

**THE  
JOURNAL**

**OF**

**THE AMERICAN  
CHESTNUT  
FOUNDATION**

A NATIONAL FOUNDATION  
DEDICATED TO  
THE RESTORATION OF

**THE AMERICAN CHESTNUT TREE**

VOLUME TWO-NUMBER ONE

December, 1987

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CHESTNUT FOUNDATION  
Volume Two, Number One  
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## THE PRESIDENT'S MESSAGE

The first thing on my mind as I sit down to write this message is an apology. We have been slow in our communications this year, slow with the Journal, and slow with other letters and news. You deserve to know how the projects are progressing, and we will strive to do better in the coming year. There is a simple reason, however, which I think you will appreciate. The interest in chestnut is simply so large that our volunteer workers (the Journal editors, for example) are often swamped, with just too much to do.

The Foundation has reached an awkward, adolescent, stage of growth. In the past year it has been necessary to move away from the "all volunteer" stage, and hire some help on occasion. For example, I am now paid, on a ¼ time basis, to pursue the administrative and fund-raising needs of the Foundation. I actually work at it considerably more than that, but that is what we can afford at the moment. We have also authorized the hiring of a ½ time scientific assistant.

And it is not enough. All the time I can spare from the other work I must do to feed my family is not enough to truly to the job right. I am embarrassed to tell you that I am looking at my desk, and there is a (small) pile of letters from interested members and potential members, that is still unanswered. These folks deserve quick, thorough replies to their questions, but at the moment there are simply not enough hours in the day to get to them all. That is why I call this an "adolescent" phase—we have some paid help, but we need some full time professional workers. We hope to mature, and be able to afford the help we need and the tree deserves in the coming year—in the meantime, please bear with us. The progress we have made so far is steady, and remarkable for such a new organization. Better days are ahead.

Some news of the stand of trees in Wisconsin: if you read the last President's Message, you know how fascinated we have been with the magnificent grove of self-established trees there. You know that they are unblighted, and look like the trees that disappeared from the original range years ago. I was very fortunate to be able to spend a week working the stand this spring with Dr. Fred Paillet, measuring some of the ecological parameters of the grove, and trying to learn more about the chestnut's natural methods of reproduction. We did learn some very interesting things, which will be reported elsewhere, but we did, also, find the blight. On the last day of work, in the last corner of the stand, there was one tree, standing dead. When we looked closely we found 2 more with beginning infections. We were heartbroken. Visions of this beautiful forest dying rapidly

from the blight, as so many others before it, came to us instantly. The loss seemed even greater to us after having almost lived among the trees for a week, and having come to believe we would be able to study, and enjoy them, for years to come. We had already set up plans for future research, in fact. We called the Wisconsin Department of Natural Resources, which promised the best help they could manage, but we did not have much hope that the blight could actually be stopped. Past experiences have all been bad.

After calling around the country to gather the best opinions of chestnut researchers on what to do, the decision was made to spray the trees with a foam, to immobilize any spores on them; cut them down, and bury them on the spot. It seemed like the best idea available. This was done, within a week of the report of blight: then all we could do was wait.

Now the good news: I visited the site again in early September, to show it to a writer working a chestnut story, and we went to see the place where the trees were buried. I counted 20 chestnuts, from saplings to mature trees, within 50' of the place where the blighted trees had been. We could not see any sign of blight, anywhere.

It is too early to relax, and think that the blight has actually been controlled but it is clear that at least there is no raging epidemic, and we will be able to study these trees for a few more years. There is much to learn, and the trees this spring seemed willing to teach us. In a way, we were lucky. If we hadn't been there, looking at the forest so intensively, the infection could easily have gone unnoticed until it was much more extensive and had become unstoppable. As it was, we caught it as early as might be. Maybe our luck will hold.

One more little story, this time about a single nut. One solitary nut sounds like an unimportant part of this grand project we work on. But it meant a great deal to me.

This spring, in looking at the young hybrid trees from our breeding stock, in a greenhouse at the University of Minnesota, I did a double-take and then greatly surprised the student I was with by letting out a whoop and jumping up and down a few times. There, among dozens of others, in a small pot (too small for the roots, in fact) was a little chestnut tree; from a nut I had made myself. This particular tree is  $\frac{3}{4}$  American and  $\frac{1}{4}$  Chinese, and may be carrying the blight resistance we seek. It was just starting to put on its third suit of leaves, that is, it was two years old, and starting its third growing season. Very young. Only about 3 feet tall. And flowering. I was thoroughly amazed, but there they were; several full size male catkins, soon to shed pollen. I arranged to have a volunteer collect such pollen as he could, and send it to me. The pollen (a very tiny amount) arrived in good time for the breeding season in late June,

and I took it to a tree in Iowa, where it was just enough to put on 10 female flowers. And then I had to wait until September to see if anything developed.

For trees to flower so early is remarkable, but not unheard of. Our guess is that the stress of being in a too-small pot, and growing in a greenhouse, somehow stimulated the tree to begin flowering years ahead of its normal schedule. Some of the scientists I have told about this tree speculate that this is an indication such early flowering might be induced on a regular basis by several artificial means. That would mean it would be possible to breed the trees much faster than we had hoped. It is always possible, of course, that such early, unusual flowers might be sterile, and useless. So I waited impatiently. One of the aggravating things about breeding chestnuts is that the burr, which contains the seed, grows and develops whether there are any seeds inside or not, making it impossible to know whether pollination attempts were successful until the last moment before the nuts are ripe.

In September, when the time came to harvest the year's hybrids, I eagerly went to the branch where I had placed the pollen from the little potted tree. Most of the burrs had already opened and dropped-empty. But one, still green, hung on. I picked it, gently opened it, and there it was; a fat, brown, healthy nut. Just one.

Nothing to get too excited about. No particularly good pollination success, in fact. But as I held the one nut in my hand, I could see a good deal more than just one seed. For one thing, the nut is 7/8 American and 1/8 Chinese, and may, just may, be carrying the resistance to the blight. That was exciting. Such hybrids should be able to grow well in our forests. And, we know now that early flowers are fertile, and can make quick generations. The one nut promises more-more nuts, and eventually, more trees. I held it in my hand much longer than was necessary to write down its data, label it, and store it.

In fact, more than once I have taken it out of storage, just to hold it again.

Philip A. Rutter

### **LETTER FROM THE EDITOR**

As the new editor of the Journal of the American Chestnut Foundation I would like to thank you for your interest in the American chestnut tree and the American Chestnut Foundation. Due to the change in editorship, this edition of the journal has been delayed. It will be the only edition of volume two. The first edition of volume three will be published by early summer 1988 and the second edition by late fall 1988. Michigan State University will handle our printing

needs. I am accepting articles on research in progress, research reviews, new thoughts and ideas and historical perspectives.

Several new organizations involved in chestnut culture have recently formed. This demonstrates the interest thousands of people still have or have developed in the American chestnut tree. This particular organization is different from other chestnut groups in that its roots are embedded in science and technology. To restore the American chestnut tree; whether in orchards, plantations or in the forest will take a combined effort of horticulturalists, breeders, foresters, entomologists and plant pathologists. But scientists are not the only requirement. It will take involved, interested and enthusiastic citizens who want a nearly endangered species returned to its natural state. We must continually remind people of the exceptional quality of the American chestnut tree and its nut, the important role it played in its habitat and that there are methods worth developing to bring back this species. The chestnut story, its splendor, devastation, biological control and the potential for breeding, is a story that is already exciting and the best is yet to come. I believe, and so do many of you, that the restoration of the American chestnut tree is a pursuit that is well worth the attempt. So let us combine our resources and efforts and support this foundation so it may begin the job of bringing back the chestnut.

Dennis W. Fulbright  
Michigan State University  
Editor

The Journal of The American Chestnut Foundation is published twice a year by The American Chestnut Foundation, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108. The Foundation is a national foundation incorporated in the District of Columbia. It has received notification from the Internal Revenue Service that it is exempt from income tax under § 501 (c) (3) of the Internal Revenue Code, and is qualified as a public supported organization under §§(a)(1) and 170(b)(1)(A)(vi) of the Code. Donors to the Foundation may deduct contributions as provided in §170 of the Code. Regular Dues are \$15.00 per year. Manuscripts to be considered for publication should be sent to Dennis W. Fulbright, Editor, Journal of the American Chestnut Foundation, Department of Botany and Plant Pathology, Plant Biology Building, Michigan State University, East Lansing, MI 48824-1312. The Journal is printed at Michigan State University, an affirmative action/equal opportunity institution.

**PROPOSED STRATEGIES TO PRESERVE AND  
RESTORE THE AMERICAN CHESTNUT  
BY L. L. INMAN**

The most advanced lines in the backcross breeding program of the American Chestnut Foundation are believed to be only one generation of crossing from the synthesis of a chestnut with the resistance of the Chinese parent combined with the forest type of the American chestnut. These are seedling trees grown from seed produced by hand pollination the past few years. They are 7/8 American chestnut. If backcrossing is continued an additional generation, trees will be produced that are 15/16 American chestnut.

Contrast this with the progress in previous efforts. Initial crosses between susceptible forest-type American chestnut and blight-resistant orchard-type Asiatic species were made by Van Fleet of the USOA and by Burbank in California before 1900. Breeding work was resumed by the USOA in the early 1920s and later by the Connecticut Agricultural Experiment Station, New Haven. Thousands of crosses were made in almost all possible combinations between Chinese, Japanese, American, and other species of chestnuts. The USOA program was discontinued about 1960, and relatively few crosses have been made in Connecticut in recent years.

One hybrid, officially named the 'Clapper' tree, a first backcross, was described as the most promising among all the hybrids; as the "longsought-for-combination" of blight resistance and excellent form. Contrary to expectations, it was not widely propagated.

From published descriptions of research, Dr. Charles Burnham recognized that the backcross method has been applied in an illogical manner. Only a few were first backcrosses and none were second backcrosses to American chestnut. Most backcrosses were to the resistant Asiatic species.

Since the backcross method has been successful in breeding disease resistant varieties of many crops, why not use it to produce blight-resistant American chestnuts? By enlisting the cooperation of private breeders and those who had been associated with earlier breeding programs, records and information were obtained about hybrids that had survived the blight and were flowering. A few hybrids were identified that could save years of work. These have been used the past few years for crosses with American chestnuts to produce the second generation backcross seedlings that are 7/8 American chestnut, as mentioned above.

If the American chestnut is again to become a useful species for coming centuries, the addition of blight resistance will not be enough. It must also be adapted to the various environments where it will be grown.

The current program is expected to produce forest-type American chestnuts that are resistant to blight, but they will have a narrow genetic base and uncertain adaptability. Very few American chestnut trees have been used for the backcrosses.

The natural range of American chestnut extended from north to south and at different elevations in the eastern third of the United States. Centuries of natural selection by climate and other environmental factors have resulted in survival of favorable hereditary combinations. It may be assumed that the species consists of a series of locally-adapted populations or geographic races. Included in the harmonious combinations of genes for adaptation to the local environment would be ones that a plant breeder would be likely to overlook unless the lines had been observed under the conditions of intended use for at least a rotation age. Environmental extremes occur infrequently. Many characteristics may become apparent only as the trees mature.

In general it may be assumed that, as a result of centuries of natural selection, nature has done a better job of breeding forest populations for adaptation to local environments than what a plant breeder can hope to do. A sound policy of hereditary forest-type improvement would be to utilize what nature has selected for survival and adaptation while the plant breeder concentrates on characteristics of obvious value to man.

The American chestnut survives throughout its natural range; as scattered large trees, as living sprouts attached to stumps, and also as pre-blight seedlings with shrub-like form as a result of repeated blight infections followed by sprouting from buds in the root collar. This germplasm is what nature has selected over centuries in the different zones before the blight. Blight-resistance must be introduced into the germplasm in those zones. American chestnuts brought by early settlers are growing outside the natural range but are not suitable for this program. Not only are there inadequate records of their source in the original range, but they are only a very limited sample of the original germplasm.

The proposed strategy has two parts. One is the preservation of the American chestnut germplasm adapted to different geographic zones. The other is the introduction of blight-resistance into each. Begin by using American chestnut survivors in each zone for crosses with Chinese-American chestnut hybrids. First, divide the natural range of the American chestnut into zones, primarily on the basis of climate, latitude, and altitude.

Preserving a representative sample of germplasm from each zone is



essential. It must be available for the later generations of breeding. All or most of the trees within each zone must be included, since the collections must be large enough to include the adapted ecotypes in each zone. Desirable gene combinations may be lost unless large collections of survivors of wild populations are preserved. It may be assumed that certain desirable genes are so rare they are unlikely to be included in a small collection. They probably can be maintained only in blight-free areas with similar climatic requirements. Various possible techniques for propagation must be considered, e.g., tissue culture, grafting on American chestnut seedlings grown in the selected areas, or from rooted sprouts from buds at the root collar. Precautions must be taken against introducing the blight.

Initiation of the second part of the strategy need not wait for completion of the current program. Trees now being used as sources of resistance for backcrosses can be crossed on fruiting survivors in each of the zones. The resulting backcross hybrids and the germplasm banks will be developing at the same time. Blight-resistant trees among the backcrosses will be used for crosses later with the trees in the gene bank for the same zone. Successive generations of breeding are needed. The resulting hybrids should be adapted for growth in the zone in the natural range from which the collection came. The sources of blight-resistance are the hybrids involving the Chinese chestnut, and the three American chestnut trees that have resistance.

Techniques must be developed for using the gene banks: crosses of large numbers of unrelated trees with a minimum of work and time. As soon as the blight-resistant backcross selections begin to produce pollen, they can be used for crosses before they produce nuts. Induction of precocious flowering, and utilization of self-incompatibility for crossing many trees on clones of a single resistant selection, development of methods of screening young seedlings for resistance are some of the possibilities to reduce the time and the amount of work.

Breeding for resistance to a disease involves dealing with two hereditary systems—that of the host and that of the parasite. A single blight-resistant selection propagated as clones has essentially zero hereditary variability. Sooner or later, the pathogen will develop a new form that overcomes that resistance. To meet this problem, genes for resistance from other sources must be introduced. The Japanese chestnut, certain chinquapins, and other *Castanea* species are known to be blight resistant. Backcrossing programs to breed lines of American chestnuts with alternative types of resistance are needed so that they are available when the need arises.

The American Chestnut Foundation is an organization of unpaid volunteers working on a limited budget from private donations to restore

the American chestnut as an important forest tree. Obviously the program must be within the limits of the available resources. A limited program can be continued with current resources; however, a program large enough to avoid delays requires a greater level of support from organizations or institutions with equipment and personnel.

## **HISTORICAL OVERVIEW OF CHESTNUT BREEDING IN THE UNITED STATES**

**CHARLES R. BURNHAM**

Chestnut breeding began before 1900 in the Office of Forest Pathology, Bureau of Plant Industry, USDA. Pollinations of native species were made using the European and Asiatic species then available (Van Fleet 1914). When the blight disease appeared in the plantings in 1907 and the American chestnut and its hybrids developed the disease, work continued only with hybrids involving the European and Asiatic species and the native American Chinquapins. Three resistant selections were given Plant Introduction numbers and were available for use when the breeding work was resumed in 1922, also in the Office of Forest Pathology, USDA. New importations of blight-resistant species were obtained. The general plan was to cross the various blight-resistant species, cross them with the American Chestnut, and cross the hybrids. A similar program, initiated at the Brooklyn Botanical Garden in 1929, was transferred to the Connecticut Agricultural Experiment Station in 1936.

The goal of those programs was a single blight-resistant selection with the desired form of growth, a timber tree. That hybrid would be propagated clonally, the same as for varieties of apples.

Thousands of hybrids between the various *Castanea* species, including the chestnuts and chinquapins, were grown. First-generation (F1) hybrids between the American chestnut and blight-resistant cultivars of the Chinese species were more resistant than the American Chestnut as stated in the legend for the photograph of a row of 15-year-old Chinese X American chestnut F1 hybrids (Berry 1954). They were less resistant than the Chinese Chestnut. Blight-resistance was incompletely dominant.

For a time, the F1's were considered suitable for forest plantings (Clapper 1951), but their resistance was inadequate for long-term survival against the blight (Jaynes 1978).

Relatively few second-generation (F2) trees were grown from the F1's. There were only a few first backcrosses to the American Chestnut [(Chinese X American) X American] and no second backcrosses. Most of the F1's were crossed with the resistant parent.

The success with hybrid corn seemingly led them to believe the same principle could be applied to chestnut breeding. Beginning with openpollinated varieties of corn, inbreeding had been used to establish many true-breeding inbred lines. These were low in yield, but when crossed, a few hybrids were higher in yield than the original open-pollinated varieties from which the inbred lines came. If the two original open-pollinated varieties of corn were crossed, a few rare individual hybrid plants would be expected to be as high in yield as the highest-yielding hybrids between inbred lines. As stated by Jones (1956), "Each individual crossbred tree is a different genotype, comparable to a first generation cross of inbred strains of corn or other plants. All that is necessary to do is to test a large number of these genotypes by intercrossing and growing the progeny".

In 1946 Diller made a backcross, a Chinese X American F1 hybrid backcrossed to the American parent tree "that produced a tree with the long-sought-for combination of excellent form and vigorous growth of the American Chestnut with apparently the high blight resistance of the Chinese parent" (Oilier and Clapper 1969). They suggested the possible use of an elite Chinese chestnut as a pollen source for nut production. (The chestnut is self-incompatible-single trees and clones of a single tree rarely produce nuts). Contrary to expectations, this hybrid, officially named the Clapper hybrid, was not widely propagated (Oilier and Clapper, 1969, based on their final 1964 report). Being from a backcross to the American chestnut, its resistance could have been no greater than that of the F1 hybrid only moderate resistance.

The breeding program in the USDA was terminated about 1960. The row of F1 hybrids mentioned earlier, along with the other breeding material in the area were on rented land. All were destroyed.

When the new breeding program based on proper use of the backcross method was proposed (Burnham 1982), a search began for hybrids that would save many years in the program.

The original Clapper hybrid in a forest-type test planting in Illinois had died. Clones of that hybrid in the Lesesne State Forest planting of hybrids had been killed by frost, but three clones growing at Hamden, Connecticut have been used to produce 3-year-old second backcrosses now growing in Virginia, Tennessee, and West Virginia in blight areas and here at Minnesota, a blight-free area. One- and two-year-old second backcross hybrids are also growing here at Minnesota. These are one backcross away from the third final backcross.

Flowering Chinese X American F<sub>1</sub> hybrids in Indiana, Tennessee, and New York have been used in Ohio and here at Minnesota.

VAN FLEET, W. 1914. Chestnut breeding experience. *J. Hered.* 5:19-25.

BERRY, F. H. Chestnut blight and resistant chestnuts. *USDA Farmers' Bull.* 2068.

JONES, D. F. 1956. Genetics of tree crops, pp. 76-79. 47th Annu. Rep. Northern Nut Growers Assoc.

DELLER, J. D., and R. B. CLAPPER. 1969. Asiatic and hybrid chestnut trees in the eastern United States. *J. For.* 67:328-331.

BURNHAM, C. R. 1981. Blight-resistant American chestnut: there's hope. *Plant Dis.* 65:459-460.

The current American chestnut breeding program is using the Chinese chestnut as the best source of resistance. The blight fungus fails to grow at the point of infection. The hybrid between the two species is moderately resistant, more resistant than the American, but less resistant than the Chinese chestnut. Resistance is incompletely dominant. By crossing that hybrid back to the American chestnut and following with successive backcrosses to the American chestnut, using blight-resistant selections each time for the next backcross, the American chestnut is recovered automatically, and at the same time resistance to the blight is being added by selection. The third backcrosses are 15/16 American chestnut and some will have the gene(s) for resistance derived from the Chinese chestnut, but only from one parent and, consequently, are only moderately resistant. Progeny from crosses between those moderately-resistant selections will include some that have received the gene(s) for resistance from both parents. They are homozygous for those genes are expected to be as resistant as the Chinese chestnut. They will "breed true" for resistance.

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

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

Charles Burnham

**BACKCROSS BREEDING:  
BLIGHT-RESISTANT AMERICAN CHESTNUTS  
II. HARDIER CHINESE CHESTNUTS**

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Backcross Breeding is the Best Method to Breed an American Timber type Chestnut that Retains the Blight Resistance Found in Other Species of Chestnuts.

Chestnut blight has eliminated the American chestnut, *Castanea dentata* as an important timber and nut tree throughout its entire natural range. That range includes southern Ontario in Canada, and in the U.S. extends from southern Maine and the other New England states, south to Georgia, and west to S.E. Michigan, Indiana, Tennessee, and Louisiana. It survives throughout that range, as scattered large trees, as living sprouts attached to stumps, and also as pre-blight seedlings that survive as shrubs as a result of repeated blight infections followed by sprouting from the root collar (Paillet, 1984). These are valuable sources of American chestnut germplasm. Many were planted outside the natural range, including Michigan, Wisconsin, Minnesota, Illinois, Iowa, Washington, and Oregon. Several stands in Michigan, one in Wisconsin and one in Minnesota are reproducing naturally.

The disease appeared in N.Y. in 1904, and, in spite of efforts at control, spread rapidly until, by 1950, most of the American chestnuts in that natural range were dead or dying. Outside the natural range, blight is widespread in Michigan, Illinois, and Iowa. It continues to spread and has been found in one site in southeastern Minnesota (French et al., this Journal, Vol 1, No 1, 1985) and also in Wisconsin.

The search for resistant survivors and tests of large numbers of seedlings identified nine that were resistant. Only recently have a few, widely-scattered blight survivors been found that have a degree of blight resistance (Griffin et al. 1982, 1983).

### *Blight-Resistant Chestnut Species*

The only possible solution at the time was to find other *Castanea* species that were resistant, and then by hybridization and further breeding obtain a blight-resistant, forest-type chestnut.

The Chinese hairy chestnut, *C. mollissima*, and the Japanese chestnut *C. crenata* said to be resistant to the "bark" disease (chestnut blight), were imported and used for crosses by the USDA, beginning in the early 1900s, and later by the Connecticut Agricultural Experiment Station, New Haven.

The general plan of both programs was to make large numbers of crosses between the various blight-resistant species and between them and the American chestnut (A). Most of the first-generation (F<sub>1</sub>) hybrids, Chinese (C) and Japanese (J), x A were crossed with C or J. A few hybrids came from F<sub>1</sub>'s crossed with A. The goal was to obtain a single tree cultivar with the desired timber-type growth, combined with adequate blight resistance.

Although thousands of hybrids between the various species and between the hybrids were obtained by controlled hand pollination, none of them had the desired combination of traits. The USDA program was abandoned as hopeless about 1960. Survivors from those programs include fifteen forest-type plantings of hybrids in blight areas in thirteen states, plantings of species and hybrids at Hamden, Connecticut, and extensive plantings in the Lesesne State Forest in Virginia (see p. 8-13 of the Journal of the Foundation, Vol. 1, No. 2). Chestnut research has continued in Connecticut, but relatively few crosses have been made in recent years.

### *The Backcross Breeding Method*

The backcross method, an efficient way of adding a desired trait to a cultivar that is satisfactory in all or most other respects, has been used successfully by crop breeders since 1922. The following shows how it can be applied to improve the American chestnut and why it is so effective. The American chestnut was not perfect, but had no chance of survival as a timber tree except by adding blight resistance from the Chinese chestnut.

The first step is to cross A with C. The second step is to cross this first generation (F<sub>1</sub>) hybrid back to A. The F<sub>1</sub> hybrids were reported to be more resistant than A, but not as resistant as C. The most blight-resistant backcross individuals are selected for the next backcross to A.

The selection and backcrossing are repeated. Note that the recurrent parent must be the one being improved. This is true even when the trait being transferred is recessive, i.e., shows no degree of dominance. Lack of at least some dominance requires additional precautions: larger numbers of backcross individuals must be

used for the next backcross to be certain that ones carrying resistance are being included for the backcrossing. The earlier chestnut breeders probably would have used recurrent backcrosses to A if the dominance of blight-resistance had been complete.

Only a few backcrossing generations to A are needed to recover the recurrent A parent. The A x C F<sub>1</sub>'s have half of their inherited traits from A. Each backcross to A increases those from A by  $\sqrt{2}$ . On the average, first backcrosses are  $\frac{3}{4}$  A, second backcrosses are  $\frac{7}{8}$  A, third backcrosses are  $\frac{15}{16}$  A. Many of the third backcross plants will resemble closely the A parent. Selecting for desired traits of A as well as for resistance at each step will be equivalent to one or more additional backcrosses.

No other conventional breeding method is as effective as backcrossing for achieving the desired goal, an A that has the disease resistance from C without the undesirable traits of C. The great advantage of the backcross method, properly used, is that recovery of the recurrent parent, the one to be improved, is automatic. It is the method to use also when acceptance of the final product depends on its being the same as the original cultivar, but is now resistant. Previous programs had very few first backcrosses to A, and no second ones.

Information on the number of genes involved in the trait being transferred, in this case, blight resistance of the Chinese chestnut, is useful in planning the numbers required at each step to obtain at least one blight resistant selection for the next backcross. The one inheritance study of the difference in blight reaction between C and A (Clapper, 1952), was based on artificial inoculation of 140 seedlings from the C x A F<sub>1</sub> backcrosses to C. He reported 103 with no cankers or very small ones and 37 with medium-sized or larger ones, a ratio of 3 resistant:1 susceptible; indicating two pairs of genes for resistance that are incompletely dominant. Had they been recessive, the ratio would have been 1:3. This provides a working model for estimating the number required in making blight resistant selections for the next backcross. In the C.A x A backcrosses, 1 in 4 is expected to have the desired genotype (heterozygous for both genes for resistance) the chance of one plant not having that genotype is  $\frac{3}{4}$ , the chance of two plants not having it is  $0.75 \times 0.75$ , or  $\frac{9}{16}$ . If 16 plants are grown, the chance of failure is  $(0.75)^{16} = .01$ , or once in about 100. In F<sub>2</sub>' the desired genotype, pure-breeding for both genes for resistance, is 1 in 16, much larger numbers will be needed, since the chance of failure for each plant is  $\frac{15}{16}$ . For the same chance of failure of .01, 71 are required. To be safe, two or three times as many should be grown. Breeding

populations of blight-resistant trees are the goal of the breeding program.

How long does such a program take? In terms of tree generations (seed to seed): three for the backcrosses, followed by two for increases, one in which the most resistant third backcross trees interpollinate to produce large numbers of  $F_2$ 's, designated BC3  $F_2$ 's, which will include trees truebreeding (homozygous) for both genes for blight-resistance, and one to bring these  $F_2$ 's into nut production. The blight-susceptible  $F_2$ 's are removed, leaving resistant ones to interpollinate. The last two generations, the ones for large seed increases can be grown in seed orchards isolated from other chestnuts.

Each tree generation requires 5 to 8 or more years, although 4 and 5 years have been reported for J x A hybrids (Detlefsen and Ruth 1922). Various techniques have been applied to other tree species to induce early (precocious) flowering. When the probable reasons for failure were discovered, the backcross method of breeding blight-resistant American chestnuts was described and proposed (Burnham 1981, 1982, Rutter and Burnham 1982). The search for hybrids suitable for beginning a backcross breeding program began. Flowering and fruiting C x A  $F_1$  hybrids and a (C x A) x A first backcross survivor known as the Clapper tree, were found and used for crosses with American chestnuts to produce first and second backcrosses to A, respectively. The latter will require three more generations: one for the third backcross to flower and produce nuts, and two for the final seed increases. The progress reported in the first two issues of this Journal has been largely through volunteer efforts of a group of chestnut enthusiasts who agree that the method should work, along with several involved in the earlier breeding program and many others.

Future plans proposed by Inman include crosses to add blight resistance to American chestnuts representing different geographic zones in its natural range.

Tissue culture cloning has been successful in Paul Read's laboratory in the University of Minnesota Horticulture Dept. Using those techniques, the backcrosses can be grown here in a blight free area, each hybrid multiplied, established on its own roots, and then grown for blight resistance tests in blight areas.

### *Hypovirulence*

Apparently, based on recent reports, blighted American chestnut trees can be saved by inoculating them with hypovirulent (diseased) strains of the fungus that check the blight. Even with the promise this approach offers, there remains the need for genetically resistant chestnuts. This goal can be reached only by the



goal can be reached only by the backcross breeding program now in progress. Selection for blight resistance must be based on using virulent strains of the fungus.

## **BACKCROSS BREEDING: II. HARDIER CHINESE CHESTNUTS**

A chestnut with orchard-type growth, primarily for nut production, was the goal of another section of the USDA. Beginning in 1927, importations of nuts and scions from outstanding chestnut nut trees in China and Japan were used in a breeding program based on selection and progeny testing of individual trees (McKay and Berry 1960). This resulted in the naming of three single-tree nut cultivars, Meiling, Kuling, and Nanking. Seedlings from the importations and also hybrids were distributed to state agencies and private experimenters who also made selections and named several single-tree cultivars. The Connecticut Experiment Station described nine single-tree cultivars, many suitable for nut production (Jaynes & Graves 1963). Two of these, Essate-Jap and Sleeping Giant, may still be available.

In 1935, the USDA, in cooperation with the Auburn University Agricultural Experiment Station in Alabama, established a planting of 2,000 seedlings from open pollinated Chinese chestnut nut cultivars. Three single-tree selections were described for release in 1980 (Harris et al.).

The Chinese chestnuts available in nursery catalogs require a climate like that of the peach. A hardier chestnut is the second goal of the current breeding program. American chestnuts growing in areas requiring greater cold hardiness can be a source of hardiness. In this program, C x A F, hybrids are backcrossed to C accompanied by selection for greater hardiness. Many of the survivors in the USDA-Connecticut forest-type test plantings mentioned earlier, are (C x A) x C first backcrosses that might be used for this backcross breeding program.

For more details on the early breeding work, the backcross breeding method, the current status of the American chestnut and current efforts, see Burnham, et al., 1986.

## **REFERENCES**

Berry, F. H. 1954. Chestnut blight and resistant chestnuts. *Farmer's Bull.* 2068.

- Burnham, C. R. 1981. Blight-resistant American chestnut: there's hope. *Plant disease* 65:459-460.
- Burnham, C. R. 1982. Breeding for chestnut blight resistance. *The Nutshell* 35:8-9.
- Burnham, C. R., P. A. Rutter, and D. W. French. 1986. Breeding blight-resistant chestnuts. *Plant Breeding reviews* 4:Chapter 11, 347-397.
- Clapper, R. B. 1952. Relative blight resistance of some chestnut species and hybrids. *J. Forestry* 50:453-455.
- Griffin, G. J., F. V. Hebard, and J. R. Elkins. 1982. Blight resistance in American chestnut. 73rd An. Rpt. Northern Nut Growers Assn. pp. 66-73.
- Griffin, G. J., F. V. Hebard, R. W. Wendt, and J. R. Elkins. 1983. Survival of American chestnut trees: Evaluation of blight resistance and virulence in *Endothia parasitica*. *Phytopathology* 73: 1084-1092.
- Jaynes, R. A. 1979. Breeding and naming nut trees. Chapt. 25, *Nut tree culture in North America*. (ed.) R. A. Jaynes. Northern Nut Growers Assn. Hamden, Conn.
- Kaufert, F. H. 1977. Prospects for American Chestnut (*Castanea dentata*) plantings in Minnesota and neighboring upper Mississippi Valley states. *Minn. Agr. Exp. Sta. Misc. Rpt.* 144. Forestry Serv.
- Paillet, F. L. 1984. Growth, form and ecology of American chestnut sprout clones in northeastern Massachusetts. *Bull. Torrey Bot. Club* 111 :316-328.
- Rutter, P. A. and C. R. Burnham. 1982. The Minnesota chestnut program-new promise for breeding a blight-resistant American chestnut. 73rd An. Rpt. Northern Nut Growers Assn. pp. 81-90.

## **PRELIMINARY STUDIES OF POLLEN TUBE GROWTH IN CASTANEA**

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In the course of helping Dr. Charles Burnham with pollination of the chestnut trees at the University of Minnesota Landscape Arboretum, I initiated studies of pollen tube growth in *Castanea*. Several questions can be asked of samples taken for pollen tube observations. Based on the progress of flowering the samples taken this year we decided to check if some crossing had occurred on the trees at the Arboretum before the female flowers were bagged.

Two sets of samples were taken. The first set of samples was taken on July 9, 1986, the day our pollinations were accomplished. Since the catkins were shedding pollen and the styles were protruding from the burrs at emasculation and bagging of females (June 27), I wanted to check if natural pollination had occurred. The second set of samples was taken on July 18 when pollen was applied again to about half of the flowers pollinated previously. If pollen tubes were found in a greater proportion of styles in the second set of samples compared with the first, then our first pollinations resulted in pollen tube growth from viable pollen. Each time several samples were taken from several flowers from several trees.

The technique I followed is commonly used in research of pollen tube growth in many species and is described by Kho and Baer (1968). Briefly, the styles are fixed in farmer's solution, softened with potassium hydroxide solution, stained with 0.1 % aniline blue in 0.1 N K<sub>3</sub>P0<sub>4</sub>, and placed on a slide and viewed under a fluorescence microscope. The one difficulty found with these samples was that upon contact with the potassium hydroxide, the samples and solution darkened. Later this was found to probably be due to tannins in the tissues.

In the first set of styles collected, six out of nine specimens had pollen tubes visible in at least one style of each specimen. Each specimen consisted of one to several styles of a burr. Of the six specimens that contained pollen tubes, three were collected from flowers that had not been bagged and in which we could expect to see pollen tubes. Of the bagged specimens, two of six showed

pollen tubes. Even from these few samples examined we found that at least some crossing had occurred before bagging.

The second set of styles consisted of eight samples from various flowers of various trees all as a result of planned crosses and one sample from an unbagged burr. Eight of the nine samples showed at least one style with pollen tubes. Styles of one of the planned crosses showed no pollen tube. Although I made no counts, styles with pollen tubes seemed to be present in greater numbers compared with those in the first sample set.

The data from these samples indicate that some pollination occurred before bagging of the burrs. Since a greater proportion of the styles of the second set of samples showed pollen tubes our pollinations were done during the receptive period and pollen was at least partially viable. Since this year's crosses at the Arboretum were with pollen of Japanese and Chinese chestnuts, non-hybrids from crossing before bagging should be distinguishable from hybrids during early growth of the seedlings.

One very interesting and important observation became apparent from the samples studied. Pollen tubes in samples of a few very good preparations showed tubes (some with pollen grains still attached) entering the style only at the very tip of the style. In no samples could any other point of entry into the style be observed. These observations proved difficult as the styles themselves exhibited fluorescence probably due to tannins present in the style tissue but the entering pollen tube or its exit from the style could be seen clearly. If the pollen grains germinate and grow into the style only at the tip of the style and not along its flank, breeders should make sure that at least this area is pollinated when crossing.

An important area that I wanted to study deals with the self-unfruitfulness of the chestnut. In the scientific literature, this is called self incompatibility. This is the inability of a plant to set seed from pollination with self pollen. Self pollen does not result in fertilization of eggs and no nuts are produced. Cross pollen from other trees usually but not always produces fertilized eggs and results in nuts.

Two major systems of incompatibility are termed the gametophytic and sporophytic systems. Correlated with sporophytic incompatibility is a barrier to self pollen tube germination and growth at the stigma surface. Gametophytic incompatibility exhibits a difference in growth rate between self and compatible pollen tubes. In either system, cross pollen tubes are not inhibited and fertilize eggs.

Genetically, there are important reasons for knowing the incompatibility system. In a population of a given size, a greater proportion of the crosses will be compatible in the gametophytic system of incompatibility. Knowledge of the incompatibility system operating can help to prevent wasted effort on incompatible crosses in a breeding program. Breeding chestnuts for nut prod-

uction will require at least two selections that are cross compatible to get nuts produced. Sweet cherries, apples and filbert are self incompatible and require interplanting of cross compatible individuals for production.

There are several reasons for continued studies of pollen tube growth in chestnut. Pollination of chestnut styles with known compatible pollen over a range of ages might help in determining very exactly the period of receptivity of the styles. Very important is the determination of the incompatibility system operating. Inasmuch as the system of incompatibility is correlated with the site of inhibition of pollen tubes, further pollen tube growth studies may tell us which system is operating. After this is found, tests can be done among breeding material or promising selections. Depending on the ease of determination of compatibility, it may be possible to test for incompatibility within a crossing season and plan crosses known to be compatible.

Kho, Y. O. and J. Baer. 1968. Observing pollen tubes by means of fluorescence. *Euphytica* 17: 298-302.

## **FOUNDATION NEWS**

### **A NATIONAL CHESTNUT RESEARCH CENTER A LONG-RANGE GOAL**

During my speaking tour last fall it became clear to me that interest in the chestnut is so broad and enthusiastic that we may not be thinking "big" enough in our current plans. As a consequence, I began raising the possibility of a National Chestnut Research Center with the people I was meeting, both laymen and scientists: I never received a negative response. On the contrary, the idea was universally received with enthusiasm and suggestions for what should be included.

I also mentioned the idea to several people in the philanthropic community, and found enthusiasm here, too. The point they raised which encouraged me to ask that this be formally adopted as a long-range goal of the Foundation is that philanthropists are often more interested in large, inclusive, sweeping programs than they are in funding a part-time worker somewhere. They prefer to fund programs that are highly visible and have high goals. It is, in fact, easier to fund grand projects than mundane ones.

The Center as currently conceived will consist of a laboratory located in

in the central part of the old chestnut range, with facilities for several permanent researchers and their support staff, and additional lab space for visiting researchers. It will also house a library, archives, and computerized breeding records. It should be located on 200 or more acres of first-rate chestnut land, suitable for intensive management.

After due consideration, I am proud to announce, the Board in February, 1987, passed the following:

**RESOLVED:** That The American Chestnut Foundation adopt as a long range goal the establishment of a **National Chestnut Research Center**, to be located within the central part of the old range of the American chestnut, with facilities for research, preservation of chestnut genetic material, and a library of published, unpublished, and archival material relating to chestnuts.

### **Reasons why the Center should be established:**

It will provide an improved focus for chestnut research, and provide a year-round forum for interaction between researchers, which is now sometimes limited.

It will be an effective tool for attacking the other questions of chestnut biology. If our goal is truly the recovery of the species, we must eventually put some research energy into ecology and other pests such as the gall wasp. We do ourselves no favor by ignoring factors other than the blight.

It will establish a "national" focus for the tree.

This future National Chestnut Research Center will require land, buildings, staff, and an endowment. We see it as providing a focus both for research and for the necessary fund raising required to see research completed and implemented. Once launched, it is firmly expected to be self-supporting, drawing on the strong interest in chestnut which is still to be found in the regions where the tree once grew. As the economic importance of chestnut is re-established, industry can be counted on to provide additional funding. For the present, the Center must take a back seat to the immediate needs of basic research funding and the day to day expenses of the Foundation. But the goal is now established, and we can work on it as it is possible to do so. The first thing to look for is the location, and the land. We must seek a donation of land, as we do not have funds for such a purchase; but once the land is acquired, it immediately becomes easier to move to the next stage. Then we can go to potential donors and show them that we have support and a start, and we can ask them to consider funding the next step. And then the next. You, our members, can play a very important role here. Do you know of land that would be suitable, and which might be donated to such

a cause? Are you willing to spend some time searching for such gifts? If this is a project which catches your imagination, and you would like to work for it, please get in touch with me.

P. A. Rutter

### **HONORS FOR OUR OFFICERS**

We regret, and are proud, to announce that Dr. Peter Raven has had to resign as an active Director of our Board. He was elected Home Secretary of The National Academy of Sciences in the past year, a great honor, and also a great addition to his work load. He will continue, however to be a part of the Foundation, as an Honorary Director, our first.

Dr. David Merrell, a Director of the Foundation, has had his textbook Ecological Genetics selected by the Chinese scientific community as the first text on that subject to be translated into the Chinese language. He has been invited to visit China to lecture on the subject, and to be honored by geneticists there.

Dr. Greg Miller, Coordinator for Ohio, was elected as Vice President of the Northern Nut Growers Association, at the July meeting.

Dr. Paul Read, a Director of the Foundation, has moved to Lincoln, Nebraska, where he has been made Head of the Department of Horticulture.