The Minnesota Chestnut Program—New Promise for Breeding a Blight-Resistant American Chestnut

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The title of this paper introduces a new word into the chestnut literature, "promise." Previously, researchers have only been willing to hold out "hope," but we feel the breeding program presented below may indeed merit using "promise" instead.

The program was initiated by Charles R. Burnham (1981) and currently consists of a loose group of some fifteen scientists and technicians, located primarily at the University of Minnesota, St. Paul, and at the University of Minnesota Landscape Arboretum, Chaska, Minnesota, who contribute time, expertise, and criticisms to the effort.

This group of researchers is in agreement on these two points: one, there are several clear reasons why the previous breeding programs failed to produce a blight-resistant American type chestnut tree; two, there is a standard plant breeding technique, untried in the previous breeding programs, which should work, given the facts established early in those programs. Those facts include:

1) The hybrids between the various species of chestnuts are fertile, except for occasional male sterility among F_1 and later generations (Jaynes, 1974).
2) There are two major sources of blight resistance, the Chinese and the Japanese chestnuts.
3) The first generation (F_1) hybrids between the blight-resistant and the American (blight-susceptible) species are more resistant than

their American parent but less resistant than the Chinese or the Japanese parent (Graves, 1950; Clapper, 1952; Berry, 1959). This is an intermediate condition known as partial dominance, where the gene from one parent and its partner gene or allele from the other parent are both expressed. A standard example in plants is snapdragons where the cross between one with red flowers and one with white flowers yields first generation (F_1) plants having pink flowers. Red flowered F_1 plants would indicate complete dominance.

The implications of realizing that blight resistance is partially dominant are these: *one*, selecting F_1's and later generation hybrids for breeding is easier since any resistance present should be visible; and *two*, the genetics of the oriental parents are clarified, making it easier to understand the variations in resistance observed within those populations.

Information on the inheritance of blight resistance is meager. The only study of inheritance of the blight resistance involved in the American and Chinese species is by Clapper (1952, 1954). He reported that the progeny from backcrosses of Chinese x American on Chinese approximate a ratio of 3 resistant:1 susceptible, i.e., two pairs of genes for resistance. A ratio of 1 resistant:1 susceptible would have indicated only one pair of genes. He did not refer to them as dominant genes. The current trend has been to consider the blight-resistant genes as recessives.

By definition, a recessive gene is not expressed at all in the presence of a dominant gene for the same trait (using the word recessive implies that the partner gene, or allele, is dominant).

If blight resistance were recessive, the ratio in Clapper's study would have been 3 susceptible:1 resistant. Clearly, the genes for blight resistance are not recessive.

One block to success for the earlier breeding programs expressed by Graves and others was the opinion formed after years of observing the hybrids, that the poor form of the Chinese chestnut was genetically linked to its blight resistance and the good timber character of the American species was linked to its blight susceptibility. The accompanying diagrams are designed to show how that opinion arose, and why it is not necessarily correct.

The opinion regarding linkage, together with the belief that many genes are involved in blight resistance, gave rise to an undue pessimism among breeders and contributed to the demise of the USDA program (about 1960). The plantings, or rented land, were destroyed.

Figure I is a simplified breeding chart, intended to show basic principles and the flow of reasoning, ignoring some of the fine points. The chart shows the average forms of the two species and a genetic system for blight resistance with one gene (R). The F_1 hybrids then are Rr, partially resistant. This resistance is inadequate, so the previous breeders decided to cross back to the Chinese parent to gain more resistance. This backcross, however, in addition to yielding some indi-

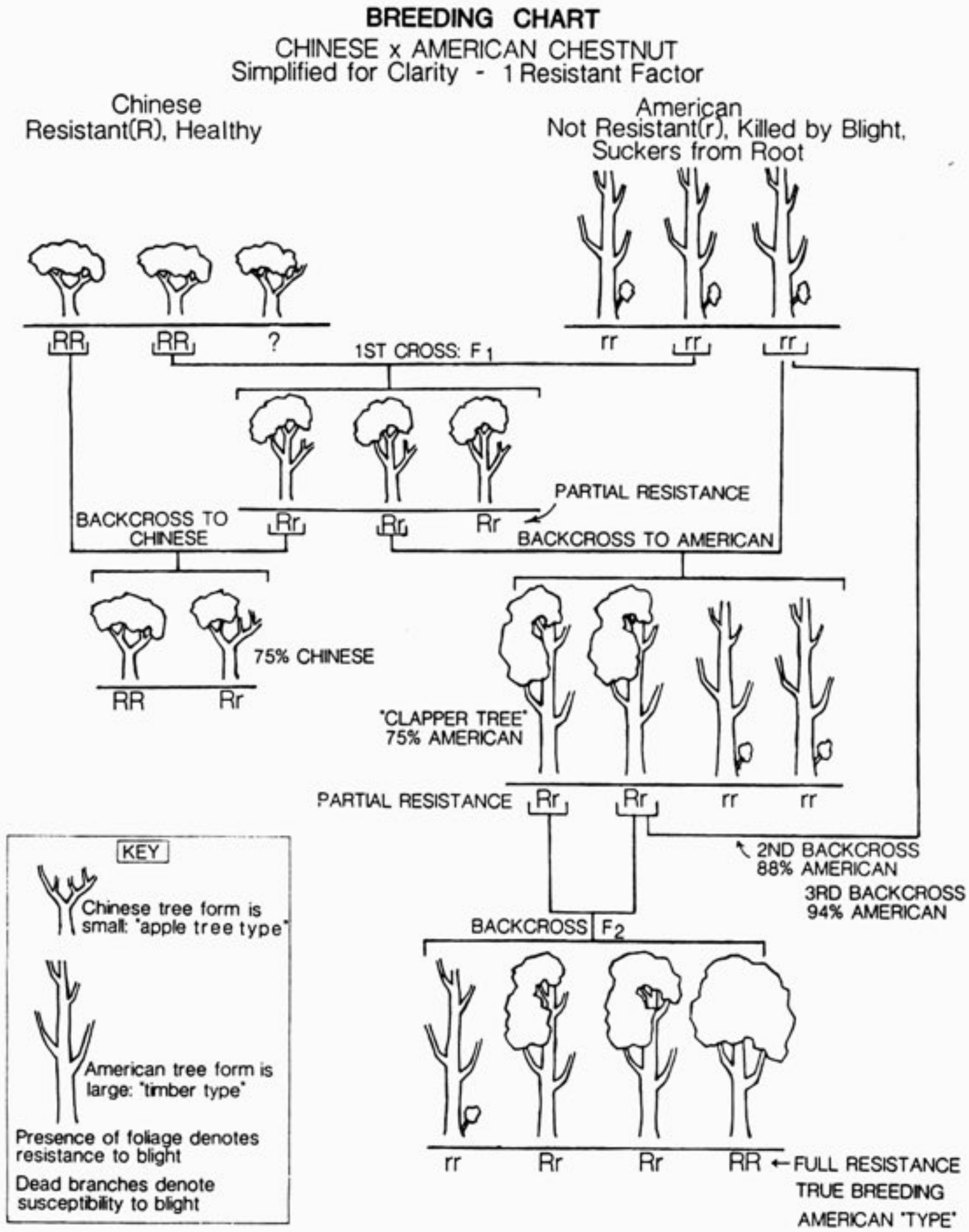

FIGURE 1

viduals with a resistant (RR) genotype, also increases the percentage of Chinese genes for all other characteristics for which the Chinese chestnut is inadequate, including tree form, vigor, cold hardiness, and general adaptability to the wide range of American climate and forest conditions. All of these traits are certainly governed by many genes. The fact that the trees from the backcross to the Chinese owe, on the average, 75% of their genotype to the Chinese species is sufficient to explain their poor form. Genetic linkage is not at all necessary to explain the observations.

Figure I additionally delineates the principles of the breeding program we have begun. Our program also relies on a backcross, but to the American species, which is well adapted to our forests and which requires only that the blight resistance trait be added to it.

This program will not immediately lead to a tree that is sufficiently blight resistant. What the first backcross to the American chestnut does is create a population of trees that owes 75% of its genes to the American species and that will show partial resistance in some of its individuals. These half-resistant trees will be tending toward the American phenotype; indeed, the "most promising USDA hybrid," the Clapper chestnut, was a first backcross to the American parent. There were only 37 of these backcrosses in the USDA program (Clapper, 1954). The Clapper tree had good form and vigor, lived for many years, but proved insufficiently blight resistant, precisely as we would expect.

From this first backcross generation those trees showing good, partial (F_1) resistance and the best form available are selected and crossed back to the American parent again. The second backcross trees will owe 88% of their genes to the American species, which means they will be 88% adapted to the American forests. This process may be repeated once more, to create the third backcross generation, which would be 94% American—virtually indistinguishable from the original parent population.

At this point the best partially resistant third backcross trees are crossed with each other to make the backcross F_2 generation (BC_3F_2). Out of this generation will come a few trees that have essentially pure American genotype and the full resistance of the best Chinese trees. Their genotype for blight resistance will be RR, just as it was in the Chinese parent.

Those BC_3F_2 trees with the RR genotype will be true-breeding for both blight resistance and the American form and vigor.

Figure II illustrates what we believe may be the actual genetics of blight resistance. All of the observations made by chestnut researchers can be explained by this hypothesis: two genes for blight resistance that are additive in their effects, the $R_1r_1R_2r_2$ F_1 hybrid being more resistant than the American parent (partial dominance) due to resistance from Chinese.

The parent Chinese population shows considerable variation in blight resistance. Part of this may be simply due to differences in the overall health of individual trees; strong, vigorous trees resist any disease better than puny ones do. It is probable that vigorous trees with genetically incomplete resistance (e.g., $R_1R_1R_2r_2$ or $R_1r_1R_2R_2$) might appear wholly resistant, but would not breed true and the progeny from intercrossing certain ones would include individuals with less resistance than the parents. This would also account for the observed variation in the resistance of Chinese x American F_1 hybrids.

The observation that trees may appear resistant for many years and then succumb to the blight might also be due to such incomplete

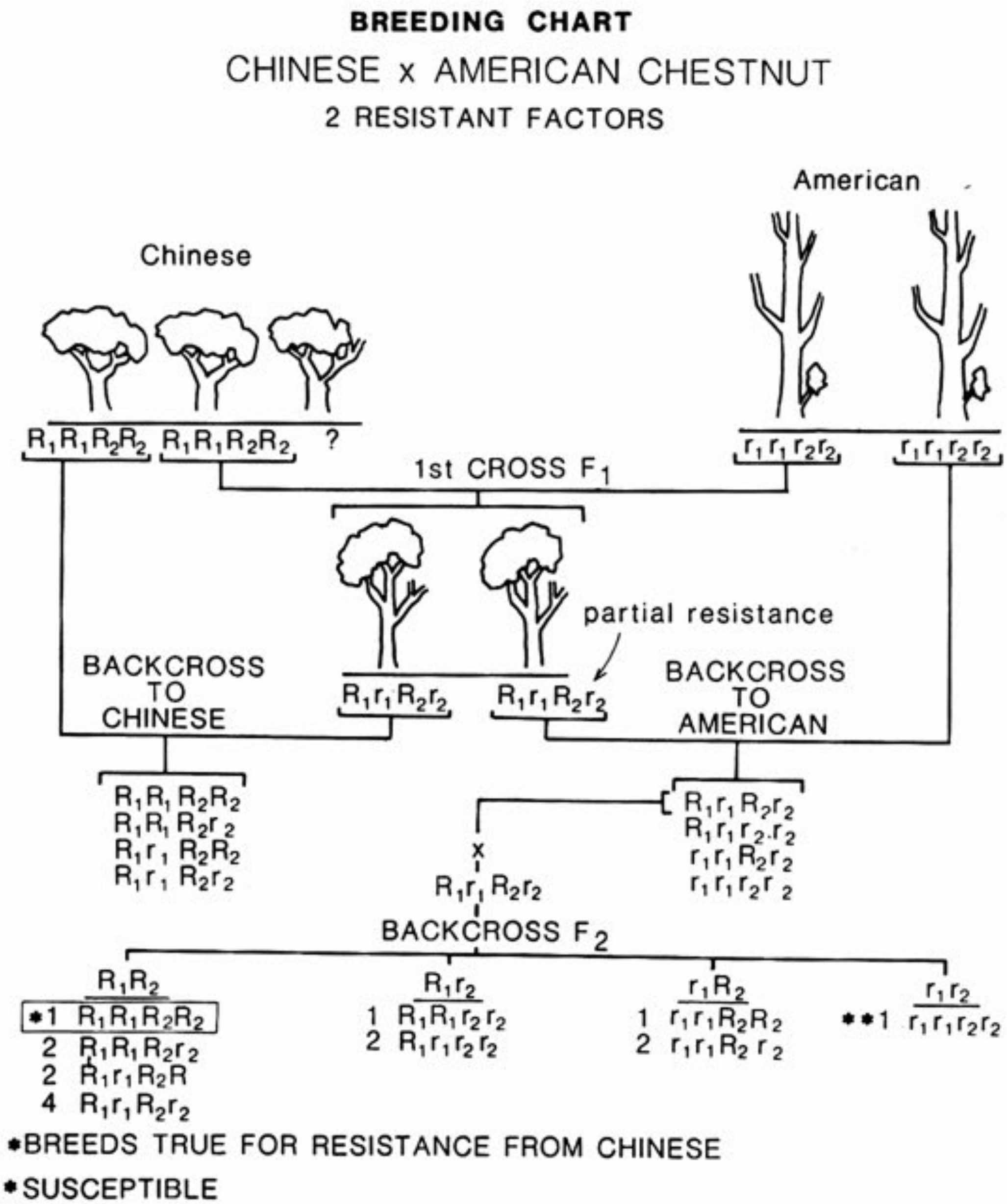

FIGURE 2

resistance. Often such losses are observed following a severe drought or winter. This, too, is consistent.

Several researchers have expressed the opinion that blight resistance may be genetically complex, i.e., caused by many genes. This was primarily because of the broad range of expressed variation in blight reaction in hybrid progenies. In fact, however, two genes for resistance are adequate to give a complete continuum from none to severe blight. In the segregating generations (F_2 or BCF_2) two genes will give rise to nine separate genotypes. That in itself would result in indistinct categories of resistance, and when the additional factors of genetic variation in vigor and random environmental stresses are added it becomes obvious that the observed continuum is to be expected.

Figure III is a series of hypothetical frequency distribution curves for tree "form," drawn from population genetics theory and experience with the behavior of multi-genic traits in other species. It illustrates why reliance on a backcross to the Chinese parent or an F_2 generation is unlikely to result in finding an American type timber tree. In the F_2 the desired tree would be found in the "very rare" part of the distribution; many thousands of trees from these crosses would have to be grown to find one with all the desirable traits. On the other hand, the backcross to the American shifts the mode of the population 50% of the remaining distance back to the parent population with each successive backcross. With continued selection for good F_1 resistance the BCF_2 generation can eventually be grown, and the fully resistant American type trees attained.

The previous breeding programs did not grow sufficient numbers of the segregating F_2 generations from the F_1 hybrids to find the "very rare" timber tree. This may sound surprising since it is generally known that many thousands of controlled pollinations were made. A summary of the crosses made in the USDA program (Clapper, 1954) reveals, however, that most of these crosses were based on hopes. In fact, the USDA grew only 228 F_2's, far too few to expect to find trees desirable in all respects.

Dr. John Shafer, Jr., of Logansport, Ind., has privately grown 100 additional F_2's from seed obtained from Diller of the USDA. These trees were grown under forest conditions and two of the few survivors (now 30 years old) appear to have good form and adequate blight resistance, since blight was certainly present in the plantation during the entire period. The odds for finding desirable trees in F_2's from the first backcross generation (BC_1F_2) should be better, much better in the BC_2F_2, and virtually a certainty in BC_3F_2.

The specific design of the Minnesota program is as follows: first backcross (to American chestnut) seed is being produced, both by hand pollinations and by mass pollination of large isolated trees. This BC_1 seed will be planted in isolated seed orchards ("A" type) where no pollen source other than these BC_1 trees in the orchard itself will be available. The first such "A" type seed orchard will be at Oberlin College, Oberlin, Ohio, on college land. The trees in the "A" orchard will be tested for blight resistance at about 6 years of age, and only the most resistant trees will be saved. Pollen from the best of these BC_1 trees will be used on American chestnut trees to create the second backcross BC_2 generation. In addition, open pollination within the "A" seed orchard will produce seed for a BC_1F_2 generation. This BC_1F_2 generation will be grown in an isolated "B" type seed orchard for this segregating generation, and will provide definite scientific information on the genetics of blight resistance. These trees also will be challenged with the blight, and only those with full resistance will be saved. Those trees should breed true for blight resistance, and will furnish a test of the hypothesis. Since some of the trees may appear to be fully resistant but be $R_1R_1R_2r_2$ or $R_1r_1R_2R_2$, some susceptible trees may occur among their future progeny.

Tree Form Frequency Distributions:
Chinese(C), American(A), and Hybrid Chestnuts

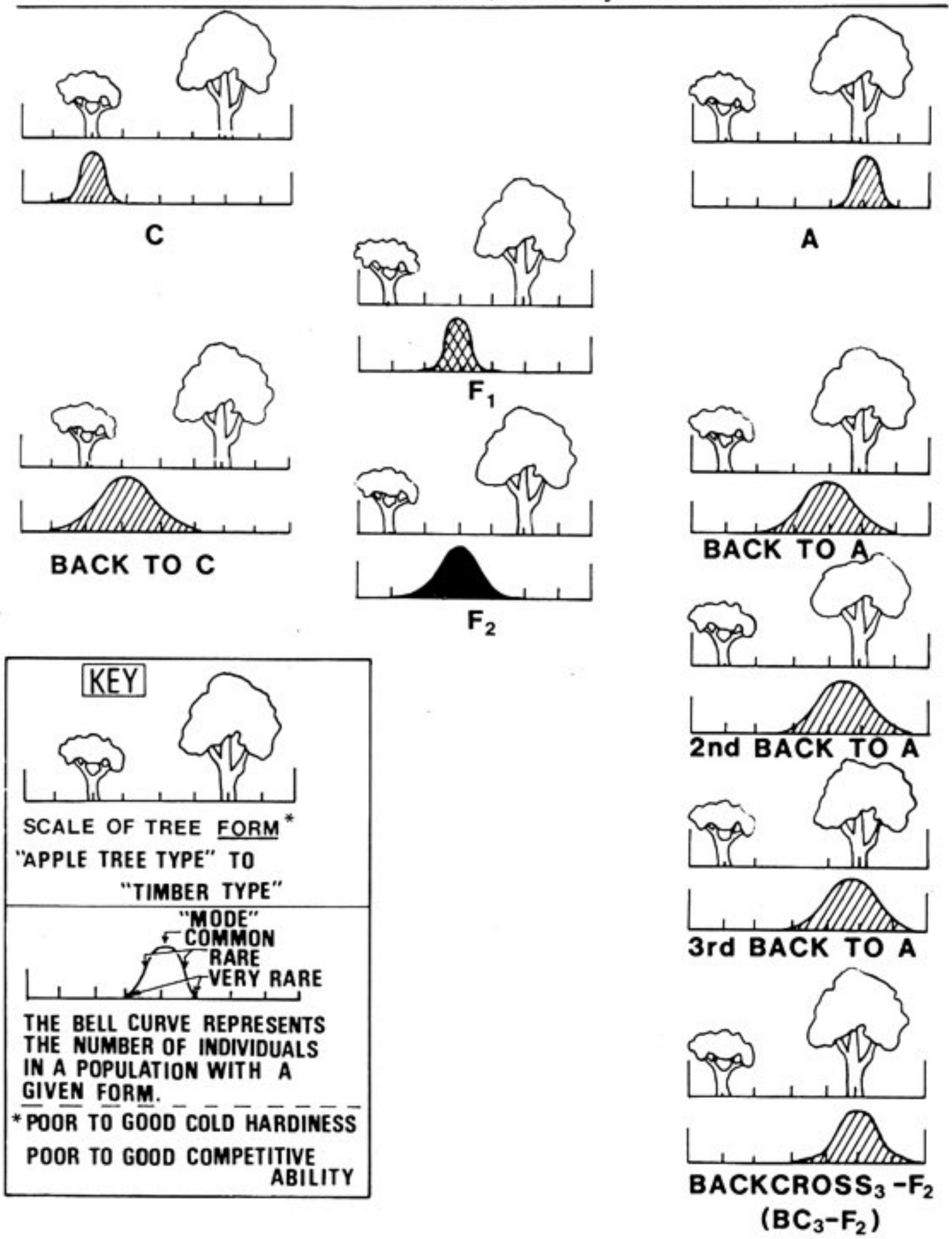

FIGURE 3

The second backcross (BC_2) generation will be grown in its own "A" type seed orchard, and the BC_2F_2 in its corresponding "B" type seed orchard. It is possible that gains in form and vigor may be sufficient at this point, and the BC_3 generation might prove unnecessary, although it seems desirable now.

The progam will continue, with modifications as we learn more, until the goal is reached. If, as is possible, the genetics of blight resistance should prove to be more complex, this program will not lose us any time. It is, in fact, designed to demonstrate, through progeny tests and pedigrees on all trees used for breeding, just what the genetic situation is. Since the two-gene hypothesis is the simplest one consistent with current knowledge, the rules of scientific research dictate that it should serve as the working hypothesis until proven or refuted. The BC_1F_2 generation will either prove or refute it. If it should be proven, the program will continue as designed. If refuted, larger numbers may be needed, but the trees in the A and B orchards will still be producing seed that could be used for that purpose.

Not to be overlooked is the possibility that the BC_1F_2's might be a source of useful nut cultivars with better cold hardiness than the Chinese chestnút cultivars currently in the market.

Needs of the Program for the Future

1. American chestnut trees with sufficient isolation that they produce few, if any, nut-bearing burrs. These trees can be mass pollinated to produce the controlled backcrosses needed in the program.
2. Permanent sites in diverse geographic areas for the seed orchards. These should be administered by an institution or organization so they will be continued regardless of changes in personnel. The first such site is located at Oberlin College, Oberlin, Ohio, on land owned by the college and dedicated for use in the project.
3. Progeny tests of individual American chestnut trees to identify superior seed parents, superior in nut quality as well as other characteristics. These will be in areas without blight.
4. Locations of flowering C x A F_1 hybrids that have been shown to be blight resistant. Pollen from these can be used on American chestnut trees.
5. A plantation for parents and hybrids (reciprocal crosses) of all *Castanea* species. This would be the source for material needed for basic scientific studies, e.g., the development of a biochemical method of screening for blight resistance.
6. New introductions of Asian chestnuts. Plant introduction records indicate there is a wide range of unsampled diversity.
7. Eventually there will be a need for financial support.

A word about hypovirulence: while it appears promising, the end result is still doubtful, both in practice and theory. Additionally, photos of European trees "cured" by hypovirulent strains still show considerable damage to the timber value of the trunk. It would be most desirable to have both genetically resistant trees and hypovirulent blight strains to minimize damage to the trees. Tests for the possible presence of (different) resistant genes in the native American population also need to be continued. It is possible also that certain of the genes for blight resistance in the Japanese chestnut may be different from those in the Chinese chestnuts. It is clearly not time to abandon any of these lines of attack on the blight.

Summary

A program of crossing Chinese x American chestnut first generation hybrids back on the American parent is outlined, which we believe is promising for the addition of blight resistance to the American chestnut. The program will furnish for each hybrid tree used for crossing a progeny that can be tested for blight resistance. A hybrid tree that did not carry resistance but was used for backcrossing will have only blight susceptible progeny. It and its progeny can then be destroyed.

The future needs of the program are listed. If you can supply any of those needs, or wish to dispute any of the theory put forth here, please contact:

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