The Use of Ionizing Radiation to Develop a Blight Resistant American Chestnut, *Castanea dentata,* Through Induced Mutations

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ABSTRACT.— Based on reported successes in developing disease-resistant crop varieties through induced mutation, the prospects for developing a blight-resistant American chestnut, *Castanea dentata,* by this technique seems very promising. Two types of radiation used to produce mutations in American chestnut are gamma rays and thermal neutrons. A total of 8243 M_1 and 10642 M_2 American chestnut trees from irradiated seed are now growing at permanent out-planting sites at 12 locations in seven states.

The loss of the American chestnut (*Castanea dentata* [Marsh] Borkh.) was the worst natural disaster ever experienced by our nation. If all of the chestnut trees alive had been in one forest at the time the blight fungus, *Endothia parasitica (* Murr.) P. J. & H. W. And. was discovered in New York State in 1904, there would have been nine million acres of pure chestnut. The present-day market value of the chestnut lumber would amount to more than $400 billion. By 1925, trees in the entire range of the American chestnut were infected and its destruction as a commercial timber species was considered inevitable.

The use of ionizing radiation to develop a blight-resistant American chestnut was first proposed in 1955 by W. Ralph Singleton at the University of Virginia.

Having learned of this proposal through a newspaper article, I contacted Singleton to offer him two quarts of American chestnut seeds I had collected along the Blue Ridge Parkway. These seeds were irradiated at the Brookhaven National Laboratory and planted at Blandy Farm, Boyce, Virginia, during the spring of 1956. This was the beginning of the irradiation program to develop a blight-resistant American chestnut.

JUSTIFICATION FOR CHESTNUT RESEARCH

There is convincing evidence that a blight-resistant American chestnut tree, with essentially all of the desirable characteristics of the native American chestnut, may be developed within a reasonable time and at a justifiable expenditure of time and money. The most convincing evidence that a blight-resistant American chestnut may exist is the recorded location of old surviving American chestnut trees. Only recently, Forest *et al.* (1977) published a bulletin which describes locations of surviving American chestnut trees in the Genesee Region of New York. This includes 11 counties.

The use of ionizing radiations as a breeding tool has come into its own during the last two decades. The periods of release of mutant varieties are shown in Table 1.

Before 1951, only three mutant crops had been released. From 1957 to 1966, a total of 29 mutant varieties were released compared to a total of 62 from 1967 to 1973.

Table 1
Released Crop Varieties Developed Through Induced Mutations (Sigurbjornsson and Micke, 1974).

Period of Release	Number of Released Crop Varieties
Before 1951	3
1952-1956	4
1957-1961	11
1962-1966	18
1967-1971	51
1972-1973	11
Total	98

The number of crop varieties developed by direct multiplication of mutants compared to crop varieties developed from mutants used in cross breeding is presented in Table 2. A total of 98 crops are listed in which induced mutations are involved.

Table 2
Crop Varieties Developed Through Induced Mutations (Sigurbjornsson and Micke, 1974).

Method of Breeding	Number of Released Varieties
Direct Multiplication of Mutants	85
Mutants Used in Cross Breeding	13
Total	98

The same 98 varieties referred to in Table 2 are shown in Table 3 but listed by crop. This information is presented to illustrate that ionizing radiations have been used to improve a wide variety of crops with different reproductive systems.

Table 3
Released Crop Varieties Developed Through Induced Mutations (Sigurbjornsson and Micke, 1974).

Type of Crop	Number of Released Varieties
Cereals	54
Legumes	21
Fruit Trees	7
Other Crops	16
Total	98

Table 4 indicates the different types of mutagens which have led to the development of superior varieties. These data show the availability of different mutagens at different times. Until recently X-rays were used almost exclusively. With the availability of gamma rays from cobalt and cesium sources more researchers have turned to the use of gamma rays. Fewer varieties have been developed from neutrons but some of the most important mutants have resulted from neutron treatment. Crosses are not included in this tabulation.

Table 4
Mutagens Used to Breed Varieties Through Induced Mutations and Direct Multiplication (Sigurborjnsson and Micke, 1974).

Type of Mutagen	Number of Released Varieties
X-Rays	78
Gamma Rays	24
Neutrons (Thermal)	12
Neutrons (Fast)	3
Other Radiations	6
Total	123

Various characteristics which are claimed to have been improved through mutagen treatment are listed in Table 5. The striking aspect of this list is that induced mutations seem capable of improving almost any character of the plant. Significantly, 24 crop varieties developed through induced mutations were for improved disease resistance.

Tables 1 through 5 are presented to support a philosophy and judgment that mutation breeding is a practical and attractive plant-breeding tool for the American chestnut program. The data in these tables allow the conclusion that the use of mutation breeding is increasing rapidly, that the number of "mutant" crops being grown indicates the economic success of this practice, that ionizing radiations are mutagens useful in producing superior varieties and that disease resistance in crop varieties is frequently achieved through induced mutations.

Table 5
Crop Varieties Developed Through Induced Mutations (Sigurbjornsson and Micke, 1974).

Improved Character	Number of Occurrences in Released Varieties			
	Cereals	Legumes	Others	Total
Higher Yield	27	10	10	47
Disease Resistance	13	9	2	24
Early Maturity	19	9	8	35
Higher Protein	2	2	—	4
Improved Plant Type	3	3	3	9
Easier Harvesting	1	2	—	3

SOURCES OF M_1 AMERICAN CHESTNUT SEEDS

American chestnut seeds to be irradiated for this project were collected from the nine geographic locations listed in Table 6. Only the Missouri and Wisconsin seed sources were from uninfected trees.

Table 6
Sources of Original (M_1) American Chestnut Seeds.

1) North Carolina and Virginia, Blue Ridge Parkway
2) Maryland, Washington County
3) Massachusetts, Orange, Franklin County
4) Missouri, Rolla, Phelps County
5) Ohio, Coshocton and Stark Counties
6) Tennessee, Monroe County
7) Virginia, Bath and Fauquier Counties
8) West Virginia, Pocahontas County
9) Wisconsin, Galesville, Trempealeau County

PROCEDURES AND RESULTS

The radiation dose applied to the seeds was selected to allow approximately 50 percent germination as measured by emerging sprouts after planting. There was no technological or theoretical justification for using 50 percent germination as a criterion.

The data shown in Table 7 were generated over a period of several years (1962 to 1971). The output of the cobalt gamma source was calibrated immediately prior to irradiating the seeds. Planting was completed within 48 hours of irradiation. Of the four trees obtained from 200 seeds irradiated at 10,000

RADS, only one has produced burs after 12 years. Each bur produced only one viable seed with no undeveloped seeds. The trees in each case appeared normal except for slow growth rate.

Radiation dosage with thermal neutrons at 500 REMS to 3,500 REMS is represented in only one experiment. At each radiation dose 52 seeds were involved. However, two separate irradiations were involved at doses of 4,000 REMS in which 1,000 and 2,000 seeds were irradiated. An average survival rate of 42 percent was obtained for all irradiations. Neither moisture content of the seeds nor time from irradiation to planting was rigorously controlled.

Table 7
Per Cent Germination vs. Radiation Dosage.

Type of Radiation	Dosage	Germination (percent)
Gamma Rays	3,000 RADS	62
Gamma Rays	5,000 RADS	40
Gamma Rays	7,500 RADS	22
Gamma Rays	10,000 RADS	2
"Thermal Neutrons"	500 "REMS"	97
"Thermal Neutrons"	1,000 "REMS"	82
"Thermal Neutrons"	1,500 "REMS"	88
"Thermal Neutrons"	2,000 "REMS"	84
"Thermal Neutrons"	3,000 "REMS"	90
"Thermal Neutrons"	4,000 "REMS"	31
"Thermal Neutrons"	6,000 "REMS"	28
"Thermal Neutrons"	8,000 "REMS"	6

Table 8 lists cooperators with interest and activity in this American chestnut project. Each participant, in his location, is making an important contribution. At present, there are approximately 8,243 M_1 trees and 10,643 M_2 trees. A total of 16,000 M_2 seeds and 430 M_3 seeds were harvested in 1977. With the current population of M_1 and M_2 trees, increasingly large number of M_2 and M_3 seeds will become available. With this impressive inventory of trees and seeds it appears that ample material will be available for use in any and all chestnut research, including re-irradiating M_2 and M_3 seeds. Permanent outplanting space for each M_2 and M_3 generation must be made available.

CONTINUING RADIATION PROGRAM

As noted in Table 8 under totals, there are listed 8,243 M_1 trees, 10,643 M_2 trees, 16,000 M_2 seeds and 430 M_3 seeds in current inventory. The number of M_2 trees and seeds will increase rapidly. We should continue to increase the M_1 population using mutagens, thermal neutrons and gamma rays, at programmed doses which will allow germinations ranging from 2 to 90 percent as measured by emerging sprouts after planting.

The effect of variation in irradiating techniques and condition of seeds should be investigated. These variations may include: state of dormancy; moisture content of seeds; elapsed time from collection to irradiation; degree of active sprouting; elapsed time from irradiating the seeds to planting.

In mutation breeding it is the M_2 or subsequent generations that may produce the blight-resistant chestnut tree. Sector mutations will appear in the

Table 8
Inventory—Trees and Seeds.

Location	Tree Ages Years (1978)		Number of Trees		Source of Information
	M_1	M_2	M_1	M_2	
Brooklyn Botanical Garden, N.Y.	—	2	—	150	C. Hibben
Johnstown, PA	—	3	—	300	H. Mackey
Lesesne State Forest, VA	9	2	3,000	2,000	T. Dieroff
Medina County, OH	5	3	600	2,200	A. Dietz
Monterey, TN	—	3	—	200	O. Williams
Monroe County, OH	6	—	1,200	—	A. Dietz
National Colonial Farm, MD	9	4	1,000	159	R. Singleton
Stronghold Inc., MD	9	5	1,000	500	R. Holland
VPI, Blacksburg, VA	—	4	—	300	G. Griffin, J. Elkins
WVU, Morgantown, WV	—	3	—	100	F. Cech
Nicholas County, WV	16	—	600	—	A. Dietz
Clements Nursery, Lakin, WV	1	1	22	1,900	A. Peaslee
Parsons Nursery, Parsons, WV	1	1	421	1,922	D. McCurdy
Wadsworth, OH	7-16	2	400	12	A. Dietz
Total - Trees			8,243	9,743	
Total - Seeds 16,000 M_2					
430 M_3					

first generation, and recessive mutations, such as blight-resistance, may appear in subsequent generations. It is the M2, M3, and Mn generations in which blight resistance may appear. As we establish large populations of M2, M3, M4 . . . Mn tree generations, tests will be developed to determine:

1) Blight resistance at an early age;
2) Decay resistance, density and strength of the wood;
3) Timber characteristics of the trees;
4) Adaptability of growing trees to a wide variety of soils, including rocky mountain ridges;
5) Ability of the stump after harvesting to sprout to reestablish the tree population;
6) Storage properties of the seeds compared to the best American chestnut seeds.

To continue this chestnut program at an accelerated rate, large acreages for permanent outplanting are needed. With the immediate availability of M2 trees and M2 and M3 seeds, 40 acres will be needed within the next two years.

In Illinois, Indiana, Kentucky, Ohio, Pennsylvania, Tennessee, Virginia and West Virginia there are vast areas of reclaimed strip mined land. The best of this land is ideal for growing American chestnut trees. Can it be made available for this chestnut program?

THE DECISION?

The American chestnut program deserves the effort of a "Manhattan Project" or a "moon Landing Project." A blight-resistant American chestnut would be a valuable renewable resource. The success of the program is within our technical and economic resources.

LITERATURE CITED

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