Use of Neutron Irradiations in the Brookhaven Mutations Program*

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1. INTRODUCTION

The cooperative program was initiated at Brookhaven National Laboratory in the early 1950's* to help plant scientists gain more knowledge of the extent of the usefulness of ionizing radiations in plant breeding. The purpose of this paper is to present a summary of some of the information obtained by the cooperators over the past 10 years.

A description of the origin, organization and facilities of the Cooperative Mutations Program has been presented by Shapiro.[1] A more detailed report by the same author was presented to the Joint Committee on Atomic Energy.[2]

The facilities available to the cooperators are the same as described in the two previous papers. In brief, these facilities consist of a 250 KvP X-ray generator; two areas of a research reactor, one a well thermalized unit of moderate capacity and a larger area with a mixed thermal and fast neutron distribution, all of which are used for brief, acute exposures. A 10 acre field, currently with almost 4000 curies of cobalt 60, serves to irradiate entire plants for either short or long periods of time. Recently, the flux density of the thermal column was increased by a factor of 5 over the original density (Table I).[3] This was accomplished by lowering the thermal column 12 inches deeper into the

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* The original program was begun in late 1952 at the instigation of Drs. Curtis, Singleton and Sparrow after preliminary discussions with a number of representatives of agricultural research institutions in Northeastern United States.

reactor shield (Fig. 1). Fast neutrons at this higher flux density are also available to the cooperator. An additional facility available to the program is the array of kilocurie gamma sources in the Nuclear Engineering Department of Brookhaven National Laboratory.

Approximately 150 scientists have performed more than 700 experiments over the past 10 years on approximately 70 different plant genera. Geographically, 45 states and 37 foreign countries are represented in the program.

2. RESULTS

A graphical representation of treatments for United States and foreign cooperators is seen in Figure 2. With regard to United States cooperators, there was a rapid increase of service irradiations from 1953 to the 1955-56 years, followed by a decrease of usage in 1957, after which the program leveled off to about 50 per cent of the 1955-56 years. Osborne,[4] who heads a similar cooperative program at the University of Tennessee AEC Research Laboratory, communicates that the same pattern of usage prevails there. However, the curve from the University of Tennessee is skewed to the right as compared to Brookhaven National Laboratory's program because their program started in 1955, which may also account for the decline of usage of the Brookhaven National Laboratory cooperative facilities in 1957.

The number of treatments for cooperators of foreign countries during the same 1953-63 period displays essentially the same type of distribution; however, three differences are evident: 1) the use of the program by foreign scientists began in 1954 and rose considerably over the next few years largely as a consequence of interest engendered by the First Atoms for Peace Conference; 2) the relatively high usage remained at that level for a longer time (5 years as opposed to 4 years in the United States); 3) the decline of usage in 1961 has continued through 1963 and will probably continue to do so in the future.

There are many reasons for these patterns of usage; however, the major accountable factor is unquestionably the widespread development of radiation facilities--for example, approximately 60 reactors have been placed in operation throughout the world since 1950, not counting the 130 reactors in the United States.[5]

Other nuclear facilities are available, such as gamma sources which are in all probability being used in increasing numbers by biologists. The most current listing has approximately 30 gamma irradiation facilities in Japan for public use.[6] The United States and Canada, at the present time, have 50 such facilities available for research.[7]

Although the number of experiments is lower, the experiments being conducted are probably better planned and more conscientiously executed than many of those undertaken during the earlier years of interest.

In the course of the past ten years of the program, many plant species were irradiated with X rays, thermal and fast neutrons, and gamma rays. A partial list of the species irradiated with X rays and thermal neutrons is given in Table II. We would like to emphasize the fact that the dose ranges given are not definitive for these species. It is well known that there may be large sensitivity differences between varieties of a species and that such things as seed moisture content and the conditions and duration of the postirradiation storage period markedly influence the radiation response. Further, the environmental conditions during early seedling growth, particularly with field grown plants, may also modify the basic radiation response.[8-13]

Some tangible results that have come from the large number of irradiation services are the newly released varieties listed in Table III.

3. DISCUSSION

Knowledge of the biological responses to neutrons is very meagre. In a thorough review of the biochemical, physiological, and morphological effects of ionizing radiations on plants, Gunckel and Sparrow present several tables which summarize the effects of various radiations.[14] One table lists the effects of various radiations upon the activity of 26 different enzymes. Of the 21 different scientific papers cited, only one was concerned with the effects of neutrons upon enzyme activity. Another table presents a compilation of the morphological effects of irradiation of higher plants and contains 538 citations. Only 12 of these are from experiments performed with neutrons. The remainder of the studies are related to the effects of X rays and gamma rays.

Despite the lack of fundamental information pertaining to biological responses to neutrons, certain facts are now fairly well known and they apply to the plant breeder or agriculturalist's interest in neutrons as a mutagenic agent. First, modifying factors such as moisture content, temperature, stage of development, oxygen level, conditions of postirradiation storage, etc., all substantially alter the responses of tissues to sparsely ionizing radiations, but most of the above factors are of minor importance with neutrons. As a result, in mutation plant breeding, neutrons yield more uniform and predictable results which may be extremely advantageous to the breeder. Secondly, there appears to be agreement that the number of mutations that can be recovered from plants that survive treatment well enough to reach maturity, is greater following neutron irradiations than from X irradiation. However, Nilan and Konzak are of the opinion that uniformity of response and not being able to alter the biological responses by modifying factors may be a deterrant in the sense that the plants cannot be experimentally modified for increasing the total mutation yield and changing the induced mutation process.[15] However, it should be pointed out that more investigations concerned with modifying factors and neutron irradiations on more plant species are needed before the above mentioned generalities become too deeply entrenched in the minds of the plant scientists.

Radiation damage from thermal neutrons appears to be more chromosomal in nature than damage produced by sparsely ionizing radiations.[16] In view of this, it would seem that the use of neutrons for microsurgery or chromosome transfer experiments would be most appropriate.

One of the first experiments involving the transfer of genetic fragments from one species into the chromosome complement of another species is the now well known experiment of Sears.[17] In that experiment, leaf-rust resistance of Aegilops umbellulata was transferred to wheat with the aid of X-ray treatment. A similar experiment concerned with the transfer of stem rust (Puccinia graminis tritici) resistance of tall wheat grass

(Agropyron elongatum) to a hexaploid wheat was performed by Elliot again with the aid of X rays.[18] However, Larter and Elliot[19] evaluated the chromosome breaking ability of X rays, thermal neutrons and radioisotopes (p^{32} and S^{35}), and found that neutrons yielded the best translocation-survival index. The reason X rays have been used in these earlier experiments and neutrons to a lesser degree is related to the relative availability of these different radiations. The availability for use of an X-ray machine or a gamma ray emitting source to a plant scientist is much greater than a neutron source. Neutrons are, for the most part, produced in nuclear reactors which, because of their size, complexity and expense, have, until very recently, been restricted to a relatively few large atomic energy centers. However, recently chromosome transfer studies have been performed with the aid of both neutrons and X rays.[20] In this study, Knott irradiated the spikes of plants which possessed 21_{II} of wheat chromosomes plus a single added Agropyron chromosome. The seeds of this plant type were irradiated with thermal neutrons. He states, "As a result of the irradiation, in at least five lines and possibly seven, a piece of the Agropyron chromosome carrying the gene or genes for rust resistance was transferred to a wheat chromosome. One of the translocations is transmitted normally through the gametes, but the remaining six show irregularities in transmission particularly through the pollen." Further experimentation by Knott (unpublished) indicates that neutron treatment is a promising mutagenic agent.

The three experiments listed above certainly do not encompass all investigations engaged in chromosome transfer studies, but they do point out that ionizing radiation as a tool is very useful for microsurgery experiments. We would like to suggest further that neutrons be used because they are more efficient chromosome breakers and, therefore, are the radiation of choice when chromosome manipulation is desired.

At a higher degree of plant organization there are induced tissue alterations in the form of chimeras[21, 22] and regeneration or reorganization studies[23-25] of the radiation damaged shoot apices. It is the opinion of some investigators that ionizing radiation promises to be a useful tool altering and studying chimeras which are frequently observed among fruit plants. It is urged that more work of this type be attempted with neutrons. In one study by Bishop,[22] thermal neutron treatments produced 42 per cent more sectorial color changes in apple than exposure to X rays.

In consideration of mutagenic agents, the investigator is confronted with another problem which has not been satisfactorily answered and probably will not be for many decades to come, namely the comparison of the incidence (frequency) and type (spectrum) of mutations or changes produced after treatment with various types of radiations. Recently, and a by-product of the efforts in experimentally produced mutations, originally promoted by radiation studies, there has been a search for other mutagenic agents. It has been suggested that chemical mutagens act on higher plants with greater efficiency than do any of the ionizing radiations.[26-28] However, this may not be true with all traits of all plants. Thus, although one investigation reports the chemical mutagen ethyleninime to be four times as effective as gamma rays or neutrons in inducing mutations at a specific locus in oats,[29] Robinson (personal communication) concluded that thermal neutrons were the most effective agent in a comparative study with neutrons, X rays, and diepoxy butane. For mutant characters in seedling stages, neutrons produced 10 times as many changes as did the chemical mutagen and 2.5 times as many as produced with X rays, at the optimal exposure for each mutagen. Further, the chemical mutagen treated plants did not display linearity of response with increased doses as did the ionizing radiation mutagens, which may support his opinion that thermal neutrons are indeed more effective for the mutations or changes observed.

Another personal communication from Forbes of the Georgia Coastal Experiment Station, Tifton, Georgia, reports that in comparing the effectiveness of thermal neutrons and X rays, the results vary according to locus or change in question. In one part of his report he studied the frequency of four different mutations of Blanco blue lupine which are as follows: big cotyledon, brilliant yellow, white plumule, and yellow green. In the cases of big cotyledons and yellow-green chlorophyll deficient, X rays and thermal neutrons were equal in effectiveness; however, neutrons were three times as effective as X rays in the frequency of occurrence of brilliant yellow and six times as effective in the case of white plumule.

Kim _et al_. report that neutrons were more effective than X rays in mutation production in Chinese radish.[30] Burton and Ourecky,[31] also of the Georgia Coastal Experiment Station, Tifton, Georgia, suggest that thermal neutrons and ethyl methane sulfonate may not always be effective in achieving variability in _Pennisetum glaucum_. In regard to one of the six characters, plant height, measured for variability after mutagenic treatment, they state ...."on the average, 30 minutes of thermal neutrons and 0.2% solution of ethyl methane sulfonate did not significantly reduce plant height. The heavier dosages did significantly reduce plant height and the greatest reduction was observed in plants from seeds treated with thermal neutrons for 90 minutes. This treatment reduced plant height about 7 inches, on the average. It is interesting that inbred 34 was not reduced in height by any thermal neutron treatment but was significantly reduced with ethyl-methane-sulfonate treatments. On the other hand, inbreds 13, 27, and 239 were reduced in height by thermal neutron treatment but were not affected by ethyl-methane-sulfonate treatments."

The above number of reports which pertain to effectiveness of various mutagenic agents is not complete, but they briefly illustrate the general variability of results. Until more studies of this nature are completed the only generalization one can make is that a generalization on effectiveness of mutagens cannot be made. It is perhaps reasonable to conclude, from data available, that some loci will be readily altered by X rays, others by neutrons and still others by one or another of the chemical mutagens. Our present knowledge is insufficient to allow us to be able to predict, or to make a sensible choice of mutagen. A well conceived mutation experiment should, therefore, be a comparative one with at least several different mutagens included.

The literature dealing with the efficiency of different mutagens reveals many conflicting statements, even where similar traits were studied in closely related varieties. One of the reasons for this may be that until relatively recently, it was not appreciated that outcrossing may be encountered in the R_1 generation of crops generally believed to be strictly self-pollinating. Thus unsuspected hybridization may occur between the partially sterile irradiated material and other varieties nearby. Consequently, in the R_1 generation, irradiated populations must be isolated from other varieties in order to fully derive a true index of radiation-induced mutants.

An experiment which clearly supports the need for such separation was performed by Caldecott, Stevens and Roberts[32] who compared the incidence of disease resistance (oat stem rust) in oats between segregated and non-segregated N_2 populations and found no stem rust resistant variants in the segregated plantings.

In a study with soybeans, Weber and Hanson found that a seed treatment with both thermal neutrons and X rays increased natural crossing 4 to 6 times (that of the control) and suggest that treated progeny should be isolated from foreign pollen sources.[33] In the three newly released cereal varieties, Florad oats,[34] Alamo X oats,[35] and Pennrad barley,[36] isolation was not practiced and therefore the exact manner in which genetic variation occurred is not known; however, outcrossing may be involved.

This question of outcrossing bears on the interpretation of the newly released varieties. They are from experiments initiated before the problem was generally realized and it is possible that at least some may be due to radiation-induced sterility rather than radiation-induced mutation. The plant breeder must, in many cases, restrict himself to the practical matters at hand. He has a responsibility to develop new varieties to meet the constantly changing requirements of modern agriculture. He cannot turn aside from such immediate problems to fully explore theoretical aspects of his work or to so design his experiments (if by so doing he increases the burden of his work unnecessarily from the standpoint of his primary practical objectives) as to get the maximum theoretical information from them. It therefore seems unlikely that the experiments from which the new varieties were derived will ever be exactly repeated, under conditions of isolation, so that one would be able to determine whether outcrossing or mutation was the causative process. To the plant breeder this information is perhaps not as important as the fact that he did get the variability he was seeking and that he was able to stabilize it in a new variety.

4. SUMMARY

In summarizing the past 10 years of the cooperative mutations program and adjunct mutation breeding, at least four major concepts and/or approaches related to the use of mutagenic agents in plant breeding have evolved.

1. Outcrossing between treated and nontreated populations must be reckoned with and consequently the two populations should be separated before a true measure of mutation induction can be ascertained.

2. Chromosome rearrangement studies have proven to be useful with particular emphasis on inducing disease resistance.

3. Work concerned with tissue reorganization and rearrangement as related to chimera production and basic understanding of tissue ontogeny, particularly with fruit crops and horticultural crops is promising.

4. The effectiveness of responses of plant tissues to neutrons and other mutagenic agents is extremely variable and more basic work is needed before the full potentialities of mutation breeding as a tool in crop improvement can be appreciated.

In closing, the eight newly released varieties are no longer mere curiosities of the interplay between nuclear energy and agriculture but evidence that mutation breeding, utilizing a variety of mutagens, is with us for years to come.

5. ACKNOWLEDGEMENTS

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TABLE I

THERMAL COLUMN RADIATION LEVELS*

	High flux position	Intermediate flux position	Low flux position
Thermal neutrons	3×10^9 $n_{th}/cm^2/sec$	6×10^8	6×10^7
Gamma component	750 R/hr	100	20

* Fast neutron dosimetry is still in progress.

TABLE II

VARIOUS RANGES OF RADIATION TREATMENTS FOR SOME OF THE PLANTS INVESTIGATED IN THE PROGRAM

Common name*	Latin name	Range of x-ray treatment in Kr	Range of thermal neutron treatments in hours with a flux density $\approx 3 \times 10^9$ $n_{th}/cm^2/sec$
Abaca	Musa textilis	15 - 20	1.2 - 3
Alfalfa	Medicago sativa	25 - 50	4 - 12
Almond (cuttings)	Prunus sp.	6 - 12	.08 - 1
Almond	Prunus sp.	8 - 12	.4 - 2
Apple (cuttings)	Pyrus malus	2 - 5	1 - 5
Apple	Pyrus malus	20 - 40	2 - 8
Apricot (cuttings)	Prunus Armeniaca	4 - 6	1.2 - 1.5
Arabidopsis	Arabidopsis thaliana		5 - 20
Asparagus	Asparagus officinalis	2 - 8	.1 - 1.6
Aster	Aster sp.		1 - 3
Azalea	Azalea sp.		.2 - 1
Barley	Hordeum vulgare	5 - 15	.5 - 1
Bean, lima	Phaseolus limensis	6 - 12	.6 - 2.4
Bean, lima	Phaseolus vulgaris	8 - 16	.5 - 5
Blueberry	Vaccinium sp.		1.2 - 2
Blueberry (cuttings)	Vaccinium sp.	6 - 8	.8 - 2
Buckwheat	Fagopyrum sp.	5 - 10	
Cantaloupe	Cucumis melo	15 - 20	1 - 1.2
Carnation	Dianthus caryophyllus	5 - 7.5	.8 - 2
Cauliflower	Brassica oleracea	20 - 40	2.5 - 5
Cherry (cuttings)	Prunus sp.	2 - 4	.2 - 1
Cherry	Prunus sp.		1 - 1.2
Cherry (pollen)	Prunus sp.		.01 - .2
Chestnut	Castanea sp.	5 - 10	.1 - 1.3
Chestnut (cuttings)	Castanea sp.	2.5 - 5	
Chestnut (pollen)	Castanea sp.		.08 - .8
Chrysanthemum	Chrysanthemum sp.	3 - 4	.8 - 3.2
Coffee	Coffea arabica		1.2 - 3
Clover	Trifolium squarrosum	25 - 35	3.2 - 6.5
Clover	Trifolium resupinatum	25 - 35	1 - 5
Clover	Trifolium subterraneum	25 - 35	1 - 5
Corn	Zea Mays	15 - 20	1.2 - 2.5
Cotton	Gossypium sp.	15 - 40	2 - 4
Cucumber	Cucumis sativa	20 - 40	2 - 3
Elm	Ulmus americana		1 - 1.2
Flax	Linum usitatissimum	15 - 30	.9 - 3
Gladiolus	Gladiolus sp.	7 - 20	2 - 6
Grape (cuttings)	Vitis vinifera	2 - 6	.1 - .33

Grasses (noncereal crops)			
Bahia, Pensacola strain	Paspalum sp.		3 - 6
Bromegrass	Bromus sp.	10 - 20	1.5 - 3
Kentucky bluegrass	Poa pratensis		3 - 8
Orchard grass	Dactylis glomerata		3 - 5
Prostrate dallisgrass	Paspalum dilatatum	10 - 30	3 - 5
Tall fescue	Festuca sp.		3 - 5
Hemp	Cannabis sativa		1 - 1.5
Iris (bulbs)	Iris sp.	10 - 35	1 - 2
Jute	Corchorus capsularis	5 - 20	1 - 1.8
Lettuce	Lactuca sativa	20 - 40	1 - 5
Lilac	Syringa vulgaris		.4 - 1
Lily	Lilium sp.	15 - 25	.6 - 1.1
Lupine, yellow	Lupinus luteus	15 - 25	1 - 1.3
Lupine, blue	Lupinus hirsutus	15 - 30	1 - 1.3
Mustard	Brassica sp.		9 - 15
Nectarine (cuttings)	Prunus persica		.1 - .6
Oats	Avena sativa	15 - 25	.8 - 1.6
Onion	Allium cepa	10 - 20	.6 - 1.2
Orchid	Odontoglossum sp.		.2 - .8
Orchid (pollen)	Odontoglossum sp.		.02 - .04
Papaya	Carica papaya	20 - 35	5 - 7
Pea	Pisum sativum	15 - 30	.5 - 1
Peach	Prunus persica	10 - 20	.5 - 1.5
Peach (cuttings)	Prunus persica	2 - 6	
Peanuts	Arachis hypogea	15 - 20	.4 - 1
Pear	Pyrus communis		4 - 6
Pear	Pyrus communis	6 - 10	.8 - 1.1
Pearl millet	Pennisetum glaucum		.5 - 1.5
Peppermint	Mentha piperita		.65 - .75
Peppermint (cuttings)	Mentha piperita	4 - 6	.3 - .6
Pepper	Piper sp.		.2 - .6
Petunia	Petunia hybrida	10 - 15	.8 - 1.2
Pine	Pinus densiflora		.6 - 1.8
Pine	Pinus rigida		.6 - 1.8
Plum	Prunus cerasifera		1.2 - 1.5
Potato	Solanum tuberosum		.2 - .8
Radish	Raphanus sativus	20 - 50	3 - 8
Rape	Brassica rapus	60 -150	
Rhubarb	Rheum Rhaponticum	15 - 40	1 - 3
Rice	Oryza sativa	20 - 25	.5 - 1.5
Rose	Rosa sp.	15 - 20	.4 - .8
Rose	Rosa multiflora	15 - 20	.4 - 1.2
Rose (cuttings)	Rosa sp.	6 - 8	.2 - .6
Rye	Secale cereale	8 - 15	.6 - 1.4
Sesame	Sesamum indicum		.6 - 1
Sorghum	Sorghum vulgare	20 - 40	2.4 - 3.6
Soybean	Glycine max	10 - 20	1.2 - 2
Squash	Cucurbita sp.	20 - 25	
Strawberry	Fragaria sp.	6 - 10	
Sugar beet	Beta vulgaris	15 - 20	1.6 - 2.8
Sugar cane	Saccharinum officinarum	4 - 10	.8 - 1.2
Tobacco	Nicotiana tabacum	30 - 40	2.6 - 4.3
Tomato	Lycopersicon esculentum	10 - 40	3 - 6
Wheat	Triticum sp.	15 - 20	1 - 1.6

* All material given is seed except where noted.

TABLE III

"RADIATION-INDUCED" VARIETIES RELEASED IN THE UNITED STATES SINCE 1953

Mutation	Mutagen	Crop	Investigator(s) and location
1. Sanilac bean (Vine to erect type of growth, disease resistance)	X ray	pea bean Michilite variety	a
2. Seaway bean (Erect type of growth, virus resistance)	X ray	pea bean Michilite variety	a
3. Gratiote bean (Better seed type)	X ray	pea bean Michilite variety	a
4. Florad oats (Disease resistance)	Thermal neutrons	oats Floriland variety	b
5. Alamo X oats (Disease resistance)	X ray	oats Alamo variety	c
6. NC4 X peanut (Tougher hull)	X ray	NC4	d
7. Pennrad barley (Better winter hardiness)	Thermal neutrons	barley Hudson variety	e
8. Yukon 1 carnation ($\approx$10 less petals and holds longer)	γ ray	"White Sim" carnation	f

[a] E. E. Downs and A. Anderson, Michigan State University, East Lansing, Michigan.

[b] W. H. Chapman, H. H. Luke, A. T. Wallace and P. L. Pfahler, University of Florida Agricultural Experiment Station, Gainesville, Florida.

[c] I. M. Atkins, M. C. Futrell, Q. J. Raab and W. E. Tyles, The Agricultural and Mechanical College of Texas, College Station, Texas.

[d] W. C. Gregory, North Carolina State College, Raleigh, North Carolina.

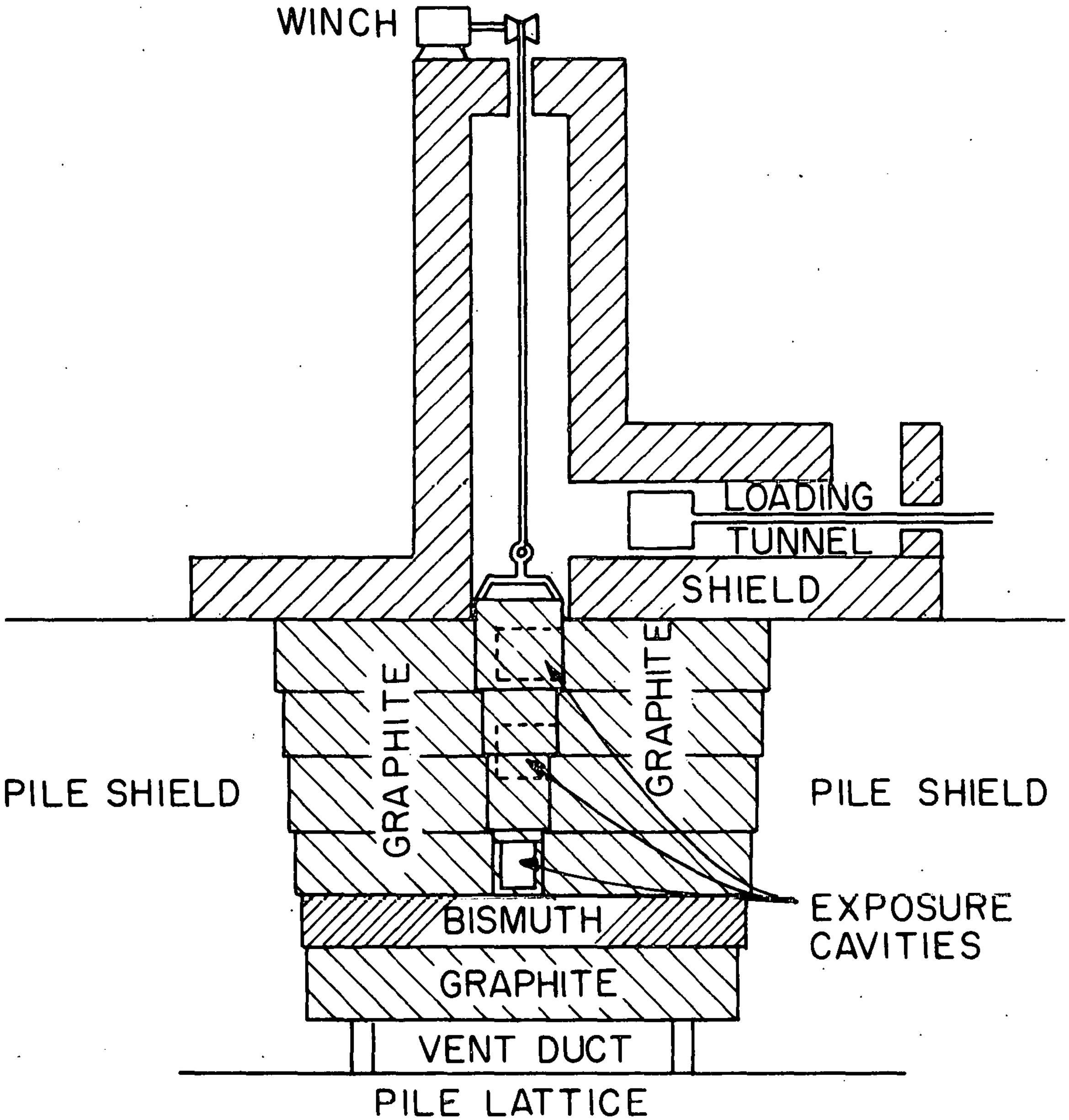
[e] R. P. Pfiefer and R. I. Schein, Pennsylvania State University Agricultural Experiment Station, University Park, Pennsylvania.

[f] G. A. L. Mehlquist, University of Connecticut, Storrs, Connecticut.

FIGURE LEGENDS

Figure 1. Diagrammatic representation of the thermal neutron facility showing three positions in the column which yield three levels of flux densities (see Table I).

Figure 2. Graphical representation of the number of service irradiations performed for United States and foreign cooperators at Brookhaven National Laboratory from 1953-1963.


WINCH
LOADING
TUNNEL
SHIELD
GRAPHITE
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EXPOSURE
CAVITIES
BISMUTH
GRAPHITE
VENT DUCT
PILE LATTICE

FIGURE 1

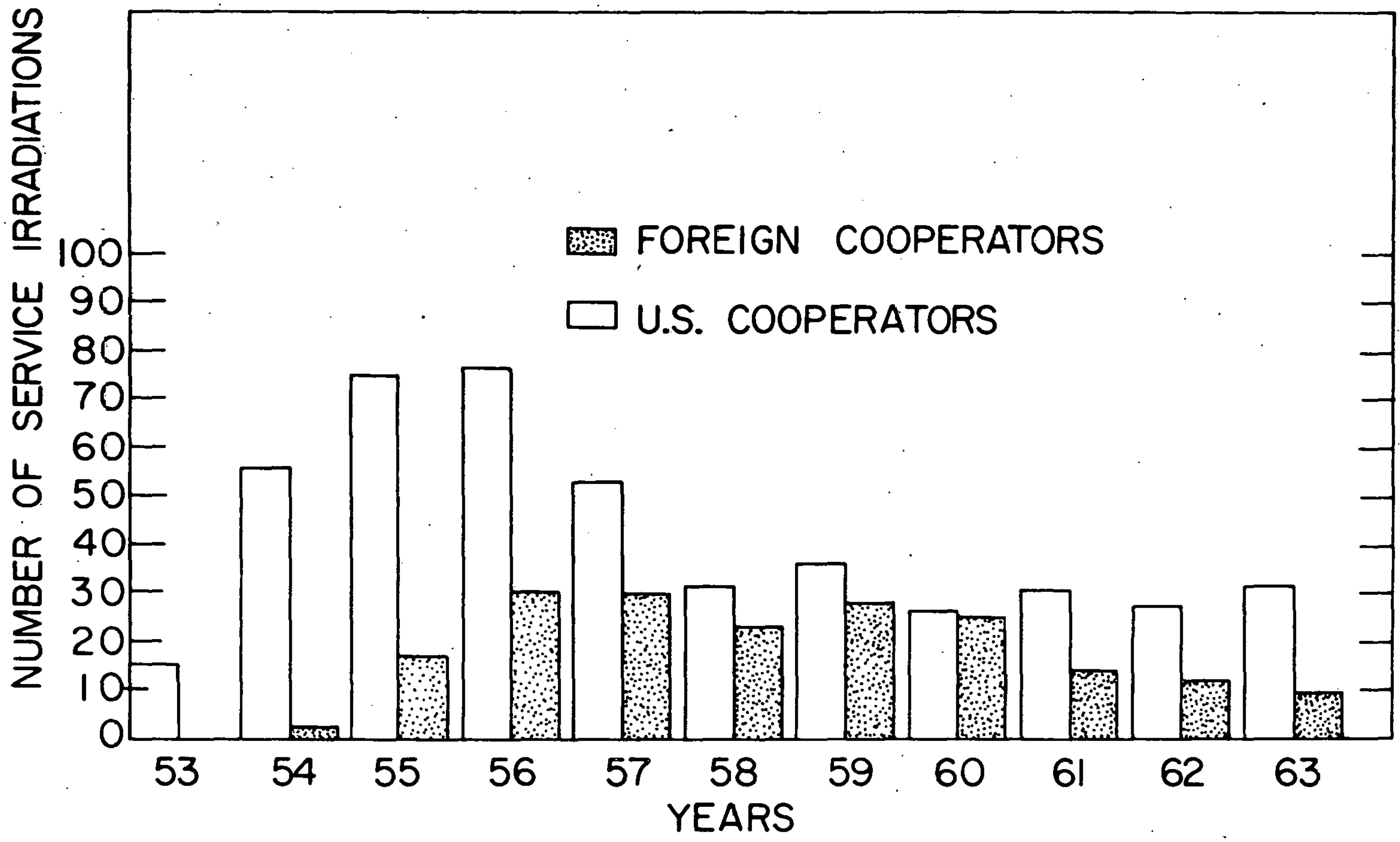
NUMBER OF SERVICE IRRADIATIONS
100
90
80
70
60
50
40
30
20
10
0
FOREIGN COOPERATORS
U.S. COOPERATORS
53
54
55
56
57
58
59
60
61
62
63
YEARS

FIGURE 2