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GAMMA-RAY SPROUT INHIBITION OF POTATOES

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CONTRACT RESEARCH PROGRESS REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
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Title of Contract: Gamma-Ray Sprout Inhibition of Potatoes

SUMMARY

Earlier limited studies at The University of Michigan[1] and elsewhere[2] indicated that low-dosage gamma irradiation of potatoes was useful in preventing sprouting and spoilage of potatoes under storage without the development of undesirable changes. Because of sprouting followed by rapid deterioration, it usually is not possible to keep northern-grown potatoes under storage for more than eight or nine months of the year. It is believed that desirable types of potatoes can, by irradiation, be made available the year around. This treatment might be particularly useful in increasing the storage life of any type of potato shipped overseas for the armed services. However, little was known about the limitations of the process of irradiation and about the optimum conditions for storage of irradiated potatoes. During the past year a study has been conducted with support from the Quartermaster Corps of the U.S. Army to explore the storage properties of irradiated potatoes. The gamma irradiations have been performed at the Fission Products Laboratory, Engineering Research Institute, The University of Michigan, and the research studies have been conducted jointly at The University of Michigan and at Michigan State University.

I. TECHNICAL OBJECTIVES

Low-dosage gamma irradiation of potatoes has been found to be very successful in preventing sprouting and spoilage of potatoes under storage without the development of undesirable changes. Northern-grown potatoes are available only eight or nine months of the year. Because of sprouting followed by rapid deterioration, it usually is not possible to keep potatoes under storage for longer periods. It is believed that desirable types of potatoes can, by irradiation, be made available the year around. This treatment might be particularly useful in increasing the storage life of any type of potato shipped overseas for the armed services.

More specifically, the general technical objective is described below:

A. A study will be made on the effect of low dosages of gamma radiation (approximately 5,000 to 25,000 rep) on at least one white-skinned and one russet-variety potato with the object of determining the dosage needed to inhibit sprouting when stored at 35°, 40°, 50°, 60°, and 80°F with 85% relative humidity.

B. An investigation will be made, using doses of gamma radiation as high as 200,000 rep on the same types of potatoes as studied in (A) above, to determine the effect of overdose.

C. A study will be made of the effect of three different relative humidities and at two storage temperatures during storage on a white-skinned and a russet-variety potato.

D. An evaluation will be made at no less than four scheduled intervals during the storage of the irradiated potatoes that have been stored. The said evaluation shall include:

1. total starch, sucrose, and reducing-sugar content,
2. sprouting and its inhibition,
3. general appearance and texture,
4. interior fleshy region of peeled and sliced potatoes for decay, black heart, blackening, and other manifestations of enzyme and/or microbial action, and
5. loss in weight, to be determined and subdivided into combined respiration and transpiration loss and loss due to sprouts.

E. As time allows, a limited study will be made on the effects of wound healing, with special emphasis on formation of cork cambium, cellular organization, and structure.

F. A quantitative respiration study will be conducted on at least a white-skinned variety and a selected russet variety of potato.

G. The effect of gamma radiation on the activity of specific enzymes involved in potato respiration will be investigated. This will be aimed at understanding the inhibition of enzyme activity as reflected by changes in starch content, total and reducing-sugar content, and color change, allowing for extended storage life of the potato.

H. A study will be made of the growth hormone and inhibitors in and around the eyes of irradiated and control potatoes to determine whether or not gamma-ray-induced inhibition of potato sprouting is caused by an increase in the quantity of sprout inhibitors.

I. A study will be conducted to determine the incidence of common storage rot in irradiated potatoes. This will include inoculation and storage studies utilizing common potato-rotting bacteria and fungi.

J. Samples of potatoes described under (A) will be made available for acceptance testing by personnel of QM F and CI.

Respiration is one of the fundamental processes of all living organisms. Therefore it was included as one of the processes to be investigated in the study of the influence of gamma irradiation on the sprout inhibition of potatoes.

II. SPRING IRRADIATION AND SUBSEQUENT STORAGE OF IDAHO-GROWN RUSSET BURBANK VARIETY (SEED) POTATOES

A. IRRADIATION

Because of the late date (May, 1955) of activation of this research program relative to the potato season, uniform potatoes of good quality and known history which were suitable for experimental studies were difficult to obtain when the study began. Twenty bags, 100 lb each, of Idaho certified seed potatoes of the Russet Burbank variety and with known history were obtained.

The potatoes were given a radiation dose of 10,000 rep on or about the first of June, 1955, and were put in storage with control lots of the same potatoes at various temperatures nominally controlled at 35°, 40°, 50°, 55°, and 65°F and at room temperature (75°-80°F) at The University of Michigan and Michigan State University. Additional lots of the same potatoes were irradiated with doses of 5,000, 15,000, 50,000, 100,000, and 200,000 rep and all were stored at 45°F. These potatoes were weighed every two weeks and checked for sprout development.

The irradiations were performed in quart cardboard containers in the radiation cave of the Fission Products Laboratory. The use of these small containers permitted more precise evaluation of the radiation dosage delivered, although somewhat more handling time was required to complete the task. Figure 1 shows a typical arrangement of these cartons, full of potatoes, in place around the outside of the source position. After the irradiation has been half completed, all cartons are rotated 180 degrees, and the upper and lower rows are interchanged. In the background, other irradiation experiments (animal food for feeding studies) may be seen in progress.

Samples of potatoes from each of the different dosage lots were put in storage at Michigan State University at 50°F (50° to 55°F) and three controlled humidities. The constant-humidity cabinets were originally to be held at 70, 80, and 90% relative humidity. However, the equilibrium established after placing the tubers in the cabinets resulted in humidities which varied somewhat and when measured were found to be 66, 74, and 90%. The average value was estimated to be 60, 75, and 90% in one measurement and 55, 75, and 95% in another measurement.

Fig. 1. Potatoes in cardboard containers in position to be irradiated.

The bulk of the potatoes was stored in the Food Service Building at The University of Michigan and it was found that the actual average temperature of the storage rooms at the location of the potatoes varied considerably and differed from the temperature at the thermostat and the temperature at which the room was reported to be maintained. Table I lists the temperatures and humidities that were observed at the location of potato storage in the various rooms used.

TABLE I

TEMPERATURE AND HUMIDITIES NEAR STORED POTATOES IN STORAGE ROOMS IN FOOD SERVICE BUILDING

Rated Temperature of Room, °F	Measured High Temperature, °F	Measured Low Temperature, °F	Average of Measured Temperatures, °F	Average Relative Humidity, %
35	39	31	35	87
40	45	40	42	82
45	42	40	41	95
50	53	46	50	85
55	54	49	52	95
60	64	62	63	53
RT	90	75	80	*

*Varies as do outside humidity and temperature.

During storage the potatoes were examined, weighed, and the sprouts measured monthly. The sprouts on the control tubers were of maximum size at the end of August. Figure 2 shows irradiated potatoes (on left) and sprouted controls stored at "60"°F.

Fig. 2. Irradiated (left) and control (right) Idaho seed potatoes stored at "60"°F, at maximum sprout growth (August 29, 1955).

B. SPROUTING

A slow but steady sprout growth was noted first on the control potatoes stored at "45" and "40" degrees. The sprouts at "45" and "40" degrees at the end of October were from two to six inches long. Control potatoes stored at "35" degrees at this time had tiny sprouts from three to five mm long. All the sprouts which formed on the control potatoes stored at "room temperature" withered and died by October 26 as a result of lack of nutrient and moisture.

None of the irradiated potatoes stored at The University of Michigan or at Michigan State University sprouted, except those which received only 5000 rep. These potatoes were stored at "45" degrees and have developed some very short sprouts (3-5 mm long), much shorter than those of the control potatoes which were stored at the same temperature. Samples of the potatoes which were irradiated to the extent of 10,000 rep were stored at all the temperatures listed and none stored in Michigan developed sprouts. However, some of the 10,000-rep irradiated potatoes which were shipped to QMC in Chicago did show some limited sprouting.

Figure 3 is a composite of the control (left) and the 10,000-rep irradiated (right) potatoes stored at "35," "40," "45," "50," "55," and "60" degrees and at room temperature (RT). These photographs were taken on September 27, 1955, when the sprouts of the control potatoes stored at "50"°F and above had reached their peak growth and had started to die. The very tiny sprouts in the eyes that were present in the potatoes at irradiation died and disappeared in the irradiated potatoes stored at "35"°F, but remained dormant in the irradiated potatoes stored at "40"°F and higher.

C. WEIGHT LOSS

Weight loss of Idaho-grown potatoes was determined as a function of radiation dosage, storage temperature, storage humidity, and storage time. Increases in the storage temperature of irradiated potatoes resulted in increases in weight loss with time. Increases in the storage temperature of the control potatoes resulted in a rate of weight loss considerably higher than that of the irradiated potatoes, probably because of the higher metabolic rate and greater surface for transpiration presented by the sprouts. Potatoes receiving increasing doses of radiation, but held at one storage temperature, showed a marked decrease of weight loss with increase in radiation dose up to 15,000 rep. Radiation doses of 50,000 rep and higher resulted in the same weight loss for the same storage conditions. It appears that 15,000 rep is essentially as effective as higher doses in checking weight loss.

In storage tests at different humidities, the potatoes lost less weight as the humidity of the air in which they were stored increased. It was also observed that irradiated potatoes lost less weight than nonirradiated potatoes when samples of each were stored at the same relative humidity.

Figure 4 is a plot of weight loss vs radiation dosage for potatoes stored for 140 days at 90% relative humidity and 50°F. It shows the general trend observed with these potatoes of the effect of increasing radiation dosage on the reduction of weight loss and an approach to an equilibrium value at about 20,000-rep dosage. Increases of radiation dosage above this level have little additional effect on the inhibition of weight loss. Figure 5 shows additional data plotted on semilogarithmic graph paper and further illustrates the phenomenon and demonstrates that the equilibrium value of "weight loss independent of radiation dose" varies with storage time and relative humidity. The potatoes lose weight for a number of reasons, but for simplicity the losses may be divided into two groups, (M) "physical" and (B) "biological."

Regardless of whether the potato tubers are living or dead, they will lose weight when stored in an atmosphere not saturated with water vapor. This is true because the skin of the potato tuber behaves as a membrane through which water, and also oxygen, carbon dioxide, etc., may diffuse. The "physical" weight loss is largely the result of the evaporation of water, which de-

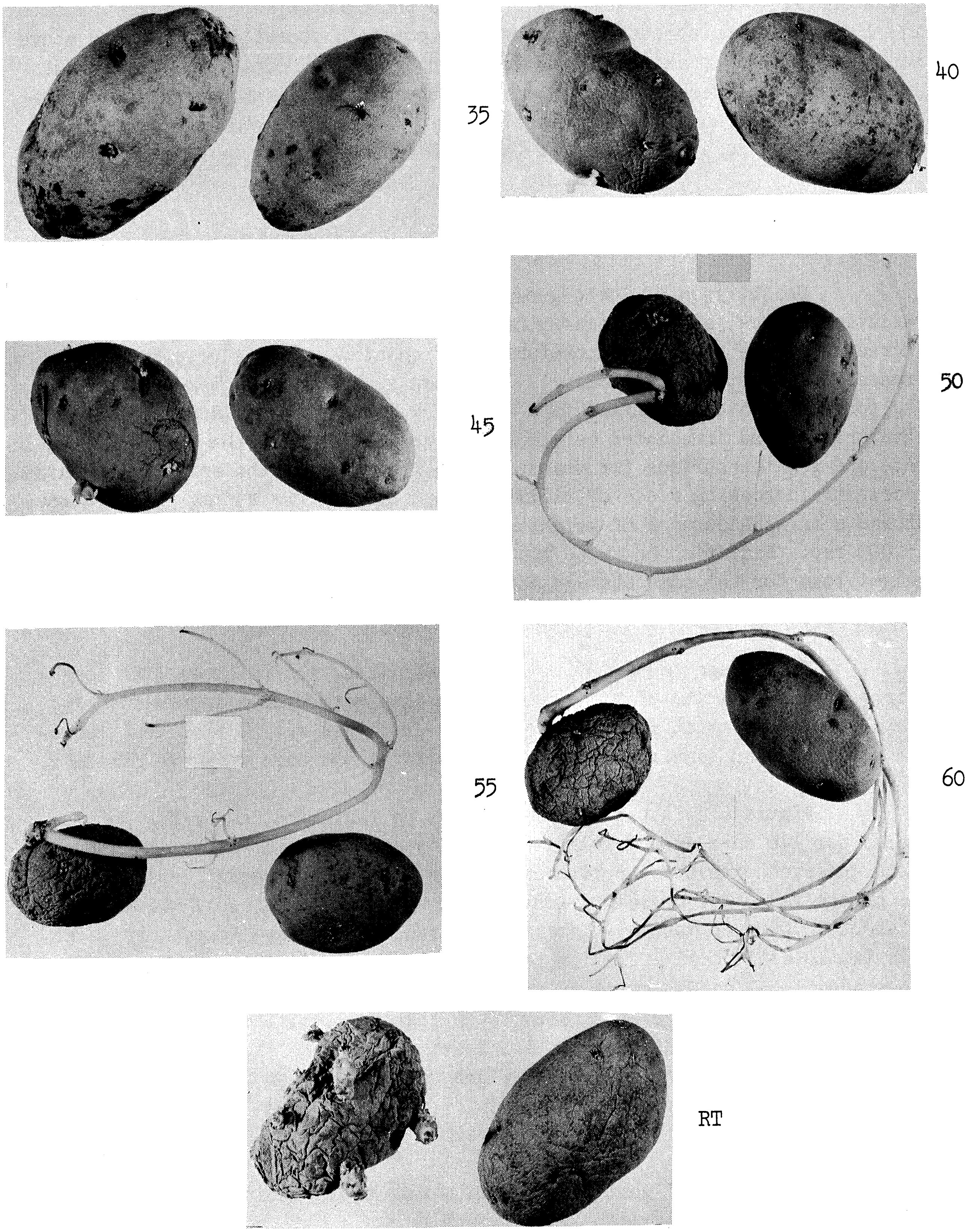


Fig. 3. Nonirradiated (left) and 10,000-rep irradiated (right) Idaho seed potatoes (1954 crop) stored at various temperatures until September 27, 1955.

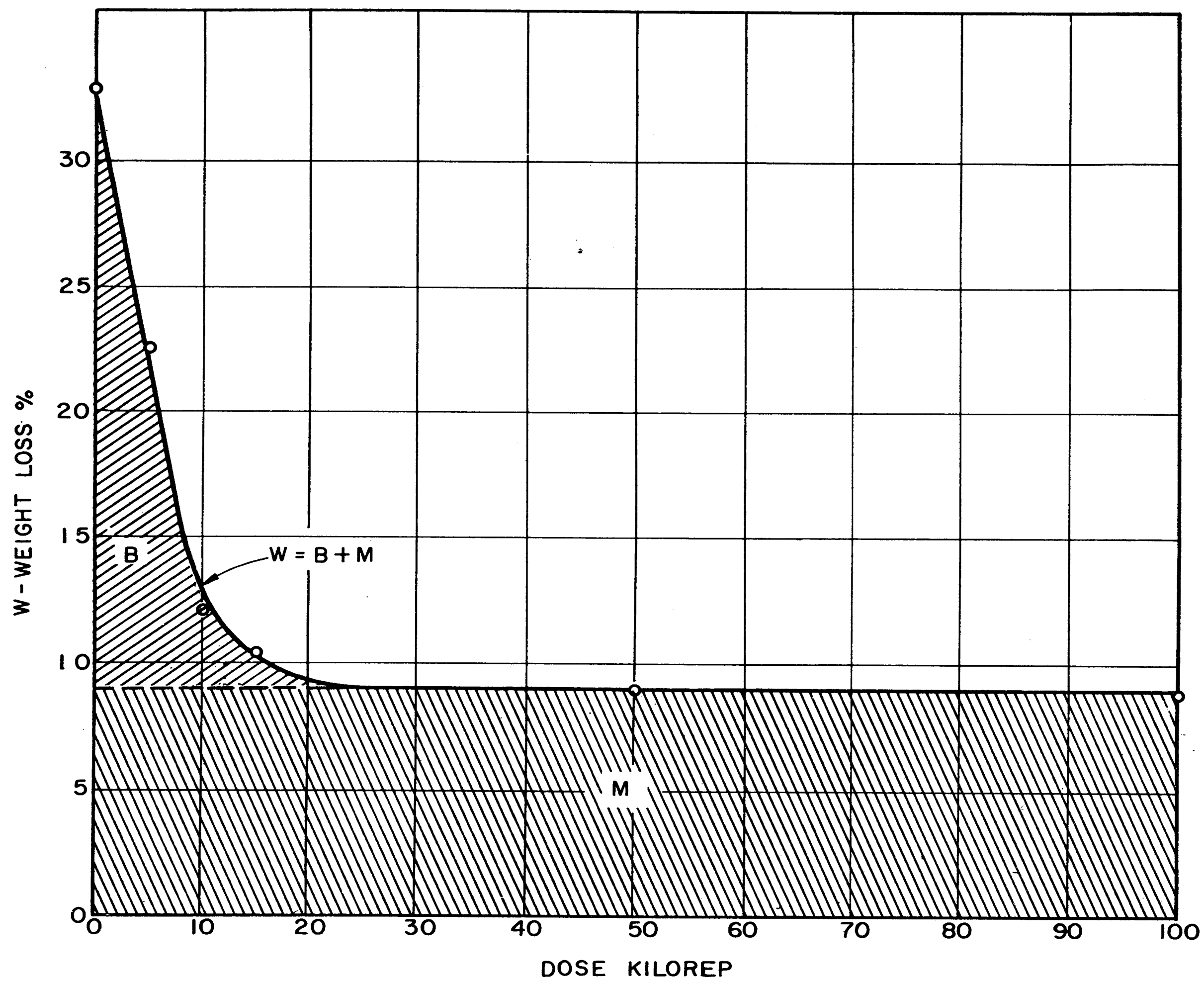


Fig. 4. The graphical relationship between the total percent weight loss, W, the biological weight loss, B, and the weight loss independent of radiation, M (potatoes stored 140 days at 50°F and 90% R.H.).

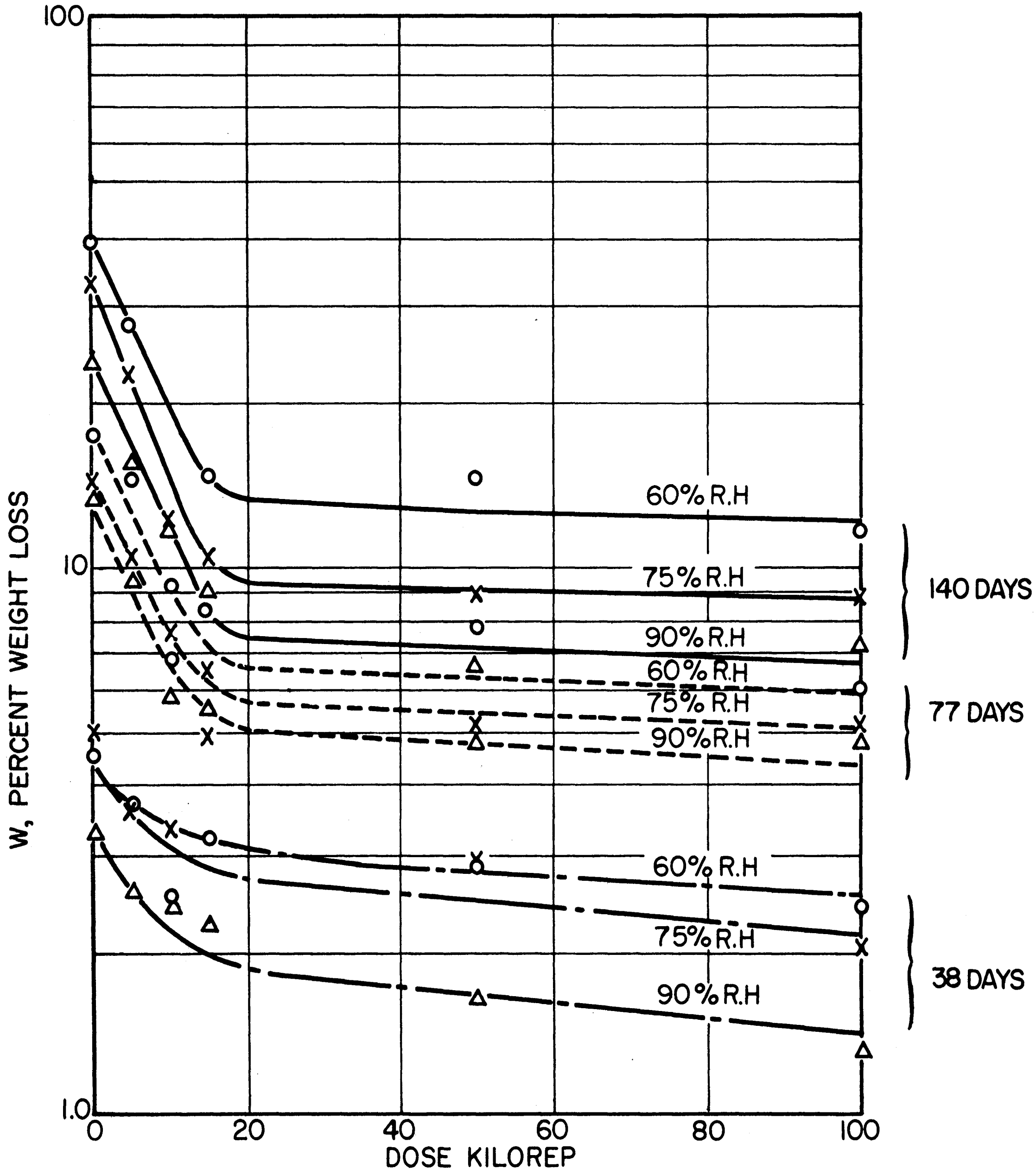


Fig. 5. Weight-loss data as a function of radiation dose, length of storage, and relative humidity.

pends in part on the diffusivity of water through the skin, the partial pressure of water vapor in the storage air, and the velocity of air circulation around the stored tubers. These and other variables which influence the mass transfer coefficients will have some effect on this "physical" or "radiation-independent weight loss." If this value of "radiation-independent weight loss," M, is subtracted from the curve for "total weight loss," W, the remaining "biological" weight loss, B, shown in Fig. 4, is radiation-dependent.

For the case of Idaho-grown potatoes used in the studies, the radiation-dependent component of weight loss demonstrates a logarithmic reduction with radiation dosage, as illustrated in Fig. 6. This phenomenon tends to support a hypothesis of weight loss proportional to the number of living cells, if the cells are inactivated by a random, statistical process. Extensive data on a wide variety of unicellular organisms from many laboratories indicate similar exponential radiation inactivation phenomena.

D. ROT AND OTHER LOSSES DURING STORAGE

Table II presents the percent of loss attributed to rot and to severe shriveling and lists the percent usable for the various doses used for the Idaho potatoes as of November, 1955. The lots stored at various conditions and which received from 10 to 200 kilorep were considered to be 84.9 to 98.5% usable, whereas none of the controls were usable on November 1. In November, 1955, no sprouts were found on any tubers receiving treatments of 10,000 rep or more, but the total losses of the controls and low-dosage-rate samples resulted in a discontinuation of the study of these potatoes and the initiation of a new study.

III. FALL IRRADIATION AND SUBSEQUENT STORAGE OF MICHIGAN-GROWN SEBAGO AND RUSSET RURAL VARIETIES OF POTATOES

A. WEIGHT LOSS

The procedure described for the Idaho-grown potatoes was repeated in November and December for Michigan-grown Sebago and Russet Rural varieties of potatoes of known history. The weight-loss data for Sebago variety potatoes are plotted in Fig. 7 and clearly indicate that, over the range studied, weight loss in the case is an <u>increasing</u> function of dosage. Comparing these data with those for Russet Burbank vairety Idaho seed potatoes shown in earlier figures indicates that a marked difference exists in the responses of the two lots of potatoes exposed to radiation treatment. Similar data for Russet Rurals which are plotted in Fig. 8 indicate a response intermediate between the other two varieties and show no gross dependence of weight loss

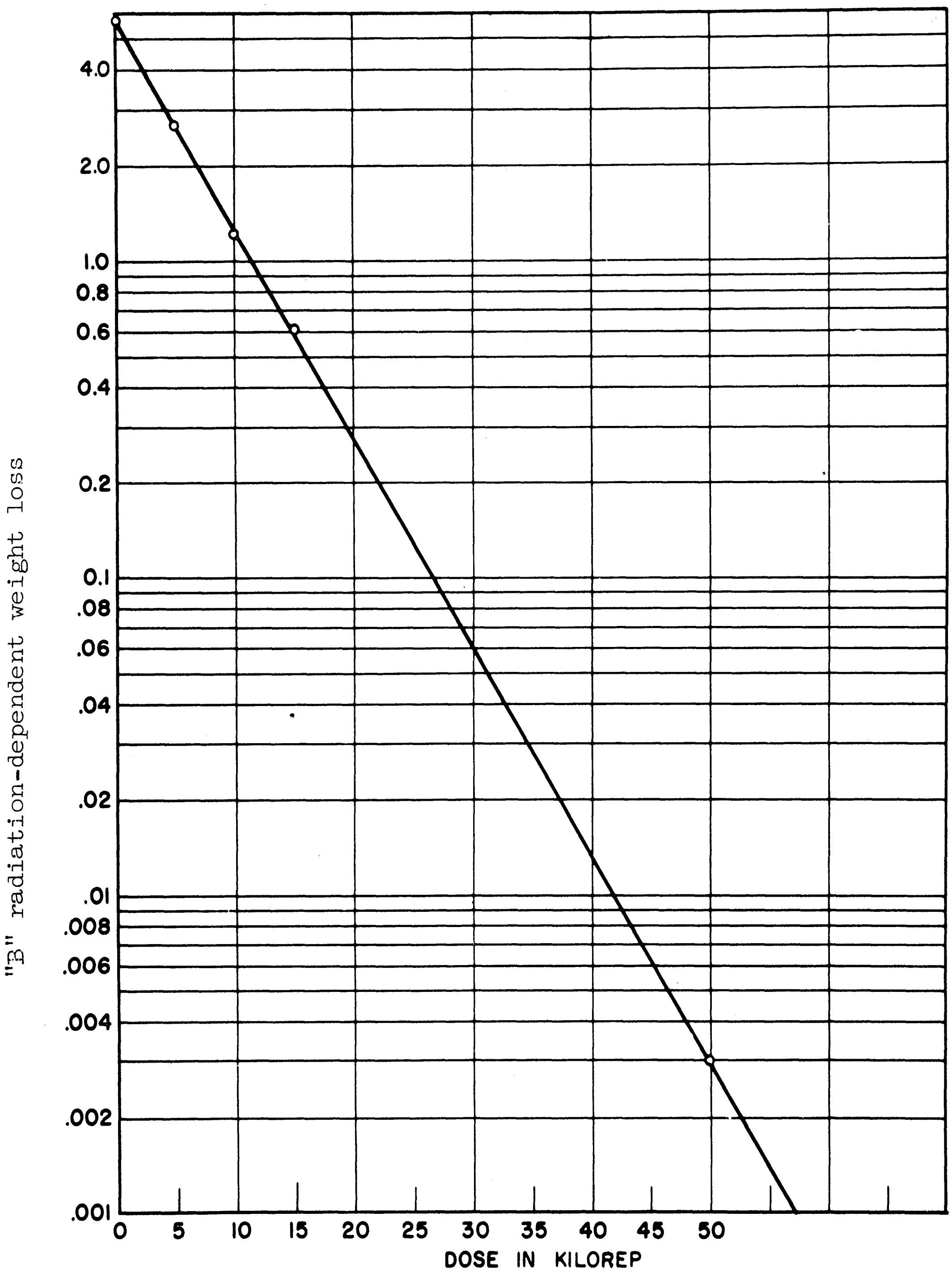


Fig. 6. Plot of "B" - Rate of weight loss dependent on radiation dosage.

TABLE II

PERCENT LOSS TO ROT AND SEVERE SHRIVELING, AND PERCENT USABLE AT FINAL SAMPLING DATE (11-1-55) FOR IDAHO POTATOES

Relative Humidity, percent	Dosage, kilorep	Rot	Severe Shriveling	Usable
60 (avg)	0	1.1	98.9	0
	5	2.1	68.2	29.7
	10	5.0	8.4	86.6
	15	6.8	5.9	87.5
	50	6.4	8.7	84.9
	100	4.6	9.3	86.1
	200	7.4	0	92.6
75 (avg)	0	0	100	0
	5	0.9	54.5	44.6
	10	1.9	11.0	87.1
	15	2.4	29.2	68.4
	50	2.2	11.1	86.7
	100	3.2	8.2	88.6
	200	4.6	6.7	88.7
90 (avg)	0	0	100	0
	5	2.5	46.1	41.4
	10	1.5	0	98.5
	15	0	5.5	94.5
	50	3.4	1.4	95.2
	100	2.3	7.1	90.6
	200	5.2	0	94.8

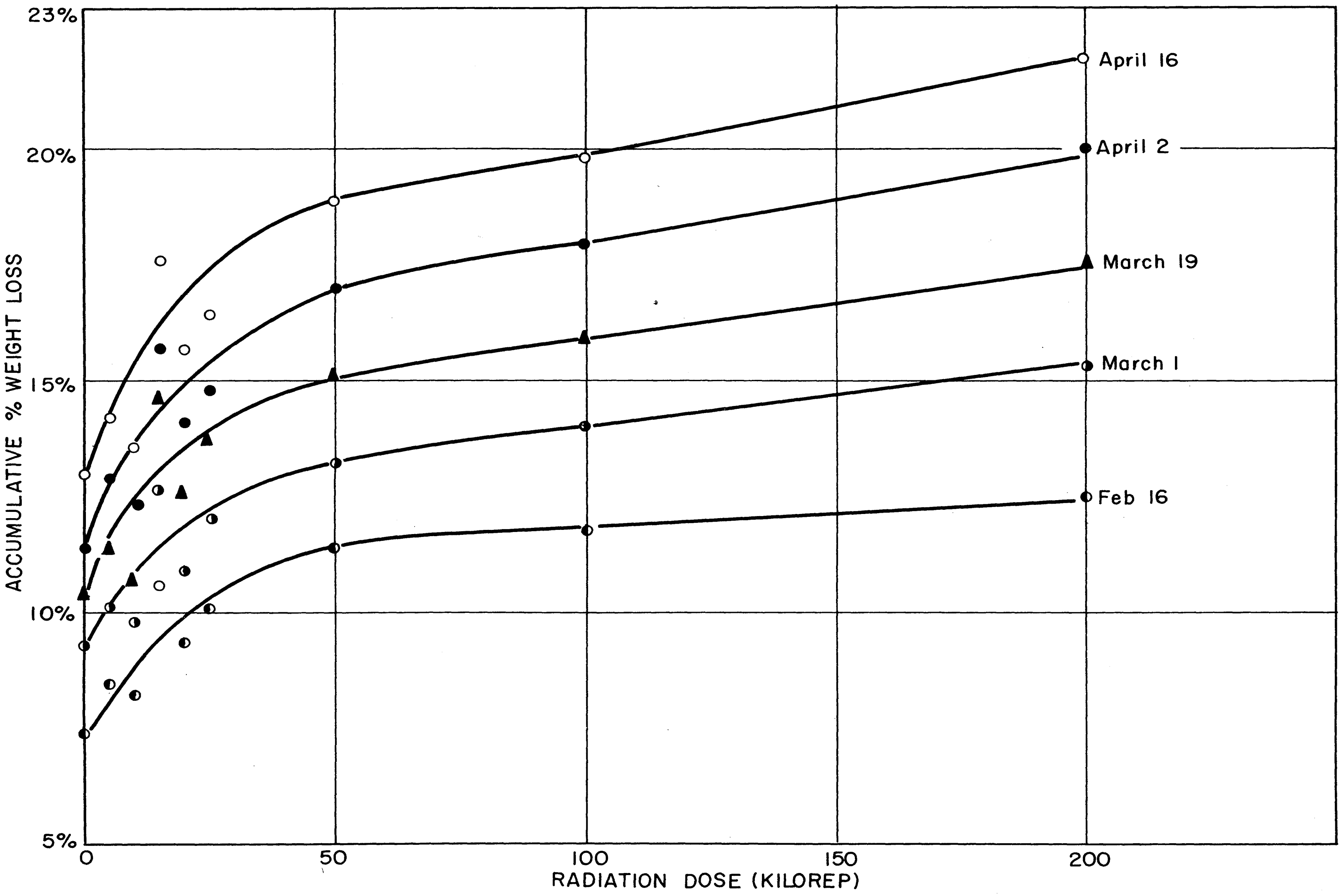


Fig. 7. Accumulative percentage weight loss vs radiation dose for Sebago variety potatoes stored at 45°F since December 7, 1955.

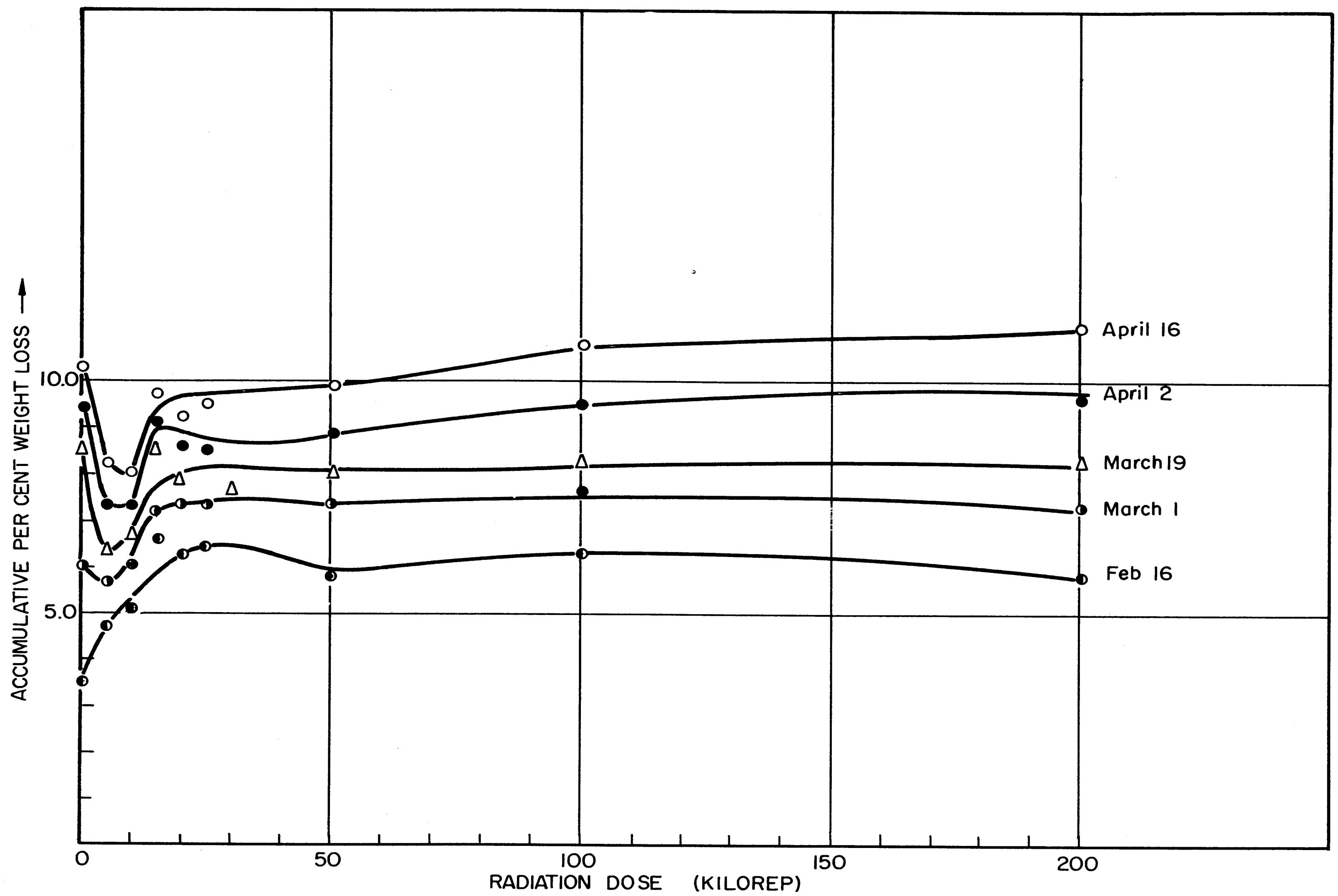


Fig. 8. Accumulative percentage weight loss vs radiation dose for Russet Rural variety potatoes stored at 45°F since December 7, 1955.

on radiation dosage. In addition to differences in variety and source it should be pointed out that the Idaho Russet Burbank variety seed potatoes were irradiated in May after storage for several months, whereas the Sebago and Russet Rural varieties were irradiated after harvest in the early winter of 1955.

These differences among the three varieties with regard to weight loss during storage as influenced by radiation dosage are exemplified by typical data in Fig. 9. More detailed evaluation of these differences as functions of storage time, temperature, and humidity could be undertaken from the data. It is clear, however, that three qualitatively distinct gross radiation responses are present in the three varieties. In all three cases, anomalous behavior is exhibited in the dose region less than 20,000 rep. More detailed data in this region might prove enlightening.

The hypothesis of radiation destruction of living organisms, assumed to explain the apparent reduction of metabolism with radiation dose in the Idaho seed potatoes, is clearly not a satisfactory explanation for the phenomena observed in the other two varieties. If a second hypothetical process of <u>radiation-induced</u> weight loss is proposed, a satisfactory model for the three responses can be constructed. Physiological differences among the three varieties must then be considered to explain the fact that one process is predominant in the Idaho seed potatoes, the other in Sebagos, and neither in Russet Rurals.

The second hypothesized process might be simply a mechanical damage to intercellular structure, facilitating moisture removal. Given a variety particularly sensitive to such destruction, such a process could conceivably overbalance the reduced metabolism due to radiation damage and produce a gross increase in weight loss with dose.

B. STORAGE OF RING-ROT-INFECTED POTATOES FOLLOWING GAMMA IRRADIATION

Field-infected Sebago potatoes were obtained on December 5, 1955, from a grower near Howard City, Michigan, and were gamma irradiated in the Fission Products Laboratory on December 12-13. The potatoes were stored at 1°C and 20°C at Michigan State University and were first examined on December 22, ten days after treatment, for incidence of ring rot and other storage rots. The results of this and subsequent inspections on incidence of ring rot for tubers stored at 20°C are plotted in Fig. 10. No trend was observed in incidence of ring rot in turbers stored at 1°C; therefore, these data were not plotted. In the inspection, tubers suspected of being rotted were cut and those showing typical ring-rot symptoms were considered to have ring rot. Stain diagnosis was not made. Other rotted tubers lacking ring-rot symptoms were placed in a second grouping classed as "storage rot" as plotted in Fig. 11.

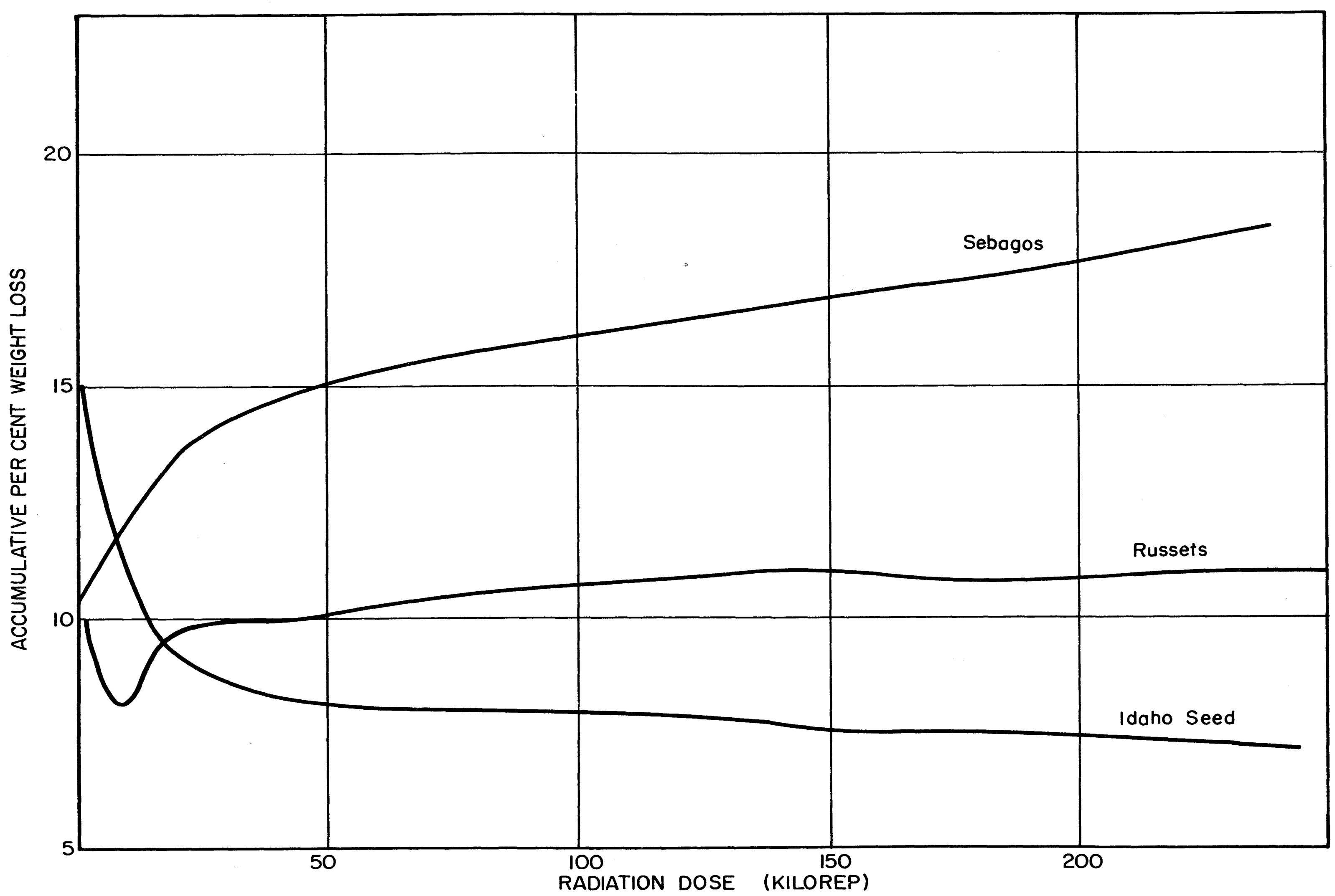


Fig. 9. Comparison of typical data for three varieties of potatoes (percentage weight loss vs radiation dose).

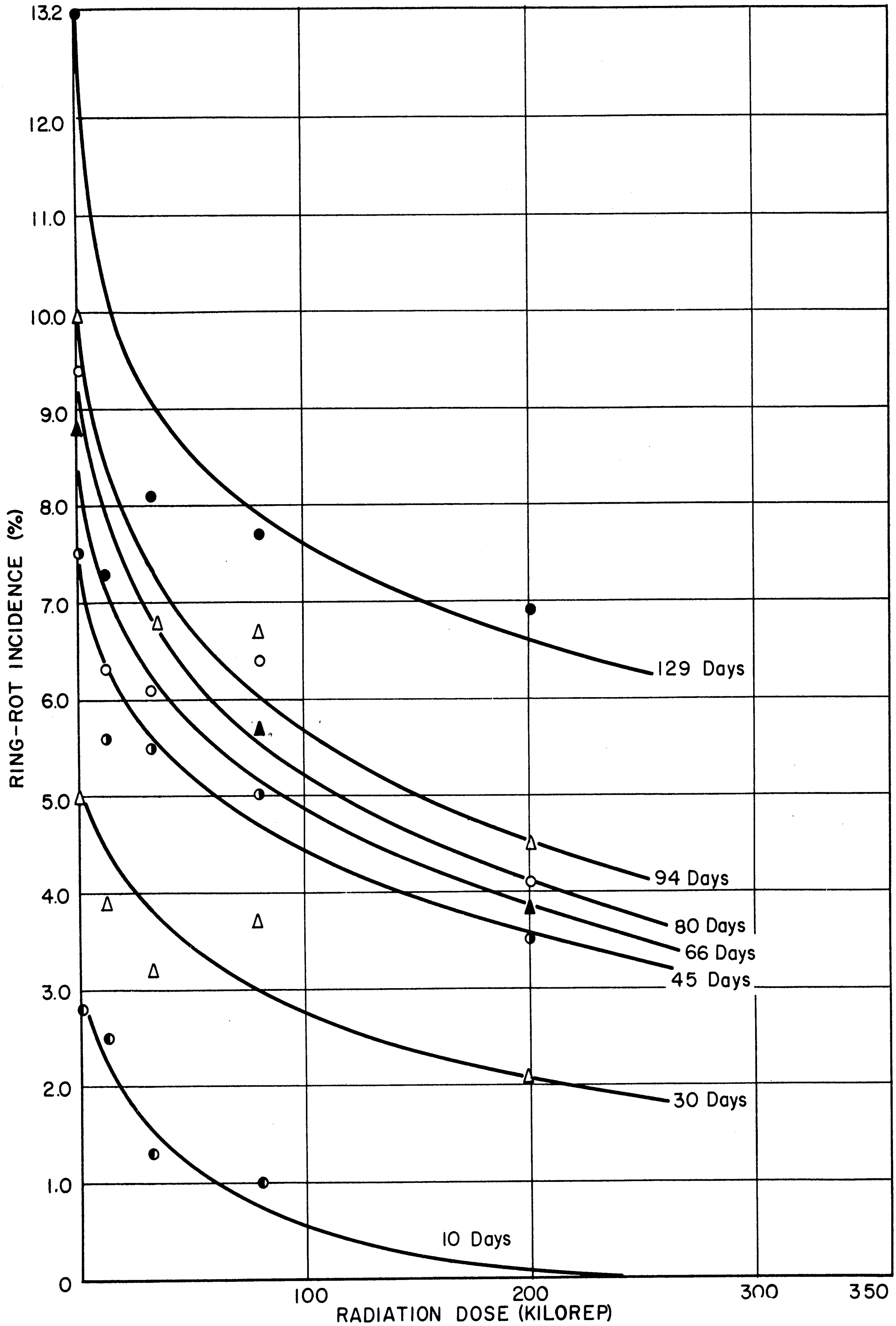


Fig. 10. Ring-rot incidence in irradiated field-infected Sebago potatoes stored at 20°C.

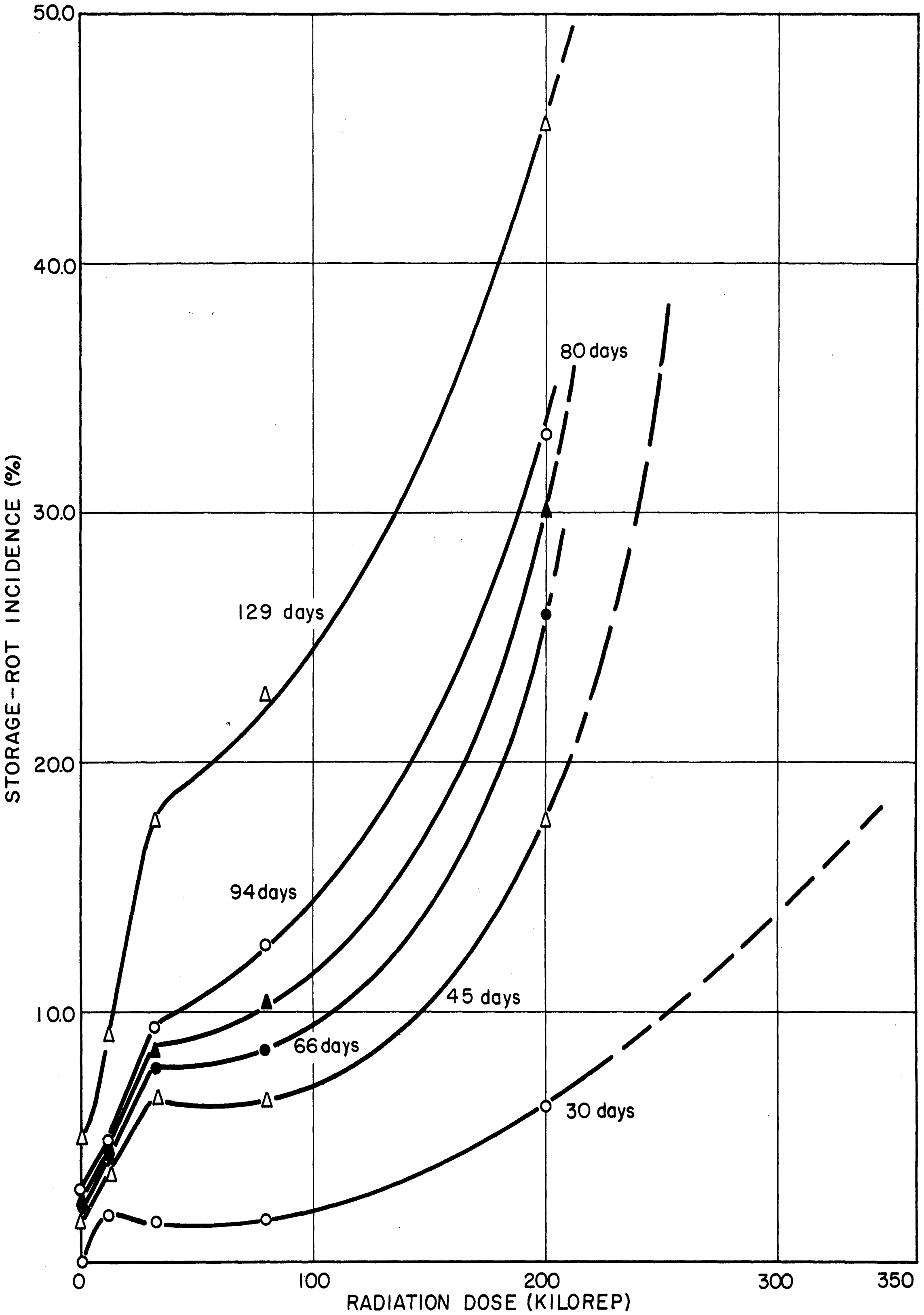


Fig. 11. Storage-rot incidence in irradiated field-infected Sebago potatoes stored at 20°C.

It should be pointed out that some of the potatoes infected with ring rot in this lot had been discarded during harvesting operations and in grading operations before shipment. Thus, the amount of ring rot shown in Fig. 10 does not reflect the total amount of infection in the lot. There was no late blight in the shipment of tubers, which made possible rather accurate diagnosis of the tuber injury.

Tuber injury as a result of irradiation was not evident following ten days in storage. By the end of 30 days, the majority of tubers receiving 500,000 rep were rotted and a few receiving 200,000 rep had broken down. Positive diagnosis of ring rot in tubers receiving the 500,000-rep treatment could not be made due to inability to identify ring rot following severe radiation injury.

Radiation injury resembled severe freezing injury in many respects. Affected tubers were often somewhat cheesy in consistency, later breaking down into a soft rot. Affected tubers often had a fermented odor, and the general appearance was more suggestive of storage rots of the sweet potato than that of the Irish potato. The alcoholic-type fermentation observed in the tubers receiving the higher dose of gamma radiation may have some connection with the sucrose analyses reported later.

Many tubers held at 20°C were rather badly wilted after 80 days in storage. Nonirradiated controls were so badly sprouted at the 129-day inspection that all tubers were cut for ring-rot evaluation. At 1°C storage there was little wilting after 133 days and tubers had not begun to sprout.

At 20°C storage (Fig. 10) there was some evidence that ring rot was developing somewhat more slowly in the irradiated tubers than in the untreated tubers. The increase in incidence of ring rot at the 129-day period is due in part to the cutting of all the tubers remaining in the sample because of sprouting.

C. WOUND HEALING, SUBERIZATION, AND PERIDERM FORMATION

Sebago, Russet Rural, and Katahdin varieties of potatoes were irradiated and then wounded to study wound healing, suberization, and periderm formation. In an initial study, suberization and periderm formation following wounding of nonirradiated Sebago tubers were observed. These tubers were peeled uniformly by hand, packed loosely in moist sphagnum moss, and held at 26°C for the two-day period during which observations were made. A one-half-inch cork borer was used to obtain tissue samples which were taken from the median region of the tubers. The cylindrical samples were then trimmed to rectangular shape, fixed in FAA, dehydrated in an ethyl alcohol series, changed to chloroform, infiltrated with and embedded in ordinary paraffin, and cut into sections 10 to 20 microns thick on a rotary microtome. The sections were strained with saffranine and fast green. This procedure was standard for all material prepared for this study.

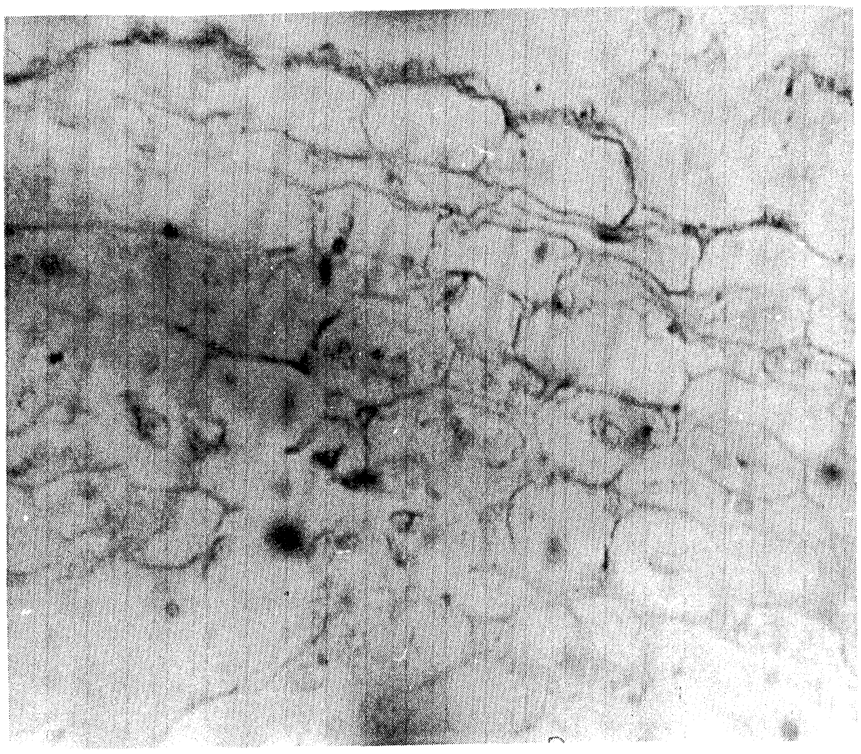
The preliminary study of untreated Sebago tubers yielded results similar to those described by Artischwager[3] in his classical study of potato histology. Six hours after wounding there is evidence of suberization of the surface layer of cells. This layer increases in depth and in density of suberin deposit until after 48 hours three to four cells from the wounded surface show distinct suberization. Periderm formation is not evident after 36 hours, but after 48 hours distinct periderm involving two to four cells is apparent. Some of these cells appear not to have completed division, but are binucleate with traces of developing wall appearing along a median plane of the parent cell.

In the second series of tests, Sebago tubers which were irradiated at 0-, 15-, and 200-kilorep dosages and stored for 50 days were then peeled and sampled as described for the initial study, but the number of sampling intervals was increased to include 0, 6, 12, 24, 48, 96, and 192 hours after wounding.

At six hours after wounding only the nonirradiated tubers showed any suberization. Traces of suberization were apparent in treated sections after 36 hours, and at 48 hours there was little to distinguish the suberization in treated sections from that in control sections. Periderm formation in controls was not evident at 48 hours, but periderm was well developed after 96 hours. There was no evidence of periderm formation in either of the two treated series even after 196 hours, and it is assumed that cell division was completely inhibited in the treated potatoes, precluding the possibility of periderm formation. Beginning at the 48-hour stage and continuing through 96 and 192 hours, the depth of the layer of suberized cells and the intensity of suberization were essentially the same in both treated and control potatoes. Figures 12 and 13 illustrate the response of tubers at each treatment level and after 6 and 192 hours, respectively, subsequent to wounding.

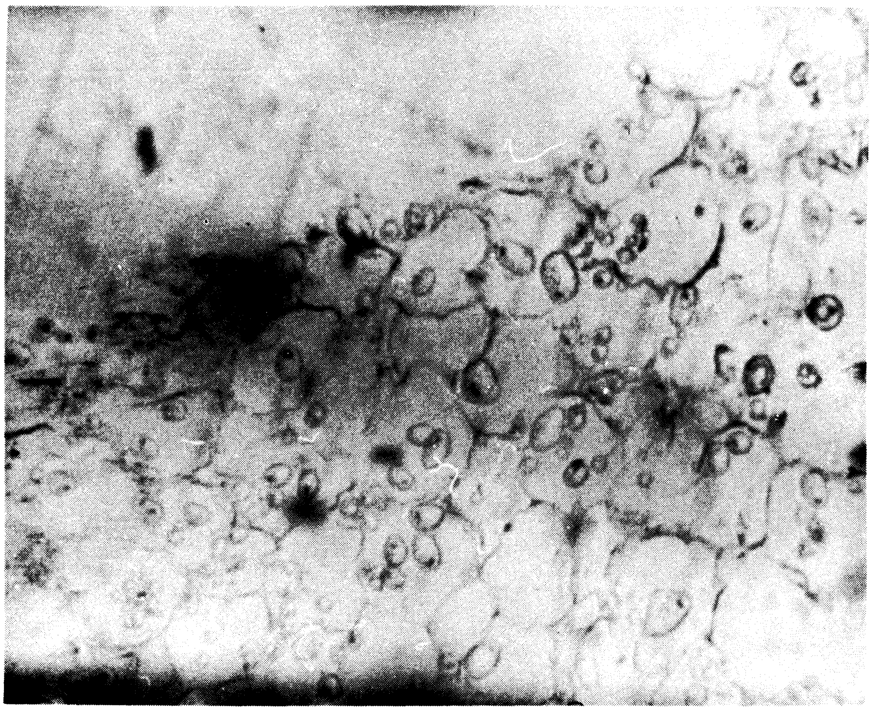
In the third series, tubers of Sebago, Katahdin, and Russet Rural varieties were irradiated at 0, 2500, 5000, 10,000, and 20,000-rep dosages and were wounded (peeled) eight days after treatment. These tubers were sampled after 36, 72, and 192 hours. Suberization occurred in all tubers with little difference evident among the various treatments and varieties. Irradiation at any level prevented formation of any distinct periderm, although in at least one sample of Katahdin treated at 2500 rep some periderm-like cells could be distinguished.

In a fourth series, 29 days elapsed between irradiation and wounding. In other respects this experiment was similar to the preceding test. In Sebago tubers, no distinct periderm could be distinguished in tubers receiving any level of irradiation. At 10,000 rep there were scattered periderm-like groups of

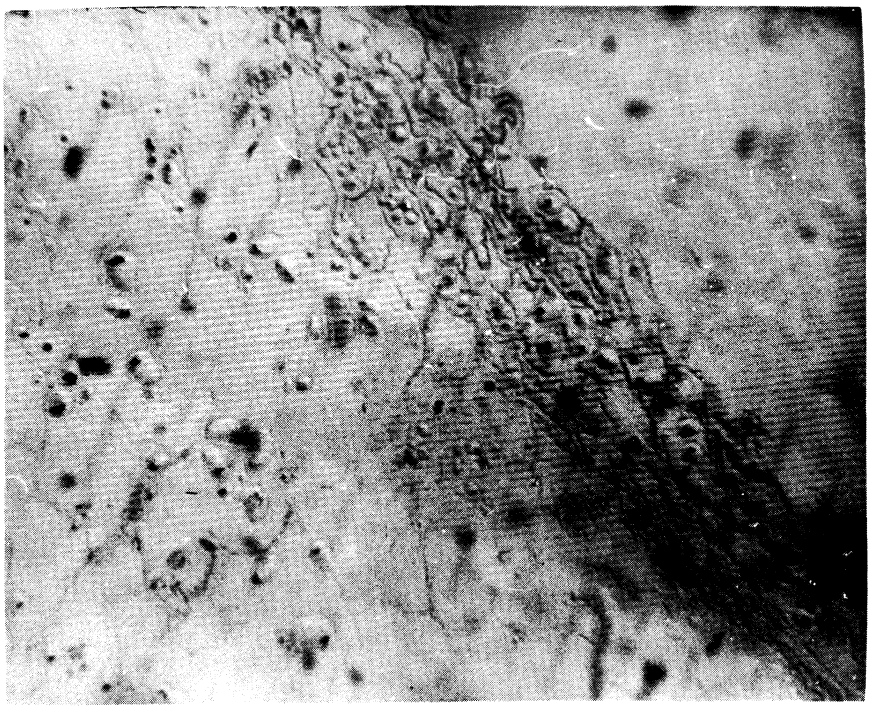


Control

Fig. 12. Photomicrographs of Sebago potato-tuber sections six hours after peeling, showing suberization of control only. 80X

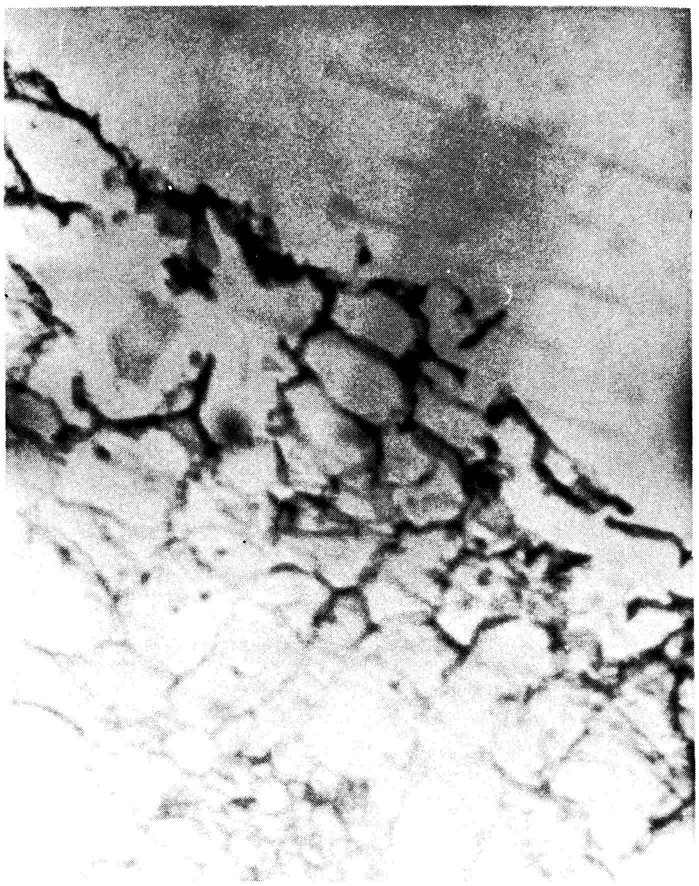


15,000-
rep
dosage



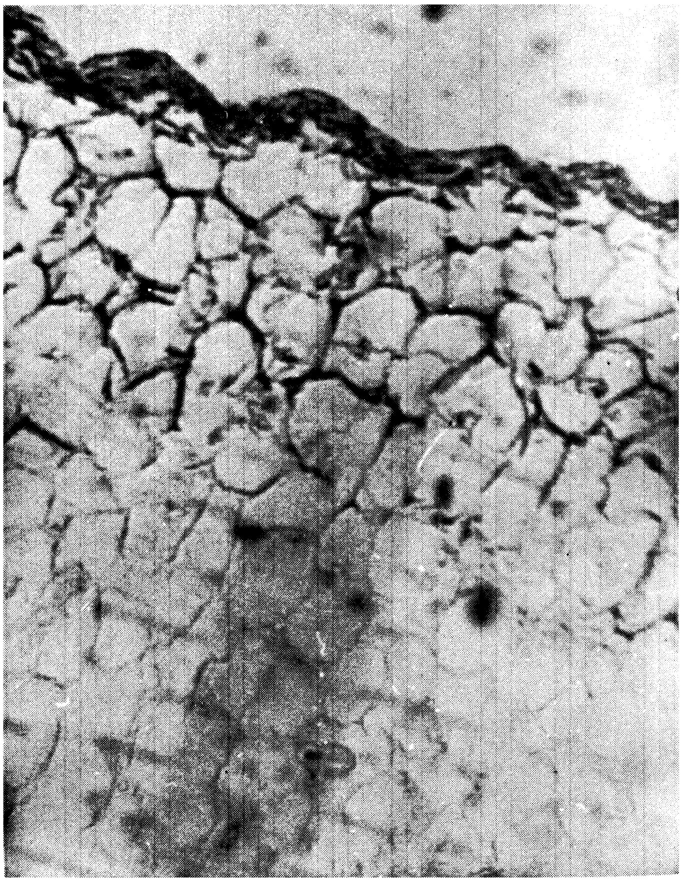
200,000-
rep
dosage

Fig. 12. Concluded.

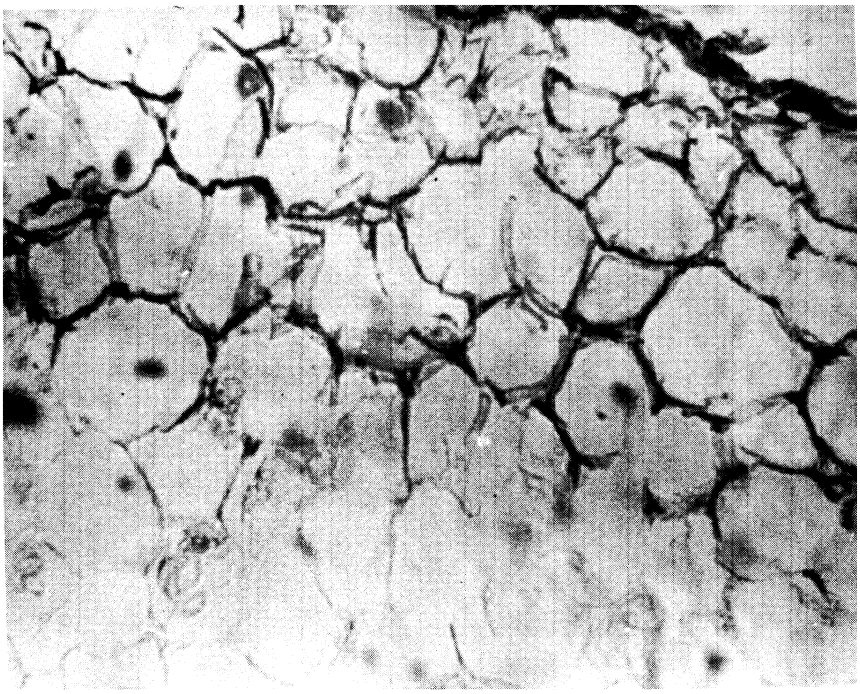


Control

Fig. 13. Photomicrographs of Sebago potato-tuber sections 196 hours after peeling, showing suberization of control and treated tubers. Extensive periderm formation has occurred in controls, none in treated tubers. 80X



15,000-
rep
dosage



200,000-
rep
dosage

Fig. 13. Concluded.

cells, and other treated tubers showed occasional pairs of periderm-like cells. Distinct periderm was evident in controls after 72 hours. Suberization was delayed but not prevented by the irradiation treatments.

Katahdin tubers showed evidence of slightly more frequent periderm-like divisions, but again there was no obvious continuous periderm such as appeared in control tubers. Suberization was delayed slightly less than in Sebago tubers.

Russet Rural potatoes showed fewer of the scattered periderm-like areas than either Sebago or Katahdin. Those which were observed were chiefly in tubers irradiated at 2500 rep, but not at higher dosages. However, suberization was not very distinctly slowed in Russet Rural, in contrast to the other two varieties.

D. STUDIES ON POTATO "HORMONES"

The observation that plants tend to grow toward the source of light is very old. That this phototropic action is related to definite chemical compounds was first clearly indicated in the classical investigations of Boysen-Jensen[4] on the coleoptile of Avena. Naturally occurring chemical compounds possessing the property of being able to regulate plant growth were given the name "hormone." Any compound, either synthetic or naturally occurring, that has this property is termed auxin. A large number of scientific articles have appeared relating to auxins.[5]

In general, if there is no auxin present, there will be no growth, but a supra-optimal concentration of auxin will also prevent growth. Only minimal quantities are required for plant growth, and the application of an excess will retard or stop growth. By the use of chemicals it is possible to prolong artificially the rest period of potato tubers. On the other hand, the normal resting period can also be shortened by using ethylene chlorohydrin. The belief prevails that the content of auxin not only varies in quantity during storage, but that the concentration also varies in the different parts of the potato. Prior to sprouting of the potatoes in the spring an increase in auxin has been observed in the fleshy part of the potato, while later the auxin, or its precursor, increases in the potato peel. The change of the precursors into auxin is generally assumed to be enzymatic in origin. One of the chief precursors may be presumed to be the amino acid tryptophane, because it has been shown that the amount of biosynthesis of auxin in a medium depends on the content of tryptophane in the medium.

One of the objectives of this research project was to increase the storage life of potatoes as a result of gamma irradiation. Gamma irradiation has been shown to slow down or halt the sprouting of the potato. Therefore, a study of the concentration of the growth-regulating substances which control sprouting was considered advisable. This study involved a comparison of concentrations of phytohormones in irradiated and nonirradiated potatoes under varying storage conditions.

In this research study an extract of the "eyes" and a small amount of surrounding tissue of potatoes was made with freshly distilled ether which is completely free of hormone-destroying hydrogen peroxide. The various growth-regulating substances were then separated by paper chromatography.

The sample consisted of the eyes of several potatoes, including a small portion of the flesh around each eye. The eyes at the apical end were not used because they seemed quite variable. About 10 grams of this material were collected and covered with peroxide-free ether. The ether and eye tissue were kept in the refrigerator (about 40°F) for a period of 20 hours. The ether was then decanted and evaporated to dryness. The residue was taken up in about 0.1 ml of alcohol, and this solution subjected to paper chromatography.

The auxins separated by chromatography were assayed by the Avena (oat) technique. In this procedure 72-hour-old plants which had been grown in the dark at a relative humidity of 85% and at a temperature of 25°-26°C were used. Plants of this age and grown under these conditions are very reactive. This assay procedure was originally developed by Went,[6] but it has been variously modified by other investigators.

A preliminary set of experiments was conducted to test the Avena assay with varying amounts of whole extract (not chromatographed) of the eyes of nonirradiated tubers, using Sebago variety potatoes. The results showed that such extracts had pronounced growth-hormone activity. A second set of experiments was conducted with Sebago variety potatoes to determine if the extract could be resolved by paper chromatography into separate components, and to determine the activity of each. In the early studies two components were separated, one which moved slowly, by paper chromatography, and one which moved fast. The latter was found to consist of essentially all the growth-promoting activity of the original mixture (positive bending of the oat coleoptile to an approximate angle of 25°). The former, however, appeared to possess slight growth-hormone-inhibiting activity, as shown by a negative bending of the oat coleoptile. The extent of the negative bending was considerably less than 25°, indicating that the hormone inhibition, if any, was considerably weaker than the positive growth-stimulating activity. Furthermore, the subsequent assays showed no evidence at all of the hormone-inhibitor activity in the eyes of nonirradiated as well as irradiated potatoes. This would be consistent with other studies which have shown the amount of growth-hormone inhibitor to decrease with cessation of dormancy.

Another set of experiments was conducted to determine the effect of irradiation of the potatoes on the hormone activity of the eye tissue directly after irradiation. Sebagos given 5, 15, 25, and 50 kilorep were used in the first assay. It was found that all doses of radiation caused a decrease in growth-hormone activity. As mentioned above, there was no hormone-inhibition action in either irradiated or nonirradiated samples. Most of the activity appeared to come from one "spot" on the paper chromatogram, although this region of the chromatogram may contain several components only partially resolved. It was not possible to distinguish differences in the extent by which different doses of radiation caused a decrease in hormone activity with respect to controls. In a set of experiments with Russet Rurals given 5-, 15-, and 25-kilorep dosages of radiation, similar results were obtained. Since samples of Sebagos and Russets have not yet been assayed in the same experiment, it is not possible to compare the two varieties.

It was also noted that throughout the course of these experiments, the amount of growth-hormone activity in the control (nonirradiated) Sebagos, which were used each time an experiment was conducted, had been slowly but steadily decreasing. Whereas the approximate average angle of bending was 25° in the first experiments, it decreased to 18° in experiments conducted two and a half months later.

When the hormone and inhibitor concentrations of the control and irradiated Sebago tubers were compared, it was found that the observed differences were of such a small magnitude that they could be the result of errors in technic and not due to any significant difference between irradiated and control tubers. This indicates that the inhibition of sprouting as a result of irradiation is not associated with the hormones and inhibitors found in or just around the eyes in Sebago variety stored potatoes.

In late March a shipment of freshly harvested Pontiac potatoes was received from Homestead, Florida. These potatoes were obtained for the purpose of investigating the hormone and inhibitor concentrations in freshly harvested tubers.

Three experiments were conducted with the Pontiac tubers. The first test was made for the purpose of determining what amounts to use in subsequent experiments. In the other two tests, dosages of 25 and 75 kilorep were given a week before the analyses. Table III gives the results for the two experiments.

TABLE III

HORMONE ACTIVITY OF PONTIAC VARIETY POTATOES

Date	Treatment	Total Hormone Activity in Degrees Curvature of Oat Seedlings	Total Inhibitor Activity in Degrees Curvature of Oat Seedlings
April 13	Control	10.0	1.9
	25-kilorep dosage	17.6	0
April 20	Control	9.2	22.8
	75-kilorep dosage	13.5	16.2

These two experiments would tend to show that irradiation increases the hormone concentrations. In the April 20 experiment there is for the first time evidence of a high concentration of inhibitor. This was found both in the control and the treated tubers, so there seems to be no error. In both experiments the treated tubers had less inhibitor.

IV. OTHER STUDIES

Extensive data were obtained on the analysis of irradiated and control tubers with respect to percent reducing sugar, percent sucrose, percent starch, and ascorbic acid content. Reducing-sugar values were obtained on samples of potatoes receiving altogether 15 different treatments. The results showed that increasing the storage temperature of potatoes irradiated with 10,000-rep gamma radiation resulted in decreasing the reducing-sugar content from a value of 2.7% for potatoes stored at 35°F for six months to 0.35% for potatoes stored at approximately 80°F. Potatoes receiving doses of radiation ranging from 0 to 200,000 rep, but stored at the same temperature for five months, showed no consistent change in reducing-sugar content. Table IV gives values of reducing sugar, sucrose, and starch for control and 15,000-rep irradiated Russet Rural variety tubers stored at various temperatures for eight weeks.

TABLE IV

CARBOHYDRATE ANALYSES ON RUSSET RURAL POTATOES

Time in Weeks	Temperature	Control				Irradiated (15 Krep)			
		% Red'g Sugar	% Sucrose	% Starch	Total	% Red'g Sugar	% Sucrose	% Starch	Total
0	All temps.	0.43	0.19			0.43	0.30		
8	35	1.71	0.47	13.1	15.3	1.25	2.71	13.4	17.4
8	45	0.50	0.28	20.7	21.5	0.40	0.81	21.4	22.6
8	55	0.27	0.31	20.4	21.0	0.43	0.45	20.4	21.3
8	65	0.37	0.25	20.9	21.5	0.55	0.20	20.9	21.7
8	room	0.36	0.46	22.5	23.3	0.37	0.49	17.1	18.0

Carbohydrate analyses for both irradiated and control tubers during the late storage period have shown that the percent reducing sugar, sucrose, and starch remained fairly constant with respect to time.

A sharp reduction in reduced ascorbic acid content at radiation doses of 10,000 rep was observed with an additional but more gradual reduction at higher doses.

Respiration studies were made both with whole potato tubers and with potato-tissue slices and a Warburg apparatus. Respiration studies on potato-tissue slices show no significant effect of gamma-radiation doses of 5,000, 10,000, and 15,000 rep. Doses of 50,000, 100,000, and 200,000 rep resulted in higher respiration rates for the slices of irradiated tubers as compared to the controls. This has continued with time of storage.

Respiration studies on whole tubers gave results similar to the tests on slices for doses of 50,000, 100,000, and 200,000 rep. Low doses of 5,000, 15,000, and 25,000 rep show (as compared to controls) a drop of about -30% in respiration rate during the first day after irradiation, followed by a sharp increase the second day to about plus 60%, followed by a gradual decline to about -5% at the 13th week of storage.

V. ANALYSIS OF RESULTS

A. CORRELATION OF WEIGHT-LOSS DATA ON IDAHO-GROWN RUSSET BURBANK VARIETY SEED POTATOES

Based on the method of analysis described and illustrated in Fig. 6, other data were analyzed, using the assumption that the weight loss at 50 kilorep was in equilibrium range and that the weight loss at the 50-kilorep dose was equal to "M," the radiation-independent weight loss.

It was desirable to express M in terms of a rate so as to compare data collected at different storage intervals. Therefore, the values of accumulative weight loss independent of dosage were divided by the storage time to give M in terms of weight loss per day. This is considered permissible because observations indicated that weight loss varied nearly directly with time if storage conditions remained constant. Table V gives the average values of M so determined as a function of humidity.

The values of M in Table V show a dependence on relative humidity during storage, as might be expected. As anticipated, the rate of weight loss

TABLE V

VALUES OF M: WEIGHT LOSS INDEPENDENT
OF RADIATION DOSAGE FOR IDAHO TUBERS

Relative Humidity, percent	"M" Weight Loss/Day Independent of Dose, lb/day per 100 lb of tubers
60	.0761
75	.0640
90	.0531

decreases as the humidity increases. To correlate these data, the mass transfer relationships for "drying" were considered.

Water was removed in any "drying" process involves the phenomenon known as mass transfer. This phenomenon, when limited to drying, consists of the transfer of mass of substance "x" from point 1 to point 2 as a result of a driving force. This driving force is often expressed as the difference in partial pressure of substance x between points 1 and 2. In the case of weight loss in potatoes by "drying," the substance x is water and the driving force is the difference between the partial pressure of water at the existing humidity and the vapor pressure of water at the existing temperature.

The water must pass through the skins of the potatoes; therefore, the greater the area of the potatoes, the greater will be the rate of weight loss. It is generally true that the mass transfer depends on the area of the mass transfer surface.

The water vapor must pass through a film of stagnant air that surrounds each tuber. The mass transfer characteristic by this film is termed the mass transfer coefficient, k', and is comparable to heat transfer coefficients in heat transfer relationships.

The relationships discussed above can be summarized by the following equation:

$$W_1 = k'a\,(p_1 - p_2) \quad , \tag{1}$$

where

W_1 = rate of weight loss by "drying," lb/day per 100 lb of tubers,
k' = mass transfer coefficient,
a = area of tubers, sq in. per 100 lb,
p_1 = vapor pressure of water at storage temperature, mm Hg, and
p_2 = partial pressure of water in bulk of air surrounding tubers, mm Hg.

The mass transfer coefficient may be correlated in terms of air velocity, diffusivity of water, temperature, and other properties of the system that affect the resistance of the stagnant-air film to mass transfer. However, as these are seldom major variables for conditions used in potato storage, k' will be considered to be a constant. Potatoes are of fairly uniform size and shape; therefore, the area per 100 lb "a" may be considered constant. It is more convenient to express partial-pressure difference of water vapor in terms of relative humidity. Combining these constants, Equation 1 may be rewritten as

$$W_1 = k\,(100 - \text{R.H.}) \quad , \tag{2}$$

where

k = a constant and

R.H. = percent relative humidity.

Analysis of the data of Table V indicated that k has a value of 0.0008. Using this value of k in Equation 2 did not account for all the weight loss independent of radiation dosage. There was a constant rate of weight loss, W_2, which is independent of both radiation dosage and humidity of storage and which is equal to 0.045 lb per day per 100 lb of potatoes or

$$\begin{aligned} \text{"M"} &= W_1 + W_2 \\ &= 0.0008\,(100 - \text{R.H.}) + 0.045 \\ &= 0.125 - 0.0008\,\text{R.H.} \quad , \end{aligned} \tag{3}$$

where

M = total rate of weight loss independent of radiation dosage, lb/day per 100 lb of tubers, and

W_2 = weight loss independent of both dosage and humidity of storage = 0.045 lb/day per 100 lb of tubers.

It is not surprising that some of the weight loss independent of radiation dosage is also independent of humidity of storage, as some of this weight loss occurs by methods other than drying. Certain chemical reactions involved in respiration and which result in weight loss may be independent of radiation dosage and, of course, would be independent of humidity of storage.

To calculate values of "B," the value of M (the rate of weight loss at 50 kilorep) was subtracted from the total rate of weight loss for data obtained with storage at 55°F and various radiation dosages and various humidities. The results of the calculations are plotted in Fig. 14, which shows considerable scattering of the data, as might be expected in such measurements. The greatest deviation occurs for 38 days, the shortest storage period analyzed. The percentage error is higher for this short period when the total weight loss was small.

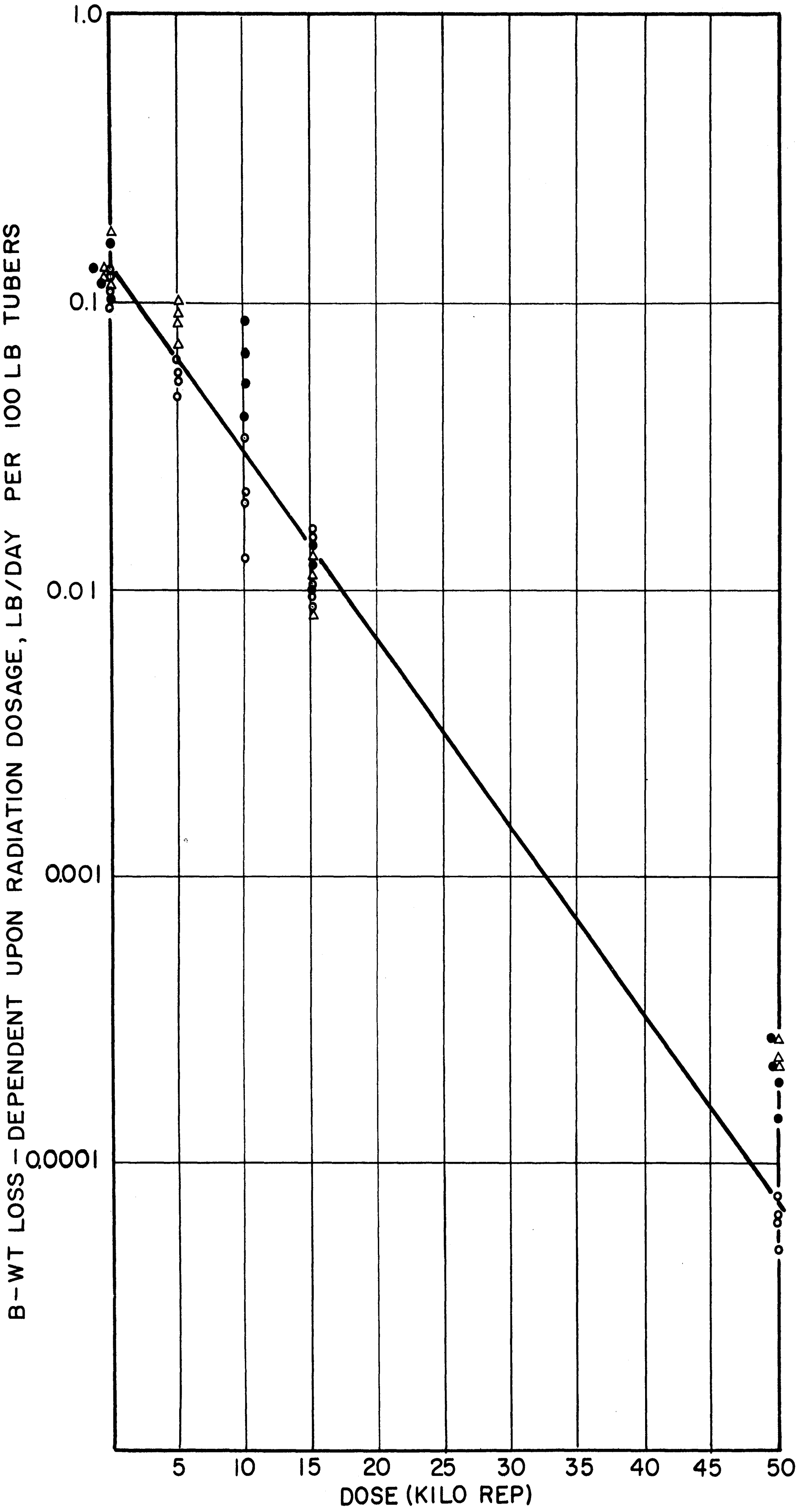


Fig. 14. "B" rate of weight loss dependent on radiation dosage.

Figures 6 and 14 show that (except for the scattering of the data in the latter figure) the logarithm of percent weight loss for a given storage period or the logarithm of the weight loss per day per 100 lb of tubers is a linear function of the radiation dosage for the Idaho potatoes used. The equation of this line is

$$\ell nB = \ell nc + mD$$

or, taking antilogs,

$$B = ce^{mD} \quad , \tag{4}$$

where

B = rate of weight loss dependent on radiation dosage, lb/day per 100 lb of tubers,
e = Naperian base, = 2.3026,
m = slope of line in Fig. 14 = 0.15,
D = radiation dose, kilorep, and
c = antilog of intercept of line in Fig. 14 = 0.13.

To solve for c and m, two points are selected on the curve at B = 0.0001 and B = 0.1:

$$2.3026\ (-4) = n(48) + \ell nc \tag{5}$$

$$2.3026\ (-1) = n(2) + \ell nc \quad . \tag{6}$$

Subtracting Equation 6 from Equation 5,

$$m = \frac{2.3026(-3)}{46} = -\ 0.15$$

$$c = \text{intercept Fig. 14}$$

$$= 0.13$$

$$\therefore \ell nB = -\ 0.15\ D + \ell n\ 0.13$$

or

$$B = 0.13\ e^{-0.15D} \quad . \tag{7}$$

The semilogarithmic relationship given by Equation 7 indicates that the weight loss "B" dependent on radiation may be proportional to the number of living cells, if the cells are inactivated by a random statistical process. This phenomenon has been termed the "target theory" and has been given appreciable consideration with regard to the effects of radiation on the killing of microorganisms.

Equations 3 and 5 may be combined to give the total weight loss for irradiated Idaho seed potatoes stored at 55°F:

$$W_{55} = (.125 - .0008\ R.H. + 0.13e^{-.15D})t \quad , \qquad (8)$$

where

W_{55} = total weight loss at 55°F, lb/100 lb,
R.H. = relative humidity, %,
D = radiation dose, kilorep, and
t = time, days.

Total weight loss as a function of the temperature of storage was measured with tubers stored in different constant-temperature rooms in the Food Service Building at The University of Michigan. Weight loss was found to increase with temperature of storage, as might be expected from a consideration of the effect of temperature on chemical reactions. The Arrhenius equation for reaction rate as a function of temperature and other variables may be stated as follows:

$$k_1 = Ae^{-E/RT} \quad , \qquad (9)$$

where

k_1 = specific reaction velocity constant,
A = frequency factor for reaction,
E = energy of activation,
T = absolute temperature, and
R = universal gas constant (8.3×10^7 ergs per degree per mole).

If storage humidities are high, the weight loss by "drying" will be proportionately small and an appreciable portion of the weight loss will result from respiration losses.

Thus, based on the concept that a major portion of the weight loss results from the chemical reactions involved in respiration, Equation 9 may be applied. As all the variables in Equation 9 may be considered constant except temperature, the data for the weight loss were plotted vs the reciprocal of the absolute temperature as shown in Fig. 15. Equation 9 is an exponential relation and, considering the form of the variables, the logarithm of the rate of weight loss (rate of reaction) should be plotted vs the reciprocal of the absolute temperature.

The logarithm of the total percent weight loss (W) for 134 days was plotted against the reciprocal of Absolute Temperature (1/T) as shown in Fig. 15. Straight-line curves were obtained for the data on the control and the irradiated tubers. Equations for the straight lines are given as follows:

CONTROL Tubers

Equation of the straight line

$$\ln W_o = \frac{m_o}{T} + C_o \quad , \qquad (10)$$

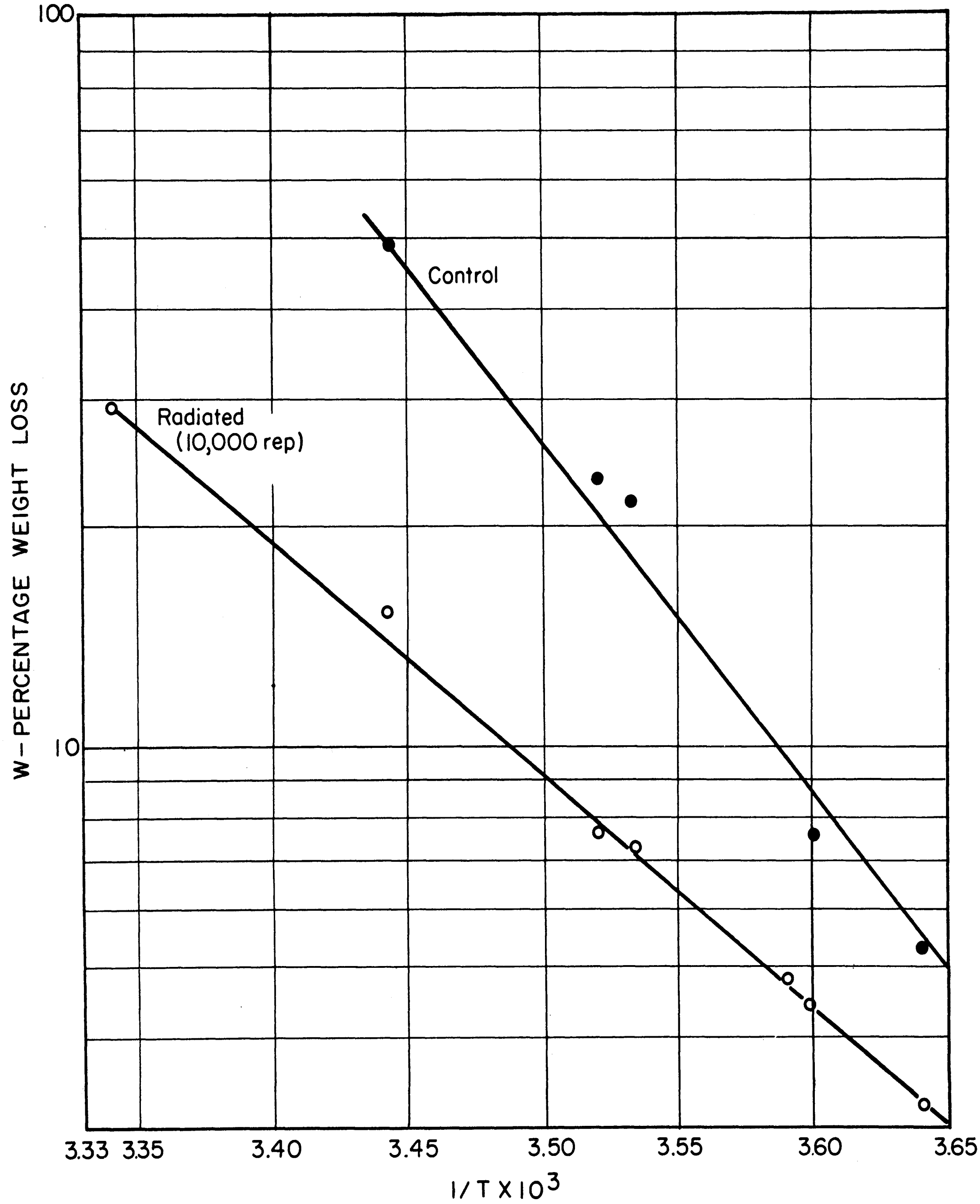


Fig. 15. Weight loss vs reciprocal of the absolute temperature (°K) of storage.

where

ℓn = Naperian logarithm,
W_O = percentage weight loss for control tubers,
m_O = slope of line for control tubers, and
C_O = intercept on y-axis.

In order to determine m_O, two points are selected on the line for the control tubers:

$$\ell n\ 50 = m_O \times 3.44 \times 10^{-3} + C_O \tag{11}$$

$$\ell n\ 8.5 = m_O \times 3.6 \times 10^{-3} + C_O \quad . \tag{12}$$

Subtracting Equation 12 from Equation 11,

$$3.91202 - 2.14007 = -0.16 \times 10^{-3} \times m_O$$

or

$$m_O = -\frac{1.77195}{0.16 \times 10^{-3}}$$

$$m_O = -11.075 \times 10^3$$

$$W_O = A_O e^{\frac{-11.075 \times 10^3}{T}} \quad , \tag{13}$$

where $A_O = e^{C_O}$.

<u>IRRADIATED</u> Tubers

$$\ell n\ W_R = \frac{m_R}{T} + C_R \quad , \tag{14}$$

where

W_R = weight loss for irradiated tubers,
m_R = slope of line for irradiated tubers, and
C_R = intercept on y-axis.

To determine m_R, two points are selected on the line for irradiated tubers:

$$\ell n\ 27.3 = m_R \times 3.35 \times 10^{-3} + C_R \tag{15}$$

$$\ell n\ 4.35 = m_R \times 3.60 \times 10^{-3} + C_R \quad . \tag{16}$$

Subtracting Equation 16 from Equation 15,

$$3.30689 - 1.47018 = 0.25\ m_R \times 10^{-3} \quad .$$

$$\therefore m_R = -7.3468 \times 10^3$$

$$W_R = A_R \, e^{\frac{-7.35 \times 10^3}{T}} , \qquad (17)$$

where $A_R = e^{C_R}$.

Rewriting Equation 17,

$$W_R = A_R \, e^{\frac{-7.35 \times 10^3}{T}} .$$

To evaluate A_R, the value of W_R at 55°F may be calculated and equated to W_{55} obtained by Equation 8 or:

$$W_R \text{ at } 55°F = A_R \, e^{\frac{-7.35 \times 10^3}{288}} \qquad (18)$$

$$W_{55°F} = [0.125 - 0.0008 \text{ R.H.} + 0.13 \, e^{-0.15D}]t . \qquad (19)$$

Equating $W_{55°F} = W_R$ at 55°F or

$$A_R = \left[0.125 - 0.0008 \text{ R.H.} + 0.13 \, e^{-0.15D} \, te^{\frac{-7.35 \times 10^3}{288}}\right] \qquad (20)$$

$\therefore$ General Equation

$$W_{RT°K} = [0.125 - 0.0008 \text{ R.H.} + 0.13 \, e^{-0.15D}] \, te^{-7.35 \times 10^3\left(\frac{1}{288} - \frac{1}{T}\right)} \qquad (21)$$

or

$$W_{RT°K} = e^{b}t \, [0.125 - 0.0008 \text{ R.H.} + 0.13e^{-0.15D}] , \qquad (22)$$

where

$W_{RT°K}$	=	percentage weight loss at T°K,
t	=	storage time after irradiation, days,
T	=	absolute temperature, °K,
D	=	radiation dose, kilorep,
R.H.	=	relative humidity, %,
e	=	Naperian base, 2.3026, and
b	=	$-7.35 \times 10^3 \, [1/288 - 1/T]$.

The data collected on Idaho seed potatoes from the 1954 crop which were treated with gamma radiation and stored at various temperatures and humidities were summarized by means of Equation 22. However, Equation 22 is applicable only to the potatoes of the variety, size, source, and age used in the experiment and for storage for periods of from 0 to 4 months after irradiation.

B. DISCUSSION OF STUDIES ON HORMONE ACTIVITY AND ON PERIDERM FORMATION

The results listed in Table III indicate that sprout inhibition as a result of irradiation is not caused by a decrease in activity of the growth hormones or an increase in the amount of growth inhibitor. In fact these results indicate the exact reverse—an increase in growth hormone and a decrease in inhibitor. Additional data will be required to establish this point. However, these results support some earlier studies made with irradiated pearl onions and irradiated seed black walnuts.[1] In both of these cases low doses of radiation from 10,000 to 50,000 rep actually stimulated the sprouting of the irradiated onions and walnuts prior to the sprouting of the controls. But these sprouts were short lived and died after reaching a length of about 1/2 inch, whereas the controls subsequently sprouted and the sprouts continued to grow. These limited observations support the idea that growth hormones may be increased and/or growth-hormone inhibitors decreased as a result of gamma irradiation.

The studies on periderm formation (see Fig. 13) indicate that cell division is inhibited by gamma irradiation. This also checks with general observations of the effect of low doses of gamma radiation on microorganisms. At the time of irradiation there is little observable effect on the microorganisms, and some time must elapse before the effects of irradiation become apparent. Most cells continue to respire normally after irradiation and to show other biological characteristics such as motility where it occurs normally. However, the irradiation appears to interfere with the process of cell division. Some individual cells may grow to giant size before dividing and when cell division does occur, the daughter cells do not redivide.

The hypothesis is proposed that sprout inhibition in irradiated potatoes is caused by a mechanism induced by irradiation which interferes with cell division. The production of growth hormones may be stimulated as a response to the damage to the cells caused by irradiation.

VI. SUMMARY AND CONCLUSIONS

The optimum effect of gamma irradiation in increasing the storage life of potatoes was obtained with Idaho-grown Russet Burbank variety potatoes irradiated in late spring. Irradiation in the spring appears to be preferable to irradiation in the fall or early winter. Russet Burbank and Russet Rural varieties of potatoes appear to have better storage properties after irradiation than does the Sebago (a white skin) variety.

The optimum radiation dose appears to be about 15,000 rep. The optimum storage conditions appear to be about 45°F and about 85%-90% relative humidity.

In the studies on Russet Burbank variety tubers, weight loss for a given storage decreased with increased dosage up to 15,000 to 25,000 rep, whereas the reverse effect was observed with the Sebago variety tubers. Length of storage prior to irradiation as well as variety may have caused these differences.

In studies in suberization and periderm formation, all levels of gamma radiation tested (15,000 to 200,000 rep) were found to inhibit periderm formation completely and to delay but not to inhibit suberization.

Hormone studies on Sebago variety irradiated and control potatoes stored for some time showed such low hormone content that no effect of irradiation could be detected. However, limited data on freshly harvested Pontiac variety (Florida grown) tubers indicate that gamma radiation increases the hormone concentration and decreases inhibitor concentration.

Studies on field-infected Sebago variety potatoes indicate that gamma radiation decreases incidence of ring rot on potatoes stored at 20°C but has no significant effect on the same tubers stored at 1°C. However, other unclassified forms of rot increased at both storage temperatures with increases in radiation dosage.

Respiration studies on potato-tissue slices show no significant effect of gamma-radiation doses of 5,000, 10,000, and 15,000 rep. Doses of 50,000, 100,000, and 200,000 rep resulted in higher respiration rates for the slices of irradiated tubers as compared to the controls. This has continued with time of storage.

Respiration studies on whole tubers gave results similar to the tests on slices for doses of 50,000, 100,000, and 200,000 rep. Low doses of 5,000, 15,000, and 25,000 rep show (as compared to controls) a drop of about -30% in respiration rate during the first day after irradiation, followed by a sharp increase the second day to about plus 60%, followed by a gradual decline to about -5% at the 13th week of storage.

Carbohydrate analyses for reducing sugar, sucrose, and starch show little effect of low radiation dose or length of storage on these analyses. Higher radiation dosages and lower storage temperature increase the sucrose concentration.

Studies on enzyme systems have shown a sharp reduction in reduced ascorbic acid content at radiation doses of 10,000 rep and an additional but more gradual reduction at higher doses.

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