

TECHNICAL SESSION

Chairman: F. Mergen

STATUS OF CHESTNUT BREEDING AT THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION

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The loss of the American chestnut, *Castanea dentata*, within a period of fifty years is the fact with which we are faced. This tree was the most valuable hardwood growing in our Eastern forests, yet it was virtually eliminated by a fungus disease. Large sums of money have been spent by state and federal agencies for research on how to control or eradicate the causal agent, *Endothia parasitica*, but these efforts have been of little aid in stemming the destruction from this noxious fungus. From research on the blight fungus it appears that externally applied chemicals would be uneconomical since the spores are present constantly and the trees are susceptible 365 days of the year. Systemic chemicals are a possibility but prohibitive from the point of view of cost.

To give up the fight for a chestnut tree at this point is rather impractical, especially since there is no replacement for the American chestnut in respect to its timber qualities. A long recognized possibility and hope has been the breeding of a tree similar to *C. dentata* in stature and quality with the added trait of blight resistance. Breeding programs have been developed on the hypothesis that the blight resistance of certain Asiatic species can be transferred by hybridization to the American chestnut.

The first chestnut breeding program aimed at producing blight resistant chestnuts was conducted by Walter Van Fleet at the Bureau of Plant Industry in Beltsville, Maryland, from 1909 to 1922. This work was later continued by G. Flippo Gravatt and Russell B. Clapper of the Division of Forest Pathology, during the period of 1925 to 1949, and recently by Frederick H. Berry of the Agricultural Research Service.

The chestnut breeding project at the Connecticut Agricultural Experiment Station stems directly from a project initiated by Arthur H. Graves in 1930 while he was a curator at the Brooklyn Botanic Garden. In 1939, the Connecticut Station began to cooperate actively with Dr. Graves on the chestnut project and the first plantings of chestnut trees at the Experimental Farm in Mt. Carmel, Conn., were established at this time. In 1947, the administration of the chestnut breeding program was formally taken over by the Connecticut Station and at that time Dr. Graves became consultant in the project, a position he continues to hold. At this time Hans Nienstaedt started at the Connecticut Station, continuing until 1950

When this project was first initiated by Dr. Graves the aim of the program was to develop a timber chestnut tree by crossing *C. crenata*, the Japanese chestnut, with *C. dentata* the American chestnut, and then select among the progeny for blight resistant trees of the desired phenotype. Since 1930, though, the scope of the program has broadened and various lines of approach have been used in an effort to produce better chestnut trees for both timber and nuts.

The early crossing work showed that blight resistance or blight susceptibility is not inherited in simple Mendelian fashion. Many genes are apparently involved.

The fact that we are dealing with such a complex problem has led us to shift some of our emphasis from the immediate development of a forest tree to some of the more basic questions at hand. That is, we are finding that we have to know more about our material, the structure of the genus, and how various quantitative characters are inherited.

At present our understanding of the crossability among the different species in the genus *Castanea* is still inadequate. As described by Aimee Camus in 1929 the genus *Castanea* is composed of twelve species arranged in three series; the series being determined primarily by the number of nuts to a bur along with the position of the nuts in the bur. In our crosses we have utilized, to a greater or lesser extent, all but one of the twelve species of the genus. Over 250 different interspecific combinations have been made, including many reciprocal crosses. From these crosses over 16,000 hybrid nuts have been harvested, or an average of approximately 70 nuts a year. In regard to these crosses it can be said that species within a series cross readily and the offspring of such crosses are usually fertile, though in particular intra-series crosses male sterile offspring may commonly arise. As for crosses among the three series less is known, Clapper listed in 1954 numerous crosses among series in the genus along with the number of seedling progeny he grew from these. He made no mention of having difficulty in making any of these crosses. Our own experience indicates, that, though most of these crosses are possible to make, an incomplete incompatibility mechanism may be working, for it is often difficult to attain appreciable numbers of offspring. Little is known of the viability of the nuts or the fertility of the progeny of these inter-series crosses.

By analyzing the progeny of various interspecific crosses we can learn about the inheritance of the various taxonomic key characters among the species. Normally one would expect these to be inherited in a polygenic or quantitative manner. However, in species that are as closely related as these are, this may not be true, Such a study should lead to a better understanding of the structure of the genus.

Progeny of several crosses are currently being grown together, with the aim of analyzing the inheritance of some of these taxonomic key characters. Seedlings are being used presently since they are considerably easier to work with in large numbers than older trees.

In making an interspecific cross it is of course advisable to compare the hybrid offspring with offspring from each of the parents. Since the chestnuts are normally self-sterile it is impossible to check the

hybrid crosses against inbred progeny of the parents. As a considerable amount of labor is involved in making a cross we are using open pollinated nuts from the parental trees rather than nuts from controlled pollinations to compare the hybrids against progeny of the respective parents.

One hypothesis which we are interested in studying further is whether heterosis or hybrid vigor actually appears in some of the interspecific crosses; and is it a rule or an exception. This is a problem not confined just to the work with chestnuts but in the breeding work of many forest programs. Heterosis can be defined as a general increase in vigor and vitality of the progeny of a cross beyond that shown by the progeny of each parent when self-fertilized. For practical purposes every chestnut is the result of a cross involving two trees of two different genotypes, since they are normally self-sterile. Because heterosis results from a heterozygous condition we see that all the progeny of a self-sterile plant are normally quite heterozygous, due to outcrossing, and thus probably show a certain amount of heterosis. Our question, then, is whether particular interspecific crosses yield offspring expressing vigor and vitality greater than intraspecific crosses of either parent. For our purpose we will call this increased luxuriance heterosis. The variation between species will naturally be greater than that within a species. Hence there will be a greater amount of heterozygosity in progeny of interspecific crosses than in progeny of intraspecific crosses. Thus interspecific crosses may show a greater degree of heterosis or hybrid vigor than the intraspecific crosses.

Observations over the years have indicated that at least certain individual trees of some of our interspecific crosses have shown heterosis when measured in terms of rapid growth and general luxuriance. In 1958 certain pollinations were set up expressly to test this hypothesis. From the data gathered from the seedlings this year (the height of many being over two feet), and on the basis of previous crosses, it appears that the success of a cross is not as dependent on the species involved in the cross as on the particular trees used as parents. More expressly, when crossing *C. crenata* with *C. dentata* the progeny will not necessarily excel both parental species in their respective rates of growth. But choosing an appropriate individual from each of the two species and by crossing them, then it is possible to attain progeny which excel, on the average, progeny of crosses within either parental species. This sort of a situation appears to be true particularly when using parents of complex pedigrees. For example, a cross of R59T39 with R7T15, both of the pedigree CJA. (*C. mollissima* x (*C. crenata* x *C. dentata*)) may give progeny which are superior to the open pollinated nuts from either parent tree. We cannot deduce from this, though, that every CJA crossed with every other tree of the same species pedigree will give good results. Some trees do appear to be especially suitable in particular crosses. In a controlled cross of a *C. crenata* tree located in Cheshire, Connecticut, with four *C. dentata* trees in Roxbury, Connecticut, we find that the offspring of all four crosses are doing better than offspring from open pollinated nuts of the parents. There are no chestnut trees other than those of the same species in the immediate vicinity of each of the respective parents. Thus the open pollinated nuts from the parental trees undoubtedly resulted from intraspecific crossing. In these four CJA crosses, then, we have "hybrid vigor" or "heterosis".

We are also currently interested in some pollen studies: namely how can it be germinated successfully and what is its longevity under various conditions? Preliminary germination tests show that high temperatures, about 30°C., and a 0.5% sucrose solution are suitable at

least for certain species or individuals. Getting consistent results among different trees has been a problem. Pollen collected on match sticks and stored over a dessicant at room temperature and at temperatures approximately 29° C. below zero have shown 70 - 80% germination after several weeks storage. Successful pollinations have been made with pollen from the early flowering *C. henryii* which was stored at this low temperature for three weeks.

Since 1953, when Nienstaedts work indicated that various tannin fractions might determine the degree of blight resistance in the different chestnut species, Bazzigher and other European workers have shown that other factors must also be involved. We are interested in growing tissue of the different host species and in testing these cultures in various ways with the blight fungus. The aim would be to develop a differential test in vitro that would be analogous to the reaction of the host and parasite under natural conditions, if this could be done then we would have a more rigidly controlled system to which we could readily apply various analytical techniques, such as chromatography, to determine the cause for resistance or susceptibility. It is also possible that such a system would allow us to test the susceptibility of a tree while it was still in the juvenile state.

So far we have had success in growing callus tissue of *C. mollissii*, the Chinese chestnut, but our success with *C. dentata* and *C. crenata* has been more limited. By refining our techniques there is little question that tissue cultures of species other than *C. mollissima* could successfully be grown. Unfortunately time has been a limiting factor in following up this particular project.

I have mentioned some of the actual hybridization work we have done along the lines of developing a good timber tree. Something should be said about our planting of these trees, along with how they are doing.

The first hybrid trees produced during the early thirties were planted on the grounds adjoining the Sleeping Giant Park in Hamden, Conn. Soon, however, this land was fully planted and a series of plots was started throughout the State of Connecticut, most of them on Park and Forest Commission land.

In 1947 Jesse D. Diller, of the Division of Forest Pathology, in cooperation with Russell B. Clapper, of the same Division, and Arthur H. Graves, of the Connecticut Station, initiated a series of chestnut plots throughout the entire range of the American chestnut. In each of these plots approximately 150 trees were planted fifty selected *C. mollissima* seedlings and fifty of the best hybrids from both Clapper and Graves. Through 1955, fourteen of these plots had been established in thirteen different states. These plots are on sites of hardwood stands that once consisted primarily of *C. dentata*. In establishing a plot the overstory is killed by girdling. In this manner the seedlings receive sufficient light, yet the disturbance of the forest floor is kept at a minimum.

Observation of the plots in Connecticut and a look at the records of trees in the other plots indicate that we have some promising material. Only time will tell us if these trees are sufficiently resistant to the blight and valuable in their other traits.

One of our biggest problems has been the vegetative propagation of our superior trees. Grafting has been the only practical method that we have had success with so far. This is a rather unsatisfactory method for setting up test plots for the comparison of different clones due to the added variability of the root stock and graft union among the trees. We have done some work on other techniques of vegetative propagation and we hope to report on a successful method in another year. As soon as we are able to clonally propagate our best trees we will set up plots to grow them on a comparative basis. By using grafts of promising trees we have set up a few seed orchards.

It would be nice to report that in our plantings we find a particular species-cross to be the most promising of our hybrids for a timber tree. The fact is that although some crosses appear particularly good, our best individual trees are of quite different pedigrees. The results are similar to those in the study of the seedlings which we are checking for the expression of heterosis, The variation within a species is considerable and the success of a cross is dependent on the particular trees used as well as the species used.

In a breeding program such as we are carrying on we cannot afford to overlook the possibility of resistance developing spontaneously within the native species. Jesse Diller has been particularly active in tracing down reports of blight resistant chestnuts. There is little doubt that there is a difference in the susceptibility of *C. dentata* to the blight. So far, however, no large trees have been found that will produce resistant offspring but large trees surviving the blight are known. These are being used as much as possible in our breeding program.

By utilizing the native and oriental species we have the necessary background variation to produce a superior chestnut tree, Progress is being made towards the goal of a good timber tree but much is left to be done. It is hoped, also, that ultimately the experience of breeding work in the genus *Castanea*. will be of help to other breeding programs, particularly those dealing with hardwood trees.

Literature Cited

- Bazzigher, G. 1957. Tannin - und phenolspaltende Fermente dreier parasitischer Pilze. *Phytopath. Z.*, 29 299-304.
- Beattie, R. Kent, and Diller Jesse D. 1954. Fifty years of chestnut blight in America. *Jour. Forestry.* 323-329.
- Camus, A. 1929. *Les chataigniers. Monographic des genres Castanea et Castanopsis.* Paul Lechevalier. Paris. 604 pp. plus atlas.
- Clapper, Russell B. 1954. Chestnut breeding, techniques and results. I. Breeding material and pollination techniques. *Jour. Heredity* 45: 107-114.
- 1954. Chestnut breeding, techniques and results. II. Inheritance of characters, breeding for vigor and mutations. *Jour. Heredity* 45: 201-208.
- Diner, Jesse B. 1947. Growing chestnuts for timber. *Northern Nut Growers Assoc. Ann. Rpt.* 37 66-70.
- Nienstaedt, Hans 1953. Tannin as a factor in the resistance of chestnut, *Castanea* spp., to the chestnut blight fungus. *Endothia. parasitica* (Murr) A. and A. *Phytopath.*, 43: 32-38.