# PART 1. BETA AND GAMMA RADIATION STUDIES IN PISUM SATIVUM

# PART 11. THE INHERITANCE OF FUSARIUM ROOT-ROT, FUSARIUM SOLANI PISI, RESISTANCE IN PISUM SATIVUM

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Dean E. Knavel
1959

This is to certify that the

thesis entitled

Part I. BETA AND GAMMA RADIATION STUDIES IN PISUM SATIVUM

Part II. THE INHERITANCE OF FUSARIUM ROOT ROT FUSARTUM SOLANI PISI, RESISTANCE IN PISUM SATIVUM

presented by

Dean E. Knavel

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

A. Clark Ballard
Major professor

Date January 29, 1959

O-169

PART I. BETA AND GAMMA RADIATION STUDIES IN PISUM SATIVUM

PART II. THE INHERITANCE OF FUSARIUM ROOT-ROT, FUSARIUM SOLANI PISI, RESISTANCE IN PISUM SATIVUM

By

Dean E. Knavel

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1959

Approved J. Clark Ballard

ABSTRACT Dean E. Knavel

Beta and gamma radiation sources were evaluated for producing mutations and resistance to Fusarium root-rot, Fusarium solani f. pisi, in peas. Dry pea seeds and seeds soaked in either water, solutions of colchicine, Endothal, or uranyl nitrate of the variety Early Perfection were irradiated. The dry seeds were exposed to radiation dosages of 7,500r and 15,000r of the gamma source and 20,000 rep and 40,000 rep of the beta source. The soaked seeds were exposed to dosages of 1,500r, 3,000r, 10,000 rep, and 20,000 rep.

Gamma irradiation was more effective than beta irradiation of dry seeds for reducing germination and increasing the numbers of plants showing dwarfing, leaf-distortion, and leaf-variegations in the $R_1$. Five per cent of the seeds germinated and all of the surviving $R_1$ plants showed visible changes at a dosage level of 15,000r. In comparison, 16 per cent of the seeds germinated, and only 58 per cent of the $R_1$ plants showed similar visible changes at 40,000 rep.

Soaking seeds in the chemical solutions prior to either radiation source did not cause any appreciable increase in the variability in the $R_2$ plants. However, soaking seeds in water prior to gamma radiation was more effective than gamma and beta irradiation of dry and chemically soaked seeds with regards to the numbers of abnormal plants in the $R_2$. Abnormal $R_2$ plants were characterized by chlorophyll deficiencies,

THE

sterility, lateness, earliness, tallness, dwarfness, and plants that branched at the base.

Two gamma radiation-induced tall plants were studied in detail. Plants from irradiated seeds in the $R_3$ generation flowered as early as Early Perfection plants from non-irradiated seeds during long days, however, when the $R_4$ was grown under short day lengths and low light intensities, flowering was delayed approximately one month longer than the non-irradiated Early Perfection plants. Non-irradiated plants flowered in 32-36 days after seeding under long days.

All $R_3$ plants grown from irradiated seed and tested for Fusarium root-rot resistance were found susceptible to the disease.

The variety Early Perfection was crossed to a Fusarium root-rot resistant Foreign pea introduction, 140165, to study the mode of inheritance for resistance to Fusarium root-rot, to determine if resistance was associated with seed-shape and seed coat color, and if resistant Perfection-type plants could be obtained.

The Fusarium root-rot test of the $F_3$ populations, Early Perfection X 140165 and 140165 X Early Perfection, indicated that resistance to Fusarium root-rot was dominant. The curve showing the distribution of the $F_3$ progeny was not normal. $F_3$ segregates from wrinkled seeds and seeds with transparent coats, which are characteristic of Early Perfection, were

THE

similar in resistance as color coated and smooth seeds. The latter seed-types are representative of the resistant parent, 140165. The short internode Perfection-type progeny of the $F_3$ populations were slightly more resistant than the long internode 140165-type.

PART I. BETA AND GAMMA RADIATION STUDIES IN *PISUM SATIVUM*

PART II. THE INHERITANCE OF FUSARIUM ROOT-ROT, *FUSARIUM SOLANI PISI*, RESISTANCE IN *PISUM SATIVUM*

By

Dean E. Knavel

A THESIS

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1959

THE

## DEDICATION

To my wife, Phyllis, in appreciation for her encouragement, assistance, and many sacrifices. To my mother-in-law, Margaret Seese, for her continuing interest and loyal support during the conduction of this investigation.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. J. C. Ballard for his generous help, valuable suggestions, and direction throughout this investigation.

The author wishes to thank the other members of the guidance committee, Drs. S. Honma, S. H. Wittwer, J. L. Lockwood, and J. E. Grafius, for their helpful review of the manuscript.

Grateful acknowledgement is expressed to Dr. J. L. Lockwood for his generous help and advice on the pathological techniques.

Sincere thanks are also extended to Dr. R. Nicholas of the Department of Agriculture Engineering, Michigan State University, and Dr. J. V. Nehemias of the Fission Products Laboratory, University of Michigan, for their generous help in performing the irradiation of seeds used in this study.

The author also wishes to thank Mrs. Mary Hillman for typing the thesis.

THE

TABLE OF CONTENTS


| | Page |
|---|---|
| I. Introduction | 1 |
| II. Literature Review | 3 |
| 1. Sources of ionizing radiations | 3 |
| 2. Irradiation of seeds | 4 |
| 3. Irradiation of dry versus water-soaked seeds | 4 |
| 4. Mutation rates | 5 |
| 5. Irradiation of chemically soaked seeds | 6 |
| 6. Beneficial mutations | 7 |
| 7. Root-rot resistance | 9 |
| III. Part I. Radiation Studies | 10 |
| A. Experimental materials and methods | 10 |
| 1. General methods | 10 |
| 2. Irradiation of seeds and results | 11 |
| a. Experiment 1. Comparison of irradiation of dry seeds under field conditions | 11 |
| (1) Results | 12 |
| b. Experiment 2. Beta irradiation of soaked seeds under field and greenhouse conditions | 15 |
| (1) Results | 15 |
| c. Experiment 3. Gamma irradiation of dry and soaked seeds under greenhouse conditions | 16 |
| (1) Results | 17 |

THE

Page

d. Experiment 4. Two dosage levels of gamma and beta radiation of dry and soaked seeds under greenhouse conditions . . . . . 17

(1) Results . . . . . 19

e. Experiment 5. Three dosage levels of gamma and beta radiation on dry and soaked seeds under greenhouse conditions . . . . . 19

(1) Results . . . . . 20

f. Experiment 6. Two dosage levels of gamma and beta radiation on dry and soaked seeds under field conditions . 20

(1) Results . . . . . 26

(a) Germination in the $R_1$ . . . . 26

(b) Abnormalities in the $R_1$ . . . 31

(c) Survival of $R_1$ plants . . . . 32

(d) Variability in the $R_2$ plants. 32

(e) Variability in the $R_3$ plants. 37

(f) Test of $R_3$ plants from irradiated seeds for Fusarium root-rot resistance . . . . . 39

(g) Observation of two tall mutants for three generations . 39

IV. Part III. Fusarium Root-rot Inheritance Studies 44

A. Experimental materials and methods . . . . 44

B. Experimental results . . . . . 46

1. Seed coat pigmentation of parental, $F_1$, backcross, and $F_2$ populations . . . . . 46

2. Test for resistance: backcross and $F_2$ populations . . . . . 50

Page

3. Test for resistance: $F_3$ populations. . 51

4. Relationship between $F_3$ progeny-types and resistance to root-rot. . . . . 51

V. Discussion. . . . . 62

A. Radiation studies. . . . . 62

1. $R_1$ data: germination . . . . . 62

2. $R_1$ data: dwarfing, leaf-variegation, and leaf-distortion . . . . . 63

3. $R_2$ data . . . . . 66

4. $R_3$ data . . . . . 69

5. Root-rot resistance test of $R_3$ plants from irradiated seeds . . . . . 71

B. Inheritance studies. . . . . 72

VI. Summary and Conclusions . . . . . 74

VII. Literature Cited. . . . . 77

# INTRODUCTION

Resistance to the various root-rots attacking pea, Pisum sativum, has not been found. Commercial varieties have been tested without success for resistance to Fusarium solani f. pisi. Six-hundred foreign pea assessions were tested at Michigan State University. The tests included seeds with pigmented and transparent coats. Resistance was associated with seeds with a pigmented seed coat, and to some extent, smooth seeds. Seeds with resistance varied from red to various shades of brown, gray, and purple, usually with a brown mottling and/or purple dotting of the seed coat.

Peas with a pigmented seed coat are not accepted for the canned-pack. The canners demand peas with a transparent seed coat, and moreover, a majority require a wrinkled or sweet pea. A frozen pea pack should contain dark-green and wrinkled seeds.

Therefore, it would be necessary either to separate the resistance from seeds with the pigmented seed coat, or induce resistance in a commercial variety by radiation techniques.

Various workers have reported success in using radiation to induce mutations in plant and animal material. Moreover, irradiation of the organism is one method for increasing

TH

the mutation rate. According to Muller (43), mutations can arise through gene and chromosome abberations, and both occur naturally. A chromosome mutation may occur through one of several ways, namely, translocation, inversion, deletion, and duplication of chromosome parts.

The objectives are as follows:

1. Irradiation of pea seeds to induce mutations;
2. Determine the relationship between plant abnormalities in the first generation and those in the second generation;
3. Correlation between Fusarium root-rot resistance and various seed and plant characters;
4. Inheritance of resistance to Fusarium root-rot.

## LITERATURE REVIEW

Irradiation of plants is not a new method for crop improvement. The value of using radiation as a tool for increasing the number of mutants has been known since Muller (41) and Stadler (55). Muller worked with the Drosophila and Stadler with corn and barley. Since then researchers in the various sciences have contributed to the present knowledge of radiation and its effect on plants and animals.

### 1. Sources of ionizing radiations

The sources of ionizing radiations are separated on the basis of their penetrating power. According to Brock (6),

> "The ionizing radiations either consist of, or produce in the cell, charged particles of high kinetic energy. Their biological effect results from the transfer of this energy to the cell either by collision or by ionization of cellular components--which results in a chain of chemical reactions.
>
> The radiations which consists of charged particles have low penetration and their uses for plant and animal material are limited. The charged particles are either electrons, e.g. beta-rays produced by radioactive phosphorous ($P^{32}$); or atomic nuclei, e.g. alpha-rays produced by the decay of radon or polonium.
>
> The radiations which produce charged particles in the cell are the electromagnetic radiations (X-and gamma-rays) which produce electrons, and neutrons which produce atomic nuclei (protons). These have high penetrating power and consequently widespread use in biological experiments."

2. Irradiation of seeds

The majority of reports on induction of mutations by radiation has been where the seeds have been irradiated dry rather than irradiating the growing plants. The use of seeds permits treating large numbers of progeny to obtain beneficial mutations (55) (23). According to Gustafson (24), irradiation of the seed is superior to treating either the ovules or the pollen grains.

3. Irradiation of dry versus water-soaked seeds.

Smith (53), in reviewing the literature on radiation, stated:

> "It is of interest to note that radiation of dry seeds was used, the method which today has been most productive of beneficial mutations."

Wheat (1), tomatoes (35), and barley (8) (14) (23) (25) (30) (55) (56) seeds with a high moisture content demonstrated greater X-radiation sensitivity than dry seeds.

Stadler (55) (56), Ehrenberg, et al. (14), and Gustafsson (25) soaked barley seeds prior to irradiation and increased the mutation rate 7 to 15 times that of dry seeds. They also found dry seeds survived dosages 15 to 20 times greater than germinating seeds. Gustafsson (23) irradiated soaked barley seeds which had moisture contents of 10 and 15 per cent. The seeds with 10 per cent moisture content produced a larger

TH

number of mutants. At the higher moisture level, more mutants were obtained at a dosage of 5,000r than at 10,000r units.

Gelin (19) found X-irradiated barley seeds with a moisture content of 10 per cent produced plants of which 12 per cent had abnormal cell divisions, while at 15 per cent moisture, 27 per cent showed abnormal cell divisions.

In comparing X-rays with neutrons, Key (30) and Ehrenberg (13) found the effect of neutrons to be independent of the moisture content in the seeds.

4. Mutation rates.

Increase in the mutation rate has been reported by Stadler (55), Muller (42), and Gustafsson (23) to be directly proportional to the dosage of X-rays. However, Gustafsson (23) found that the number of chromosome irregularities was not directly proportional to dosage. Nevertheless, as the number of chromosome rearrangements were increased, there was an increase in the mutation rate (23) (25).

Gustafsson (24) also reported that mutations in cereals were most frequently close to lethal dosages of X-rays. At such dosages cell divisions contained nearly 100 per cent fragments and bridges, and germination was reduced to about 10-20 per cent of normal.

TH

Tedin (57) irradiated 3,528 seeds of yellow sweet lupine and obtained 1.6 per cent mutations. From 8,616 $X_2$ barley plants, Akerberg (2) was able to select 6 plants which produced lines superior to the maternal strain in yield, weight, straw strength, and earliness. Muller (42) found the rate of desirable mutations in rye to be about 1 in 800 of those plants surviving the radiation treatment.

5. Irradiation of chemically soaked seeds.

Chromosome re-arrangements and other mitotic disturbances have been increased by pre-treating or soaking seed in metallic salt solutions and other metabolic inhibitors prior to irradiation.

Stadler (54) reported 0.5M solutions of $Pb(NO_3)_2$, $Ba(NO_3)_2$, and $UO_2(NO_3)_2$ to be effective for increasing mutations in barley. A three-fold increase was obtained by Damato and Gustafsson (10) with $H_2O_2$, KCN, and Butter Yellow when used prior to X-irradiation of barley seeds.

Colchicine, a mitotic poison, used at the concentration of 0.1 per cent prior to X-irradiation of barley seeds increased the mutation rate. Damato and Gustafsson (10) noted that at 0.005 per cent colchicine increased the number of "very rare mutants."

6. Beneficial mutations.

Much of the radiation on plants in this country has been to control the oat stem rust organism, Puccinia graminis, var. avenae. Konzak (33) treated the oat variety Mohawk and obtained plants which were resistant to race 7a of stem rust. About the same time Frey (15) X-rayed oat seeds of the variety Huron and produced three strains which were resistant to race 7 of stem rust. Others who were successful in producing resistance to stem rust in oats were Meyers, et al. (40), Frey and Browning (17), Singleton, et al. (50), and MacKey (38). Resistance has been found for other cereal diseases. Konzak, et al. (32) obtained wheat mutants which were resistant to stripe rust, Puccinia glumarum. Singleton, et al. (50) irradiated the oat variety Tamma and induced resistance to Helminthosporium Victoria blight. Resistance to both diseases appeared to be recessive.

Irradiation of the cereals has resulted in beneficial mutants which were earlier, higher yielding, and had greater straw strength than non-irradiated (23) (25). Gustafsson called these mutants erectoids. Similar types of mutants have been reported in barley (2) (5) (18) (26) (51), oats (16), and wheat (38). Froier (18) selected mutant barley plants which yielded better at a medium level of nitrogen than at a high level.

. Gregory (22) working with X-ray treated peanuts was able to select plants resistant to stem rust and leaf spot. He also selected five best-mutants and conducted yield tests with the $X_5$ generation in 1953 and 1955 and found an increase in yields. This was one of the first works demonstrating the use of radiation to improve a quantitative character.

There are three X-radiation induced mutant crop varieties on the market. These are the Stralart pea, Primex white mustard, and the Sanilac navy bean. The Stralart pea is commercially important in Sweden because of its ability to out-yield the mother strain by 6 per cent and all commercial Swedish varieties by 10 per cent (20). Primex white mustard of Svalof is important for its high oil yield. A mutation was found that had a 7 per cent increase over the mother strain (3). Down and Anderson (12) made use of a bush-type mutation which was produced by X-irradiation of navy bean seeds of the variety Michelite. After 14 generations of crossing and back-crossing they improved its quality and resistance to anthracnose. The improved bean was released in 1957 as the variety Sanilac.

Progressive mutations have been induced in cotton by X-ray treatment of dry seeds (27).

Mertens and Burdick (39) X-irradiated seeds of the tomato variety Red Cherry and reported a mutation for early fruit maturation.

7. Root-rot resistance.

A review of the literature revealed little information on resistance to root-rot diseases of peas. Wade (59) listed Fusarium root-rot as one in which the genetic basis for resistance had not been worked out. Earlier, Jones (29) reported that two varieties of peas, Rice 330 and Horal, have some resistance to Aphanomyces euteiches. According to Jones, resistance in these varieties was a "very small factor in relation to total resistance."

TH

# PART I. RADIATION STUDIES

## A. Experimental materials and methods

### 1. General methods.

Preliminary experiments were conducted in the field during the summer of 1956 and the greenhouse during the fall and winter of 1956-1957. The objective was to establish chemical and/or radiation treatments which would give low germination and high numbers of plant-changes, as leaf-variegation, leaf-distortion, and dwarfed pea-seedlings in the $R_1$ (first generation). Seeds of the variety Early Perfection were used in these studies.

Gamma and beta radiation were used in these studies. Gamma irradiation was accomplished by placing the bags of dry seed in the center well of a colbalt-60 radiation source where the dosage was approximately 250,000 rep/hr. The dosage, measured in time of exposure and intensity, was in roentgens and will be designated hereafter as "r." The seeds were exposed to gamma radiation at the Fission Products Laboratory at the University of Michigan. Beta irradiation was accomplished by placing the dry seed, one layer thick within the bag, on a metal tray which travelled beneath the electron beam at the rate of 12.2 cm/sec. The

distance from the window of the electron tube to the base of the tray was 47 cm. The dosage, measured by intensity, is in roentgen-equivalen-physical and will hereafter be designated as "rep." The seeds were exposed to high-speed electron beta radiation of thecathode source at the Agriculture Engineering Department at Michigan State University.

Treatments prior to irradiation were performed by soaking the seed in either water or the chemical solutions. The seeds were placed in covered plastic dishes and soaked in tap-water at room temperatures for six hours. At the end of the six hours, the water was removed and either fresh water or chemical solutions were added and the seeds allowed to soak for an additional six hours. All seeds were covered with the solution during the period. At the termination of the 12-hours soaking period the seeds were washed in tap-water and placed in polyethylene bags for irradiation. In order not to introduce error because of the length of time-lapse between the termination of the soaking period and the time of irradiation, all irradiations were made approximately six hours after the soaking treatments were completed. The seeds were sown the same day they were soaked and irradiated.

2. Irradiation of seeds and results.

a. Experiment I. Comparison of gamma and beta irradiation of dry seeds under field conditions.

One hundred dry seeds per treatment were irradiated and planted in the field. The per cent germination and the percentage of the variegated, distorted, and dwarfed seedlings which survived the radiation treatments were determined.

(1) Results.

Gamma irradiation of dry seeds did not reduce germination at dosage levels of 1,000r to 7,000r as greatly as 10,000 rep and 20,000 rep of beta irradiation (Figures 1 and 2). However, 21,000r of gamma radiation was more lethal than 80,000 rep of beta radiation. A dosage level of 10,000 rep appeared to be comparable to a dosage level somewhere between 7,000r and 9,000r of gamma radiation with regards to seed germination.

The numbers of deformed $R_1$ seedlings were not equivalent for both sources of radiation (Figures 1 and 2). The $R_1$ seedlings showing the immediate affects from radiation were slow growing and greatly deformed. The color of the leaves on the visibly affected plants were variegated and mottled with large irregular areas devoid of chlorophyll (Figure 3). At 40,000 rep of beta radiation, 52 per cent of the $R_1$ seedlings were deformed;

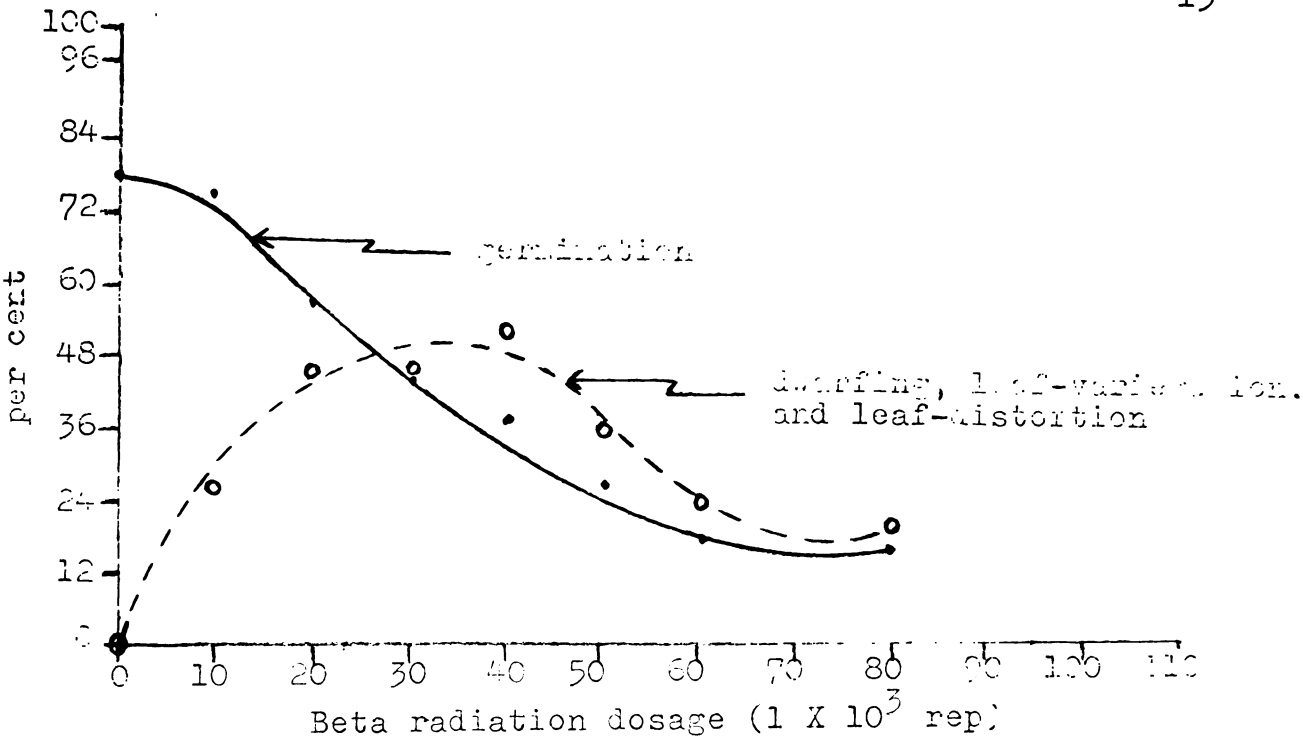


Figure 1. Influence of beta radiation on pea seeds with regards to per cent germination and the percentage of surviving $M_2$ plants showing dwarfing, leaf-variegation, and leaf-distortion.

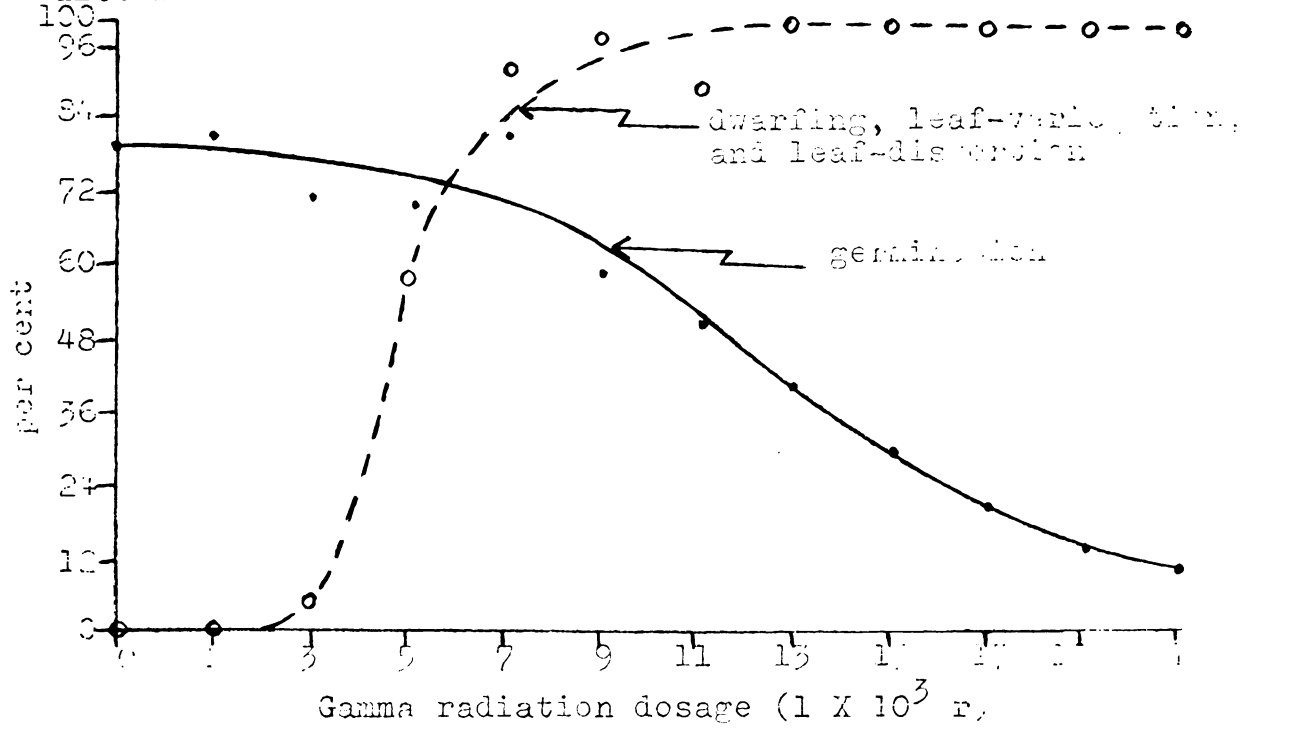


Figure 2. Influence of gamma radiation on pea seeds with regards to per cent germination and the percentage of surviving $M_2$ plants showing dwarfing, leaf-variegation, and leaf-distortion.

TH

Figure 3. (top): normal plants. (bottom): dwarfed $R_1$ plants with variegation and distorted leaves as result of gamma and beta radiation on dry and soaked seeds, and seeds soaked in colchicine.

whereas, at a dosage level of 80,000 rep, only 21 per cent of the seedlings were visibly changed. In comparison, all plants surviving dosage levels of 13,000r or greater of gamma radiation were deformed.

b. Experiment 2. Beta irradiation of soaked seeds under field and greenhouse conditions.

To determine if soaking seeds prior to irradiation increased the effectiveness of beta radiation, 100 seeds per treatment were irradiated following soaking in either water, 0.01, or 0.03 per cent solutions of colchicine.

The seeds were sown in the greenhouse in flats of non-sterilized soil, and a duplicated planting was made in the field. The treatments were not replicated. The per cent germination was determined.

(1) Results.

Seeds did not germinate in the field as well as seeds in the greenhouse (Table 1). The reduced germination in the field was evident in all treatments, regardless if beta-irradiated or not. A dosage of 15,000 rep on soaked seeds appeared to be too high for field studies. Colchicine solutions of 0.01 and 0.03 per cent reduced germination and appeared to be too high.

Table 1. Influence of beta radiation with regards to per cent germination of pea seeds under field and greenhouse conditions as result of irradiation of seeds after soaking in water or solutions of colchicine.

| Soaking Treatment | Radiation dosage (1 X $10^3$ rep) | Germination | |
|---|---|---|---|
| | | Field (per cent) | Greenhouse (per cent) |
| Water-soaked | | | |
| | --- | 70 | 98 |
| | 15 | 0 | 4 |
| | 25 | 0 | 4 |
| Colchicine (0.01%) | | | |
| | --- | 61 | 68 |
| | 15 | 0 | 4 |
| | 25 | 0 | 0 |
| (0.03%) | | | |
| | --- | 4 | 12 |
| | 15 | 0 | 0 |
| | 25 | 0 | 0 |

--- without radiation.

c. Experiment 3. Gamma irradiation of dry and soaked seeds under greenhouse conditions.

To compare the effect of gamma radiation on dry and soaked seeds, seeds were soaked in 0.01 and 0.005 per cent colchicine solutions and 0.001 and 0.005 per cent Endothal solutions prior to irradiation. Wilson (60)

suggested that Endothal (disodium 3,6-endoxohexahydrophthalate) might be a mutagentic agent. A sample of 100 seeds were used in each treatment. The seeds were sown in flats of non-sterilized soil in the greenhouse.

(1) Results.

Only 8 per cent of the dry seeds which were exposed to a dosage of 15,000r germinated (Table 2). Therefore, it appeared that 15,000r might be the upper dosage level for satisfactory germination under field conditions.

d. Experiment 4. Two dosage levels of gamma and beta radiation on dry and soaked seeds under greenhouse conditions.

To compare the effect of gamma and beta radiations on dry seeds and seeds soaked in various chemical solutions, seeds were soaked in either water, 0.002 per cent colchicine, 0.002 per cent Endothal, or 0.25 per cent uranyl nitrate solutions prior to irradiation. Dry seeds were irradiated at either 10,000r, 15,000r, 20,000 rep, or 40,000 rep. Soaked seeds were irradiated at either 2,000r, 3,000r, 10,000 rep, or 20,000 rep. A sample of 100 seeds were used in each treatment. The seeds were sown in flats of non-sterilized soil

Table 2. Influence of gamma radiation with regards to per cent germination of pea seeds under greenhouse conditions as result of irradiation of seeds dry or after soaking in water or solutions of colchicine and Endothal.

| Treatment | Radiation dosage ($1 \times 10^3$ r) | Germination (per cent) |
|---|---|---|
| Dry seed | --- | 96 |
| | 5 | 76 |
| | 8 | 80 |
| | 10 | 64 |
| | 12 | 12 |
| | 15 | 8 |
| | 20 | 4 |
| | 25 | 0 |
| Water-soaked | --- | 97 |
| | 1 | 96 |
| | 2 | 76 |
| | 3 | 52 |
| | 4 | 64 |
| Colchicine (0.01%) | --- | 72 |
| | 0.5 | 32 |
| | 1 | 20 |
| | 3 | 16 |
| (0.005%) | --- | 80 |
| | 0.5 | 76 |
| | 1 | 76 |
| | 3 | 40 |
| Endothal (0.001%) | --- | 92 |
| | 0.5 | 88 |
| | 1 | 88 |
| | 3 | 48 |
| (0.005%) | --- | 72 |
| | 0.5 | 68 |
| | 1 | 64 |
| | 3 | 48 |

--- without radiation.

in the greenhouse. The per cent germination was determined.

(1) Results.

Germination in all treatments appeared to be satisfactory and, therefore, the treatments appeared applicable for field studies (Table 3). The concentrations of 0.002 per cent of Endothal and colchicine, and 0.25 per cent uranyl nitrate gave satisfactory germination. Each chemical treatment resulted in germination similar to the water-soaked control where 60 per cent of the seeds germinated.

e. Experiment 5. Three dosage levels of gamma and beta radiation on dry and soaked seeds under greenhouse conditions.

To determine the effect of gamma and beta radiation in combination with treatment with chemicals, another experiment was conducted using three dosage levels of each radiation source. Dry seeds were exposed to dosages of 7,500r, 15,000r, 22,500r, 20,000 rep, 40,000 rep, and 60,000 rep. Soaked seeds were exposed to dosages of 1,500r, 3,000r, 4,500r, 10,000 rep, 20,000 rep, and 30,000 rep. The percentage germination and the percentage of plants surviving the radiation treatments, which exhibited dwarfing, leaf-variegation, and

leaf-distortion, were determined. A sample of 100 seeds was used in each treatment. The seeds were sown to flats in the greenhouse.

(1) Results.

Germination in this experiment was extremely high for all treatments (Table 4). However, the percentage of plants showing dwarfing, leaf-variegation, and distortion appeared satisfactory. Soaking seeds in Endothal and uranyl nitrate, not followed with radiation, did not cause visible changes in the $R_1$ plants.

When the data on germination in Table 4 is compared with that in Table 3, there is an indication that from experiment to experiment the same treatments do not produce similar results under greenhouse conditions. The experiment was not repeated because greenhouse space was limited.

f. Experiment 6. Two dosage levels of gamma and beta radiation on dry and soaked seeds under field conditions.

The results of the previous experiments conducted in the greenhouse indicated that 7,500r, 15,000r, 20,000 rep, and 40,000 rep on dry seeds and 1,500r, 3,000r, 10,000 rep, and 20,000 rep on soaked seeds might give satisfactory results in the field with

Table 3. Influence of gamma and beta radiation with regards to per cent germination of pea seeds under greenhouse conditions as result of irradiation of seeds dry or after soaking in water or solutions of colchicine, Endothal, and uranyl nitrate.

| Treatment | Radiation dosage ($1 \times 10^3$) | Germination (per cent) |
|---|---|---|
| | (Gamma radiation r) | |
| Dry seeds | --- | 77 |
| | 10 | 57 |
| | 15 | 50 |
| Water-soaked | --- | 60 |
| | 2 | 52 |
| | 3 | 14 |
| Colchicine (0.002%) | --- | 51 |
| | 2 | 38 |
| | 3 | 15 |
| Endothal (0.002%) | --- | 63 |
| | 2 | 36 |
| | 3 | 17 |
| Uranyl nitrate (0.25%) | --- | 76 |
| | 2 | 41 |
| | 3 | 16 |
| | (Beta radiation rep) | |
| Dry seeds | 25 | 66 |
| | 50 | 30 |
| Water-soaked | 10 | 48 |
| | 20 | 32 |
| Colchicine (0.002%) | 10 | 44 |
| | 20 | 29 |
| Endothal (0.002%) | 10 | 46 |
| | 20 | 38 |
| Uranyl nitrate (0.25%) | 10 | 47 |
| | 20 | 35 |

--- without radiation.

Table 4. Influence of gamma and beta radiation with regards to per cent germination of pea seeds and the percentage of surviving $R_1$ plants showing dwarfing, leaf-variegation, and leaf-distortion under greenhouse conditions as result of irradiation of seeds dry or after soaking in water or solutions of colchicine, Endothal, and uranyl nitrate.

| Treatment | Radiation dosage ($1 \times 10^3$) | Germination (per cent) | Dwarfing, leaf-variegating, and leaf-distortion (per cent) |
|---|---|---|---|
| | (Gamma radiation r) | | |
| Dry seeds | --- | 99 | 0 |
| | 7.5 | 93 | 100 |
| | 15.0 | 77 | 100 |
| | 22.5 | 70 | 100 |
| Water-soaked | --- | 96 | 0 |
| | 1.5 | 100 | 9 |
| | 3.0 | 87 | 60 |
| | 4.5 | 63 | 100 |
| Colchicine (0.002%) | --- | 97 | 23 |
| | 1.5 | 91 | 53 |
| | 3.0 | 93 | 48 |
| | 4.5 | 74 | 100 |
| Endothal (0.002%) | --- | 93 | 0 |
| | 1.5 | 98 | 18 |
| | 3.0 | 86 | 23 |
| | 4.5 | 45 | 51 |
| Uranyl nitrate (0.25%) | --- | 97 | 0 |
| | 1.5 | 99 | 12 |
| | 3.0 | 88 | 36 |
| | 4.5 | 71 | 68 |
| | (Beta radiation rep) | | |
| Dry seeds | 20.0 | 99 | 26 |
| | 40.0 | 86 | 42 |
| | 60.0 | 52 | 69 |

(Continued on page 23)

Table 4. (Continued)

| Treatment | Radiation dosage (1 X $10^3$) | Germination (per cent) | Dwarfing, leaf-variegation, and leaf-distortion (per cent) |
|---|---|---|---|
| (Beta radiation rep) | | | |
| Water-soaked | 10.0 | 76 | 13 |
| | 20.0 | 53 | 9 |
| | 30.0 | 46 | 5 |
| Colchicine (0.002%) | 10.0 | 89 | 39 |
| | 20.0 | 48 | 46 |
| | 30.0 | 46 | 17 |
| Endothal (0.002%) | 10.0 | 75 | 7 |
| | 20.0 | 73 | 6 |
| | 30.0 | 63 | 5 |
| Uranyl nitrate (0.25%) | 10.0 | 81 | 9 |
| | 20.0 | 56 | 7 |
| | 30.0 | 50 | 4 |

--- without radiation.

regards to germination and variability. Hence these radiation dosages were used in the 1957 field experiment. The seeds were exposed to gamma radiation on May 29 and beta on May 31. A sample of 500 seeds were used in each treatment. The treatments were randomized in a split-plot design. The main plots were the sources of radiation, while the sub-plots were the soaking treatment and/or radiation treatments. The rows in each replication were 36 feet long. Each treatment was replicated four times.

The $R_1$ seedlings which showed visible leaf and plant changes were tagged and harvested separately from the normal-appearing plants to observe if any relationship existed between the variability in the $R_1$ plants and the variability obtained in the $R_2$ (second generation) plants. The per cent germination, percentage of abnormal plants, and the percentage of plants which survived were determined in the $R_1$. The near-dry plants were harvested by hand. Further drying was accomplished in a forced warm-air drying oven at 80° F. The pods were threshed by machine.

The $R_2$ generation was grown in the field in the summer of 1958. A sample of 300 seeds, where seed was available, from the separated $R_1$ plants was planted for each treatment. Seeds of the $R_1$ plants which showed visible differences were sown adjacent to the seeds from plants of the same treatment which showed no visible effects of radiation in the $R_1$. The treatments were randomized in a paired split-plot design. Four replications were used.

The $R_2$ plants were observed for visible differences. Seeds of the plants differing from the normal perfection-type plant were harvested separately. Five seeds from each plant were sown in pots in the greenhouse

for additional observation in the $R_3$ (third generation), and an additional five seeds from the same plants were saved and tested for Fusarium root-rot resistance.

One pod was harvested from each of the remaining $R_2$ plants for each treatment. The seeds were bulked and saved to test for root-rot resistance. Since testing large numbers of seeds is desirable and space was limited in the greenhouse, four-200 seed samples from each of the following treatments were tested for resistance to Fusarium root-rot:

| Treatment | Radiation dosage ($1 \times 10^3$) |
|---|---|
| | (Gamma r) |
| Dry seeds | 7.5 |
| | 15.0 |
| Water-soaked | 1.5 |
| | 3.0 |
| Colchicine | 1.5 |
| | 3.0 |
| Endothal | 1.5 |
| | 3.0 |
| Uranyl nitrate | 1.5 |
| | 3.0 |
| | (Beta rep) |
| Dry seeds | 20.0 |
| | 40.0 |

The seeds were sown in quartz sand in wooden flats in the greenhouse. Each flat contained a row of non-irradiated Early Perfection as a control.

The plants were inoculated with isolate 48 of Fusarium solani f. pisi. The technique of Lockwood and Ballard (37) was used. Spore suspensions were calibrated to 70 per cent light transmittance with a photoelectric colorimiter. When the suspensions were diluted to 1:4, there was approximately 200,000 spores per ml. Ten ml. of the diluted suspension was pipetted to each row with the inoculum being directed to the base of the seedlings when all seedlings had emerged. Some seedlings were larger than others at the time of inoculation because of the difference in rate of emergence.

(1) Results.

(a) Germination in the $R_1$.

As the radiation dosage levels were increased, there was a reduction in germination (Figures 4, 5, and 6). The effect of beta radiation on germination was more pronounced at the lower dosages of gamma radiation in both dry and soaked seeds. Eighty-two per cent of the seeds germinated in the dry control. At a dosage of 20,000 rep, 28 per cent of the seeds germinated.

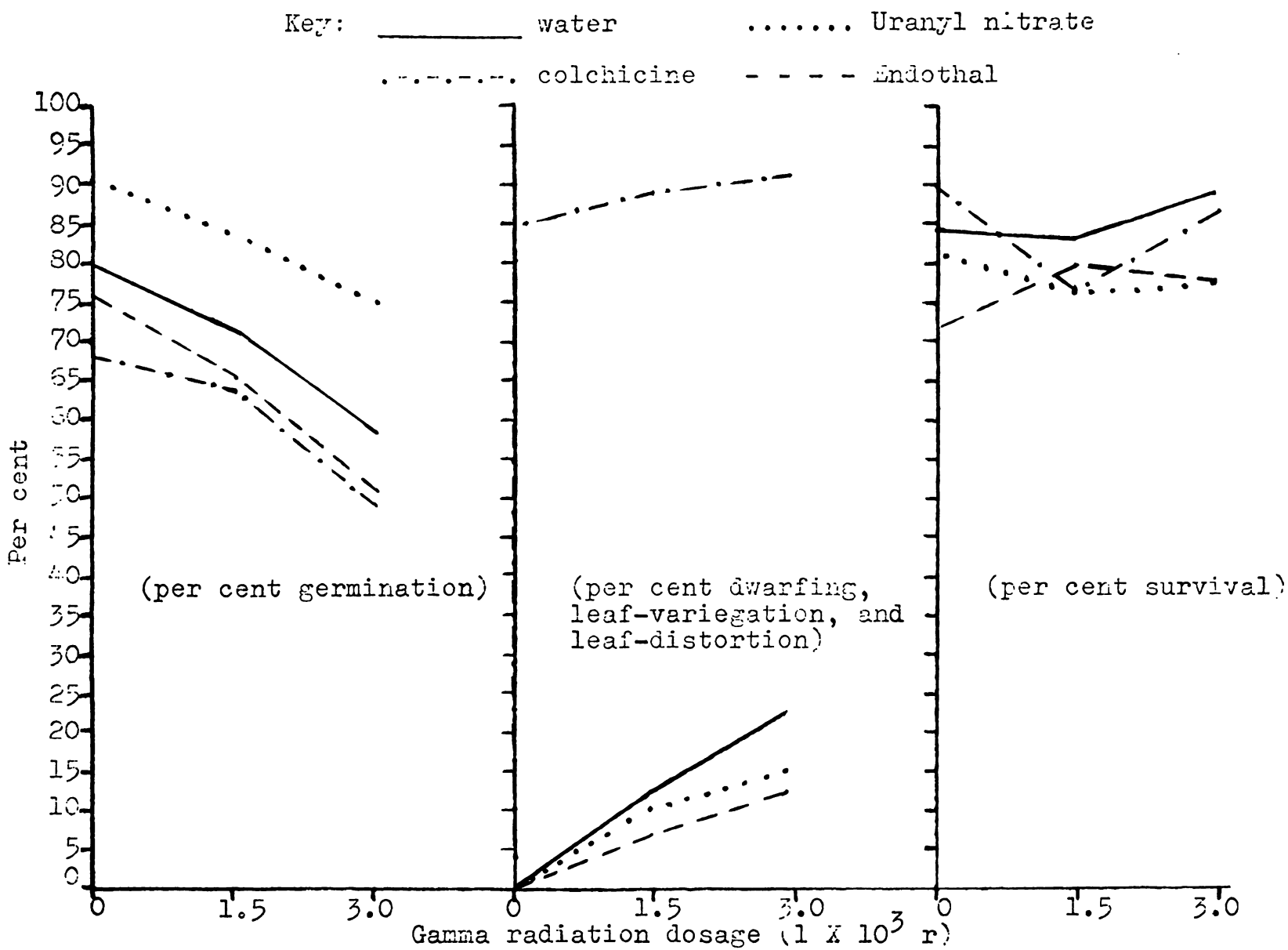


Figure 4. Influence of gamma radiation with regards to per cent germination, percentage of surviving $R_1$ pea plants showing dwarfing, leaf-variegation, and leaf-distortion, and per cent survival under field conditions as result of irradiation of the seeds after soaking in water or solutions of colchicine, Endothal, and uranyl nitrate.

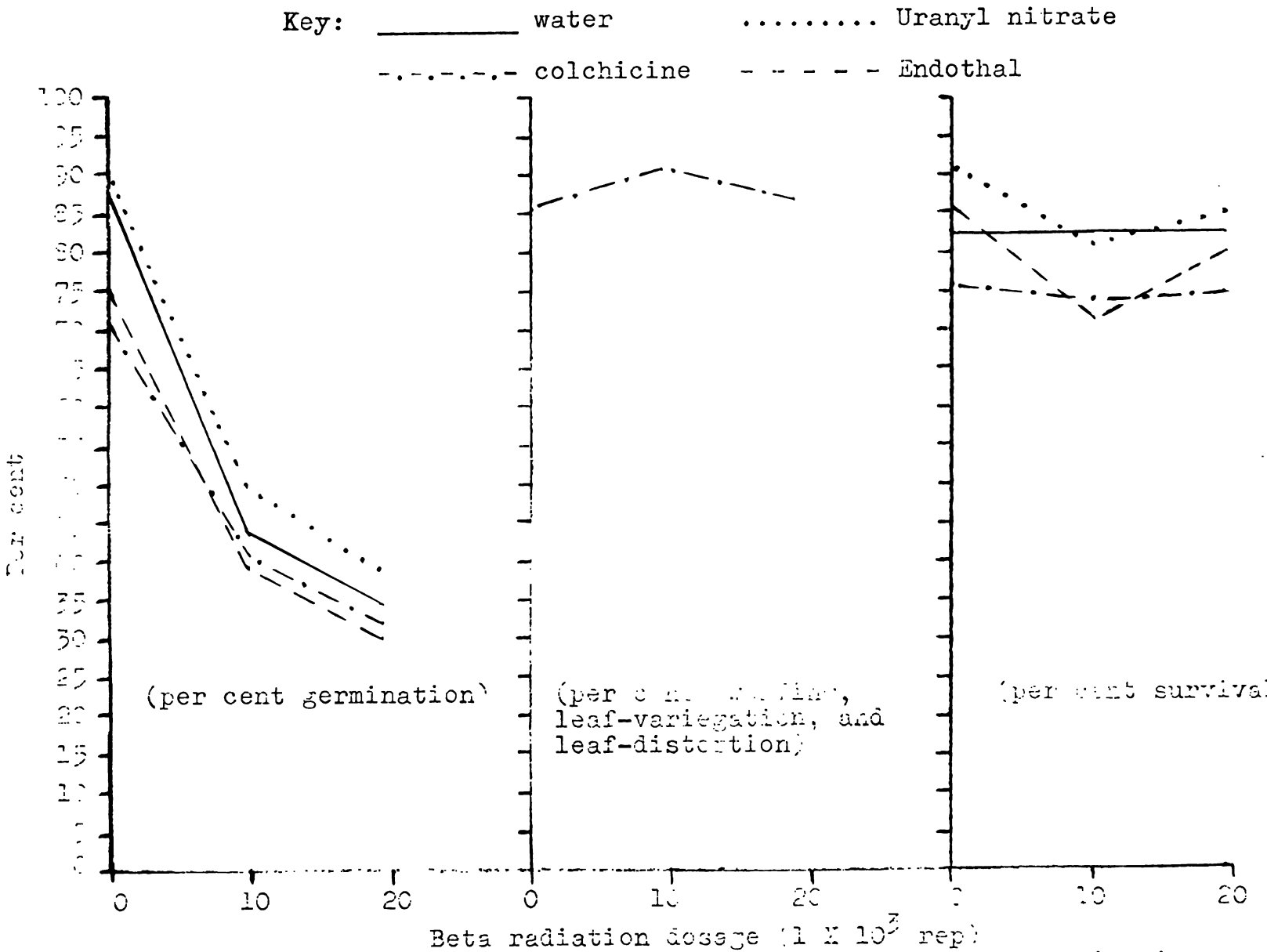


Figure 5. Influence of beta radiation with regards to per cent germination, percentage of surviving $R_1$ pea plants showing dwarfing, leaf-variegation, and leaf-distortion, and per cent survival under field conditions as result of irradiation of seeds after soaking in water or solutions of colchicine, Endothal, and uranyl nitrate.

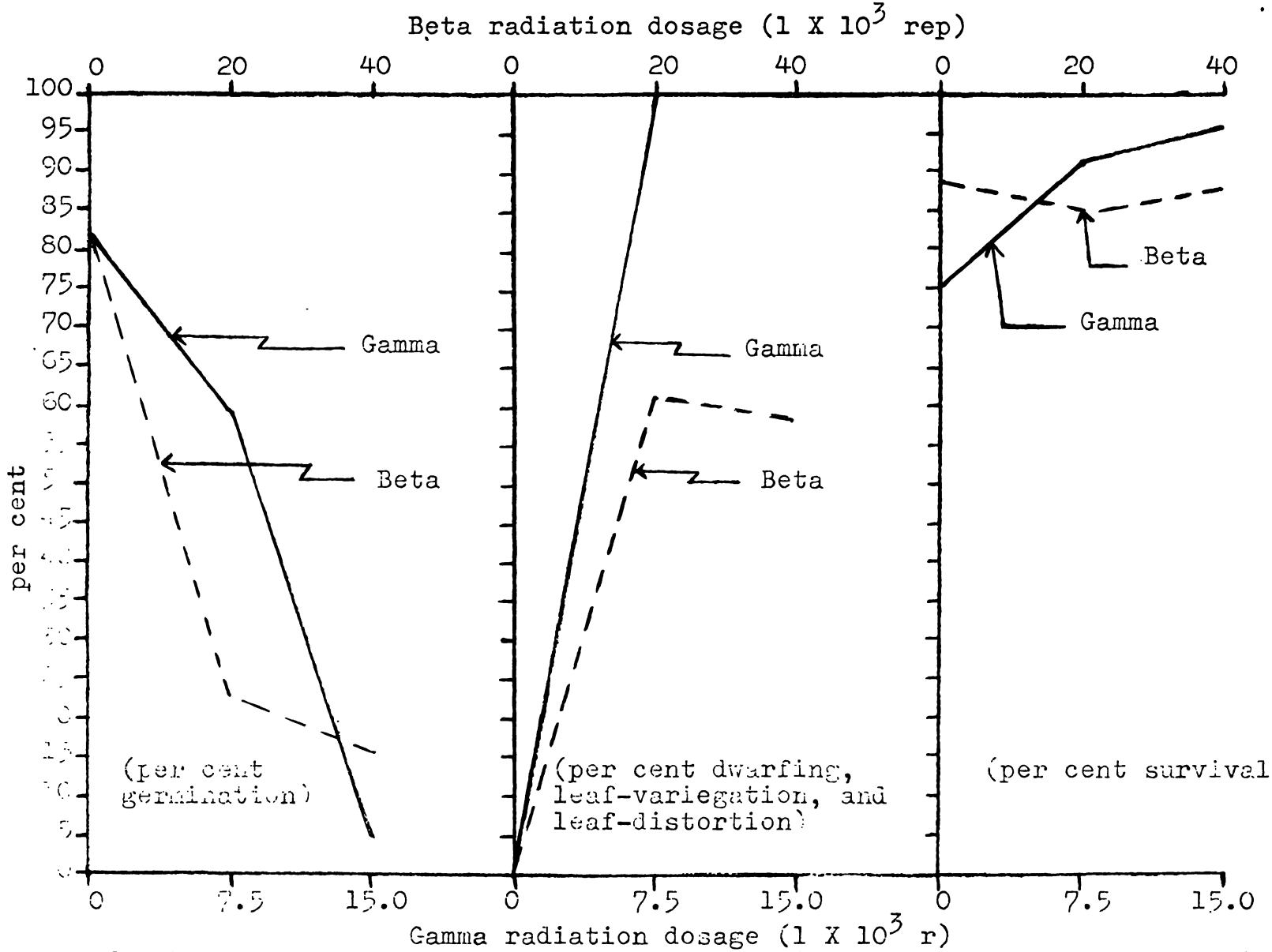


Figure 6. Influence of gamma and beta radiation with regards to per cent germination, percentage of surviving $R_1$ pea plants showing dwarfing, leaf-variegation, and leaf-distortion, and per cent survival under field conditions as result of irradiation of dry seeds.

When the dosage was doubled to 40,000 rep, 16 per cent of the seeds germinated. In comparison, a dosage of 7,500r gave 59 per cent germination. However, when the dosage was doubled to 15,000r, only 5 per cent of the seeds germinated.

A similar reduction in germination was found in irradiated soaked seeds; however, dosages of 1,500r, 3,000r, 10,000 rep, and 20,000 rep on soaked seeds did not cause as great a reduction as 7,500r, 15,000r, 20,000 rep, and 40,000 rep on dry seeds. Seeds exposed to 10,000 rep of beta radiation after soaking in either water, Endothal, colchicine, or uranyl nitrate gave less germination than seeds which received the same soaking treatments and exposed to 1,500r of gamma radiation. A similar decrease was obtained in both sources of radiation for the water and chemical treatments at dosages of 3,000r and 20,000 rep. Soaking seeds in the chemical solutions and subjecting them to either source of radiation did not appear to increase germination over water-soaking and irradiation.

Treatment with colchicine and Endothal without subsequent radiation reduced germination, when compared with the water-soaked control. On

the other hand, soaking seeds in uranyl nitrate appeared to stimulate germination over the water-soaked control. Ninety and 92 per cent of the uranyl nitrate soaked seeds germinated, whereas only 79 and 87 per cent of the water-soaked seeds germinated (Figure 4).

(b) Abnormalities in the $R_1$.

Dry and soaked seeds exposed to gamma radiation resulted in more leaf and plant abnormalities than exposing seeds to beta radiation (Figures 4, 5, and 6). The exposure of seeds to dosage levels of 7,500r and 15,000r of gamma radiation resulted in visible changes in 100 per cent of the plants, while beta radiation produced similar changes in only 62 per cent of the plants at 20,000 rep and 58 per cent at 40,000 rep (Figure 6).

Soaking seeds prior to gamma radiation slightly increased the number of visibly changed plants (Figure 4). In comparison, beta treatment did not produce any changes in the $R_1$ plants from soaked seeds (Figure 5).

Colchicine alone produced greater changes than either radiation treatment on pre-soaked seeds for the production of leaf-variegation and distortion in the $R_1$ plants (Figures 5 and 6). In both

sub-plots, colchicine-treatment of seeds resulted in 85 per cent of the $R_1$ seedlings exhibiting leaf changes. No changes were noted where Endothal and uranyl nitrate were used alone.

(c) Survival of $R_1$ plants.

Plant survival was not appreciably increased or decreased by the action of gamma and beta radiation on dry and soaked seeds.

(d) Variability in the $R_2$ plants.

Abnormal plants observed in the $R_2$ were distinguished as chlorophyll deficiencies, sterility, lateness, earliness, tallness, dwarfness, and branching at the base (Table 5). Seventy-two per cent of the changes observed were the result of gamma irradiation, while only 16 per cent can be associated with beta irradiation.

The chlorophyll deficiencies, which comprised 19 per cent of observed off-type plants, included viridis, chlorina, xantha, and albino types (Table 5). Seventy-eight per cent of the chlorophyll deficient plants were viridis and albinos.

Forty-four per cent of the abnormal plants exhibited degrees of fertility (Table 5). Nine per cent of these plants were sterile,

Table 5. Numbers of chlorophyll deficient, sterile, and otherwise morphologically or physiologically different $R_2$ pea plants as result of gamma and beta irradiation of seeds dry or after soaking in water or solutions of colchicine, Endothal, and uranyl nitrate.

| $R_1$ Treatment | Radiation dosage, ($1 \times 10^3$) | Chlorophyll deficient | | Sterile | | Otherwise*** different | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | I* | II** | I | II | I | II | I | II |
| | (Gamma radiation r) | | | | | | | | |
| Dry seeds | ---- | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | 7.5 | 0 | 2 | 0 | 2 | 0 | 5 | 0 | 9 |
| | 15.0 | 0 | 3 | 0 | 8 | 0 | 6 | 0 | 17 |
| Water-soaked | ---- | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| | 1.5 | 1 | 4 | 1 | 8 | 1 | 3 | 3 | 15 |
| | 3.0 | 1 | 0 | 4 | 5 | 2 | 2 | 7 | 7 |
| Colchicine | ---- | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 4 |
| | 1.5 | 0 | 1 | 1 | 0 | 1 | 0 | 2 | 1 |
| | 3.0 | 0 | 0 | 1 | 3 | 3 | 3 | 4 | 6 |
| Endothal | ---- | 1 | 0 | 2 | 0 | 0 | 0 | 3 | 0 |
| | 1.5 | 1 | 0 | 2 | 1 | 1 | 0 | 4 | 1 |
| | 3.0 | 0 | 0 | 0 | 3 | 2 | 2 | 2 | 5 |
| Uranyl nitrate | ---- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1.5 | 2 | 1 | 3 | 2 | 2 | 0 | 7 | 3 |
| | 3.0 | 0 | 0 | 1 | 0 | 5 | 4 | 6 | 4 |

(Continued on page 34)

Table 5. (Continued).

| $R_1$ Treatment | Radiation dosage ($1 \times 10^3$) | Chlorophyll deficient | | Sterile | | Otherwise*** different | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | I* | II** | I | II | I | II | I | II |
| | (Beta radiation rep) | | | | | | | | |
| Dry seeds | ---- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20.0 | 2 | 0 | 3 | 0 | 3 | 0 | 8 | 0 |
| | 40.0 | 3 | 2 | 4 | 0 | 1 | 0 | 8 | 2 |
| Water-soaked | ---- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10.0 | 1 | 0 | 0 | 0 | 3 | 0 | 4 | 0 |
| | 20.0 | 2 | 0 | 2 | 0 | 1 | 0 | 5 | 0 |
| Colchicine | ---- | 0 | 0 | 2 | 1 | 1 | 0 | 3 | 1 |
| | 10.0 | 0 | 0 | 3 | 0 | 1 | 0 | 4 | 0 |
| | 20.0 | 0 | 0 | 2 | 0 | 2 | 1 | 4 | 1 |
| Endothal | ---- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10.0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | 20.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Uranyl nitrate | ---- | 1 | 0 | 3 | 0 | 2 | 0 | 6 | 0 |
| | 10.0 | 2 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| | 20.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | | 18 | 14 | 37 | 35 | 32 | 27 | 87 | 76 |
| Percentage | | 11 | 8 | 23 | 21 | 20 | 17 | | |

---- without radiation.
I* plants appeared normal in the $R_1$.
II** plants showed dwarfing, leaf-variegation, and leaf-distortion in the $R_1$.
*** plants characterized with dwarfing, over-vegetative growth, branching at base of stem, earliness, and lateness.

vegetative, and flowered continuously without setting pods. One per cent of the sterile plants had petaloidic flowers with as many as 15 petals per flower. These flowers, which were without pistils and stamens, were usually in clusters. Types of sterile plants are shown in Figure 7.

The differences observed in the $R_2$ plants were not associated with the visibly changed $R_1$ plants, except for the following treatments: 7,500r and 15,000r on dry seeds, and 1,500r on water-soaked seeds (Table 5). Nine off-type plants were found in the 7,500r, and 17 in the 15,000r treatments. However, the water-soaked-1,500r treatment, had 15 off-type plants which originated from the changed $R_1$ plants, as compared to only three from the non-changed $R_1$ plants.

Chemical soaking of seeds prior to irradiation did not increase the variability in the $R_2$ as compared to irradiation of dry seeds. On the other hand, soaking with water prior to gamma irradiation increased the number of off-type plants. At a dosage of 1,500r, 18 plants or 11 per cent of the abnormal plants came from seeds which had been soaked in water prior to irradiation.

**Figure 7.** Degrees of sterility in $R_2$ pea plants as result of gamma and beta irradiation of seeds.
**Left:** petaloidic flowers; no pods.
**Middle:** normal-appearing flowers; pods later drop.
**Right:** normal-appearing flowers, pods with rudimentary ovules.

(e) Variability in the $R_3$ plants.

Plants of Lines 2-B, 5, 6-A, 6-B, 7-A, 7-B, 8, and 9 bred true for tallness in the $R_3$ and were significantly taller than the non-irradiated plants (Table 6). Plants of Line 3 bred true for dwarfness, and were significantly smaller. Plants of Line 1 were homozygous for dark green seed-coat. Plants of Line 2-C were homozygous for small pods and small seeds. The fifth internode of the plants of Line 2-C were non-measurable without histological observation, and instead of the usual type of double flowering where the peduncle branches dichotomously, these plants had two peduncles from the leaf-axila, each producing a single flower.

The 9-15th internodes of plants in Line 3 averaged 0.5 inches in length as compared to 1.5 inches for the non-irradiated Early Perfection plants.

Plants of Line 4, from seeds treated with colchicine, had thick stems and leaves and heavy short pods and low fertility. The plants had the appearances of polyploids.

The plants of Line 7-B were tall, averaging 45 inches as compared to 17 inches for the non-irradiated plants. The foliage was gray rather than the normal green. The leaflets were narrower than the Perfection-type.

Table 6. Comparison of $R_3$ Perfection pea plants from seeds gamma or beta irradiated dry or after soaking in water or solutions of colchicine and uranyl nitrate with regards to days to flower, plant heights, and the description of the plants in the $R_2$ and $R_3$.

| $R_1$ seed treatment | Selection line No. | Description of selected plants in $R_2$ | Description of selected plants in $R_3$ | | |
|---|---|---|---|---|---|
| | | | Days to flower | Plant heights | Other characteristics |
| Gamma radiation (1 X $10_3$ r) | | | | | |
| Non-irradiated | | | 43 | 17 ± 1.92 | |
| Dry seeds 7.5 | (1) | Tall; late; dark-green seed coats. | 43 | 29 ± 1.11 | Seed coats dark-green. |
| 15.0 | (2-A) | Tall; semi-sterile. | 55 | 11 ± 2.23 | One plant with dark-green foliage and excessive gray mottling. |
| | (2-B) | Tall. | 43 | 27 ± 2.02 | |
| | (2-C) | Normal height with small pods and seeds. | 51 | 17 ± 1.26 | Small pods and seeds; fifth internode micro in size; double flowering. |
| Water-soaked 1.5 | (3) | Dwarf; late; branched at base. | 45 | 13 ± 1.02 | Internodes, 9-15, averaged 0.5 inches long. |
| Colchicine | (4) | Tall. | 44 | 20 ± 1.47 | Thick leaves and stems; semi-sterile. |
| Uranyl nitrate | (5) | Tall. | 43 | 25 ± 1.37 | |
| 3.0 | (6-A) | Tall. | 43 | 25 ± 1.84 | |
| | (6-B) | Tall; late. | 58 | 25 ± 2.37 | |
| Beta radiation (1 X $10_3$ rep) | | | | | |
| Dry seeds 20.0 | (7-A) | Tall. | 43 | 28 ± 3.42 | |
| | (7-B) | Tall. | 52 | 45 ± 1.22 | Gray foliage; narrow leaflets. |
| Water-soaked 10.0 | (8) | Tall; late. | 43 | 25 ± 2.33 | |
| 20.0 | (9) | Tall; late. | 43 | 26 ± 2.15 | |
| Colchicine 20.0 | (10) | Tall. | 46 | 20 ± 1.32 | Plants branched at base. |

Double branching at the base of the main stem was characteristic in the plants of Line 10.

(f) Test of $R_3$ plants from irradiated seeds for Fusarium root-rot resistance.

None of the $R_3$ plants exhibited resistance to Fusarium root-rot.

(g) Observation of two tall mutants for three generations.

Two tall plants were found in the preliminary studies. One in the $R_2$ and the other in the $R_3$. These plants originated from seeds which were exposed to 20,000r of gamma radiation. These plants were increased and observed in subsequent generations. Means and standard deviations are presented in Table 7 for height, number of nodes to the first flower, and internode length. The comparative height of plants of Line A and Early Perfection plants from non-irradiated seeds are shown in Figures 8 and 9. The $R_3$ of Line A which was grown from January to March flowered a month later than the non-irradiated plants and attained a height of 64 inches, as compared to 28 inches for the control. The $R_4$ of the same line, grown in April, May, and June, flowered with non-irradiated Early Perfection and averaged 41 inches in height. The control plants

Table 7. Comparison of non-irradiated pea plants and plants of Lines A and B of Early Perfection which resulted from gamma irradiation of dry seeds with regards to plant heights, numbers of nodes to first flower, and the length of internodes.

| Population | Generation | Height (in.) | Nodes to flower | Internode length (in.) |
|---|---|---|---|---|
| Non-irradiated** | | 28 ± 4.3 | 15 ± 1.4 | 1.5 ± 0.83 |
| Line A | $R_2$* | 46 --- | 21 --- | 2.0 --- |
| | $R_3$** | 64 ± 5.0 | 22 ± 2.4 | 2.5 ± 0.48 |
| | $R_4$*** | 41 ± 2.4 | 18 ± 0.3 | 2.3 ± 0.22 |
| Line B | $R_3$** | 43 --- | 18 --- | 1.8 --- |
| | $R_4$*** | 42 ± 5.8 | 20 ± 1.3 | 1.8 ± 0.81 |
| Non-irradiated*** | | 29 ± 5.1 | 16 ± 1.0 | 1.5 ± 0.24 |

--- only one plant observed.
* plants grown from Feb.-Mar.
** plants grown from Jan.-Mar.
*** plants grown from Apr.-June.

averaged 29 inches when grown from April to June. The $R_4$ of Line B was similar to the $R_4$ of Line A when grown under the same conditions. The plants can be considered homozygous for tallness because the lines were no more variable than the non-irradiated control.

Cytological examination of mother-megaspores in the $R_4$ plants showed meiosis to be normal.*

* Verification by G. B. Wilson, Professor of Botany, M.S.U.

Measurements of pollen grains, stomata, and cell-sizes of epidermis tissue indicated no significant differences between Line A and the control.

**Figure 8.** Early Perfection (three plants on the left) and $R_3$ plants of the Line A tall mutant of Early Perfection which resulted from gamma irradiation of dry seeds.

Figure 9. Early Perfection (two plants on the left) and $R_4$ plants of the Line A tall mutant of Early Perfection which resulted from gamma irradiation of dry seeds.

## PART II. FUSARIUM ROOT-ROT INHERITANCE STUDIES

### A. Experimental materials and methods

Plants of an Afghanistan pea-introduction, 140165, which exhibited resistance to Fusarium solani f pisi in a screening test, were crossed to the root-rot susceptible commercial parent, Early Perfection. Parental crosses and backcrosses were made in the following ways:

(1) 140165 X Early Perfection

BC (a) $F_1$ X 140165

BC (b) $F_1$ X Early Perfection

(2) Early Perfection X 140165

BC (c) $F_1$ X Early Perfection

BC (d) $F_1$ X 140165

Hybridizations were made without bagging the flowers. However, since the stigmas were exposed, frequent spray applications were made to control greenhouse insects. All crosses were marked with tags. Naturally selfed seeds were saved from both parents and the $F_1$ populations.

The $F_2$ populations were grown in an air-cooled greenhouse room during the summer of 1958 to facilitate a larger pod-set and higher seed-yields. Sixty seeds of each $F_1$ population were distributed among 10 pots. Each $F_2$ population represented a random sample of seed harvested from selfed $F_1$ plants.

The seeds of parental, $F_1$, backcross, and $F_2$ populations were classified according to various seed characteristics to determine whether the resistance as found in the parent was associated with any specific seed character. The seed characters were wrinkled and smooth, either with or without purple dotting of the seed coats.

The testing of seeds for resistance was conducted in the greenhouse. The seeds were mechanically scarified and surface sterilized for 15 minutes in a chlorox solution, diluted 1:10. The seed was sown approximately one and one-half inches deep in washed quartz sand in metal pans. Twenty seeds, from the larger lots were sown in each row. Each pan included a check row of Early Perfection. The pans were placed in tanks in which the water was maintained at 82° F. The test contained a sample of the parents and all seed-classes of both $F_1$, both $F_2$, and the four backcross populations. The test of the $F_3$ progenies were from both $F_2$ populations. The seedlings were inoculated by the previously described technique of Lockwood and Ballard (37).

The plants were carefully lifted approximately three weeks after inoculation and the roots examined for Fusarium root-rot symptoms. Plants of the parental, backcross, and $F_2$ populations were divided into four classes depending on their severity of symptom expression. These classes were

none-0, slight-1, moderate-2, and severe-3. To determine whether resistance was either qualitative or quantitative, plants of the $F_3$ test were classified according to the severity of the disease on three areas of the plant, top, collar (point of seed attachment), and the roots. One to three points were allowed for each area of the plant depending upon the severity of the disease symptoms. The top of the plant was classified by the degree of wilting. The collar was classified by the amount of brown discoloration. The roots were classified by the amount of discoloration and the number of rootlets. The total number of points, summation of points for the three areas, served as the disease rating. A rating system of 0 to 9 was used with 0 representing disease-free plants. Rated plants are shown in Figure 10.

## B. Experimental Results

### 1. Seed coat pigmentation of parental, $F_1$, backcross, and $F_2$.

The seed of the cross, Early Perfection X 140165, had a transparent seed coat. The reciprocal cross, 140165 X Early Perfection, produced seeds with pigmented coats, similar to seeds of the resistant parent. Seeds in each $F_2$ and each backcross to Early Perfection were separated into eight classes (Table 8). Backcrosses to 140165, as well as the $F_1$, 140165 X Early Perfection, had only two classes. No

Figure 10. $F_3$ pea plants rated according to Fusarium root-rot incidence on top of plant (left number), collar-point of seed-attachment (middle number), and roots (right number). 0 represents disease-free; 3 represents a badly diseased area. Left-right:

(Top) 0-0-0;
0-0-2;
0-1-0.

(Middle) 0-1-1;
0-2-1;
0-3-1.

(Bottom) 0-3-2;
1-3-2;
3-3-3.

Table 8. Numbers of plants per disease severity rating as related to seed classification for $F_1$, backcross, and $F_2$ populations which originated from crosses of Early Perfection to the Fusarium root-rot resistant parent, 140165.

| Population | Seed classification: Seed-surface Smooth (S) Wrinkled (W) | Seed classification: Cotyledon color Yellow (Y) Green (G) | Seed classification: Purple dotting Present (P) Absent (A) | Total of seed population (percent) | Disease Severity Ratings (No. of Plants): None | Slight | Moderate | Severe |
|---|---|---|---|---|---|---|---|---|
| Early Perf. | W | G | A | 100 | 2 | 1 | 112 | 317 |
| 140165 | S | Y | P | 36 | 47 | 3 | 8 | 0 |
| | S | Y | A | 64 | 50 | 8 | 4 | 2 |
| ($F_1$) Early Perf. X 140165 | S | Y | A | 100 | 45 | 39 | 48 | 53 |
| ($F_1$) 140165 X Early Perf. | S | Y | P | 54 | 83 | 1 | 6 | 6 |
| | S | Y | A | 46 | 34 | 11 | 0 | 9 |
| (BC) Early Perf. X 140165 $F_1$ X Early Perf. | S | Y | P | 24 | 23 | 0 | 3 | 4 |
| | S | Y | A | 7 | 4 | 2 | 0 | 0 |
| | W | Y | P | 14 | 13 | 2 | 1 | 0 |
| | W | Y | A | 10 | 4 | 0 | 5 | 2 |
| | S | G | P | 13 | 16 | 0 | 2 | 0 |
| | S | G | A | 7 | 4 | 3 | 0 | 0 |
| | W | G | P | 15 | 0 | 0 | 10 | 5 |
| | W | G | A | 10 | 3 | 0 | 0 | 7 |
| (BC) Early Perf. X 140165 $F_1$ X 140165 | S | Y | P | 72 | 59 | 1 | 0 | 0 |
| | S | Y | A | 28 | 29 | 11 | 0 | 0 |
| (BC) 140165 X Early Perf. $F_1$ X 140165 | S | Y | P | 54 | 46 | 7 | 1 | 0 |
| | S | Y | A | 46 | 52 | 3 | 4 | 0 |

Table 8. (Continued)

| Population | Seed classification: Seed-surface Smooth (S) Wrinkled (W) | Cotyledon color Yellow (Y) Green (G) | Purple dotting Present (P) Absent (A) | Total of seed population (percent) | Disease Severity Ratings (No. of Plants): None | Slight | Moderate | Severe |
|---|---|---|---|---|---|---|---|---|
| (BC) 140165 X Early Perf. $F_1$ X Early Perf. | S | Y | P | 17 | 10 | 0 | 1 | 0 |
| | S | Y | A | 10 | 8 | 0 | 0 | 0 |
| | W | Y | P | 15 | 1 | 0 | 0 | 9 |
| | W | Y | A | 13 | 4 | 0 | 6 | 0 |
| | S | G | P | 12 | 6 | 0 | 2 | 0 |
| | S | G | A | 9 | 5 | 0 | 0 | 0 |
| | W | G | P | 16 | 7 | 0 | 0 | 0 |
| | W | G | A | 8 | 3 | 0 | 1 | 0 |
| ($F_2$) Early Perf. X 140165 | S | Y | P | 29 | 125 | 20 | 2 | 1 |
| | S | Y | A | 22 | 96 | 11 | 18 | 13 |
| | W | Y | P | 16 | 69 | 12 | 27 | 27 |
| | W | Y | A | 8 | 37 | 6 | 1 | 6 |
| | S | G | P | 12 | 65 | 5 | 12 | 10 |
| | S | G | A | 7 | 38 | 1 | 0 | 0 |
| | W | G | P | 4 | 20 | 1 | 4 | 9 |
| | W | G | A | 2 | 11 | 0 | 2 | 1 |
| ($F_2$) 140165 X Early Perf. | S | Y | P | 34 | 128 | 13 | 6 | 7 |
| | S | Y | A | 19 | 74 | 1 | 4 | 1 |
| | W | Y | P | 14 | 67 | 5 | 9 | 1 |
| | W | Y | A | 9 | 25 | 3 | 3 | 8 |
| | S | G | P | 11 | 39 | 12 | 7 | 5 |
| | S | G | A | 7 | 40 | 0 | 0 | 0 |
| | W | G | P | 4 | 8 | 4 | 0 | 8 |
| | W | G | A | 2 | 15 | 0 | 3 | 3 |

transparent Perfection-type seed was obtained in the $F_2$ and backcross populations. Seeds with transparent coats were obtained from $F_2$ plants irrespective of flower color. This can be seen where data in Table 10, which lists the classified $F_3$ seeds, is compared with data in Table 9. No white-flowered plants produced seeds with either colored and/or purple dotted seed coats.

2. Test for resistance: backcross and $F_2$ populations.

Disease ratings of the backcross and $F_2$ populations were grouped on the basis of seed-type to determine if wrinkled seeds and non-purple-dotted seeds were as resistant as smooth seeds and purple-dotted seeds (Table 8). Plants in the ratings of 0 and 1 were considered as resistant. The ratios, based on the number of resistant plants to the total number of plants tested for each type of seed, are presented in Table 11. The smooth-seeded plants had considerably more resistance than those from wrinkled seeds within each population. Plants from purple-dotted and non-dotted seeds were equal in resistance (Table 11).

Seed coats were removed from seeds of 140165 to determine whether the seed coat itself, other than color, was influencing resistance to Fusarium root-rot. It was found that seed-coat removal did not influence resistance. Cuttings made of disease-free plants of the resistant parent were inoculated

to further test the role of the seed coat in resistance. Plants from cuttings were as resistant as those plants grown from seed.

3. Test for resistance: $F_3$ populations.

Both $F_3$ populations were approximately equal with regard to resistance (Figure 11). The 140165 parent had slightly more resistance than either $F_3$, while Early Perfection was highly susceptible to the disease. The data were based on the total number of plants for the combined classes of seed in each population. Resistance was not associated with the color of the seed coat, nor with the seed-shape. The data showing this relationship is shown in Figures 12, 13, 14, and 15. Seeds with transparent coats were as resistant as pigmented seeds.

4. Relationship between $F_3$ progeny-types and resistance to root-rot.

The intermediate length internode (0.8cm) $F_3$ plants appeared to be more resistant than the long internode (2.0 cm) 140165-type plants. Ratios were obtained by rating each plant on a point system in order to establish relative degrees of resistance. The total number of points was obtained by multiplying the number of short plants in each disease rating in Table 10 by their disease rating. The total number of points was divided by the total number of plants to give a

ratio. The following ratios were obtained: 0.33 for 140165-type plants as compared to 0.45 for the Perfection-type plants in the cross, 140165 X Early Perfection; 0.30 for the 140165-type plants as compared to 0.44 for the Perfection-type plants in the reciprocal cross, Early Perfection X 140165.

Table 9. Description of $F_2$ seeds and $F_2$ plants of the populations, Early Perfection X 140165 and 140165 X Early Perfection, used in the Fusarium root-rot inheritance study.

| | $F_2$ seeds | | | $F_2$ plants | |
|---|---|---|---|---|---|
| | Seed-surface | Cotyledon color | Purple dotting | Flower color | Node color |
| Seed lot | Smooth (S) Wrinkled (W) | Yellow (Y) Green (G) | Present (P) Absent (A) | Pigmented (P) White (W) | Pigmented (P) Colorless (C) |
| 140165 X Early Perfection | | | | | |
| 101 | W | G | P | P | P |
| 102 | S | G | P | P | P |
| 103 | S | G | P | P | P |
| 104 | S | G | A | W | C |
| 105 | S | G | A | P | P |
| 106 | S | G | A | P | P |
| 107 | W | G | A | P | P |
| 108 | W | Y | A | P | P |
| 109 | W | Y | A | W | C |
| 110 | W | Y | A | P | P |
| 111 | W | Y | A | P | C |
| 112 | S | Y | A | P | C |
| 113 | S | Y | A | W | C |
| 114 | S | Y | A | P | P |
| 115 | S | Y | A | P | C |
| 116 | W | Y | P | P | C |
| 117 | W | Y | P | P | P |
| 118 | W | Y | P | P | C |
| 119 | W | Y | P | P | P |
| 120 | W | Y | P | P | P |
| 121 | W | Y | A | W | C |
| 122 | S | Y | P | P | P |
| 123 | S | Y | P | P | P |
| 124 | S | Y | P | P | P |
| 125 | S | Y | P | P | C |
| 126 | S | Y | P | P | C |
| 127 | S | Y | A | W | C |
| Early Perfection X 140165 | | | | | |
| 128 | W | G | A | P | P |
| 129 | S | G | P | P | C |
| 130 | W | G | P | P | P |
| 131 | W | G | P | P | P |
| 132 | W | G | P | P | C |
| 133 | W | G | P | P | C |
| 134 | W | Y | A | P | P |
| 135 | W | Y | A | P | C |

Table 9. (Continued)

| Seed lot | $F_2$ seeds: Seed-surface Smooth (S) Wrinkled (W) | $F_2$ seeds: Cotyledon color Yellow (Y) Green (G) | $F_2$ seeds: Purple dotting Present (P) Absent (A) | $F_2$ plants: Flower color Pigmented (P) White (W) | $F_2$ plants: Node color Pigmented (P) Colorless (C) |
|---|---|---|---|---|---|
| 136 | W | Y | A | W | C |
| 137 | S | Y | A | W | C |
| 138 | S | Y | A | P | P |
| 139 | S | Y | A | P | C |
| 140 | S | Y | A | P | P |
| 141 | S | G | P | P | P |
| 142 | S | G | A | P | P |
| 143 | S | G | A | P | C |
| 144 | S | G | A | W | C |
| 145 | S | G | P | P | C |
| 146 | S | Y | P | P | P |
| 147 | S | Y | P | P | P |
| 148 | S | Y | P | P | C |
| 149 | W | Y | P | P | P |
| 150 | W | Y | P | P | C |
| 151 | W | Y | P | P | P |
| 152 | W | Y | P | P | P |
| 153 | W | Y | P | P | P |
| 154 | W | Y | P | P | C |
| 155 | S | Y | P | P | P |
| 156 | S | Y | P | P | P |

Table 10. Numbers of F3 progeny in each Fusarium root-rot disease severity rating as related to numbers and description of F3 seeds.

| Seed lot | F3 seeds: Seed-surface, Smooth (S) Wrinkled (W), Smo. (No. of seeds) | F3 seeds: Seed-surface, Smooth (S) Wrinkled (W), Wri. (No. of seeds) | F3 seeds: Cotyledon color, Yellow (Y) Green (G), Yel. (No. of seeds) | F3 seeds: Cotyledon color, Yellow (Y) Green (G), Gr. (No. of seeds) | F3 seeds: Purple dotting, Present (P) Absent (A) | F3 seeds: Seed coat color, Colored (C) Transparent (T) | Disease severity ratings, 0 (No. of progeny) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 140165 X Early Perfection | | | | | | | | | | | | | | | | |
| 101 | 12 | 4 | | G | P | C | 1 | 1 | 4 | 8 | 1 | 1 | 0 | 0 | 0 | 0 |
| 102 | 5 | | | G | A | C | 0 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 0 |
| 103 | 13 | | | G | A | T | 6 | 2 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 104 | 8 | | | G | A | T | 0 | 1 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 |
| 105 | 10 | | | G | A | T | 0 | 0 | 0 | 3 | 2 | 3 | 1 | 0 | 1 | 0 |
| 106 | 44 | 5 | | G | A | C | 13 | 7 | 8 | 10 | 6 | 2 | 1 | 0 | 0 | 0 |
| 107 | | 6 | | G | A | T | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 108 | | W | 6 | 6 | A | C | 3 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |
| 109 | | W | 41 | 7 | A | T | 5 | 12 | 6 | 10 | 6 | 3 | 0 | 0 | 0 | 2 |
| 110 | | 20 | Y | | A | C | 0 | 3 | 4 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| 111 | | W | 4 | 2 | P | C | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 1 |
| 112 | S | | 11 | 7 | A | T | 1 | 0 | 3 | 6 | 2 | 1 | 1 | 1 | 0 | 0 |
| 113 | 10 | 4 | Y | | A | T | 0 | 0 | 7 | 4 | 2 | 0 | 0 | 0 | 1 | 0 |
| 114 | 66 | 15 | 71 | 10 | (4WG) A | C | 4 | 15 | 11 | 19 | 13 | 6 | 2 | 1 | 4 | 4 |
| 115 | 39 | 6 | 43 | 2 | A | C | 4 | 10 | 9 | 12 | 5 | 1 | 0 | 0 | 1 | 1 |
| 116 | | W | 7 | 3 | P | C | 1 | 2 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 |
| 117 | | 6 | Y | | A | T | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| 118 | | W | 17 | 1 | A | C | 7 | 4 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 119 | | W | 3 | 3 | P | C | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 120 | | W | 11 | 4 | A | C | 2 | 5 | 4 | 1 | 2 | 0 | 0 | 1 | 0 | 0 |
| 121 | | W | 4 | 1 | A | T | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 122 | 62 | 23 | 74 | 11 | (3WG) P | T | 16 | 18 | 16 | 21 | 8 | 4 | 1 | 0 | 0 | 0 |
| 123 | 45 | 8 | 48 | 5 | P | C | 14 | 14 | 5 | 8 | 3 | 5 | 0 | 0 | 0 | 2 |
| 124 | 24 | 4 | 25 | 3 | A | C | 0 | 6 | 3 | 5 | 9 | 1 | 1 | 1 | 0 | 1 |
| 125 | S | | 22 | 3 | A | C | 0 | 4 | 11 | 4 | 4 | 0 | 0 | 0 | 1 | 0 |
| 126 | 16 | 4 | 13 | 7 | P | C | 3 | 3 | 5 | 1 | 1 | 2 | 0 | 2 | 1 | 0 |
| 127 | 99 | 10 | 90 | 19 | A | T | 14 | 13 | 14 | 28 | 15 | 12 | 2 | 1 | 1 | 1 |

Table 10. (Continued).

| Seed lot | $F_3$ seeds: Seed-surface, Smooth (S) Wrinkled (W), Smo. (No. of seeds) | $F_3$ seeds: Seed-surface, Wri. (No. of seeds) | $F_3$ seeds: Cotyledon color, Yellow (Y) Green (G), Yel. | $F_3$ seeds: Cotyledon color, Gr. | $F_3$ seeds: Purple dotting, Present (P) Absent (A) | Seed coat color, Colored (C) Transparent (T) | Disease severity ratings, 0 (No. of progeny) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Early Perfection X 140165 | | | | | | | | | | | | | | | | |
| 128 | | 5 | | G | A | C | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 129 | 6 | | | G | P | T | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 130 | | 6 | | G | A | C | 0 | 2 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| 131 | | 2 | | G | P | T | | | | | | | | | | |
| 132 | | 3 | | G | P | C | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 133 | | 5 | | G | A | T | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 134 | | W | 17 | 9 | A | C | 6 | 7 | 3 | 6 | 0 | 2 | 0 | 0 | 0 | 0 |
| 135 | | W | 5 | 3 | A | C | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 136 | | W | 18 | 8 | A | T | 0 | 2 | 11 | 6 | 3 | 0 | 0 | 0 | 0 | 0 |
| 137 | 126 | 31 | 123 | 34 | (5WG) A | T | 8 | 20 | 34 | 19 | 27 | 13 | 6 | 4 | 12 | 8 |
| 138 | 6 | 3 | Y | | A | T | 0 | 1 | 1 | 3 | 2 | 2 | 0 | 0 | 0 | 0 |
| 139 | 32 | | Y | | A | C | 5 | 4 | 5 | 5 | 8 | 1 | 2 | 0 | 2 | 0 |
| 140 | 44 | 7 | 41 | 10 | A | C | 4 | 7 | 12 | 14 | 10 | 1 | 0 | 0 | 1 | 0 |
| 141 | 11 | | | G | A | C | 1 | 1 | 1 | 3 | 1 | 2 | 1 | 0 | 0 | 0 |
| 142 | 5 | 2 | | G | A | C | 0 | 0 | 0 | 1 | 1 | 1 | 3 | 0 | 1 | 0 |
| 143 | 12 | 3 | | G | A | C | 0 | 3 | 3 | 5 | 1 | 1 | 1 | 0 | 0 | 0 |
| 144 | 14 | | | G | A | T | 4 | 5 | 2 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 145 | 10 | 4 | | G | A | C | 2 | 3 | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 146 | 10 | | Y | | P | T | 2 | 2 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 147 | 18 | 2 | 16 | 4 | P | C | 5 | 6 | 6 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| 148 | S | | 9 | 1 | A | C | 0 | 0 | 6 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| 149 | | W | 18 | 7 | P | T | 2 | 10 | 4 | 2 | 1 | 1 | 3 | 0 | 2 | 0 |
| 150 | | 7 | Y | | A | C | 1 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| 151 | | W | 4 | 2 | A | T | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 152 | | W | 7 | 1 | A | C | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 153 | | W | 27 | 6 | P | C | 1 | 5 | 5 | 11 | 4 | 1 | 1 | 1 | 0 | 1 |
| 154 | | 4 | Y | | P | T | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 155 | S | | 13 | 3 | P | C | 0 | 8 | 0 | 2 | 4 | 1 | 0 | 0 | 0 | 1 |
| 156 | 10 | | Y | | A | C | 0 | 3 | 1 | 3 | 1 | 2 | 0 | 0 | 0 | 0 |

Table 11. Ratios of Fusarium root-rot resistant plants to total number of plants tested on basis of seed-shape and purple dotting of the seed coats for $F_1$, backcross, and $F_2$ populations.

| Population | Seed-shape | | Purple dotting | |
|---|---|---|---|---|
| | Smooth | Wrinkled | Present | Absent |
| ($F_1$) 140165 X Early Perfection | ----- | ----- | 0.885 | 0.833 |
| (BC) Early Perfection X 140165, $F_1$ X Early Perfection | 0.852 | 0.423 | 0.748 | 0.589 |
| (BC) Early Perfection X 140165, $F_1$ X 140165 | ----- | ----- | 1.000 | 1.000 |
| (BC) 140165 X Early Perfection, $F_1$ X 140165 | ----- | ----- | 0.981 | 0.932 |
| (BC) 140165 X Early Perfection, $F_1$ X Early Perfection | 0.906 | 0.484 | 0.556 | 0.741 |
| ($F_2$) Early Perfection X 140165 | 0.866 | 0.670 | 0.789 | 0.826 |
| ($F_2$) 140165 X Early Perfection | 0.911 | 0.784 | 0.865 | 0.878 |

----- all seeds were smooth and round.

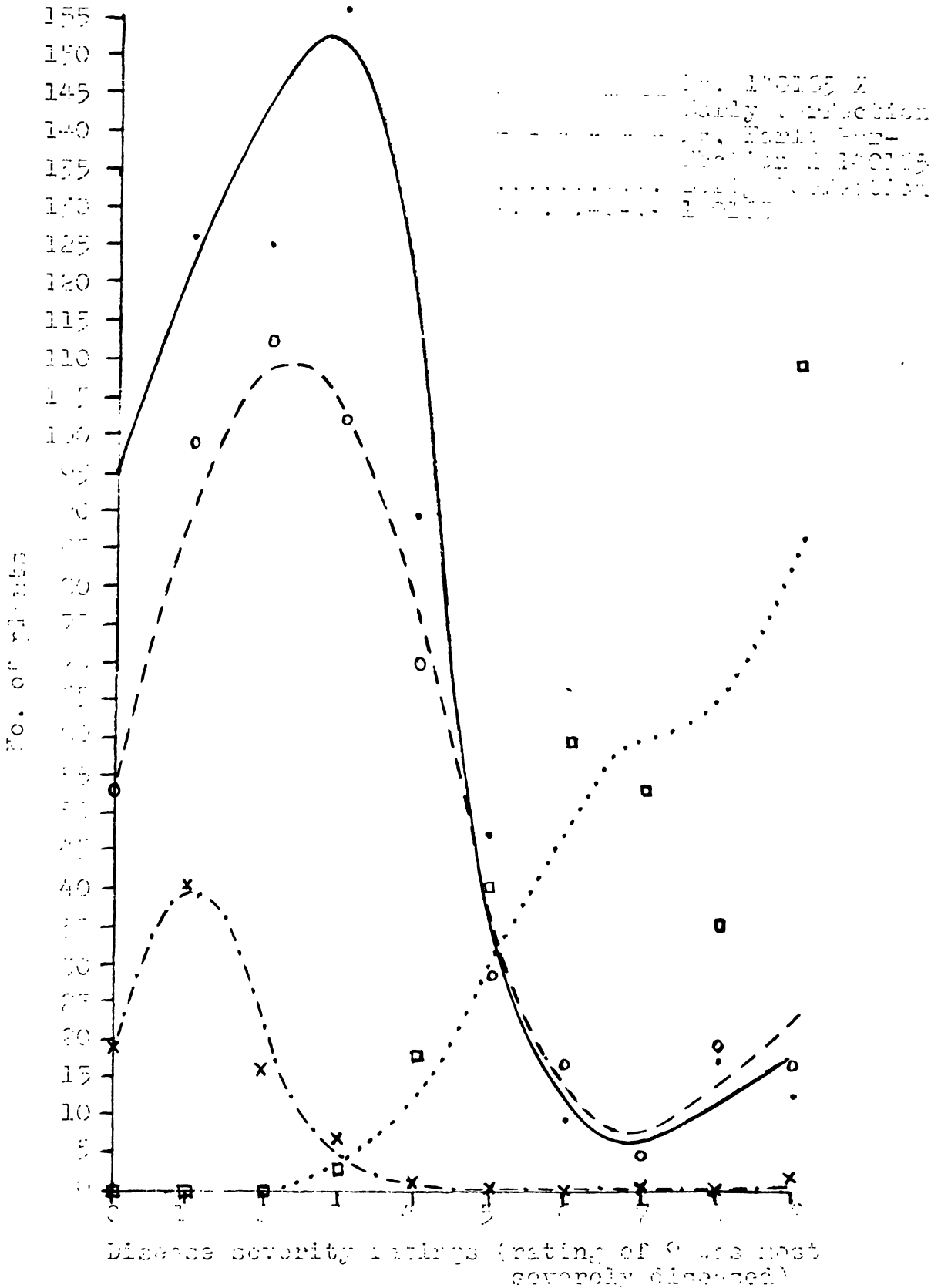


Figure 11. Distribution of progeny of Early Perfection and plant introduction, PI 0[illegible], and the F2 populations, Early Perfection × PI 0[illegible] and PI 0[illegible] × Early Perfection, with regards to performance in the Fusarium root-rot test.

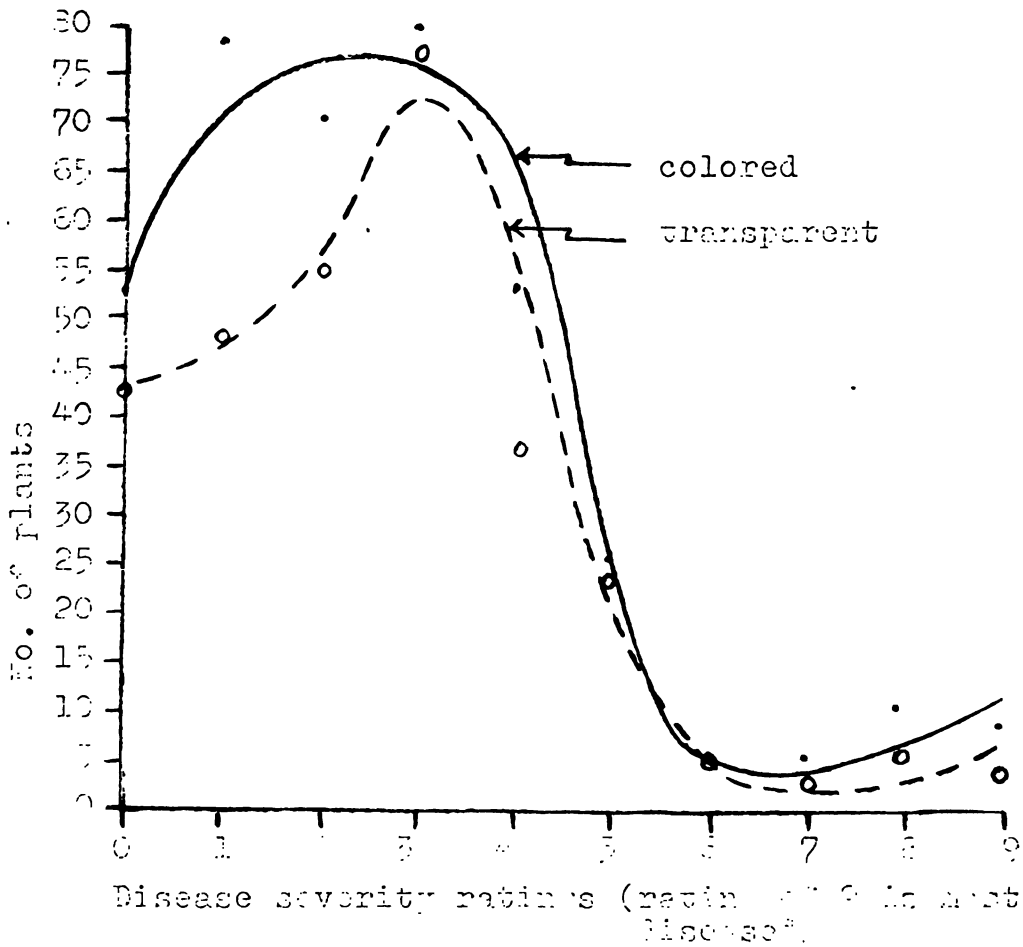


Figure 13. Distribution of progeny from colored and transparent coated seeds of the F2, 1-0155 / Early Perfection, with regards to performance in the Fusarium root-rot test.

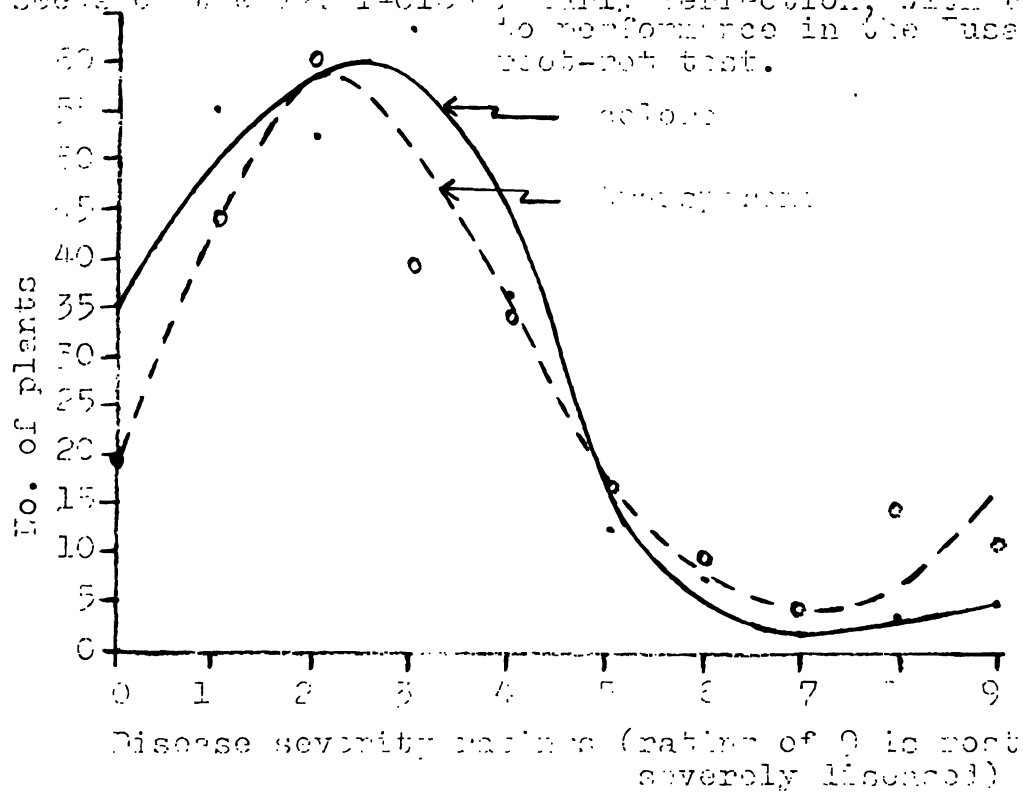


Figure 14. Distribution of progeny from colored and transparent coated seeds of the F2, Early Perfection / 140155, with regards to performance in the Fusarium root-rot test.

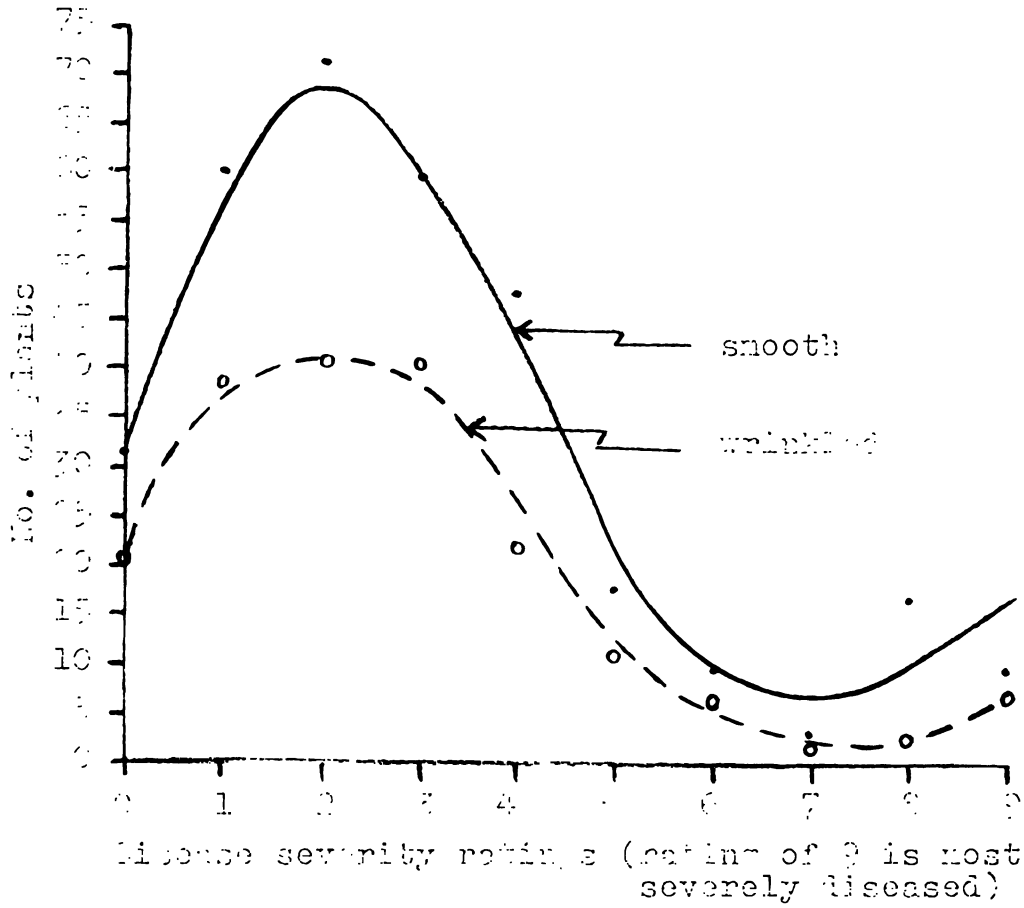


Figure 14. Distribution of the progeny from smooth and wrinkled seeds of the F2 Early Perfection x 140165, with regards to performance in the Fusarium root-rot test.

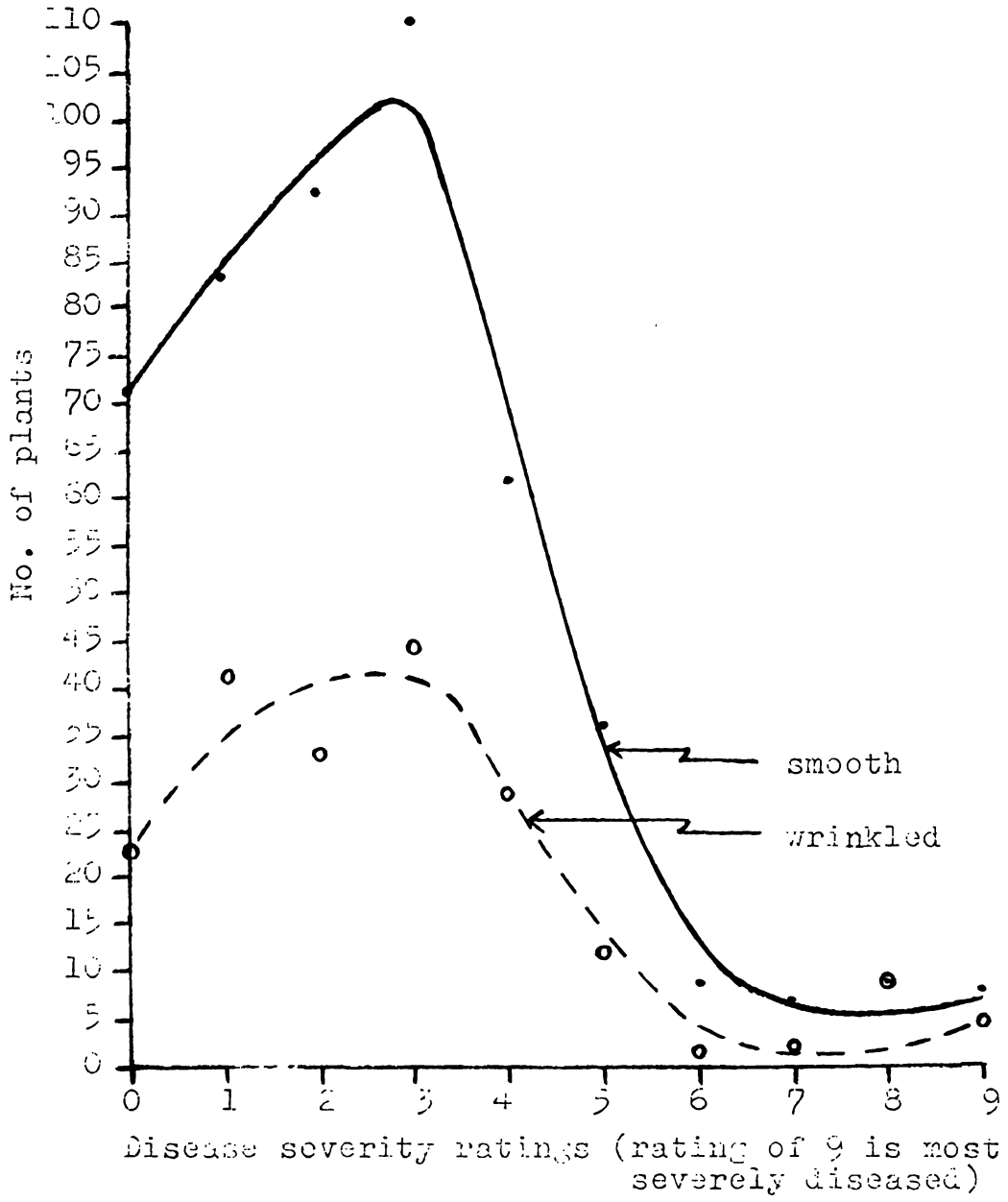


Figure 15. Distribution of the progeny from smooth and wrinkled seeds of the F , 1-0165 7 Early Perfection, with regards to performance in the Fusarium root-rot test.

## DISCUSSION

### A. Radiation Studies

#### 1. $R_1$ data: germination.

Gamma radiation dosages of 1,500r and 3,000r on soaked seeds were too low for maximum reduction in germination (Figure 4). An interaction of genotype and environment was evident in both field and greenhouse tests. Therefore, the results obtained at any one time are dependent upon the action of radiation on genes and chromosomes and the interaction of gene products with the existing environmental conditions. Results of radiation treatment cannot be predicted, since mutations and chromosome irregularities occur at random and the same changes are infrequently repeated (42). Higher radiation dosages could have been used in these studies on seeds soaked in water or chemical solutions, but since irregularities and mutations are more frequent at high dosage levels (42), there are also great detrimental changes with most favorable mutations (55). Therefore, it was reasoned that low dosage levels might produce beneficial changes with fewer deleterious ones.

The dosages of 7,500r and 15,000r of gamma radiation and 20,000 rep and 40,000 rep on dry seeds, and 10,000 rep and

20,000 rep of beta irradiation on soaked seeds gave satisfactory results in the $R_1$, as measured by the low germination (Figure 5). The concentrations of Endothal and uranyl nitrate used in this study were low, 0.002 per cent and 0.25 per cent, respectively. As a result, these chemicals appeared to have no visible radiomimetic effects on $R_1$ plants. However, the fact that the chemicals did not produce visible effects does not eliminate the possibility that Endothal and uranyl nitrate may be radiomimetic at higher concentrations, since the concentrations used were adjusted to give germination comparable to that of the water-soaking treatments. On the other hand, colchicine appeared to have radiomimetic properties at a concentration as low as 0.002 per cent. This is less than that which is effective in producing chromosome doubling and induction of mutations (10).

2. $R_1$ data: dwarfing, leaf-distortion, and leaf variegation.

The abnormal distortion and variegation observed in the early stages of growth in the $R_1$ plants did not continue with growth and eventually disappeared with the withering and dropping of the lower leaves. Similar plant and leaf abnormalities were obtained from X-irradiated maize seeds by Anderson, et al. (4) and Randolph (47). They observed the typical plant dwarfing and mottling of the lower leaves.

Similar leaf changes were found when tomato seeds (28) and Fodder pea seeds (21) were X-rayed. Since these changes were not carried over in the later generations as hereditary characters, there is an indication that the abnormalities might have been the result of either the effect of radiation on cell differentiation, effecting only the older, outer layer of cells of the embryo, or to a temporarily disturbed metabolism and the inhibition of plant auxins. It has been postulated that the first compound formed in irradiated cells is hydrogen peroxide (58). According to Quastler and Baer (46) and Skoog (52), the delay in growth of irradiated plants is the result of auxin inactivation by the peroxide. Clark (9) attributes the reduced growth-response in irradiated plants to a delay in mitosis, while death of irradiated seeds, and seedlings from irradiated seeds is caused by the inactivation of enzymes (33).

Histological examination of mottled leaves of plants from X-rayed seeds of sunflower by Noyucki (45) revealed that the chloroplasts were in clusters and that the chlorophyll was diffused throughout the leaf-cells. It was concluded that the distortion was the result of wavy, irregular-shaped epidermal cells with no distinction between the palisade and the parenchyma tissue. The mottled leaves were the result of a differential concentration of chloroplasts and intercellular spaces. Dark areas were composed of great

concentrations of chloroplasts with little or no intercellular spaces, while the light areas contained few chloroplasts with large intercellular spaces.

Beta irradiation of dry seeds resulted in 62 per cent plant-abnormalities in the $R_1$ at a dosage of 20,000 rep (Figure 6). At the higher dosage of 40,000 rep there was a decrease in the percentage of plants showing dwarfing, leaf-distortion, and leaf-variegation. The data presented in Figure 2 support the results with regards to the action of beta radiation on seeds. With regards to the number of plant abnormalities in the $R_1$, 52 per cent of the plants showed leaf-changes at 40,000 rep, while 21 per cent showed changes at 80,000 rep.

The inability of electrons to penetrate deeply into living cells, may explain the large number of plants surviving the high dosages of beta radiation and the small percentage of plants exhibiting leaf-changes in the $R_1$. By comparison, seeds which were irradiated with high dosages, 7,500r and 15,000r, of gamma rays produced plants which were all visibly changed (Figure 6). Gamma is a high ion penetrating radiation source (5). If the seeds receiving the beta radiation had been oriented so that the young embryos were toward the radiation source, equal penetration might have occurred and the effect might have been equal to that of gamma radiation. However, the cotyledons of the seeds

probably absorbed the rays and protected the embryo, since the electrons have a low penetrating power. Seeds which were exposed to 80,000 rep (Figure 1) and germinated and showed visible changes in the $R_1$ mighthave originated from seeds that had their embryos oriented to the side, perpendicular to the electron beam, and within the penetration range of the electrons. The depth-dose function relative to the per cent penetrance of the ions per unit density of material is shown in Figure 16. The specific gravity of dry Early Perfection seed is 1.4 and the seeds average 0.70 X 1.0 cm. through the short and long axis. The unit density of pea seed was determined by multiplying specific gravity by unit density (centimeters). At a depth of 0.1 cm., which is the depth of the embryo in Perfection seeds, the unit density is 0.14. This gives approximately 85 per cent penetrance of the ions from its source. Virtually no ions can penetrate the embryo to a depth of 0.3 cm. An embryo at the base of the seed, 0.7 cm. from the point of 100 per cent penetrance, would be unaffected by ionization.

3. $R_2$ data.

Only a few "possible-mutations" were found in this study (Table 5). The frequency of abnormal $R_2$ plants increased with an increase in the dosage rate on dry seeds. However,

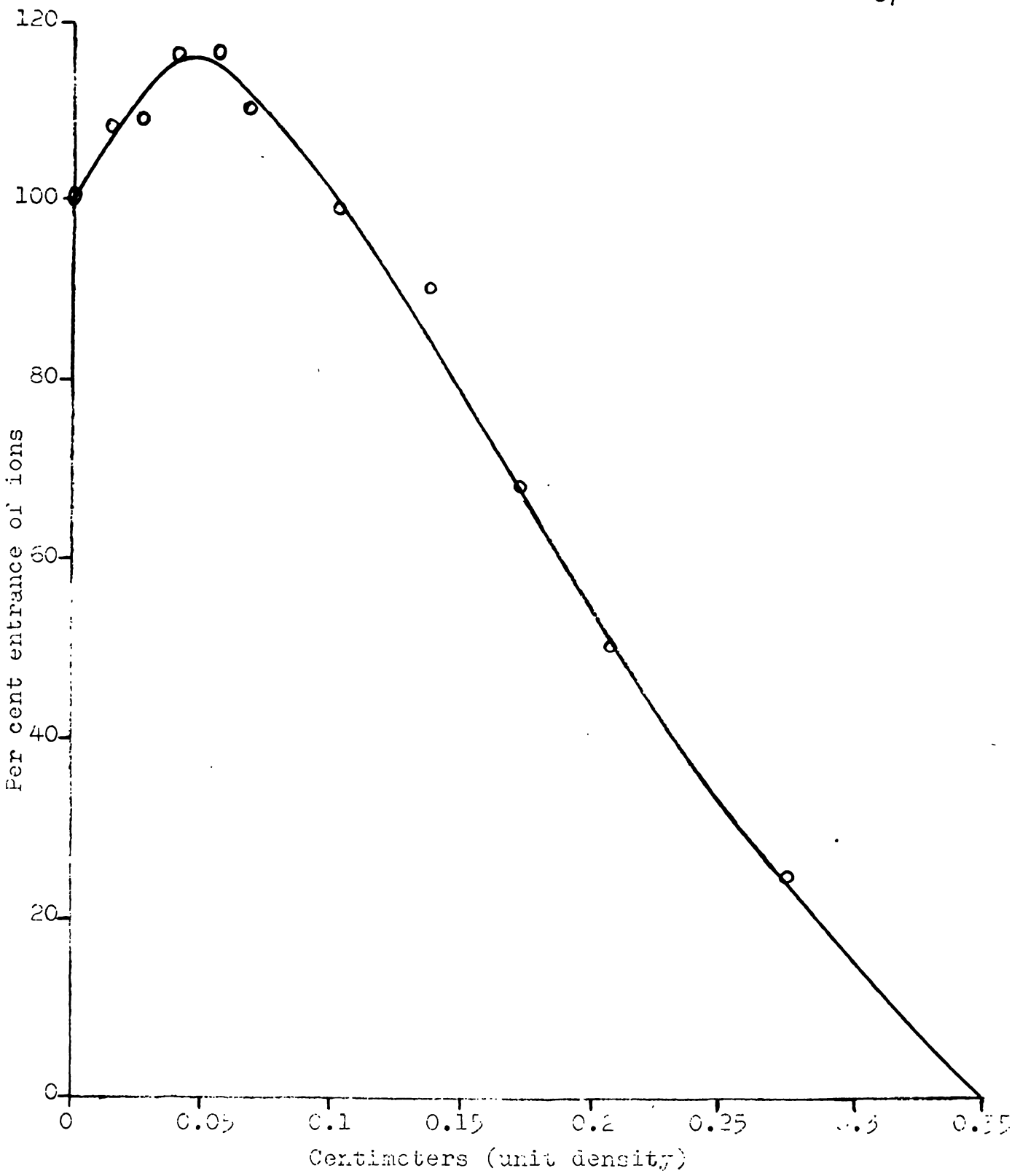


Figure 16. Depth-dose function, MSU General Electric 1-Mev electron accelerator at 1 Mev.

Courtesy of R. C. Nicholas, (44).

there appeared to be little change when the dosage was increased on soaked seeds, except where water was used. In contrast, Freisleben and Lein reported the actual mutation frequency falls continuously from 18.4 X $10^{-6}$ at 4,000r to 5.6 X $10^{-6}$ at 14,000r in X-irradiated dry barley seeds (25). This frequency is considerably lower than a frequency of 14.2 X $10^{-3}$ obtained in this study where the dry seeds were exposed to 15,000r. However, the data presented do not include the lethals which may or may not be considered as mutations. Gustafsson (25) suggested that Freisleben and Lein may not have considered the non-germinated seeds as lethals. He postulated that the mutation rate might have been greater at the dosage of 14,000r if lethals had been included.

A conclusion might be made that the visible changes observed in the $R_1$ plants were not hereditary, since there appeared to be no association between the $R_1$ plants and the number and kind of abnormal $R_2$ plants. Therefore, seeds from $R_1$ plants could be used without regard to their appearance in the $R_1$ when radiation is used for the production of mutations.

The infertility observed in some plants, as measured by the occurrence of pods with rudimentary ovules, indicated that fertilization may have occurred, but as result of

possible differences in the chromosomes, the ovules aborted. Radiation induced sterility has been reported in tomatoes (7), Fodder peas (21), and corn (4). Anderson and co-workers explained sterility on the basis of chromosome irregularities which gave rise to pollen grains deficient for some portion of the chromosome. Lamprecht (34) obtained partially sterile plants from X-irradiated pea seeds which were characterized by an interchange in the $X_1$, and segregation in the $X_2$ into 50 per cent fertile and 50 per cent semi-sterile plants. He also observed plants with a chromosome duplication which resulted in segregation into 75 per cent fertile and 25 per cent semi-sterile in the $X_2$ plants.

Forty-four per cent of the chlorophyll deficient plants found in the $R_2$ were virides, and 34 per cent albino types. Rosen (49) did not find any albinos in plants from X-irradiated pea seed, but frequently found xantha, lutescens, chlorina, and albo-virides types. Gustafsson (26) classified chlorophyll mutations in X-irradiated pea material as xantha and virides types.

4. $R_3$ data.

Gamma radiation appeared to be more effective than beta radiation for producing mutations (Table 6). Gamma irradiation of dry seeds was responsible for the production of the less common mutations, for example: dark-green seeds in Line 1 and small seeds and pods in Line 2-C.

Plants of Line 1 were tall in the $R_2$ generation and produced dark-green seeds. There was no change in the $R_3$ plants. This indicates that a mutation had occurred for these characters.

The mutation in plants of Line 2-C, which had small pods and seeds, might be explained on the basis of either a "position effect," or some other chromosome change. Since plants of Line 7-B were tall and had normal type foliage in the $R_2$, while the plants in the succeeding generation were all tall and narrow-leafed. Thus, a recessive gene mutation might have been responsible for the different leaf-type. Recessive mutations occur in the $R_3$ of self-pollinated plants which originated from seeds which were exposed to radiation (55).

Tall $R_3$ plants, as illustrated in Figures 8 and 9, appeared to be the most frequent type of mutation found. This mutation appears to be a dominant character. According to the review of pea-genetics by Wade (59), a series of genes or modifying genes control plant-height which is determined by the number of internodes prior to flowering, and the length of the internodes. However, other growth-inhibiting genes are present which condition the action of the genes for long internodes. According to DeHann (10) at least one of the inhibiting genes must be in the recessive state with the gene which controls long internodes in order to have a

tall plant. A mutation of the genes for tallness and of one or several of the growth-inhibiting genes might have occurred for an intermediate height Early Perfection plant to mutate to tall. Plants of Lines A and B had longer internodes and a greater number of nodes prior to flower emergence (Table 7).

Plants of Line A responded differently at different times of the year, either the length of day or the light intensity may have influenced the action of the genes which determine vine-height. Investigations by Reath and Wittwer (48) indicated that the pea variety Early Perfection responded to day lengths.

The work by Lewis (36) on heterosis in tomato may help support the supposition that light intensity is an influencing factor. He found the growth rate of the cultivated and the wild tomato to be similar at low light intensities. On the otherhand, at a high light intensity, the wild species had a greater growth rate than the cultivated one. Nevertheless, even though the reaction in tomato is in reverse to that of the supposition presented, there is an indication that light intensities may induce differential growth-response in plants.

5. Root-rot resistance test of $R_3$ plants from irradiated seeds.

No resistance was obtained to Fusarium root-rot in the $R_3$ plants from seeds which were exposed to radiation. Furthermore, no $R_3$ plant rated better than any non-irradiated

Early Perfection control. However, it must be considered that only a small population was tested, because of inadequate space and facilities in the greenhouse.

## B. Inheritance Studies

Resistance appeared to be highly associated with smooth seeds where the plants from the smooth-seeded classes in each $F_2$ population were combined into the following two groups: plants of ratings 0 and 1 as resistant plants; plants of ratings 2 and 2 as susceptible plants (Table 11). However the $F_1$ Early Perfection X 140165, had a distribution intermediate to the parents, even though the seeds were smooth. The reciprocal cross, where the seeds were also smooth, produced an $F_1$ in which the plants had resistance similar to 140165. A comparison of the two $F_3$ populations (Figures 14 and 15) indicated that plants from wrinkled seeds were as resistant as plants from smooth seeds. Similarly, plants from seeds with transparent coats were as resistant as plants from pigmented-coated seeds (Figures 12 and 13). Therefore, on the basis of $F_3$ data, a conclusion can be made that resistance is not associated with either the seed-shape nor with the seed coat color.

Resistance to Fusarium root-rot appeared to be a quantitative character. However, the distribution of $F_3$ plants

was skewed (Figure 11) in favor of the resistant parent. Two possible explanations for the skewness of the curve are as follows:

1. It may be possible that resistance is linked or associated with a character or characters other than seed coat color, seed-shape, and purple dotting of the seed coat.
2. A series of allelic genes with modifiers may determine the degree of resistance to Fusarium root-rot.

## SUMMARY AND CONCLUSIONS

Soaking seeds with either Endothal, uranyl nitrate, or colchicine, prior to either source of radiation, did not appreciably increase the variability. However, colchicine was similar to radiation in producing dwarfing, leaf-distortion, and leaf-variegation in the $R_1$ plants. Soaking seeds in water prior to gamma irradiation was as effective as any treatment for producing abnormal plants in the $R_2$. These abnormal $R_2$ plants were characterized by chlorophyll deficiencies, sterility, lateness, earliness, tallness, dwarfness, and branching.

The exposure of dry seeds to gamma, as compared to exposure to beta irradiation, resulted in a greater reduction in germination and a greater increase in the numbers of plants showing leaf and plant abnormalities in the $R_1$. Data are presented to support the theory that the low penetrating power of beta ions is responsible for the ineffectiveness of the cathode radiation source.

No association was noted between the variability in the $R_2$ plants with that in the $R_1$ plants. Therefore, there would be no advantage in saving seed from abnormal $R_1$ plants rather than from the normal-appearing plants.

All $R_3$ plants from the radiation treatments which were tested for Fusarium root-rot resistance were found to be susceptible.

The study revealed that precise results from radiation treatments cannot be predicted and that environmental conditions, such as temperature and soil moisture, exert a profound influence on plant-growth and tend to confound the reaction to radiation. Optimum specifications with regards to dosage levels, as established under greenhouse conditions, are not directly transferable to the field.

Reciprocal crosses were made between the variety Early Perfection and a Fusarium root-rot resistant plant introduction, 140165, to determine the mode of inheritance of resistance to this disease. The backcross and $F_2$ populations produced only colored seeds. Therefore, no definite conclusions could be made, on the basis of $F_2$ data, concerning the mode of inheritance. Segregation for seed coat color occurred in the $F_3$ populations. On the basis of $F_3$ data, transparent coated and wrinkled seeds were as resistant as pigmented and smooth seeds.

Resistance to Fusarium root-rot appeared dorminant. The distribution of the Fusarium root-rot tested $F_3$ progeny was not normal. Two suppositions were presented to explain the skewed distribution.

Clear-cut results were not possible in the inheritance studies, primarily because the resistant parent, 140165 was not immune.

Resistance was obtained in seeds with transparent coats and in desirable plants. One should be able to initiate a breeding program with reasonable assurance that Fusarium root-rot resistant commercial-type plants could be produced.

## LITERATURE CITED


1. Afanasene, A. S. 1944. The action of X-rays on air-dry, moist, and germinated wheat grains. Chem. Abst. 5529.

2. Akerberg, E. 1954. Mutations in X-rayed material of the six-rowed barley variety, Edda. Acta. Agr. Scand. 4:544-548.

3. Anderson, G. and G. Olsson. 1954. Svalofs Primex white mustard--A market variety selected in X-ray treated material. Acta. Agr. Scand. 4:574-577.

4. Anderson, E. G., A. E. Longley, C. H. Li, and K. L. Retherford. 1949. Hereditary effects produced in maize by radiation from the Bikini Atomic Bomb I. Studies on seedlings and pollen of the exposed generation. Genetics 34:639-646.

5. Arnason, T. J., C. O. Person, and J. M. Naylor. 1952. Radiation-induced mutations in wheat and barley. Can. J. Bot. 30:743-754.

6. Brock, R. D. 1957. Mutation plant breeding. J. Aust. Inst. Agr. Sci. 23:39-50.

7. Burdick, A. B. 1956. Mutagenic effect of thermal neutrons on wet and dry tomato seeds. Nature 178:360-361.

8. Caldecott, R. S. 1956. Ionizing radiations as a tool for plant breeders. Peaceful Uses of Atomic Energy 12: 40-45.

9. Clark, Janet, H. 1939. The biological effects of radiation. Ann. Rev. Pl. Physiol. 1:21-40.

10. Damato, F. and G. Gustafsson. 1948. Studies of the experimental control of the mutation process. Hereditas 34:181-192.

11. DeHaan, H. 1930-31. Contributions to the genetics of Pisum. Genetica 12:321-439.

12. Down, E. E. and A. L. Anderson. 1956. Agronomic use of an X-ray-induced mutant. Sci. 124:223-224.

13. Ehrenberg, L. 1955. Factors influencing radiation induced lethality, sterility, and mutations in barley. Hereditas 41:123-146.

14. Ehrenberg, L., A. Gustafsson, U. Lundquist, and N. Nybom. 1953. Irradiation effects, seed soaking, and oxygen pressure in barley. Hereditas 39:493-504.

15. Frey, K. J. 1954. Artificially induced mutations in oats. Agron. Jour. 46:49.

16. Frey, K. J. 1955. Agronomic mutations in oats induced by X-ray treatment. Agron. Jour. 47:207-210.

17. Frey, K. J., and J. A. Browning. 1955. Mutations for stem rust resistance induced in oats by X-ray treatment. Phytopath 45:490-492.

18. Froier, K. 1954. Aspects of the agriculture value of certain barley X-ray mutations produced and tested at the Swedish Seed Association, Svalof, and its branch stations. Acta Agr. Scand. 4:515-543.

19. Gelin, O. E. 1941. The cytological effect of different seed treatments in X-rayed barley. Hereditas 27:209-219.

20. Gelin, O. E. 1953. Weibull's Stral pea. An X-ray mutation from the Kloster pea. Pl. Br. Abst. 23:2280.

21. Gelin, O. E. 1954. X-ray mutants in peas and vetches. Acta Agr. Scand. 4:558-568.

22. Gregory, W. C. 1955. X-ray breeding of peanuts (Arachia hypogaea). Agron. J. 47:396-399.

23. Gustafsson, A. 1941-a. Mutation experiments in barley. Hereditas 27:225-242.

24. Gustafsson, A. 1941-b. Preliminary yield experiments with ten induced mutations in barley. Hereditas 27:337-359.

25. Gustafsson, A. 1947. Mutations in agriculture plants. Hereditas 33:1-100.

26. Gustafsson, A. 1951. Induction of changes in genes and chromosomes II. Cold Spring Harbor Sym. Quant. Biol. 16:263-281.

27. Horlacher, W. R., and D. T. Killough. 1933. Progressive mutations induced in Gossypium hirssutum by radiations. Am. Nat. 67:532-538.

28. Johnson, Edna L. 1931. Effect of X-irradiation upon growth and reproduction of tomato. Pl. Physiol. 6:685-694.

29. Jones, R. F. 1926. Resistance of peas to root-rot. Phytopath 16:459-465.

30. Key, J. M. 1951. Neutron and X-ray experiments in barley. Hereditas 37:421-464.

31. Konzak, C. F. 1954. Stem rust resistance in oats induced by nuclear radiation. Agron. Jour. 46:538-540.

32. Konzak, C. F., N. E. Borlang, A. Acosta, and J. Gibler. 1956. Stripe rust resistant mutants obtained from irradiation of Gabo wheat. Phytopath 46:(Note) 525.

33. Lambou, M. G., R. Y. Mayne, B. E. Proctor, and S. A. Goldblith. 1952. Effects of high-voltage cathode-ray irradiation on cottonseed. Science 115:269-271.

34. Lamprecht, H. 1957. Rontgeninduzierte spezifische Mutationen bei Pisum in ihrer Abhangigkeit von der genotypischen Konstitution. (X-ray induced specific mutations in Pisum and their dependence of the genotypical constitution). Agri. Hort. Genetica 15:169-193.

35. Lesley, J. W., and Margaret M. Lesley. 1956. Effect of seed treatments with X-ray and phosphorous 32 on tomato plants of first, second, and third generations. Genetics 41:575-588.

36. Lewis, D. 1955. Gene interaction, environment, and hybrid vigor. Royal Soc. London Proc. 144:178-185.

37. Lockwood, J. L., and J. C. Ballard. (Unpublished).

38. MacKey, J. 1954. Mutation breeding in polyploid cereals. Acta Agr. Scand. 4:549-557.

39. Mertens, T. R., and A. B. Burdick. 1957. On the X-ray production of "Desirable" mutations in quantitative traits. Am. J. Bot. 44:391-394.

40. Meyers, W. M., E. R. Ausemus, F. K. S. Koo, and K. J. Hsu. 1955. Resistance to rust induced by ionizing radiations in wheat and oats. Peaceful Uses of Atomic Energy 12:60-62.

41. Muller, H. J. 1927. Artificial transmutation of the gene. Science 46:84-87.

42. Muller, H. J. 1954-a. The manner of production of mutations by radiation. Radiation Biology 1:475-626.

43. Muller, H. J. 1954-b. The nature of the genetic effects produced by radiation. Radiation Biology 1:351-473.

44. Nicholas, R. C. 1958-b. The application of high energy electrons to some grain-infesting pests. Unpublished thesis for the PhD. M.S.U. 154p.

45. Noyuchi, Y. 1935. Modification of leaf structure by X-rays. Plant Physiol. 10:753-762.

46. Quastler, H. and Marlanne Baer. 1950. Inhibition of plant growth by irradiation. J. Cell. and Comp. Physiol. 35:75-94.

47. Randolph, L. F. 1950. Cytological and phenotypical effects induced in maize by X-rays and the Bikini Test Able Atomic Bomb. J. Cell. and Comp. Physiol. 35:103-117.

48. Reath, A. N., and S. H. Wittwer. 1952. The effects of temperature and photoperiod on the development of pea varieties. Am. Soc. Hort. Sci. 60:301-310.

49. Rosen, G. von. 1942. Effect of X-rays on Pisum sativum. Hereditas 28:313-338.

50. Singleton, W. R., C. F. Konzak, S. Shapiro, and A. H. Sparrow. 1955. The contribution of radiation genetics to crop improvement. Proc. Int. Conf. Peaceful Uses of Atomic Energy 12:25-30.

51. Shebiski, L. H., and T. Lawrence. 1954. The production of beneficial mutations in barley by irradiation. Can. J. Agr. Sci. 34:1-9.

52. Skoog, F. 1935. The effect of X-irradiation on auxins and plant growth. J. Cell. and Comp. Physiol. 7:227-270.

53. Smith, H. H. 1958. Radiation in the production of useful mutations. Bot. Rev. 24:1-24.

54. Stadler, L. J. 1928. Mutations in barley induced by X-rays and radium. Science 68:186-187.

55. Stadler, L. J. 1930. Some genetic effects of X-rays in plants. J. Heredity 21:3-19.

56. Stadler, L. J. 1931. The experimental modification of heredity in crop plants. Sci. Agr. 11:645-661.

57. Tedin, O. 1954. X-irradiation of Lupinus leutens. Acta. Agr. Scand. 4:569-573.

58. Thoday, J. M. and J. Read. 1949. Effect of oxygen on the frequency of chromosome aberrations produced by alpha-rays. Nature 168:133-134.

59. Wade, B. L. 1937. Breeding and improvement of peas and beans. U.S.D.A. Yearbook. 251-282.

60. Wilson, G. B. 1953. Cytological and potential mutagentic effects of a number of organic compounds. Final report on project carried out under contract NR164203 between the Office of Naval Research and MSU.

ROOM USE ONLY