8-1959

The effects of soaking and storage on the radiosensitivity of barley seeds

Philip E. Hoskinson

To the Graduate Council:

I am submitting herewith a thesis written by Philip E. Hoskinson entitled "The effects of soaking and storage on the radiosensitivity of barley seeds." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

Lawrence N. Skold, Major Professor

We have read this thesis and recommend its acceptance:

Thomas S. Osborne, F. H. Norris

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 6, 1959

To the Graduate Council:

I am submitting herewith a thesis written by Philip E. Hoskinson entitled "The Effects of Soaking and Storage on the Radiosensitivity of Barley Seeds." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

Lawrence N Skold

Major Professor

We have read this thesis and recommend its acceptance:

Thomas S. Osborne

Fred H. Norris

Accepted for the Council:

Dale Wantling

Dean of the Graduate School

THE EFFECTS OF SOAKING AND STORAGE ON THE RADIOSENSITIVITY OF BARLEY SEEDS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

by

Philip E. Hoskinson

August 1959

ACKNOWLEDGEMENTS

The writer wishes to express his gratitude and appreciation to:

Professor L. N. Skold and Dr. H. A. Fribourg for their helpful suggestions.

Dr. T. S. Osborne for direction, advice, and counsel in this study.

Mr. James Bacon for conducting the irradiation treatments.

A number of employees, Mr. Fred Mowery, Mr. Jesse Sweat, and Mr. Kyle Young for help in conducting the experiments.

Other members of the staff for their cooperation and helpful suggestions.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

Man has attempted to modify the harmful effects of electromagnetic radiation in seeds since 1921. In quantity, at least, he has been fairly successful, for numerous physical and chemical agents have been reported as exhibiting modifying properties.

Considerable data have been reported concerning the use of moisture as a modifying agent. More recently, several storage studies have been published. But there is a paucity of information concerning the interactions of the two modifiers when they are used together in the same experiment.

The purpose of this study was to determine the extent to which the effects of irradiation on barley seed could be modified by varying the moisture content of the seed and the storage time.

CHAPTER II

REVIEW OF THE LITERATURE

Studies concerning the radiosensitivity of dry and soaked seeds have shown that there is a positive correlation between the moisture content of tissues and their radiosensitivity. As early as 1921, Petry reported (Nilan, 24) that soaking of wheat seeds increased the damage caused by X-rays as measured in terms of decreased germination and seedling height. Johnson (19) compared the survival rates of pre-soaked and dormant wheat grains that had been exposed to X-rays. All pre-soaked wheat seedlings died within three weeks after they had been exposed to more than 5,000 r. Survival of seedlings from dry grains that had been exposed to 10,000 r was as high as that obtained from the unirradiated controls. The surviving seedlings from the soaked grains made less growth in every respect than did the controls. In a similar study, MacKey (21) reported that germinability was normal in dry, dormant seeds even after the heaviest dosage had been applied. Pre-soaking, followed by X-ray treatment caused distinctly lower germination and increased the frequency of early death. A pronounced retardation of initial seedling growth in the X_1 generation was also noted.

Stadler (28) calculated that the killing effects of X-radiation were fifteen to twenty times as high for germinating seeds as they were for dormant seeds. He steeped barley in water for six hours at 27° C and aerated it eighteen hours on moist blotting paper in covered dishes at 27° C. Tolerance of the seeds decreased during a soaking period of fourteen hours to about half that of dormant seeds and was approximately halved again by the first thirty minutes of aeration, but its further decrease during the next 4.5 hours of aeration was less marked.

Stadler (28) also compared the mutation rate of germinating and dry dormant seeds that had received identical irradiation dosages. Mutations in the pre-soaked series were about eight times as frequent as they were in the dormant series, when mutants were calculated per r-unit. Subsequently, Ehrenberg, et al. (12) confirmed Stadler's work on relative numbers of mutations from germinating and dormant seeds. They used eighteen pre-soaked and twenty-five dormant seed treatments of barley in dosages ranging from 313 r to 20,000 r. In 1951, the mutation rate for 625 r was seven times as high in soaked as in dormant seed. The difference was less pronounced in 1952, but still the mutation rate was two or three times higher in the soaked series. In addition, their data showed a conspicuous decrease in the mutation rate with rising dosage, calculated per r-unit and spike progeny. This phenomenon was especially pronounced in the pre-soaked seed series. With very high dosages the pre-soaked seed gave low mutation rates, often lower than in dormant seeds.

In 1947 Gustafsson (15) reported that the proportion of certain induced chlorophyll mutations differed following irradiation treatments of dry and soaked barley seed. Subsequent data by D'Amato and

Gustafsson (9) show that the peculiar alboxantha* mutation arose only after irradiation of hydrated seeds, whereas the tigerina mutation occurred more frequently following irradiation of dry seeds.

D'Amato and Gustafsson (9) soaked barley in several chemicals at various concentrations prior to irradiation. X-ray effects on sterility, chromosome aberrations, and chlorophyll mutations were increased by solutions of potassium cyanide, hydrogen peroxide, uranyl nitrate, and colchicine. Although the more concentrated series of potassium cyanide produced more chromosome aberrations when followed by X-radiation than did the controls, they produced only a fraction of the number of visible mutants produced by more dilute concentrations.

Still more remarkable was the Swedish authors' discovery that the proportion of chlorophyll mutations was altered in the colchicine series. A more elaborate experiment reported by Gustafsson and Nybom (16) the following year indicated that treatment with colchicine prior to X-irradiation decreased the number of viridis types, greatly increased rare and very rare mutants, and caused no change in the number of albinos. Colchicine did not especially augment the total visible mutation rate. The weakest colchicine solution (0.001 per cent) affected the seeds in the same manner as that of the water treatment, above.

---

* For a detailed description of chlorophyll mutants see: Gustafsson, A. The plastid development of chlorophyll mutations. Hereditas 28:483-492. 1942.

Gelin (14) studied the chromosomal damage sustained by barley seeds of different water content at the time of X-irradiation with 10,000 r. Seeds with 15 per cent water contained approximately twice as much distrubed cell division as those seeds with 10 per cent water. Seeds soaked twenty-three hours (water content not stated) in water contained about four times as many. He found a marked parallelism between sterility, mutation rate, and frequency of cells with chromosomal irregularities. Ehrenberg, et al. (12) reported little correlation between chromosome disturbances and mutation rates at higher dosages. They obtained equal numbers of mutations from pre-soaked seeds exposed to 2,500 r and dormant seeds exposed to 15,000 r. Eighty-four per cent of the cells of the pre-soaked seeds were disturbed as compared with only five per cent in the dormant series. They concluded that marked correlation between chromosome damage and mutation rates of pre-soaked barley seed exists at less than 1,250 r.

Numerous workers have studied the effects of various gases on the radiosensitivity of actively metabolizing plant tissue since Mottram (22) discovered the "oxygen effect" in 1935. His data showed that there was less inhibition of Vicia faba root growth when X-rayed in anaerobic rather than aerobic conditions. According to Thoday and Reed (30), bean seedlings made the most growth after they had been X-rayed in nitrogen, medium growth after air, and were most heavily damaged after oxygen. Identical dosages were administered to the seedlings in each treatment series. Hayden and Smith (17) X-rayed germinating barley seeds in a vacuum and in air. They reported that consistantly better germination and growth occurred following irradiation in a vacuum. Root-tip cells from seeds irradiated in air averaged 4.7 times as many chromatinic bridges as root-tip cells from seeds irradiated in a vacuum. Sieburth (26) soaked barley seeds for twenty-four hours in (a) boiled distilled water, (b) untreated distilled water, and (c) distilled water to which oxygen was added. She applied 15,000 r of X-rays and measured injury in terms of survival and height of seedlings. Cytological observations on root-tips and pollen mother cells were made. Seeds supplied with additional oxygen were injured considerably more than those with a normal or reduced amount. Seeds with a reduced amount of oxygen were injured slightly less than those with the normal oxygen supply obtained in water. On the other hand, Tanada (29) reported that the inhibitory effect of gamma radiation on growth of barley roots increased with increasing oxygen tension up to about 2 per cent. From 2 to 100 per cent the inhibition increased only slightly.

Nybom, Gustafsson, and Ehrenberg (25) successfully used hydrogen and a 2.5 per cent aqueous solution of mercaptoacetic acid as presumed protectors of germinating barley. Although hydrogen sulfide had afforded remarkable protection to dormant barley seeds, it proved to be highly deleterious *per se* to germinating seedlings. Apparently, chemical protection is afforded by the exclusion of oxygen rather than by any intrinsic properties of the "protective" gases, themselves.

There has been little agreement concerning the effects of oxygen on mutation frequencies. Hayden and Smith (17) found mutability to be slightly increased, Ehrenberg, et al. (12) reported it greatly increased, and Nilan (23) reported it not increased at all.

As early as 1952, Nybom, Gustafsson, and Ehrenberg (25) had found that water content per se did not entirely explain the differential radiosensitivity exhibited by plant tissue. They varied the physiological state and water content of barley independently. Certain anaesthetics, notably magnesium chloride, afforded considerable protection to the pre-soaked barley seed. Ehrenberg, et al. (12) noted that mutability results were modified by mode of pre-soaking, methods of irradiation, temperature, and field conditions.

Caldecott (3,6) demonstrated an inverse relationship between the water content of dormant embryos and their sensitivity to X-rays at certain moisture levels. Embryos decreased in sensitivity as their water content changed from about 4 per cent to about 8 per cent. There was little change in their X-ray sensitivity, between 8 and 16 per cent, but there was a steep rise at about 20 per cent. Oat seeds containing 5.0 per cent moisture were more sensitive to X-irradiation than seeds with either 13.8 or 18.2 per cent, according to Abrams and Frey (1). Seeds of the intermediate moisture level showed the greatest germination, seedling height, and seedling dry weight at all dosages listed. Dormant seeds of highest water content exhibited intermediate sensitivity except at very high dosages when they became most sensitive to X-rays. Ehrenberg (11) published data generally agreeing with those of other workers, but he found that the mutation rate showed a parallel dependence on moisture only at levels of less than 11.5 per cent. Similar results by Ehrenberg and Nybom (13) led them to suggest that protective substances may be produced in dormant seeds with a high water content.

Somewhat earlier, Nybom, Gustafsson, and Ehrenberg (25) had reported that air-dry seeds (about 10 per cent moisture) were less sensitive to X-radiation after they had been soaked for two hours at 21° C. Caldecott's data indicated that dormant barley seeds were most resistant to X-irradiation after one hour of soaking at 22° C.

Konzak (20) studied the effects of temperature and soaking on the radiosensitivity of barley seeds. He used five soaking periods and maintained temperatures of 0° C and 22° C for each soaking period. Although all soaked seeds were more sensitive to gamma rays than were dormant ones, those seeds soaked at the lower temperature were much less sensitive. Caldecott (6) shed further light on this question when he showed that the sensitivity of pre-soaked seeds, as modified by the temperature of the water used for soaking, was probably related to increased physiological activity of the embryos. He soaked dormant seeds containing 4 per cent moisture in the embryos at 0° C and 22° C for different periods of time and then subjected them to 15,000 r of X-rays. Seedlings were grown seven days and their average heights were compared with irradiated, unsoaked controls. Seeds soaked four hours at 0° C were less sensitive to radiation than the unsoaked controls. More than eight hours of soaking at 0° C were needed to increase the radiation damage noticeably.

The influence of temperature under which seeds are soaked seems to affect the X-ray sensitivity of seeds even after desiccation.

Caldecott (5) obtained more growth from seeds that had been soaked for sixteen hours at 0° C, desiccated over phosphorus penta-oxide from five to about 100 hours, and X-rayed than from dormant seeds receiving identical irradiation. Seeds soaked at 22° C were several times as sensitive as unsoaked seeds, or those seeds soaked at 0° C. Caldecott (4) had previously shown that barley seeds that had been soaked for various periods, then desiccated over dry calcium chloride until they had attained their original moisture level were more sensitive to X-radiation than unsoaked seeds of similar moisture levels. Ivanoff (18), who used air as the drying medium for pre-soaked oats, confirmed Caldecott's data completely. Caldecott believed that there were at least two possible explanations for these results: either the treatments modified some biological component (s) sensitive to X-rays, or the molecular stability of sites sensitive to X-rays was greatly modified.

Several workers have shown recently that X-radiation effects can be modified by post-treatments of storage, gases, moisture, or a combination of the group. Adams, Nilan, and Gundhardt (2) reported that storage of dormant barley seeds after X-radiation increased the amount of radiation damage as measured by the frequency of chromosome bridges, seedling height, and rate of germination. Further, oxygen enhanced these deleterious effects and nitrogen retarded them. The post-irradiation oxygen effect was emphasized by Caldecott, et al. (7) and Curtis, et al. (8). Their data indicated that the moisture content of the seeds at the time of irradiation greatly influenced the modifying effects of storage and of oxygen. Seeds with very low amounts of water (4 per cent) were consistently more heavily damaged than were dormant seeds containing more moisture.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

This study was undertaken to elucidate some of the effects of soaking, storage and gamma radiation on barley seeds. The experimental design was a randomized complete block with seven soaking periods, six storage periods, and three radiation levels, used in all possible combinations, for a total of 126 treatments. Four replicates were used, each treated on separate dates. Each treatment was performed on a one hundred-seed sample of mechanically de-awned Holston barley.

The seven soaking periods consisted of: 0, 1, 2, 4, 8, 16 and 24 hours. The storage periods were: 0, 2, 7, 15, 24 and 36 hours. The levels of radiation used were: 0, 2,000, and 10,000 r.

The four replicates were irradiated and planted on January 26, February 16, March 2, and March 23, 1959, respectively.

Seed Soaking and Storage

One hundred-seed samples were wrapped in cheesecloth and submerged in two liters of distilled water. A Thiberg Aerater, model number one, bubbled air through the water continuously at the rate of 288 milliliters per minute. Time of entry into the water was staggered so all samples within a soaked series of a given radiation dosage would complete the alloted soaking and storage times simultaneously. For each replication, elapsed time from start of soaking to end of irradiation was sixty hours and seventeen minutes. The water was completely replaced thirty-eight hours after the beginning of each experiment.

At the conclusion of each soaking period, the samples were removed from the water, the excess blotted off, and the seeds stored in packages of saran wrap. The storage packets previously had been perforated with four pin holes which permitted the exchange of oxygen and carbon dioxide but were not large enough to permit appreciable dehydration of the seeds. All sample packets from each soaking period were stored in one large perforated saran packet to facilitate later handling. Prior to irradiation, the packets were stored in a one-gallon thermos jug maintained at a temperature of approximately 70° F throughout the storage period.

Irradiation and Planting

This phase of the study was performed at the University of Tennessee Atomic Energy Commission Irradiation Facilities at Oak Ridge. The radiation source is an internal-sample, Cobalt-60, gamma-ray source with an intensity of about 650 r per minute.* All six samples of seed soaked for a specified period of time and then stored for various lengths

---

* Osborne, T. S., Personal Communication.

of time were irradiated simultaneously with 2,000 r. Then six similar samples were irradiated with 10,000 r.

The seeds were planted in standard 18 x 14 inch wood, greenhouse, flats immediately after irradiation. One hundred seeds were planted in each row, there being nine rows in each flat. Treatment locations were completely randomized. The seeds were covered with approximately one-half inch of sand.

Greenhouse Care, Plant Harvest, and Measurements

The flats were placed at random on two tables near the center of the greenhouse. They were rotated daily to ensure equal effects of temperature and light. All flats were watered twice each day. Plants of each treatment were harvested eleven days ± seven hours after the soaking treatment of the seed was initiated. Thus, three days were allotted harvesting, paralleling the soak period. All plants within a sample were cut at the surface of the sand at the appropriate time. Measuring was accomplished by placing the base of the culms against a straightedge and marking the apex of the seed leaf if it had appeared or the coleoptile on ordinary bond paper. Approximately fifteen minutes sufficed to harvest and record each treatment. Since each paper contained the sample name, treatment, time of cutting, and baseline, the actual measuring was later effected to the nearest millimeter.

Calculations and Data Analysis

The cumulative height of all plants in a sample and the germination per cent of each sample were determined. Treatment totals from the four replicates were averaged. A viability-growth index was used to compare treatment effects. The index is the product of the cumulative height (centimeters) of all seedlings from one treatment multiplied by the germination per cent and divided by equivalent data from the control (0 hours soaked, 0 hours stored, 0 radiation). This figure was multiplied by 100 so that treatment effects were expressed as per cent of control. Data shown in Table II are the product of height times germination.

The data were subjected to an analysis of variance, according to the method described in Snedecor (27). Duncan's (10) Multiple Range Test at the .01 level was used to determine the significant differences between group means. The L.S.D. was used to determine the significant differences between individual means.

CHAPTER IV

RESULTS

Results Involving All Radiation Levels

Electromagnetic radiation has been shown to be injurious to plant tissue. Pre-soaking of seeds amplifies such injury. One would expect pre-soaking, followed by storage, to further enhance those deleterious effects. Slight differences in techniques may modify the results. At the conclusion of somewhat similar studies Caldecott (5) remarked, "The striking thing about these studies is the fact that very slight differences in the way seeds are treated resulted in pronounced differences in their response to X-rays." Since each replicate was treated on a separate date, it may be interesting to note replicate variations of barley responses as shown in Table I.

Average germination percentage for all treatments was very uniform throughout; however, the average height consistently declined through the first three replicates. Possible explanations of this phenomenon are: temperature, light, daylength, or a combination of two or more of these environmental components. Since these studies occurred between January 26 and April 3, 1959, all three factors varied widely. The daylength averaged two hours longer during the period March 23-April 3 than it did on January 26-February 6. Each succeeding replicate received more total sunlight. The first replicate received only about fifty per cent as much daily sunlight as the other replicates

TABLE I

PER CENT GERMINATION, SEEDLING HEIGHT, AND VIABILITY-GROWTH INDEX. DATA REPORTED ARE AVERAGES OF ALL 126 TREATMENTS

Responses	Replication 1	2	3	4	Average
Average germination percentage	77.2	77.8	75.8	72.5	75.8
Average height (millimeters)	79.8	65.3	54.5	57.3	64.2
Viability-growth index	74.7	59.5	63.7	62.0	64.2

as the skies were cloudy during much of the period, January 26 to February 6. Appendix B shows the hours of sunlight during this period. Average temperature was nearly identical for the first three replicates. Apparently, the growth of treated and untreated samples was similarly affected as the viability-growth index was rather uniform for replicates two, three, and four.

The results of the experiment and a statistical analysis are shown in Table II. The "viability-growth mean" reported is the product of the total height in centimeters of the seedlings multiplied by the per cent germination. Data are the means of four replications. An analysis of the data revealed that all treatments and treatment interactions were significantly different at the .01 level of probability.

Treatment Differences

Figure 1 shows the average effects of radiation as compared with control. This figure clearly depicts an inverse linear relationship between radiation dosage and plant growth, as measured by the viability-growth index previously defined.

Pre-soaking makes barley seeds more radiosensitive, as shown in Figure 2. The curve is especially steep during the first four hours of soaking.

Figure 3 clearly shows that moisture plus radiation severely inhibits plant growth at 2,000 r, and at 10,000 r essentially eliminates the entire plant population. Other workers (5, 25) have shown that barley seeds are more resistant to radiation after one to two hours of

TABLE II

VIABILITY-GROWTH MEANS OF BARLEY FROM SEED THAT HAD BEEN SUBJECTED TO SEVEN SOAKING PERIODS, SIX STORAGE PERIODS, AND THREE LEVELS OF RADIATION

Hours Soaking	0 Hours Storage Radiation In 1000 r			2 Hours Storage Radiation In 1000 r			7 Hours Storage Radiation In 1000 r			15 Hours Storage Radiation In 1000 r			24 Hours Storage Radiation In 1000 r			36 Hours Storage Radiation In 1000 r		
	0	2	10	0	2	10	0	2	10	0	2	10	0	2	10	0	2	10
0	656	639	538	625	600	544	602	565	554	545	607	622	610	658	513	659	680	530
1	578	603	560	607	616	481	659	672	218	683	584	424	570	617	439	517	515	430
2	636	610	518	591	562	369	638	586	9	609	569	202	611	623	541	562	549	405
4	608	569	522	569	534	193	635	521	1	684	467	1	634	531	76	620	489	106
8	545	567	251	584	444	8	630	364	1	697	368	1	655	286	2	625	315	2
16	524	491	12	534	366	1	624	357	1	607	216	2	619	167	3	565	260	4
24	463	375	1	443	358	1	522	289	1	627	137	2	602	98	5	431	209	13

L.S.D. .05 level = 108.6

.01 level = 145.4

TABLE II (continued)

VIABILITY-GROWTH MEANS OF BARLEY FROM SEED THAT HAD BEEN SUBJECTED TO SEVEN SOAKING PERIODS, SIX STORAGE PERIODS, AND THREE LEVELS OF RADIATION

Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F. Value
Total	503	32,596,731.4		
Replicates	3	2,886,384.3		
Treatments	125	26,545,866.4		
Soak	6	7,440,543.9	1,240,090.65	661.88**
Storage	5	435,537.1	87,107.42	8.07**
Radiation	2	12,311,022.4	6,155,511.20	49.00**
Soak x Storage	30	519,703.1	17,323.44	3.36**
Soak x Radiation	12	3,390,314.3	282,526.19	61.03**
Storage x Radiation	10	877,461.9	87,746.19	15.00**
Soak x Storage x Radiation	60	1,571,283.7	26,188.06	4.26**
Error	375	3,164,480.7		
Soak error	18	337,247.6	18,735.98	
Storage error	15	161,895.5	10,793.03	
Radiation error	6	753,755.0	125,625.83	
Soak x Storage error	90	463,609.9	5,151.22	
Soak x Radiation error	36	166,668.2	4,629.67	
Storage x Radiation error	30	175,444.3	5,848.14	
Soak x Storage x Radiation error	180	1,105,860.2	6,143.67	

** Significant at the .01 level of probability.

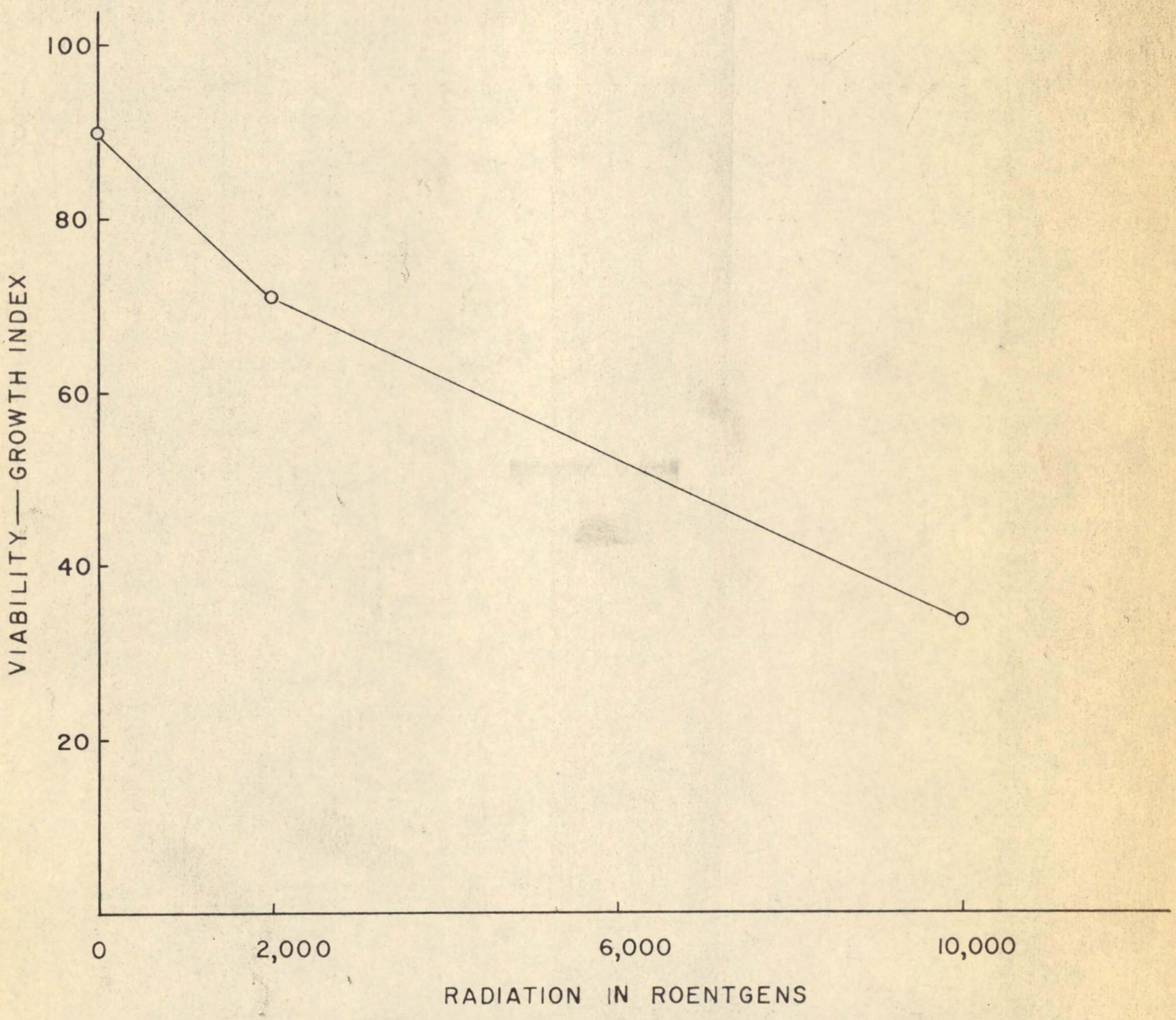

Figure 1. The viability-growth index of barley at three levels of radiation. Each point on the graph is the mean of 168 samples representing seven periods of soaking and six of storage.

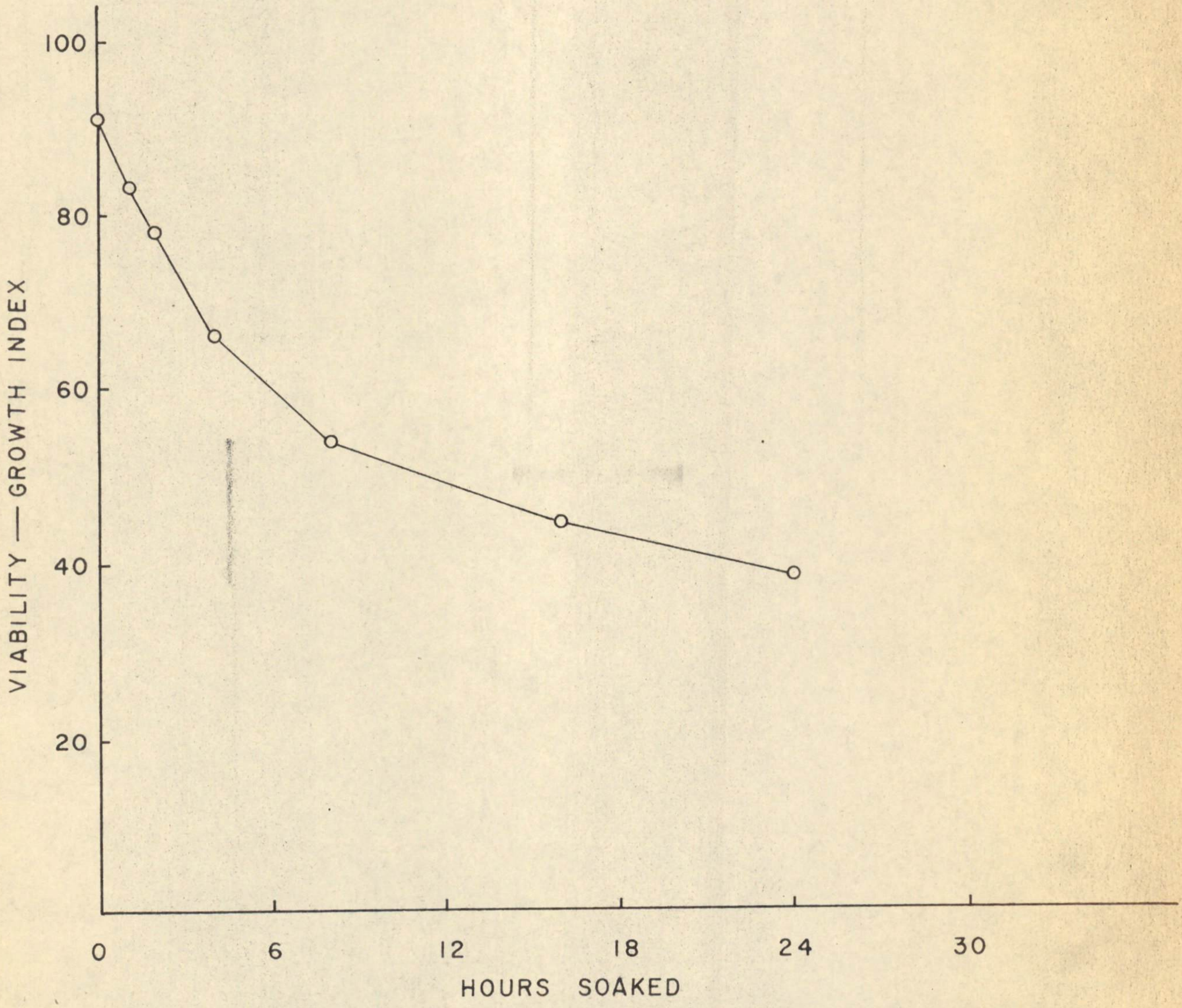

Figure 2. The viability-growth index of barley at seven periods of soaking prior to irradiation. Each point on the graph is the mean of seventy-two samples representing three levels of radiation and six periods of storage following soaking.

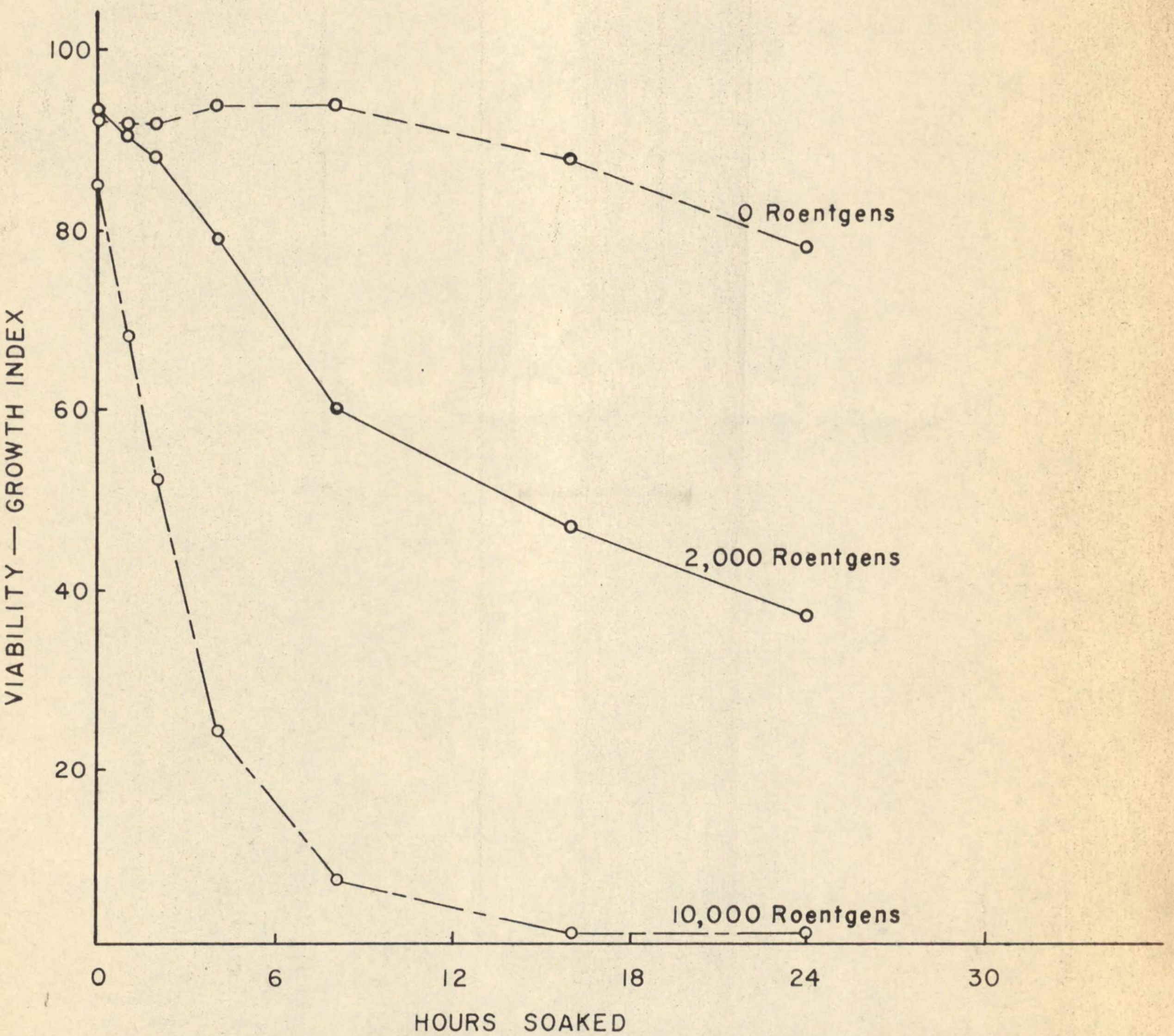

Figure 3. The viability-growth index of barley at three levels of radiation and seven periods of soaking prior to irradiation. Each point on the graph is the mean of twenty-four samples representing six periods of storage prior to irradiation.

soaking. The apparent disagreement between these studies and published data may be resolved by the introduction of the variable, storage, into this problem.

Although storage treatments caused highly significant differences, the results of storage alone were not outstanding. The slight but perceptible drop in plant growth after seven hours' storage (Figure 4) becomes more prominent in later figures.

Figure 5 shows that storage interacted with soaking to produce some unexpected results. Two to seven hours' storage increased the sensitivity of barley that had been soaked less than sixteen hours. The two shortest soak series, however, became less sensitive to radiation when stored fifteen through twenty-four hours. Both one- and two-hour soaked samples were less sensitive to gamma rays after twenty-four hours' storage than they were after thirty-six hours' storage, but there was virtually no difference between those two storage periods after the other soaking treatments.

The results of storage-radiation interaction are shown in Figure 6. There was little difference in the growth of unirradiated, but soaked samples. Seeds slowly become more sensitive to 2,000 r with increased storage up to fifteen hours, followed by virtually no change through thirty-six hours of storage. On the other hand, seeds of all soaked levels rapidly became more sensitive to 10,000 r with increased storage to seven hours, followed by a sharp drop in sensitivity at longer storage periods. This phenomenon was especially pronounced in the one- and two-hour soaked series.

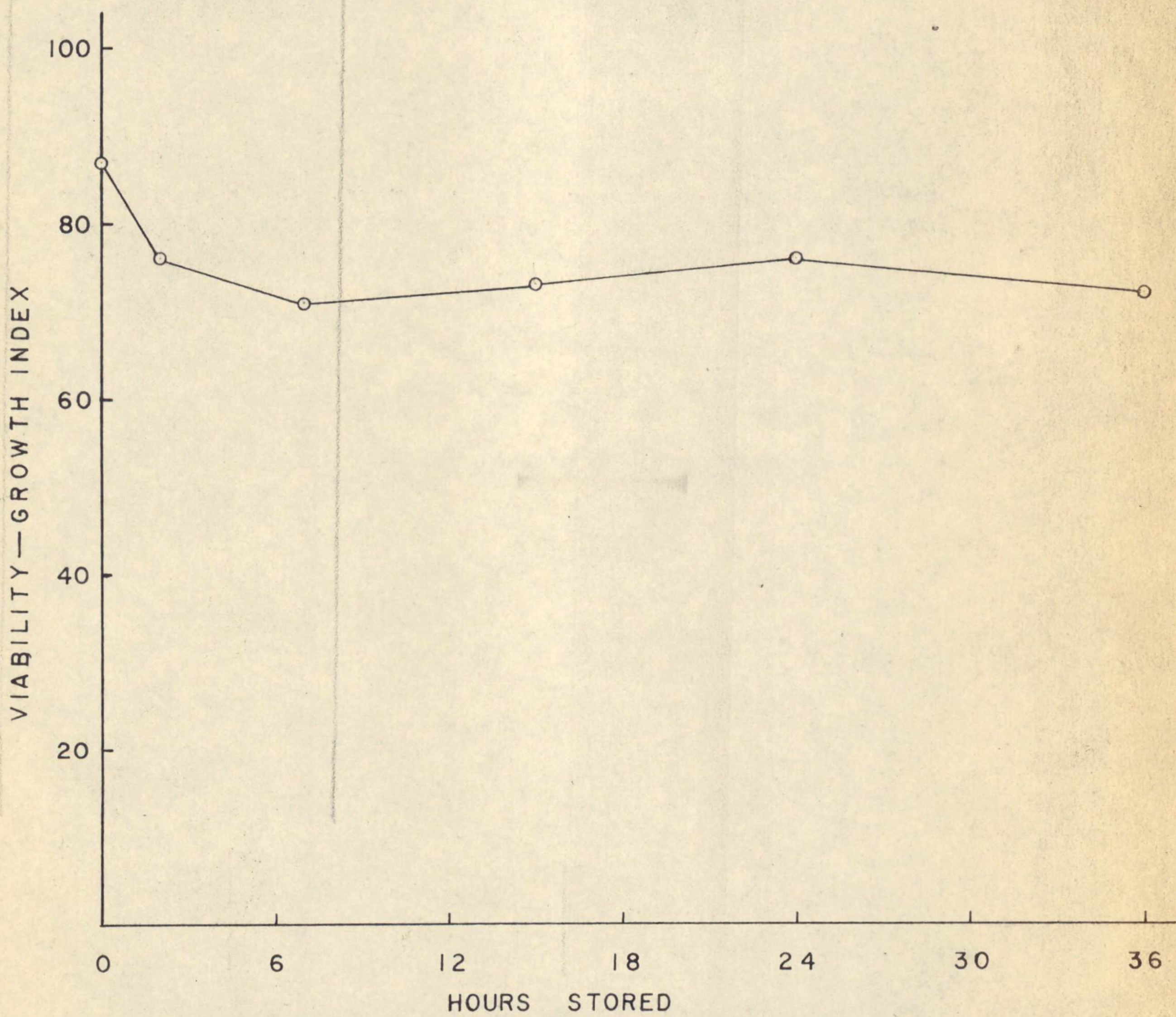

Figure 4. The viability-growth index of barley at six periods of storage prior to irradiation. Each point on the graph is the mean of eighty-four samples representing three levels of radiation and seven periods of soaking prior to irradiation.

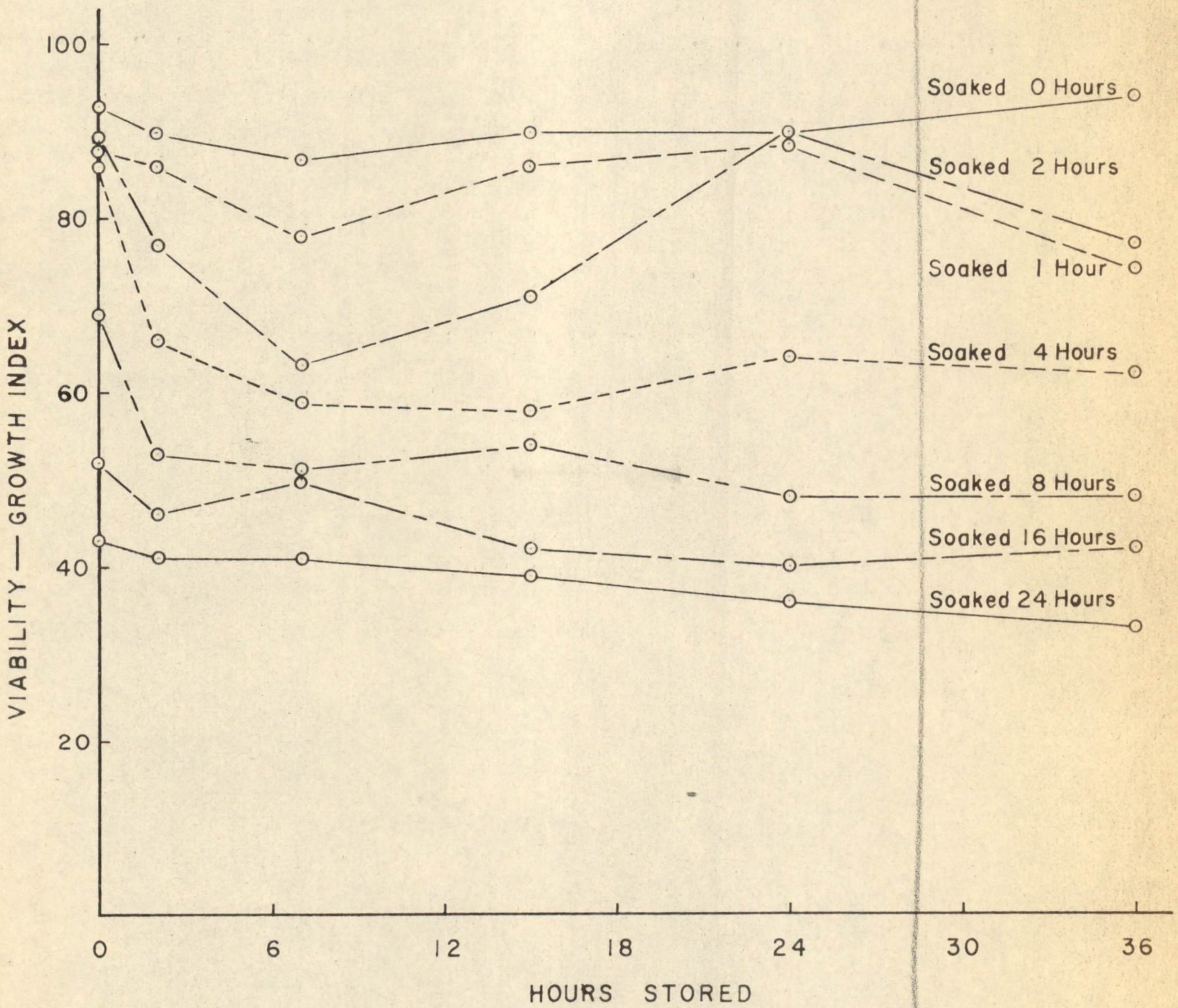

Figure 5. The viability-growth index of barley at seven periods of soaking followed by six periods of storage. Each point on the graph is the mean of twelve samples representing three levels of radiation.

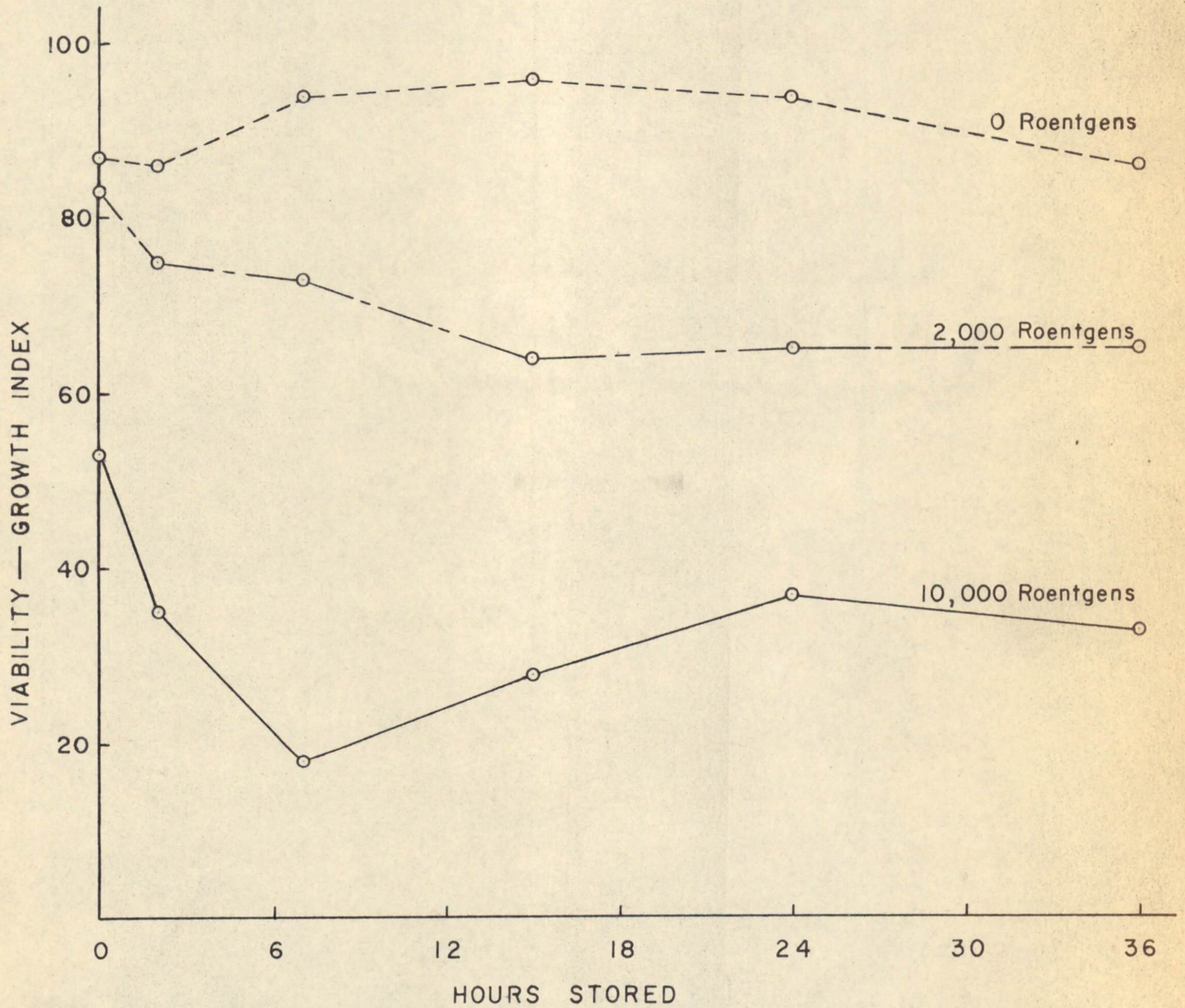

Figure 6. The viability-growth index of barley at three levels of radiation and six periods of storage prior to irradiation. Each point on the graph is the mean of twenty-eight samples representing seven periods of soaking prior to storage.

Results Involving Zero Level Radiation

It seemed desirable to study the interactions of the various soak and storage treatments at constant radiation levels. Viability-growth means are given for every treatment series in Table II.

The viability-growth index is composed of the total height of the plants multiplied by the germination percentage and expressed as per cent of control. Thus germination and seedling growth are both reflected in the data. Since variations of both were multiplied together, the product is less uniform than comparable data from either component, but is generally within the expected deviation.

Table II illustrates the soak-storage interaction when radiation is not present. The twenty-four-hour soak series is definitely inferior to the others, averaging only 79 per cent of control. Storage effects within soaking periods were erratic, but largely within expectations. Thirty-six hours' storage following one hour of soaking caused significantly more injury than seven or fifteen hours. Within the eight-hour soaked series, samples with fifteen hours' storage produced significantly more growth than did those not stored prior to seeding. Greatest differences occurred within the twenty-four hour soaked series. The viability-growth means of samples stored fifteen hours was higher at the .01 level of probability than those stored two or thirty-six hours and higher than zero storage at the .05 level. Twenty-four hours' storage gave more growth than zero, two, or thirty-six hours' storage. These differences were significant at the .05 level.

Results Involving 2,000 r Radiation

Although germination was quite uniform for seeds that had been exposed to 2,000 r after the various soaking and storage treatments and was approximately the same as for the seed receiving no irradiation, 2,000 r definitely reduced the growth of germinating seedlings, especially after longer soaking treatments. Figure 7 shows the viability-growth index of each soaked series receiving this radiation dosage. Seedlings from the one- and two-hour soaked series made essentially the same growth as those from dormant seeds. Four hours in water prior to radiation inhibited growth, though not significantly so. Much less growth occurred after longer soak periods. Within the longer soaked series, samples stored prior to radiation were always damaged more than those that were irradiated immediately after soaking. Samples within the zero, one, and two-hour soaked series were more resistant to radiation than were samples within the three longest soaking series. Radiosensitivity of barley soaked over four hours and stored for fifteen to twenty-four hours is striking. Less growth was obtained from seed stored for those two periods of time than from unstored seed in all three soak series ($p > .01$). Seeds stored an additional twelve hours had become somewhat less sensitive, but still produced less growth than those that were not stored in the eight- and sixteen-hour soak series.

Soaking up to eight hours without subsequent storage prior to radiation did not increase the sensitivity of barley to 2,000 r of gamma irradiation. However, storage following soaking increased radiation damage in all barley soaked longer than two hours.

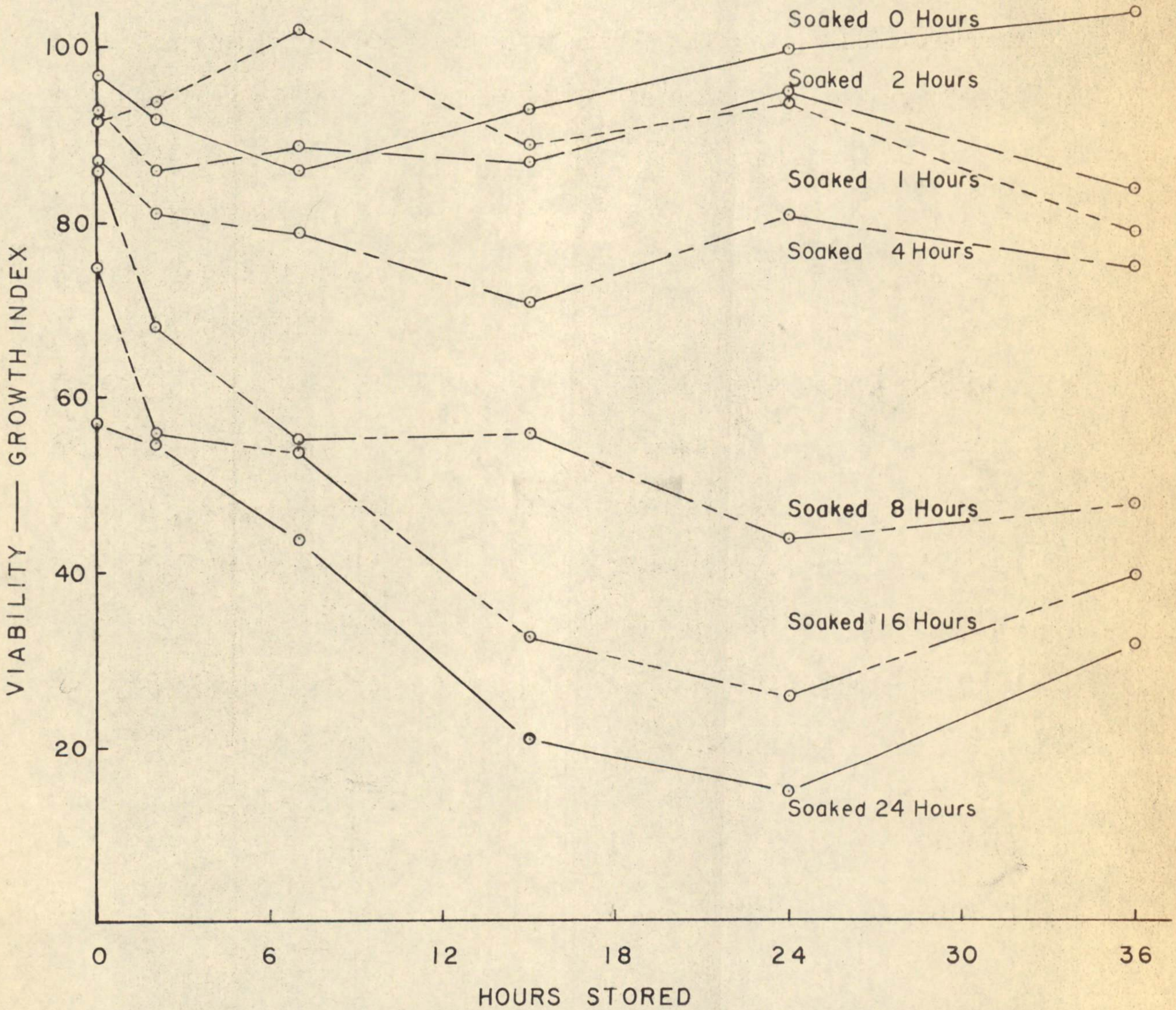

Figure 7. The viability-growth index of barley at seven periods of soaking followed by six storage periods, prior to irradiation with 2000 r of gamma rays.

Results Involving 10,000 r Radiation

Increasing damage with increasing radiation was expected. Influences of soaking and storage prior to radiation were different from those expected. Radiation with 10,000 r affected soaked, stored barley seeds very differently than with 2,000 r. This is shown in Figures 8 through 10. All pre-soaked samples were far more sensitive to 10,000 r of gamma radiation after they had been stored seven hours than after shorter storage treatments. Figure 8 illustrates the effects of soak-storage interaction on the germination of barley. With the exception of the four-hour soak series, all samples stored fifteen to thirty-six hours germinated better than those stored seven hours. Dry unsoaked, grains showed no obvious germination or growth differences due to storage. After one hour of soaking, the storage phenomenon had become pronounced. The greatest effects are shown to be after two hours of soaking. Eighty per cent germination was obtained from seeds that had been soaked two hours and stored two hours. Germination percentage had dropped to eighteen with seven hours storage following two hours soaking then rose to ninety-two with twenty-four hours of storage. Storage influenced average height similarly, though to a lesser degree as shown by Figure 9. Growth, as measured by the viability-growth index and reported in Figure 10 was essentially eliminated by two hours' soaking and seven hours' storage. That period of extreme sensitivity may well last for several hours since seed soaked four hours were as sensitive to radiation after fifteen hours' storage as they were after seven hours' storage.

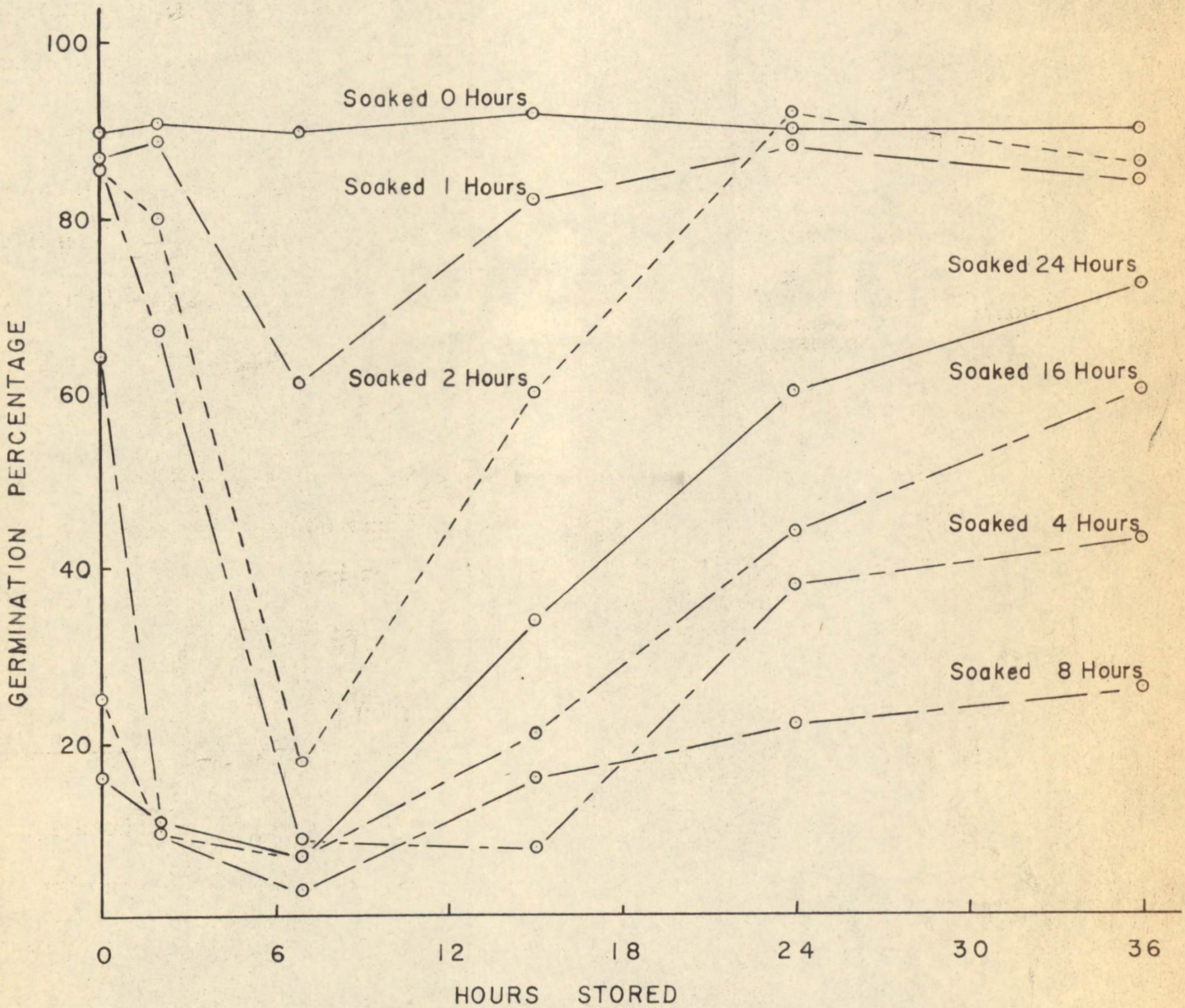

Figure 8. Average percentage germination of barley seeds at seven periods of soaking, followed by six storage periods prior to irradiation with 10,000 r of gamma rays.

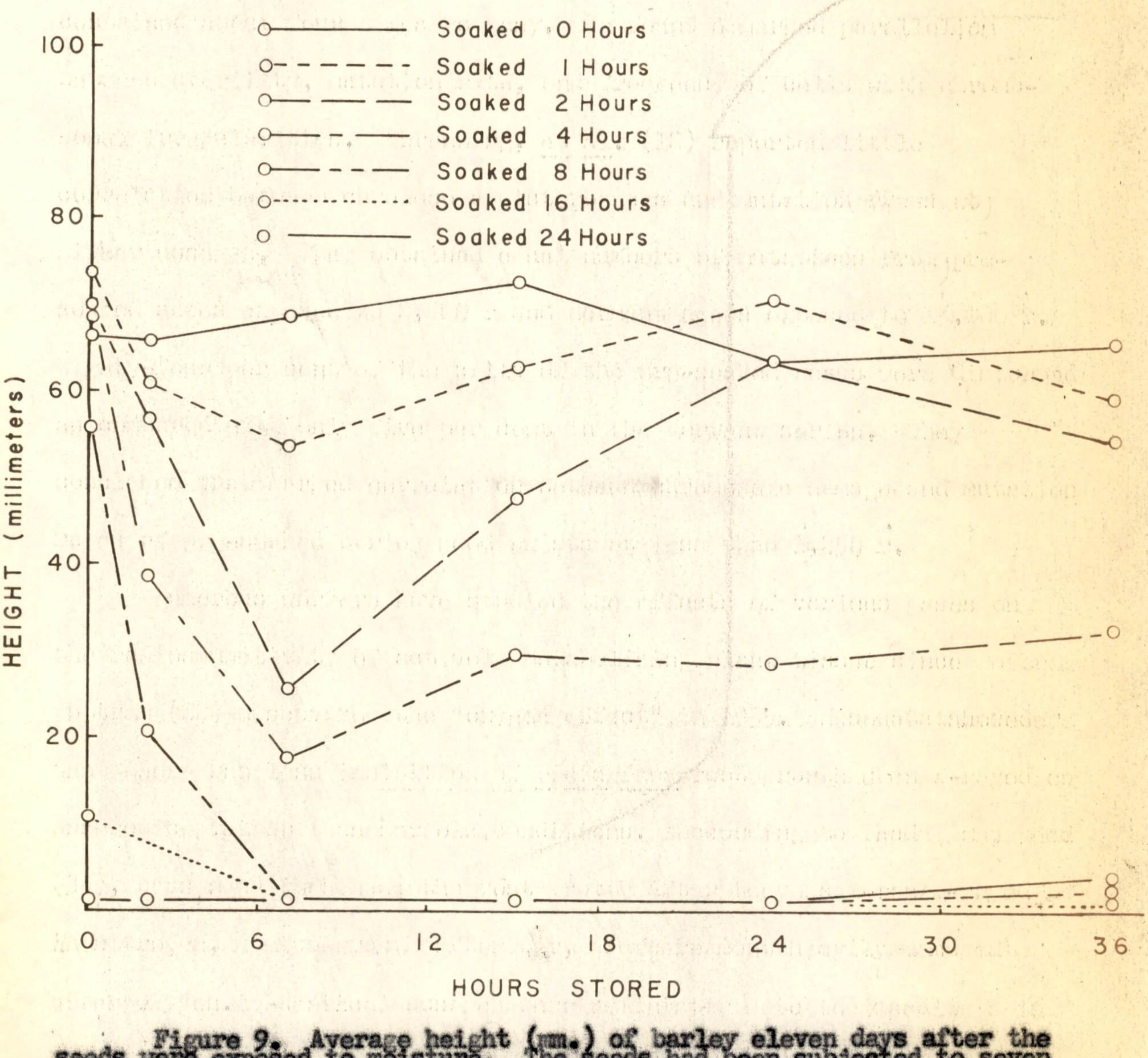

Figure 9. Average height (mm.) of barley eleven days after the seeds were exposed to moisture. The seeds had been subjected to seven soaking periods, followed by six storage periods prior to irradiation with 10,000 r of gamma rays.

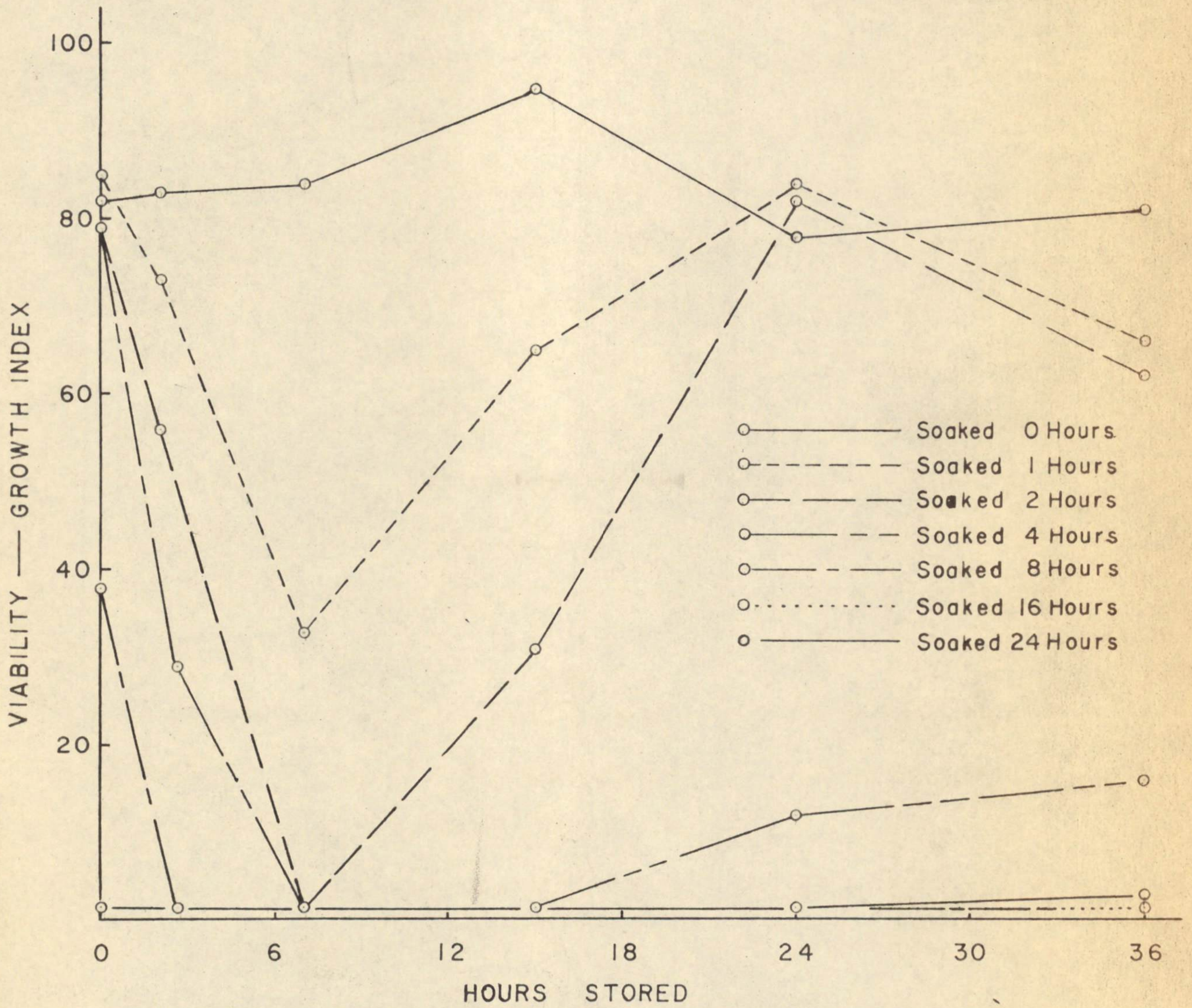

Figure 10. The viability-growth index of barley at seven soaking periods, followed by six periods of storage prior to irradiation with 10,000 r of gamma rays.

Figure 8 reveals that less germination occurred after the barley had been soaked eight hours than after any other soak period.

Average height of the seedlings was related to length of the soaking period as seedlings from seeds soaked eight hours were greatly reduced and little or no growth occurred after the barley had been soaked sixteen hours.

Duncan's Multiple Range Test revealed no differences due to storage periods or radiation treatments for unsoaked seeds as shown in Table III. Multiple range differences between groups in Tables IV and V emphasize the increased radiosensitivity of barley that was stored seven hours after it had been soaked one or two hours. Zero and twenty-four hours' storage were most resistant to irradiation, though not significantly so. The viability-growth mean of unstored seeds in the four-hour soak series was larger than that of other storage periods. Tables VII, VIII, and IX show that storage treatments superimposed on the three longest soak periods essentially eliminated plant growth.

The effects of various storage periods on barley seeds that had been subjected to 10,000 r of gamma radiation and stated periods of soaking are shown in Figures 11 and 12. The germination percentage and average height, as compared with control, are almost identical for samples that were soaked one and two hours. However, Figures 8 and 9 show that growth was inhibited to a far greater degree by the longer soaking periods.

TABLE III

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF UNSOAKED BARLEY SEED AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST. TREATMENTS JOINED BY A CONTINUOUS LINE ARE NOT SIGNIFICANTLY DIFFERENT

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
24	10,000	513	│
36	10,000	530	│
0	10,000	538	│
2	10,000	544	│
15	0	545	│
7	10,000	554	│
7	2,000	565	│
2	2,000	600	│
7	0	602	│
15	2,000	607	│
24	0	610	│
15	10,000	622	│
2	0	625	│
0	2,000	639	│
0	0	656	│
24	2,000	658	│
36	0	659	│
36	2,000	680	│

TABLE IV

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED ONE HOUR AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (percent)	Viability - Growth Means: Multiple Range Groupings
7	10,000	218	
15	10,000	424	
36	10,000	430	
2	10,000	481	
36	2,000	515	
36	0	517	
24	10,000	549	
0	10,000	560	
24	0	570	
0	0	578	
15	2,000	584	
0	2,000	603	
2	0	607	
2	2,000	616	
24	2,000	617	
7	0	659	
7	2,000	672	
15	0	683	

TABLE V

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED TWO HOURS AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
7	10,000	9	
15	10,000	202	
2	10,000	369	
36	10,000	405	
0	10,000	518	
24	10,000	541	
36	2,000	549	
2	2,000	562	
36	0	562	
15	2,000	569	
7	2,000	586	
2	0	591	
15	0	609	
0	2,000	610	
24	0	611	
24	2,000	623	
0	0	636	
7	0	638	

TABLE VI

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED FOUR HOURS AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
7	10,000	1	
15	10,000	1	
24	10,000	76	
36	10,000	106	
2	10,000	193	
15	2,000	467	
36	2,000	489	
7	2,000	521	
0	10,000	522	
24	2,000	531	
2	2,000	534	
0	2,000	569	
2	0	569	
0	0	608	
36	0	620	
24	0	634	
7	0	635	
15	0	684	

TABLE VII

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED EIGHT HOURS AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
7	10,000	1	
15	10,000	1	
24	10,000	2	
36	10,000	2	
2	10,000	8	
0	10,000	251	
24	2,000	286	
36	2,000	315	
7	2,000	364	
15	2,000	368	
2	2,000	444	
0	0	545	
0	2,000	567	
2	0	584	
36	0	625	
7	0	630	
24	0	655	
15	0	697	

TABLE VIII

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED SIXTEEN HOURS AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
2	10,000	1	
7	10,000	1	
15	10,000	2	
24	10,000	3	
36	10,000	4	
0	10,000	12	
24	2,000	167	
15	2,000	216	
36	2,000	260	
7	2,000	357	
2	2,000	366	
0	2,000	491	
0	0	524	
2	0	534	
36	0	566	
15	0	607	
24	0	619	
7	0	624	

TABLE IX

INTERACTION BETWEEN STORAGE LEVELS PRIOR TO IRRADIATION AND LEVELS OF RADIATION OF BARLEY SEED SOAKED TWENTY-FOUR HOURS AS INDICATED BY DUNCAN'S MULTIPLE RANGE TEST

Storage (hours)	Radiation (r)	Viability - Growth Means: Height (cm.) x Germination (per cent)	Viability - Growth Means: Multiple Range Groupings
0	10,000	1	
2	10,000	1	
7	10,000	1	
15	10,000	2	
24	10,000	5	
36	10,000	13	
24	2,000	98	
15	2,000	137	
36	2,000	209	
7	2,000	289	
2	2,000	358	
0	2,000	375	
36	0	431	
2	0	443	
0	0	463	
7	0	522	
24	0	602	
15	0	627	

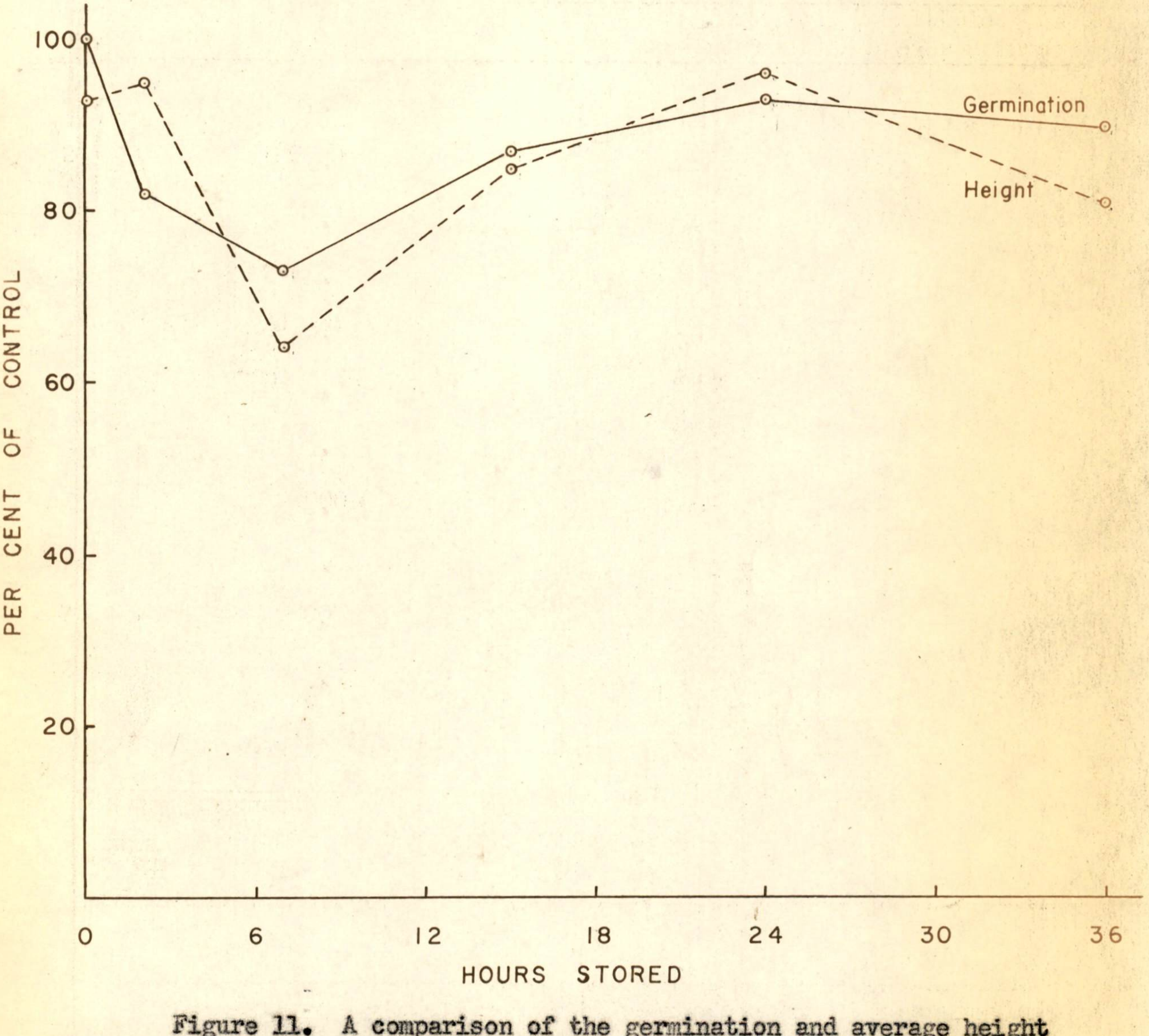

Figure 11. A comparison of the germination and average height of barley expressed as per cent of control. The barley was soaked one hour and stored for various periods prior to irradiation with 10,000 r of gamma rays.

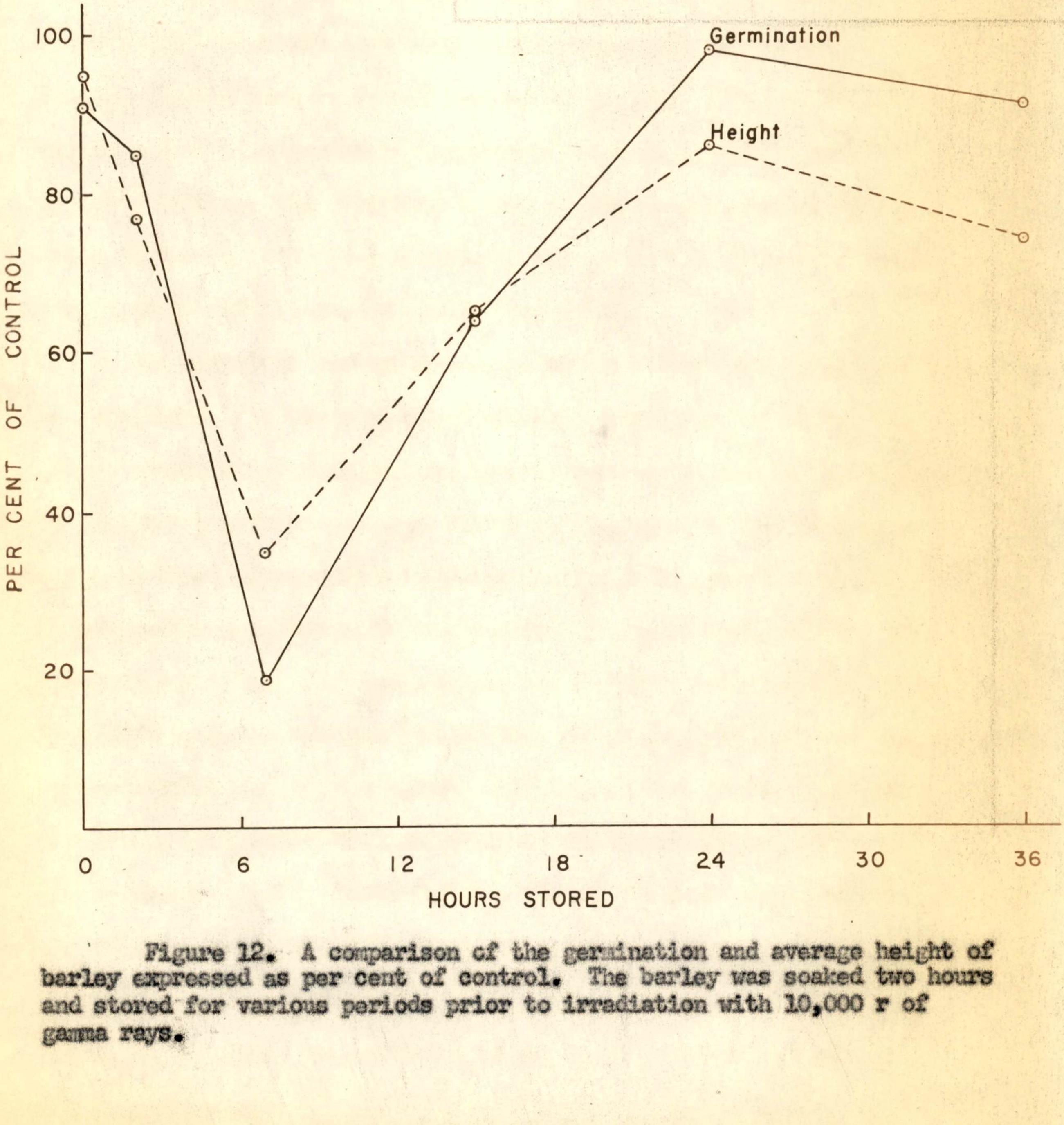

Figure 12. A comparison of the germination and average height of barley expressed as per cent of control. The barley was soaked two hours and stored for various periods prior to irradiation with 10,000 r of gamma rays.

CHAPTER V

DISCUSSION

Numerous workers (Ehrenberg 11, and others, 12, 21) have shown that electromagnetic radiation retards initial seedling growth and causes pronounced physiological killing in the X_1 generation. Petry's (24) discovery that seed soaking increases radiation sensitivity has been confirmed repeatedly (19, 20, 28). So it was not surprising that moisture treatments prior to irradiation severely retarded the growth of barley in the present study. However, the selective effects of the various storage periods following soaking and prior to radiation were wholly unexpected.

The period of storage was expected to augment the harmful effects of radiation. The seeds received adequate quantities of oxygen for active metabolism during storage and remained moist and in an atmosphere of almost one hundred per cent humidity. The germination process was expected to continue with concurrent increases in radiation sensitivity to some unknown point; then remain constant or, at most, decline relatively little. That germination did continue was evidenced by the fact that some seeds at the longer soak and storage periods had visibly sprouted before being irradiated. Although killed by the radiation treatment, they would still contribute to the germination score, but little or none to the growth totals. On the basis of this explanation the shape of the germination curves for the two longest soaked series in Figure 8 is readily understandable. This, however, emphasizes the advantages of using the product of seedling height and germination as an index of growth.

Barley seeds soaked for a few hours and stored for seven hours more were very much more sensitive to 10,000 r than were comparably soaked seeds stored for either a shorter or longer period of time. On the other hand, samples soaked longer than four hours were most sensitive to 2,000 r after twenty-four hours of storage. This confirms the results of the Swedish workers (9, 25) who have shown that modifiers may act one way at a low level of radiation, and in an entirely different way at a higher dosage.

Another interesting variation was the increased sensitivity of seeds to both levels of radiation when stored for thirty-six hours following short periods of soaking. There were no significant differences between the viability-growth means of barley that had been soaked one and two hours and stored for the two longest periods. Less growth was obtained after the longer storage period in thirteen of sixteen replicate comparisons. Even when seeds were not irradiated there was decreased growth after thirty-six hours' storage in all soaked series. This decrease was not significant for any treatment except after twenty-four hours of soaking. Nevertheless, the growth index dipped at this point with amazing regularity. Since none of these seeds had visibly sprouted at planting time, and the seeds did not contain any great excess of water, damage is believed to be attributable to soak-storage interaction rather than to radiation.

The most interesting data obtained from these studies show the greatly increased sensitivity of pre-soaked barley that had been stored seven hours prior to treatment with 10,000 roentgens of gamma radiation.

This sensitivity does not seem to have a simple explanation. Whatever happened must have had drastic effects on the embryo since both germination and growth were greatly reduced. There are two possible explanations for this, both assuming that the rate of seedling metabolism is increased upon removal from water: (a) that cell division and/or elongation had reached a radiosensitive peak after seven hours' storage or (b) enzymes, hormones, or other regulatory metabolites and probable precursors apparently are more susceptible to gamma radiation at this point of plant growth. This period may well last for several hours since those samples soaked four hours did not become more resistant to radiation until some time after fifteen hours of storage. It is clear that moisture content per se does not explain the differential radiosensitivity of plant tissue. Seed soaked one hour would not likely absorb as much water as they would if they were soaked several hours.

Previous reports (5, 25) that one to two hours of pre-soaking barley seeds confers protection from electromagnetic rays were not sustantiated in this study. Differences in techniques may well explain the disparity of the results. Air was bubbled through the water in which seeds were soaked in this study. Hayden and others (17, 25, 26) have shown that air modifies the deleterious effects of radiation. The fact that germination remained nearly constant for unirradiated seeds that were soaked for varying lengths of time indicated that the barley received enough oxygen to initiate the germination process.

Present data showed no significant differences between the growth of air-dry dormant barley seeds exposed to two levels of gamma radiation and barley that had been soaked one and two hours prior to irradiation.

Soaking period of eight hours were required prior to the application of 10,000 r before sensitivity clearly increased, sixteen hours' soaking were required to materially increase their sensitivity to 2,000 r.

CHAPTER VI

SUMMARY AND CONCLUSIONS

One-hundred-seed samples of mechanically de-awned Holston barley were subjected to seven soaking treatments, six storage treatments, and three gamma irradiation levels, used in all possible combinations for a total of 126 treatments. Germination counts were made and each seedling was measured eleven days after its treatment (s) began.

Treatment sensitivity was calculated from a viability-growth index in which total height of a sample (cm.) was multiplied by germination percentage of that sample and expressed as percentage of control.

An analysis of the data revealed that differences in results of all treatments and treatment interactions were highly significant. The following conclusions may be drawn from this experiment.

The germination and height of barley were not seriously damaged by the heaviest dosage used when dry dormant seeds were irradiated.

More than eight hours of soaking prior to irradiation was required to make seeds more sensitive to 2,000 r; eight hours of soaking made them noticeably more sensitive to 10,000 r. Twenty-four hours' soaking reduced the germination, even in the case of unirradiated seeds.

Effects of storage following soaking and prior to irradiation were erratic. Storage may have stimulated growth of some unirradiated seeds, but distinctly augmented radiation sensitivity of soaked seeds.

Soak-storage-radiation interactions were complex, and seeds reacted differently when any of the three treatments were varied.

Seeds soaked eight to twenty-four hours became more sensitive to 2,000 r with increasing storage to a maximum of twenty-four hours prior to irradiation. An additional twelve hours of storage decreased this sensitivity slightly.

Seven hours storage greatly increased radiation damage when pre-soaked seeds were exposed to 10,000 r of gamma rays.

There was a tendency for seeds soaked two hours or less to produce less growth after thirty-six hours' storage as compared with twenty-four hours' storage and comparable soaking and radiation treatments. Though not significant this phenomenon occurred regularly at all radiation levels.

These growth differences were presumably caused by differences in the metabolism of the seeds. The exact effects of the various treatments on the metabolism of barley seeds are unknown at this time.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abrams, R., and Frey, K. J. The relationship between moisture content and X-ray sensitivity of oat seeds *Avena sativa*. Proc. Iowa Acad. Sci. 64: 155-159. 1957.

2. Adams, J. D., Nilan, R. A., and Gundhardt, H. After effects of ionizing radiation in barley. I. Modification by storage of X-rayed seeds in oxygen and nitrogen. A preliminary report. Northwest Sci. 29: No. 3: 101-108. 1955.

3. Caldecott, R. S. Inverse relationship between the water content of the seeds and their sensitivity to X-rays. Sci. 120: 809-810. 1954.

4. ______, Effects of ionizing radiations on seeds of barley. Rad. Res. 2: 339-350. 1955.

5. ______, Effects of hydration on X-ray sensitivity of *Hordeum*. Rad. Res. 3: 316-330. 1955.

6. ______, Reduction in X-ray sensitivity of seeds by hydration. Nature 176: 306. 1955.

7. ______, Johnson, E. B., North, D. T., and Konzak, C. F. Modification of radiation-induced injury by post treatment with oxygen. Proc. Natl. Acad. Sci. U.S. 43: 975-983. 1957.

8. Curtis, H. J., Delihas, N., Caldecott, R. S., and Konzak, C. F. Modification of radiation damage in dormant seeds by storage. Rad. Res. 8: 526-534. 1958.

9. D'Amato, F., and Gustafsson, Å. Studies on the experimental control of the mutation process. Hereditas 34: 181-192. 1948.

10. Duncan, D. B. Multiple Range and Multiple F Tests. Biometrics 11: 1-42. 1955.

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

12. ______, Gustafsson, Å., Lundquist, U., and Nybom, N. Irradiation effects, seed soaking and oxygen pressure in barley. Hereditas 39: 493-504. 1953.

13. ______., and Nybom, N. Ion density and biological effectiveness of radiations. Acta. Agr. Scand. 4: 396-418. 1954.

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

15. Gustafsson, Å. Mutations in agricultural plants. Hereditas 33: 1-100. 1947.

16. ______., and Nybom, N. Colchicine, X-rays and the mutation process. Hereditas 35: 280-284. 1949.

17. Hayden, B., and Smith, L. The relation of atmosphere to biological effects of X-rays. Genetics 34: 26-41. 1949.

18. Ivanoff, S. S. Radiosensitivity factors in oat seeds: Dormancy, water, and development. Sci. 123: 1125-1126. 1956.

19. Johnson, E. L. Growth of wheat plants from dry and soaked irradiated grains. Plant Physiol. 14: 493-504. 1939.

20. Konzak, C. F. Radiation sensitivity of dormant and germinating barley seeds. Sci. 122: 197. 1955.

21. MacKey, J. Neutron and X-ray experiments in barley. Hereditas 37: 421-464. 1951.

22. Mottram, J. C. On the alteration in the sensitivity of cells toward radiation produced by cold and by anaerbiosis. Brit. J. Radiol. 8: 32-39. 1935.

23. Nilan, R. A. Relations of carbon dioxide, oxygen, and low temperature to the injury and cytogenetic effects of X-rays on barley. Genetics 39: 943-954. 1954.

24. Nilan, R. A. Factors governing plant radiosensitivity. In Radioactive Isotopes in Agriculture. U. S. Atomic Energy Commission T.I.D. 7512 (Washington: Government Printing Office, 1956). pp. 151-162 citing Petry, E. Zur Renntnis der bedingungen der biologischen Wirkung der Röntgenstrahlen. Biochem. Zeitshr 119: 23-44. 1921.

25. Nybom, N., Gustafsson, Å., and Ehrenberg, L. On the injurous actions of the ionizing radiations in plants. Botaniska Notiser. Häfte 4: 343-365. 1952.

26. Sieburth, L. R. The effect of (presumably different) oxygen concentrations in germinating seeds of barley on the biological effects of X-rays. Northwest Sci. (Abstract) 25: 48-49. 1951.

27. Snedecor, G. W. Statistical Methods. Iowa State College Press. Ames, Iowa. 5th Ed. 1956.

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

29. Tanada, T. Effect of oxygen concentrations on the sensitivity of barley seedlings to gamma rays. Am. J. Bot. 39: 705-706. 1952.

30. Thoday, J. M., and Reed, J. Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. Nature 160: 608. 1947.

APPENDIX

APPENDIX A

AVERAGE DAILY TEMPERATURES IN DEGREES FAHRENHEIT REPORTED AT THE U. S. DEPARTMENT OF COMMERCE, WEATHER BUREAU, MCGHEE TYSON AIRPORT, KNOXVILLE, TENNESSEE

Day	Month: January	February	March	April
1	45	40	42	64
2	39	36	41	55
3	42	42	42	61
4	31	40	47	50
5	15	40	47	56
6	24	36	45	66
7	29	39	39	63
8	36	46	42	71
9	27	54	43	74
10	27	63	46	68
11	30	45	44	58
12	34	43	38	48
13	51	45	45	39
14	55	59	55	47
15	46	46	51	52
16	24	43	41	59
17	17	53	41	62
18	21	42	40	63
19	29	29	45	64
20	47	31	54	70
21	54	31	50	58
22	29	34	45	51
23	30	52	51	52
24	38	44	58	55
25	45	45	63	62
26	53	45	60	68
27	49	53	50	72
28	49	55	45	70
29	50	..	41	64
30	50	..	54	66
31	45	..	60	..

APPENDIX B

DAILY SUNSHINE REPORTED IN TOTAL HOURS AND MINUTES AND IN PER CENT OF POSSIBLE AT THE U. S. DEPARTMENT OF COMMERCE WEATHER BUREAU, MCGHEE TYSON AIRPORT, KNOXVILLE, TENNESSEE

	Month							
	January		February		March		April	
Day	Total Hrs.-Mins.	Per Cent of Possible	Total Hrs.-Mins.	Per Cent of Possible	Total Hrs.-Mins.	Per Cent of Possible	Total Hrs.-Mins.	Per Cent of Possible
1	0:00	0	0:00	0	0:00	0	7:12	57
2	1:20	14	0:00	0	10:54	95	6:36	52
3	9:24	96	2:21	22	2:50	25	2:22	19
4	2:56	30	0:00	0	11:31	100	12:39	100
5	9:49	100	9:17	88	0:00	0	12:44	100
6	9:50	100	10:27	99	0:53	8	10:08	79
7	0:00	0	9:13	87	11:37	100	10:05	79
8	0:00	0	0:00	0	11:40	100	7:41	60
9	0:57	10	0:00	0	0:38	5	3:54	30
10	9:30	96	9:18	3	9:01	77	0:30	4
11	9:55	100	0:49	8	0:00	0	0:00	0
12	0:00	0	0:00	0	1:47	15	0:00	0
13	1:05	11	0:00	0	11:47	99	0:00	0
14	1:35	16	2:01	19	4:50	40	9:54	76
15	0:00	0	3:41	34	6:34	55	7:37	58
16	1:39	16	4:33	42	8:17	69	10:24	79
17	3:39	36	2:37	24	10:56	91	10:34	80
18	1:28	15	0:00	0	12:03	100	0:00	0
19	0:00	0	10:37	96	5:46	48	0:39	5
20	0:00	0	7:28	67	1:53	16	7:39	58
21	0:00	0	11:06	100	0:00	0	0:00	0
22	8:22	82	1:03	9	11:49	97	0:00	0
23	10:11	100	1:30	13	12:14	100	10:01	75
24	9:53	97	9:43	87	9:54	81	9:54	74
25	10:13	100	4:23	39	12:19	100	6:47	50
26	6:16	61	11:17	100	0:00	0	4:17	32
27	0:00	0	5:30	49	0:10	1	0:57	7
28	2:01	20	3:37	32	10:23	84	1:46	13
29	3:53	38	....	...	0:00	0	7:57	59
30	0:00	0	....	...	10:36	85	5:04	37
31	1:04	17	....	...	12:33	100	....	...