backcrossed into American chestnut. For American chestnut, this was a new method of plant breeding that had not been used in previous attempts to develop blight-resistant, timber-type chestnut trees. In 1983, The American Chestnut Foundation was established as a not-for-profit corporation to help fund work on Burnham's proposal (Burnham, Rutter and French, 1986). In 1989, the foundation had accumulated sufficient resources to hire a part-time researcher at a new research farm in Meadowview, VA, in the heart of the range of the American chestnut tree.

Subsequent to 1989, the foundation has grown to the point where it is supporting a large breeding effort in Meadowview, with four full-time workers tending trees on three farms totaling 130 acres. Additional workers are employed in Asheville, NC and at Penn State University to assist volunteer breeding efforts at thirteen state chapters. The administrative headquarters in Bennington, VT, also supports volunteer breeding efforts in CT and VT.

The purpose of this paper is to describe progress to date in this breeding program.

## MATERIALS AND METHODS

### BREEDING METHOD

To transfer blight resistance from Chinese to American chestnut, individuals of the two species are first crossed. The progeny from this cross, first hybrids, or  $F_1$ s, usually are exactly one-half American and one-half Chinese chestnut. An  $F_1$  is backcrossed to another American chestnut, decreasing the proportion of Chinese chestnut genes by a factor of one half, on average. The progeny of this second cross, the first backcross, are known as  $B_1$ s. Two more backcrosses again decrease the proportion of Chinese chestnut genes by a factor of one half each time, to one-eighth followed by one-sixteenth, on average, with the remaining fraction of genes being from the American parent.

At each step of backcrossing, resistant trees are selected by observing canker symptoms after inoculation of the progeny with the chestnut blight fungus (see below for details). The progeny also vary in the fraction of Chinese genes remaining, and selection against Chinese morphological type is made to accelerate recovery of the American type, using traits identified by Hebard (1995). Burnham estimated that three backcrosses to the American parent, with selection against Chinese morphological type, would be sufficient to recover trees that look and grow like the American chestnut of old.

The  $F_1$  trees, and any subsequent backcross progeny, would be heterozygous, at best, for the genes conferring blight resistance. Thus, they would not breed true for blight resistance, producing both susceptible as well as resistant progeny. To recover trees homozygous for blight resistance, third backcross trees are intercrossed among themselves, so the progeny have a chance of inheriting the genes for blight resistance from both parents. The progeny of this first intercross of third backcross trees are known as  $B_3$ - $F_2$ s.

Blight resistance is only partially dominant, so  $F_1$ s and backcrosses are, at best, intermediate in resistance between the two parent species. High levels of blight resistance, comparable to those found in the Chinese parent, are only recovered after intercrossing  $F_1$  hybrids and backcrosses. This facilitates recovery of trees reasonably homozygous for blight resistance, since they test out as more resistant than heterozygotes.



To avoid inbreeding, and its consequent decrease in genetic diversity, a different American chestnut parent is used at each step of backcrossing. Thus, in an ideal situation, four American parents are used to produce a third backcross tree. The third backcross progeny from a unique set of four American parents are termed a recurrent parent line or line for short. At the intercrossing stage, more than one line is needed in order to minimize sib crosses and their resulting inbreeding. Hebard (1994a) estimated that 20 lines would be needed to minimize loss of alleles from inbreeding. With four American parents per line, 20 lines require 80 separate American parents.

In practice, only one line was used until the first backcross with the 'Graves' and 'Clapper' sources of blight resistance. These two first backcross trees then were crossed with 20 American parents to yield the second backcross generation, and with 20 additional parents to yield the third backcross. Thus, the third backcross progeny are half first cousins rather than half third cousins.

To ensure that the progeny from intercrossing third backcross trees are homozygous for blight resistance loci, only one Chinese chestnut parent is used to make a set of 20 lines.

### SOURCES OF BLIGHT RESISTANCE

The availability of the named first backcross, 'Clapper' (Little and Diller, 1964), and the undescribed 'Graves' first backcross at the Connecticut Agricultural Research Station plantings in Hamden gave a jump start to the breeding program in 1989. They were chosen because they were the most advanced crosses available and appeared to have resistance similar to that of first hybrids between Chinese and American chestnut. These two first backcross trees were backcrossed again onto about 30 American chestnut trees each between 1989 and 1995 to yield second backcross trees, or B<sub>2</sub>s. Thirty American chestnut lines of third backcrosses were produced between 1996 and 2003 for both the 'Clapper' and the 'Graves' lines. From 2001 until present, second generation third backcross progeny, or B<sub>3</sub>-F<sub>2</sub>s, have been collected and planted from intercrosses within sources of blight resistance. The Chinese chestnut grandparent of 'Graves' is an undescribed selection known as 'Mahogany,' made by Arthur Graves of the Brooklyn Botanic Garden and Connecticut Agricultural Experiment Station.



In 1989, breeding also was started with the Chinese chestnut cultivar, Nanking, crossing it with 20 American chestnut trees to start 20 recurrent parent lines at F<sub>1</sub>. Cultivar Nanking was chosen because it had shown the highest blight resistance of any Chinese chestnut tree evaluated by Headland and Griffin (1976) and was noted as having high blight resistance when first released.

As available, other Chinese and Japanese chestnut trees, and F<sub>1</sub> hybrids between these species and American chestnut, were crossed with American chestnut trees, in these later cases with only a few American chestnut trees rather than assembling 20 lines. Table 1 lists the sources of blight resistance at their most advanced stage of backcrossing as of April, 2004, and the number of American parent lines at the most advanced stage. As indicated above, additional lines occur at less advanced stages of backcrossing for some sources of blight resistance.

**TABLE 1**

Oriental sources of blight resistance being used at The American Chestnut Foundation's Research Farms in Meadowview, VA, their most advanced stage of backcrossing into American chestnut, and the number of American parent lines at that stage as of April, 2004.

| Source of Blight Resistance     | Stage of Backcrossing          | Number of American Parent Lines |
|---------------------------------|--------------------------------|---------------------------------|
| 'Clapper'                       | B <sub>3</sub> -F <sub>2</sub> | 12                              |
| 'Mahogany' ('Graves')           | B <sub>3</sub> -F <sub>2</sub> | 5                               |
| 'Douglas'                       | B <sub>3</sub>                 | 2                               |
| 'Nanking'                       | B <sub>3</sub>                 | 2                               |
| Sleeping Giant South Lot R11T14 | B <sub>3</sub>                 | 1                               |
| Sleeping Giant South Lot R1T4   | B <sub>3</sub>                 | 1                               |
| Sleeping Giant South Lot R1T7   | B <sub>3</sub>                 | 3                               |
| 'Meiling'                       | B <sub>2</sub>                 | 1                               |
| MusickChinese                   | B <sub>2</sub>                 | 2                               |
| Greg Miller 72-211              | B <sub>1</sub>                 | 3                               |
| mollissima7                     | B <sub>1</sub>                 | 1                               |
| mollissima10                    | B <sub>1</sub>                 | 1                               |
| mollissima13                    | B <sub>1</sub>                 | 1                               |
| PI#104016                       | Japanese B <sub>1</sub>        | 1                               |

**TABLE 1 (continued)**

| Source of Blight Resistance | Stage of Backcrossing   | Number of American Parent Lines |
|-----------------------------|-------------------------|---------------------------------|
| Dunstan seedling            | F <sub>1</sub>          | 1                               |
| FP7284                      | F <sub>1</sub>          | 1                               |
| Greg Miller 65-18           | F <sub>1</sub>          | 3                               |
| Greg Miller 65-4            | F <sub>1</sub>          | 6                               |
| 'Kuling'                    | F <sub>1</sub>          | 4                               |
| 'Orrin'                     | F <sub>1</sub>          | 4                               |
| mollissima11                | F <sub>1</sub>          | 1                               |
| mollissima18                | F <sub>1</sub>          | 1                               |
| MAJ7Japanese                | Japanese F <sub>1</sub> | 1                               |
| 'Jayne'                     | mollissima x pumila     | 1                               |
| AbbsValley                  | Chinese                 |                                 |
| Altamont                    | Chinese                 |                                 |
| 'Armstrong'                 | Chinese                 |                                 |
| 'Eaton'                     | Chinese                 |                                 |
| MacBoyd                     | Chinese                 |                                 |
| MAJ                         | Chinese                 |                                 |
| MAJ4                        | Chinese                 |                                 |
| MAJ5                        | Chinese                 |                                 |
| Waynesboro                  | Chinese                 |                                 |
| mollissima12                | Chinese                 |                                 |
| mollissima14                | Chinese                 |                                 |
| mollissima15                | Chinese                 |                                 |
| mollissima16                | Chinese                 |                                 |
| mollissima17                | Chinese                 |                                 |
| mollissima19                | Chinese                 |                                 |
| mollissima20                | Chinese                 |                                 |
| mollissima8                 | Chinese                 |                                 |
| PI#7284                     | Chinese                 |                                 |
| PI#97853                    | Chinese                 |                                 |
| Richwood                    | Chinese                 |                                 |
| Wilkinson                   | Chinese                 |                                 |
| YardChinese                 | Chinese                 |                                 |
| FPGlenDaleID:GS             | Japanese                |                                 |

**AMERICAN CHESTNUT PARENTS**

In addition to the breeding at Meadowview, the American Chestnut Foundation also has an extensive network of state chapters staffed primarily by volunteers, and advised by staff officers stationed in North Carolina and Pennsylvania (Paul Sisco and Sara Fitzsimmons, respectively). The chapters have been crossing pollen of 'Graves' and 'Clapper' second and third backcrosses from Meadowview onto local American chestnut trees to produce third and fourth backcross trees. The intent is to produce a viable breeding population of 20 individuals for each source of blight resistance, adapted to the local conditions, and also to increase the genetic diversity of the breeding population, as originally proposed by Inman (1987). Table 2 depicts the number of third backcross trees in the various states as of 2004.

**TABLE 2**

Number of third-backcross (B<sub>3</sub>) chestnut at TACF breeding orchards in 2004, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

| Chapter                | Number of     |                       |                 |
|------------------------|---------------|-----------------------|-----------------|
|                        | Nuts or Trees | Sources of Resistance | American Lines* |
| Maine                  | 1445          | 2                     | 29              |
| Massachusetts          | 3076          | 2                     | 28              |
| Pennsylvania           | 5350          | 2                     | 36              |
| Maryland               | 33            | 1                     | 1               |
| Indiana                | 1496          | 1                     | 11              |
| Kentucky               | 150           | 2                     | 2               |
| Virginia (Meadowview)  | 5275          | 8                     | 73              |
| North & South Carolina | 1049          | 2                     | 9               |
| Tennessee              | 745           | 5                     | 6               |
| Alabama                | 566           | 1                     | 5               |
| <b>Total</b>           | <b>19179</b>  |                       |                 |



Following Inman's recommendation (Inman, 1989), attempts have been made to limit the range of American chestnut parents to within 20 miles of each other in building local populations. This has been easier near Meadowview than elsewhere, since the required numbers of flowering chestnut trees can be found within such a small area.

## POLLINATION

First hybrids and straight backcrosses are produced using the controlled pollination techniques described by Rutter (1991). Subsequent experience indicates that the best time to bag chestnut flowers for controlled pollination is when the styles begin to emerge from the bur, rather than to assess the time by observing the onset of anthesis, as recommended by Rutter (1991). Experience also suggests that the slide technique using dried pollen described by Rutter (1991) is more efficient than pollinating with fresh catkins. Flat surfaces other than microscope slides have been found preferable for applying pollen, such as the lid of the pollen container. In general, about one nut is produced per pollination bag placed over female flowers.

The intercross generations are produced by open pollination, where possible. Thus, breeding orchards containing straight third backcross trees ( $B_3$ ) from one source of blight resistance are isolated as much as possible from orchards with other sources of blight resistance or trees at other stages of breeding. Likewise, seed orchards, such as of  $B_3$ - $F_2$  trees, are isolated as much as possible from other orchards. A distance between orchards of about 1 kilometer (about 1/2 mile) is estimated to be sufficient to isolate orchards. Pollen from undesired trees also is eliminated by emasculation, pruning at ground level, and removal of the undesired trees.

## CULTIVATION

The cultivation methods employed are standard orchard practices adapted to screening chestnut trees for blight resistance. Hebard (1991) discussed locating flowering American chestnut trees, and Rutter and Hebard (1991) outlined cultivation methods suitable for breeding orchards. Hebard (1994a) described the techniques for inoculating chestnut trees to test their blight resistance, and the orchard spacings used to grow trees. More recently, Hebard presented designs for seed orchards and methods for producing seed in them (2002) and methods for introducing additional

sources of blight resistance into our chapter breeding programs (2001).

Orchards where backcross progenies are to be screened for blight resistance are arranged in completely randomized designs with controls consisting of 6 to 12 individuals each of pure American and pure Chinese chestnut trees, and their  $F_1$  hybrid. This experimental design was chosen because each genotype is unique, with no replication of genotypes.

In a test of the response of trees of various ages to direct inoculation, the intermediate blight resistance of  $F_1$  hybrids as young as 1 year old was distinguished from the high resistance of pure Chinese and from susceptible pure American chestnut trees. However,  $F_1$  hybrids did not survive the test unless they were at least 3 years of age (Hebard, unpublished data). Thus straight second backcrosses, which also have blight resistance up to the intermediate level found in  $F_1$  hybrids, are screened for blight resistance when they are 3 or 4 years old. At those ages and under our growing condition, their diameter at breast height (1.5 m) ranges from 3 to 7.5 cm (1 to 3 inches) and their height from 3 to 5 m (10 to 15 feet).

In order to avoid crowding prior to blight resistance screening, trees to be screened at 3 years of age are grown at a spacing of 1.2 m (4 feet) within rows. Trees screened for blight resistance at 4 years of age are grown at a spacing of 2.1 m (7 feet) within rows. Originally, straight backcross trees were screened for blight resistance at 4 years of age. Currently, straight backcross trees are screened for blight resistance when they are 3 years old, except for third backcross trees, which are screened when 4 years old (we did not wish to change methods for our most valuable breeding material). Progeny of large, surviving American chestnut trees also are screened for blight resistance when they are 4 years old. To provide access for equipment, the between-row spacing in these orchards is 6 m (20 feet).

Progenies expected to contain blight-resistant individuals, such as  $F_2$  generations, are screened for blight resistance when they are 1 or 2 years old. The blight-resistant progeny generally survive inoculation at that young age. These are spaced within rows at 30 or 60 cm (1 or 2 feet). The between-row spacing for  $F_2$  progeny varies from 2.1 to 6 m (7 to 20 feet) depending upon the location and intent of the test.

Nuts are sown directly at orchard spacing. Prior to planting, orchard rows are subsoiled, plowed and rototilled, and 31.75- $\mu$ m (1.25-mil) black plastic mulch lain in 1.22-m-wide (4 feet) strips. Holes are drilled with handled bulb planters through the mulch into the soil and filled with



a mix of one-third each ground, milled peat moss, perlite and coarse vermiculite. Nuts are planted 1-cm deep (0.5 inches) and protected from voles with aluminum cylinders 25.4-cm tall (10 inches) and 5 to 7 cm wide (2 to 3 inches). After planting, the cylinders are jammed down around the nuts to a depth of about 5 cm (2 inches). The aluminum is painted to reduce aluminum toxicity should it dissolve into the soil. Soil is mounded around the cylinders to prevent them from being blown away by wind. Styrofoam cups are inverted over cylinders until shoots emerge from the cylinders. At that point, the bottom of the cup is removed, and the cup replaced, to diminish breaking of the young shoots on the edge of the cylinders.

The seedlings generally outgrow the width of the cylinders during their third growing season. At the beginning of the third growing season, the cylinders are removed. The mulch also is removed to reduce vole damage. Prior to this time, the cylinders prevent vole damage. Voles can be harbored under mulch.

While black plastic mulch is in place, trees are fertilized with soluble fertilizer with a major nutrient composition of 30-10-10 (N-P-K) plus cationic trace elements (MirAcid™ or equivalent). Liquid fertilizer is used in order to place the fertilizer under the impermeable mulch. Approximately 2 liters (2 quarts) of fertilizer solution is applied every 2 weeks between mid May and early August. The fertilizer concentration is 3.26 ml per liter (1.25 tablespoons per gallon of water). Fertilizer is pumped directly down the cylinders or applied through a drip irrigation system. Once plastic mulch is removed, granular fertilizer is broadcast around the trees. The rate for granular fertilizer usually is 224 kg per hectare (200 lbs per acre) of N as ammonium nitrate and diammonium phosphate, 67 kg per hectare (60 lbs per acre) of P as diammonium phosphate and 67 kg per hectare of K as potash. These amounts are applied twice a year, in mid May and late June. In seed orchards, to avoid having to apply liquid fertilizer underneath plastic mulch, landscape fabric is used for mulching and granular fertilizer is broadcast at the above rates. The rates were formulated from soil and foliar mineral analysis for the soils typical of Meadowview and might differ on other soils. The rates also are adjusted depending upon the results of soil mineral analysis.

On trees 5 years of age and younger, weeds are managed with herbicides and mulch. In general, no weed management is performed on trees older than 5 years of age, other than mowing. Currently, in April,



Surflan(tm) A.S. (oryzlin) is applied at 9.35 liters per hectare (4 quarts per acre), simazine 4L at 7.02 liters per hectare (3 quarts per acre) and Roundup Ultra(tm) (glyphosate) at 3.07 liters per hectare (42 oz per acre). A supplemental spray of Roundup Ultra(tm) at 3.07 liters per hectare (42 oz per acre) is applied in July to trees younger than 3 years old. These herbicides are applied as a directed spray using TeeJet(tm) 8005 standard flat-fan nozzles operated at 2.07 bars (30 psi) in a water solution of 608 liters per hectare (65 gallons per acre). The combination of low pressure with high volume spray nozzles increases droplet size, reducing drift. A strip 152.4 cm wide (3 nozzles at 50.8-cm or 20-inch spacing, 45.72 cm or 18 inches above the ground) is sprayed down each side of a row. The nozzle closest to the trees is directed with a hand wand, the other two nozzles are mounted on the boom of the spray rig.

Grass strips are maintained between rows to reduce erosion. Fire hazard is reduced by regular mowing with rotary cutters. In B<sub>3</sub>-F<sub>2</sub> seedling seed orchards, which are sown at much higher densities (0.3 x 2.1 m, 1 x 7 feet), maintenance is performed with a riding lawn mower. Weeding of seedling seed orchards is done as above, but using a 25-gallon tow-behind sprayer attached to the lawn mower rather than a 65-gallon herbicide spray rig mounted on the three-point hitch of the standard orchard tractors used in the larger orchards. Only two nozzles are used in seedling seed orchards. The lawn mower-mounted nozzle is attached to the front of the mower. The mower operator also can manipulate a hand wand fairly easily on the lawn mower, whereas on the larger orchard tractors it is best if the hand nozzle is operated by a person walking behind. A pressure regulator needs to be added to most tow-behind sprayers. Their pumps are driven by electric motors powered from the lawn mower's electrical system, whereas the power take off drives the pumps on the orchard tractors. Thus, it is important that the lawn mower produce enough electric current to power the pump.

Using an airblast sprayer, aphids are controlled with a single application of dormant oil during bud break at 56 liters per hectare (6 gallons per acre) in 2807 liters per hectare (300 gallons per acre) of water solution. In July, Japanese beetles are controlled with 2 to 3 applications of Sevin XLR Plus(tm) at 5.8 liters per hectare (0.625 gallons per acre) in 935 liters per acre (100 gallons per acre) of water solution. Spray amounts have been reduced considerably by employing a Durand-Wayland Smart





Spray 1000(tm) attached to a Durand-Wayland model AF500CPS air-blast sprayer. This device cuts off banks of nozzles depending upon tree height and occurrence.

The pesticide application methods, composition, and rates were formulated in consultation with extension specialists from the Virginia Polytechnic Institute and State University and the "Spray Bulletin for Commercial Fruit Growers," which is issued annually (Virginia, West Virginia & Maryland Cooperative Extension Services, 2004).

Straight backcross trees have been irrigated in the year of inoculation during dry years. Since the year 2000, all young chestnut trees have been irrigated, except B<sub>3</sub>-F<sub>2</sub> seedlings, using a drip irrigation system. Soil moisture is maintained at field capacity (about 10-20 kiloPascals of soil moisture deficit). We plan not to irrigate B<sub>3</sub>-F<sub>2</sub> seedling seed orchards.

Trees are not pruned for shaping or for removal of lower branches, as is often done in commercial fruit and nut orchards to facilitate passage down the rows and weeding with herbicides, among other objectives. Not pruning results in a crown that extends to the ground on the trees (and necessitates a second person walking behind the herbicide sprayer to prune off portions of branches that are sprayed inadvertently). This larger crown may promote early and heavier bearing. For the most part, our trees produce male catkins when they are 2 to 4 years old and bisexual catkins when they are 3 to 5 years old. This early flowering also has been seen in other hardwood trees grown under intense cultivation (Wright, 1976).

Using the above methods, the trees at Meadowview have averaged 0.56 m (1.8 feet) tall after one growing season, 1.5 m (4.9 feet) tall after two, 2.4 m (7.9 feet) after three, and 3.7 m (12.1 feet) after four growing seasons. There can be considerable variation in height growth within orchards and between growing season, genotype, and location.

### **SCREENING FOR BLIGHT RESISTANCE**

The cork-borer, agar-disk method is used to inoculate chestnut trees with the blight fungus (Griffin, et al., 1983). Agar disks are obtained from the margins of growing cultures that have not reached the edge of the Petri plate. Inoculations are performed in early June. This is the earliest in the season when cool weather (daily high temperatures below 15 to 20 C) can be avoided reliably. Cool weather occurs every few years in late May in Meadowview and can lead to inoculation failure.

Two strains of the blight fungus are used, known as Ep155 and SG1 2-3. Ep155 is a widely used strain of the blight fungus (ATCC 38755), while SG1 2-3 was isolated near Meadowview by the author. When tested for pathogenicity in American chestnut, the distribution of lengths of cankers incited by virulent strains of the blight fungus follows a bell-shaped curve; it is approximately normally distributed, and variances are equal for the various canker lengths (Griffin, et al., 1983). When replicated five times each over 3 years, or 15 total replicates, Ep 155 was among the most pathogenic of 21 tested virulent strains, having significantly ( $p < 0.05$ ) larger cankers than six of the least pathogenic test strains. Likewise, SG1 2-3 was among the least pathogenic of the 21 tested strains, having significantly smaller cankers than seven of the most pathogenic test strains.

Blight resistance can be determined quantitatively by measuring the length and width of cankers. Canker depth or superficiality is not determined at Meadowview, since the intermediate to very high levels of blight resistance being sought can be distinguished using length and width measurements alone. Until 1999, the length and width of cankers was measured on all tested trees. Because this was taking too much time, beginning in 1999, blight resistance in most tests was determined using a qualitative assessment.

**TABLE 3**

Blight resistance classes distinguished qualitatively by various canker length classes for two strains of *Cryphonectria parasitica* one year after inoculation in early June.

| Numeric Blight Resistance Class | Verbal Blight Resistance Class      | Length of Canker incited by |               |
|---------------------------------|-------------------------------------|-----------------------------|---------------|
|                                 |                                     | Ep 155<br>cm                | SG1 2-3<br>cm |
| 1                               | highly blight resistant             | 2-3                         | 2-3           |
| 2                               | blight resistant                    | 3-6                         | 2-3           |
| 3                               | intermediately blight resistant     | > 6                         | 2-3           |
| 4                               | slightly blight resistant           | >> 6                        | 3-6           |
| 5                               | not blight resistant or susceptible | >>> 6                       | >6            |

The qualitative assessment is based on the following observations. In general, 1 year after inoculation, SG1 2-3 incites small cankers (2-3 cm long) on trees with intermediate levels of blight resistance or higher. It incites medium-sized cankers (3-6 cm long) on trees with low levels of blight resistance, and large cankers (> 6 cm long) on normal American chestnut trees. In contrast, Ep 155 incites large cankers on trees with intermediate levels of blight resistance or less, medium-sized cankers on trees with high levels of blight resistance, and small cankers on trees with very high levels of blight resistance. Thus five blight resistance classes can be distinguished on trees inoculated with both strains. This is depicted visually in Table 3.

Table 3 depicts idealized canker lengths for various blight resistance classes seen in average years. Depending upon the season, slightly blight-resistant trees might show small SG1 2-3 cankers or blight-resistant trees might show large Ep 155 cankers. Additionally, the responses to the two strains do not always move in parallel with each other. These various unusual patterns of response can be detected by the response of the pure American and Chinese chestnut trees and their F1 hybrids planted as control trees in the orchard and the scale adjusted accordingly.

In addition to artificial inoculation, trees in Meadowview also are exposed to naturally occurring inoculum. Blight incidence due to natural infections on straight backcross progeny exceeds 50% by the beginning of the fifth growing season, when trees are four years old. When screening artificially inoculated trees for blight resistance, the severity of these naturally occurring cankers is considered in the overall assessment of a tree. Thus, while only two strains of the blight fungus are used for direct inoculation, a larger number of strains is involved in the overall assessment.

## RESULTS AND DISCUSSION

### RECOVERY OF HIGHLY BLIGHT-RESISTANT BACKCROSS PROGENY AT F<sub>2</sub>

The first screening of progeny segregating for blight resistance in Meadowview occurred in 1993. One set of progeny consisted of B<sub>1</sub>-F<sub>2</sub>s obtained from reciprocal crosses of the 'Graves' and 'Clapper' trees. A second set of progeny consisted of straight F<sub>2</sub>s obtained from a one-way



cross of two  $F_1$ s. The  $F_1$  parents were half sibs from crosses of the 'Mahogany' Chinese chestnut tree with pollen from two American chestnut trees. A third set of progeny segregating for blight resistance consisted of straight  $B_2$ s composited from three crosses of pollen from the 'Graves' tree onto three American chestnut trees. The trees were 2 years old when inoculated in June, 1993, and the data in Table 4 summarize canker dimensions when measured in September, 1993. Each tree was inoculated once with strain Ep 155 and once with strain SG1 2-3, using the cork borer, agar-disk method with holes 2 mm in diameter. Highly blight-resistant progeny were recovered from the  $F_2$  and the  $B_1$ - $F_2$  crosses, and progeny with intermediate levels of blight resistance were recovered from the  $B_2$  crosses. The  $B_1$ - $F_2$  crosses may have had higher blight resistance than the straight  $F_2$ s. Figure 1 depicts one of these highly blight-resistant  $B_1$ - $F_2$ s.

**TABLE 4**

Mean and standard deviation and distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for straight  $F_2$ ,  $B_1$ - $F_2$  and  $B_2$  American x Chinese chestnut progeny and controls.

| Cross Type                         | Canker Size Class (cm) |           |           |           |           |            | Mean | Standard Deviation |            |
|------------------------------------|------------------------|-----------|-----------|-----------|-----------|------------|------|--------------------|------------|
|                                    | 1.0 to 2.6             | 2.6 to 4. | 4.2 to 5. | 5.8 to 7. | 7.4 to 9. | 9.0 to 10. |      |                    | 10.6 to... |
| Seedling American                  |                        |           |           |           | 3         | 5          | 2    | 9.6                | 1.1        |
| $F_1$ 'Nanking'                    |                        |           |           | 2         | 4         | 3          |      | 8.4                | 1.0        |
| Seedling Chinese                   |                        | 2         | 7         | 3         |           |            |      | 5.2                | 1.0        |
| 'Meiling' Chinese                  |                        | 1         | 2         | 2         |           |            |      | 5.5                | 1.1        |
| 'Nanking' Chinese                  | 3                      |           | 2         |           |           |            |      | 2.9                | 1.4        |
| $F_2$ 'Mahogany'                   |                        | 5         | 23        | 48        | 48        | 29         | 15   | 7.7                | 1.9        |
| $B_1$ - $F_2$ 'Clapper' x 'Graves' | 4                      | 25        | 84        | 116       | 112       | 54         | 4    | 6.9                | 1.9        |
| $B_2$ 'Graves'                     |                        |           | 2         | 4         | 15        | 26         | 6    | 9.1                | 1.5        |



Figure 1. Highly blight-resistant Chinese to American  $B_1$ - $F_2$ , 13 years old, 11 years after inoculation with *Cryphonectria parasitica*. The tree is to the left of and behind the dog.

Three-year-old  $B_2$ - $F_2$  progenies from controlled crosses between selected straight  $B_2$ s (backcrossed to American chestnut) were inoculated in June, 2003, and cankers measured in November. 'Clapper'  $B_2$ - $F_2$  progeny were from a single cross between two half sibs, while 'Graves'  $B_2$ - $F_2$  progeny were a composite of three crosses between half sibs. Depending upon their size, these trees were inoculated once or twice each with strains Ep 155 and SG1 2-3, using the cork borer, agar-disk method, but the holes were 4 mm in diameter. A larger cork borer and number of inoculations were used in 2003 than in 1993 because 2003's 3-year-old trees were larger than 1993's 2-year-old trees. Again, highly blight-resistant progeny were recovered, this time from second backcross  $F_2$ s (Table 5). Thus, not only could highly blight-resistant progeny be recovered by intercrossing  $F_1$  interspecific hybrids or by intercrossing first or second backcrosses to American chest-

nut, but high levels of blight resistance were retained through the second backcross. These results suggest very strongly that the blight resistance of Chinese chestnut can be backcrossed into American chestnut.

Canker sizes were smaller in the 2003 than in the 1993 test, possibly because of cooler, wetter weather in the later year, so there was not as much separation of canker sizes among the controls. However, the cankers on some of the  $B_3$ - $F_2$  progeny have remained small through the 2004 growing season, as illustrated in Figure 2. An earlier test, performed in 1999 on open-pollinated progeny of 'Clapper'  $B_2$ s, presumably pollinated by other 'Clapper'  $B_2$ s, gave results similar to those presented in Table 5 (Hebard, et al, 2000).

### BLIGHT RESISTANCE IN STRAIGHT BACKCROSSES

Tables 6, 7, and 8 report typical results of rating straight second and third backcross trees for blight resistance. An entire family derived from a second backcross tree has not yet been rejected based on the performance of its third backcross progeny. In general, the blight resistance of third backcross progeny is comparable to that observed in second backcross trees, again supporting the inference that there is no diminution of resistance as backcrossing proceeds.

**TABLE 5**

Mean and standard deviation and distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for B<sub>2</sub>-F<sub>2</sub> American x Chinese chestnut progeny and controls.

| Cross Type                               | Canker Size Class (cm) |            |            |            |            |            |            | Mean | Standard Deviation |
|--|------------------------|------------|------------|------------|------------|------------|------------|------|--------------------|
|  | 1.0 to 2.0             | 2.0 to 3.0 | 3.0 to 4.0 | 4.0 to 5.0 | 5.0 to 6.0 | 6.0 to 7.0 | 7.0 to 8.0 |      |                    |
| Seedling American                        |                        |            | 4          | 2          | 2          | 2          | 1          | 5.0  | 1.4                |
| F <sub>1</sub> 'Nanking'                 |                        | 1          | 2          | 3          | 1          |            |            | 4.1  | 1.0                |
| Seedling Chinese                         | 3                      | 3          | 3          | 6          |            |            |            | 3.3  | 1.2                |
| B <sub>2</sub> -F <sub>2</sub> 'Clapper' | 3                      | 11         | 15         | 37         | 16         | 12         | 3          | 4.5  | 1.4                |
| B <sub>2</sub> -F <sub>2</sub> 'Graves'  | 3                      | 11         | 21         | 31         | 14         | 14         | 1          | 4.4  | 1.3                |



Figure 2. Left, chestnut blight cankers after two growing seasons on a highly blight-resistant 'Clapper' B<sub>2</sub>-F<sub>2</sub>. Top left, canker incited by strain SG1 2-3. Bottom left, canker incited by strain Ep 155. Right, 4-year-old 'Clapper' B<sub>2</sub>-F<sub>2</sub>. Similar cankers on blight-susceptible American chestnut would be expected to exceed 40 cm in length; these cankers were 2 to 3 cm long.

**TABLE 6**

Blight resistance ratings of 'Clapper' and 'Graves' second backcross trees and controls in 1999.

| Cross Type               | Blight Resistance Rating |   |    |    |    |
|--------------------------|--------------------------|---|----|----|----|
|                          | 1                        | 2 | 3  | 4  | 5  |
| Seedling American        |                          |   |    | 2  | 3  |
| F <sub>1</sub> 'Nanking' |                          | 4 |    |    |    |
| Seedling Chinese         | 3                        | 5 |    |    |    |
| 'Nanking' Chinese        | 1                        | 1 |    |    |    |
| B <sub>2</sub> 'Clapper' |                          | 5 | 27 | 29 | 12 |
| B <sub>2</sub> 'Graves'  |                          | 3 | 42 | 47 | 25 |

**TABLE 7**

Blight resistance ratings of 'Clapper' third backcross trees and controls in 2000.

| Cross Type               | Blight Resistance Rating |    |     |     |    |
|--------------------------|--------------------------|----|-----|-----|----|
|                          | 1                        | 2  | 3   | 4   | 5  |
| Seedling American        |                          |    |     | 3   | 3  |
| F <sub>1</sub> 'Nanking' |                          | 2  | 10  |     |    |
| Seedling Chinese         | 3                        | 2  | 1   |     |    |
| B <sub>3</sub> 'Clapper' | 1                        | 19 | 139 | 383 | 95 |

**TABLE 8**

Blight resistance ratings of 'Graves' third backcross trees and controls in 2001.

| Cross Type               | Blight Resistance Rating |   |     |     |     |
|--------------------------|--------------------------|---|-----|-----|-----|
|                          | 1                        | 2 | 3   | 4   | 5   |
| Seedling American        |                          |   | 1   | 3   | 8   |
| F <sub>2</sub> 'Nanking' |                          | 2 | 5   |     |     |
| Seedling Chinese         | 7                        | 8 |     |     |     |
| B <sub>3</sub> 'Graves'  |                          |   | 124 | 124 | 122 |

Family effects have occurred in second backcross progeny fathered by both the 'Graves' and 'Clapper' trees, where the American mother of second backcross progeny influenced their phenotypic blight resistance. This is illustrated in Table 9, where the Bu3C1C x 'Clapper' family had cankers closer in size to cankers on Chinese chestnut than on F<sub>1</sub>s or Americans. It is unclear whether or not the Bu3C1C American parent

**TABLE 9**

Distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for progeny of second backcrosses of the 'Graves' and 'Clapper' first backcross trees to American chestnut and controls, in 1998.

| Cross Type                                   | Canker Size Class (cm) |        |        |         |          |          |          |
|--|------------------------|--------|--------|---------|----------|----------|----------|
|  | 2 to 4                 | 4 to 6 | 6 to 8 | 8 to 10 | 10 to 12 | 12 to 14 | 14 to 16 |
| Seedling American                            |                        |        |        |         | 4        | 1        |          |
| F <sub>1</sub> 'Nanking'                     |                        |        | 1      | 3       | 1        |          |          |
| Seedling Chinese                             |                        | 3      | 4      |         |          |          |          |
| 'Nanking' Chinese                            | 2                      | 2      |        |         |          |          |          |
| Bu <sub>2</sub> B <sub>2</sub> C x 'Clapper' |                        |        | 2      |         | 3        | 2        |          |
| Bu <sub>2</sub> B <sub>3</sub> C x 'Clapper' |                        |        | 3      | 4       | 4        | 1        |          |
| Bu <sub>3</sub> C <sub>1</sub> C x 'Clapper' |                        | 15     | 33     | 8       |          |          |          |
| Bu <sub>1</sub> C <sub>1</sub> G x 'Graves'  |                        |        | 4      | 8       | 17       | 10       | 1        |
| Bu <sub>1</sub> C <sub>2</sub> G x 'Graves'  |                        |        | 2      | 1       | 1        | 1        |          |
| Bu <sub>3</sub> B <sub>1</sub> G x 'Graves'  |                        |        |        | 1       |          |          |          |
| Bu <sub>3</sub> B <sub>2</sub> G x 'Graves'  |                        |        |        |         | 2        |          |          |
| Bu <sub>3</sub> C <sub>3</sub> C x 'Graves'  |                        | 4      | 8      | 15      | 25       | 19       | 2        |
| Bu <sub>3</sub> D <sub>1</sub> G x 'Graves'  |                        |        | 1      |         | 2        |          |          |
| Bu <sub>3</sub> F <sub>1</sub> G x 'Graves'  |                        |        |        |         | 1        | 1        |          |
| Bu <sub>3</sub> F <sub>5</sub> G x 'Graves'  |                        |        | 2      | 2       | 5        | 2        |          |
| Bu <sub>3</sub> R <sub>1</sub> G x 'Graves'  |                        |        | 2      | 7       | 13       | 4        | 1        |



was contributing genes for blight resistance by itself or contributing genes that modulated the expression of blight resistance genes from Chinese chestnut. The Bu3C1C tree did not appear to have more blight resistance than typical American chestnut trees; it died from blight the year after this cross was made, like most of the other American chestnut trees at that site.

#### **NUMBER OF GENES CONDITIONING BLIGHT RESISTANCE**

The standard deviations of canker size in Table 4 were greater for the progeny expected to be segregating for blight resistance than for the controls, and, for the  $F_2$ s, were compatible with models for one or two incompletely dominant genes controlling blight resistance, using Wright's method for estimating the number of factors controlling a segregating trait (Falconer, 1960, p. 218). (In this computation, the total genetic variance of the  $F_2$ s was substituted for the additive genetic variance; the former was computed by subtracting the mean variance of the controls from the variance of the  $F_2$ s. The broad sense heritability calculated from these variances was about 70%). The distributions of canker size in segregating progeny in Table 4 were compatible with the distributions of canker size expected for two or three incompletely dominant genes of equal effect on blight resistance, among other models for gene action. Similar models with more than three factors or fewer than two did not fit the observed values (chi-square  $p < 0.05$ ). The expected distributions were constructed from the mean response for the control trees, assuming a normal distribution of canker size with the average standard deviation of the controls shown in Table 4; missing cells, such as for trees with only one allele for resistance, were estimated by linear interpolation between the relevant observed values. Unfortunately, vegetatively propagated (grafted) individuals of 'Mahogany' were not available for inclusion in the test, nor the actual  $F_1$  parents; otherwise stronger inferences might have been possible concerning the mode of inheritance of blight resistance. Subsequent experience suggests that 'Mahogany' has a high level of blight resistance, comparable to that of 'Nanking.' This suggests in turn that two genes are involved in blight resistance. A three-gene model would be more compatible with the data if 'Mahogany' Chinese chestnut had a "normal" level of blight resistance like the 'Meiling' and seedling Chinese in Table 4 rather than the high level of blight resistance observed in 'Nanking.'

Kubisiak, *et al.* (1997) prepared a genetic map of the 'Mahogany' F<sub>2</sub>s whose canker sizes are shown in Table 4. Their results indicated that three regions of the genome (linkage groups B, F, and G) were associated with blight resistance. The Kubisiak, *et al.* (1997) map was constructed with randomly amplified polymorphic deoxyribonucleic acid markers (RAPDs), restriction fragment length polymorphic markers (RFLPs), and isozymes. Subsequent genotyping of the mapping population with markers based on simple sequence repeats (SSRs) indicated that 17 of the 185 progeny were outcrosses, not pollinated by the supposed male parent (Sisco, Kubisiak and Hebard, unpublished). These individuals are not included in Table 4. One of the three regions of the genome previously associated with blight resistance (located on Kubisiak *et al.*'s (1997) linkage group G) was no longer associated with blight resistance in the revised mapping population. However, another region (1997's linkage group E) was associated with blight resistance, although we do not know whether linkage group E is on the same chromosome as linkage group B, and we do not know whether the resistance associated with these two linkage groups is conferred by one locus or two. Molecular mapping of backcrosses of 'Mahogany' F<sub>1</sub>s to American chestnut also suggested that the same two or three regions of the genome condition blight resistance (Kubisiak and Hebard, unpublished). The molecular mapping data thus supported a model of two or three incompletely dominant genes conditioning blight resistance in these progeny.

Highly blight-resistant 'Clapper' x 'Graves' B<sub>1</sub>-F<sub>2</sub> individuals were test crossed to American chestnut to determine whether or not they were homozygous for blight resistance. Screening of these 'Clapper' x 'Graves' test cross progenies indicated that they were segregating for blight resistance (data not shown), and hence that the B<sub>1</sub>-F<sub>2</sub> parents were not homozygous. This finding suggests that some of the genes conditioning blight resistance in 'Clapper' and 'Graves' are at different loci. Highly blight-resistant 'Mahogany' F<sub>2</sub> progeny also had been test crossed to American. Unfortunately, all of the test-crossed individuals turned out to be outcrosses, as indicated by the SSR markers, invalidating this second set of tests.

There are numerous patterns of inheritance possible when a trait is controlled by more than one gene, including complementary inheritance, epistasis, etc (Grant, 1975). The model here of two (or three) incompletely



dominant genes, where each of the four (or six) Chinese alleles has an equal effect, is only one among these models, albeit one that fits the data. If further improvement of backcross chestnut trees for blight resistance is necessary beyond the  $B_2$ - $F_2$  stage of breeding, it might be best to use breeding methods for quantitative traits, such as recurrent selection.

The fact that the variance or range of canker sizes for the  $F_1$  controls in Tables 4 to 9 were similar to those of the pure species indicates that 'Nanking' Chinese chestnut trees are homozygous for blight resistance. Similar data suggest that the named Chinese chestnut cultivars Orrin and Meiling, and the Greg Miller selections, 64-4 and 72-211, likewise are homozygous for blight resistance.

### **OUTBREEDING AND INBREEDING DEPRESSION**

Not infrequently, specific Chinese x American chestnut crosses fail to produce nuts. Sometimes, nuts are produced, but fail to grow after germinating a radical. These failures may be considered extreme instances of outbreeding depression. Chinese x American  $F_1$  hybrids that do germinate often exceed pure species in size up to 10 to 20 years after planting, exhibiting hybrid vigor. For instance, after three seasons of growth,  $F_1$  hybrids in four orchards were significantly ( $p < 0.0001$ ) taller than pure species, having a least squares mean height of 2.2 m (7.2 feet) versus 1.8 m (5.9 feet) for the pure species. The  $F_1$  hybrids also were significantly taller than any of the individual pure species.

The 'Mahogany'  $F_2$ s of Table 4 came from the only intercross of Chinese x American  $F_1$  hybrids that has yielded well (greater than 1.0 nuts per pollination bag). Other  $F_1$  intercrosses have yielded fewer than 0.6 nuts per pollination bag, sometimes much less. Attempts to use Chinese x American  $F_1$  hybrids to pollinate American or Chinese chestnut trees also have produced low yields, in general. Even some intercrosses among half-sib  $B_2$ s have yielded sound nuts that failed to produce seedlings. The failures of some of these more advanced crosses may be due to inbreeding depression rather than outbreeding depression. The failures (and pollen contamination in our early crosses) bedeviled attempts to repeat the early experiments. Similar failures also may have hindered attempts of earlier researchers to test hypotheses regarding the inheritance of chestnut blight resistance.

As mentioned previously in the section on blight resistance, the

'Clapper' x 'Graves' B<sub>1</sub>-F<sub>2</sub>s of Table 4 had more apparent blight resistance than the Mahogany F<sub>2</sub>s. They also grew to be larger, more vigorous trees, perhaps because they did not suffer from inbreeding depression and/or had hybrid vigor (four-hundred, nineteen 'Clapper x Graves' and 'Graves x Clapper' progeny had a mean height at the end of the 1993 growing season of 2.43 m (8.0 feet) while 191 'Mahogany' F<sub>2</sub>s had a mean height of 2.13 m (7.0 feet), significantly shorter,  $p < 0.0001$ ; a similar trend,  $p = 0.001$ , was observed in 1992, prior to inoculation). The relative contributions of general vigor versus specific genes for blight resistance to the greater phenotypic blight resistance of the 'Clapper' x 'Graves' B<sub>1</sub>-F<sub>2</sub>s are unclear.

**Summary.** We have been able to recover highly blight-resistant chestnut trees after backcrossing blight resistance from Chinese into American chestnut for two cycles of backcrossing. Three cycles of backcrossing are expected to produce chestnut trees that, for the most part, look and grow like American chestnut. We currently are starting to test the blight resistance of second-generation, third backcross trees (B<sub>3</sub>-F<sub>2</sub>s), and currently expect some of them to have high levels of blight resistance. By 2008, we hope to begin planting their progeny (B<sub>3</sub>-F<sub>3</sub>s) back into the forest to confirm these expectations and to begin restoring the American chestnut tree to Appalachian forests.

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