Progress Report No. 7

GAMMA-RAY SPROUT INHIBITION OF POTATOES

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Project 2386

QUARTERMASTER ACTIVITIES, DEPARTMENT OF THE ARMY
CONTRACT NO. DA19-129-qm-349, PROJECT NO. 7-84-01-002

November 1956

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CONTRACT RESEARCH PROGRESS REPORT

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FOR THE ARMED FORCES, CHICAGO

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Office of the Quartermaster General

Fission Products Laboratory
The University of Michigan
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Ann Arbor, Michigan

Project No. 7-84-01-002
Contract No. DA19-129-qm-349
File No. S-527
Report No. 7 (Progress)
Period: 1 May 1956 to
30 June 1956
Initiation Date: 20 April 1955

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Title of Contract: Gamma-Ray Sprout Inhibition of Potatoes

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 8 or 9 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 himidity.

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 potatoes.

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 personnal of QMF 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. Sussman[4] has previously reported the results of short-time experiments with gamma-irradiated potatoes.

II. MATERIAL AND METHODS

Two varieties of potatoes have been used, the Sebago and the Pontiac. The former were obtained from Michigan State University in November and have been stored at 45°F since then; the latter were obtained from Homestead, Florida,* in the middle of March and have also been stored at 45°F.

The irradiation was performed in the radiation cave of the Fission Products Laboratory, The University of Michigan. The potatoes, in paper bags, were placed in the center well, i.e., within the circle of cobalt rods, and irradiated for various lengths of time to give the desired dosages. The tubers, still in the bags, were returned to the storage room and removed as needed. Before treatment the tubers had been carefully selected for uniformity. Dosages of 5, 15, 25, 50, 100, and 200 kilorep were used.

Both carbon dioxide production and oxygen consumption were measured, but in different experiments and on different tubers.

A. Carbon Dioxide Production.—The carbon dioxide production was determined on whole tubers, all of which were used throughout the investigation. On January 11 four lots of Sebago tubers were selected, each lot consisting of 7 or 8 tubers and weighing a total of about one kilo. These potatoes were given the following dosages of gamma irradiation: 0, 5, 15, and 25 kilorep. On January 18 four other lots were given dosages of 0, 50, 100, and 200 kilorep. At intervals, for a period of twenty weeks, the CO_2 production of these potatoes has been determined.

The CO_2 was determined by passing it through a $Ba(OH)_2$ solution, allowing it to react with the latter. The $Ba(OH)_2$ was placed in long Pettenkofer tubes, through which the air from the respiration jars was drawn slowly, allowing the CO_2 to be completely absorbed.

Figure 1 is a schematic picture of the setup. Laboratory air was slowly drawn through the apparatus by the aid of an asperator. The air first entered the

*We are indebted to Dr. John C. Noonan for these potatoes.

soda-lime towers (2), where the CO_2 from the air was removed, then through the cooling coil (4) into bottle (5) containing $Ba(OH)_2$ to test for the presence of CO_2. The CO_2-free air next passed into the respiration jars (6) with the potatoes. The cooling coil and the jars were inside a large refrigerator kept at 45°F to maintain the storage temperature. From the respiration jars the air, now containing CO_2 produced by the tubers, was passed into long Pettenkofer glass tubes (7) and through a narrow glass tube shown in detail. This broke the air stream into small bubbles, which passed through the $Ba(OH)_2$ solution, where the CO_2 was absorbed. To be certain that all the CO_2 had been absorbed a test bottle (8) was placed at the end of each tube. This bottle contained phenolphthalein in a solution of pH 8.5. If all of the CO_2 was not absorbed the color in the detector tube changed.

For the CO_2 determination the tubers were removed from the storage room and placed in the jars in the refrigerator. This was done early in the morning and CO_2-free air was drawn through the apparatus for two hours to allow equilibration to be reached. During this time the Pettenkofer tubes contained distilled water. After equilibrium had been reached two collections, each extending over a period of three hours, were made. The $Ba(OH)_2$ was titrated and calculations made to determine the amount of CO_2 produced. The respiration has been expressed as CO_2 per kilogram fresh potato tubers.

Oxygen Consumption.—The oxygen consumption was determined by the manometric technique in the usual manner. Respiration vessels of approximately 90 ml were used instead of the usual 25-ml vessels, to facilitate the use of a larger amount of plant material. All the determinations were made at 28°C (82°F) because no facilities were available to use the storage temperature of 45°F.

For an experiment three tubers from a lot to be studied were used. Sections 0.8 mm thick and 12 mm in diameter were cut with a microtome from cylinders cut around an "eye." Two such cylinders were cut from each tuber, midway between the stem end and the apex. Seven disks were cut from each cylinder in sequence, the first one containing the surface. Each assay (determination) was made in duplicate and 21 disks were used for each assay. In all experiments the disks were used as soon as cut. During the assay they were immersed in 0.01 M phosphate buffer of pH 6.0. A period of 50 minutes was allowed for equilibration to be reached, and the oxygen consumption then was determined for 3 consecutive periods of 20 minutes each.

Oxygen consumption was determined for both the Sebago and the Pontiac varieties. The Sebagos were selected and irradiated separately from those used for CO_2 production.

III. RESULTS

Figure 2 and Table I give the results for whole tubers. With the exception of the tubers that received 5 kilorep, there was a considerable increase in respiration two days after irradiation. This was followed by a decrease after two or four weeks, and seven weeks after irradiation the rate was low for all dosages. This low point was followed by an increase, which was dependent upon the dosage applied; the tubers receiving a dosage of 5 kilorep increased the least and those receiving the 200 kilorep, the most. The latter continued to show an even higher rate at the next analyses (13 weeks). By the thirteenth week the CO_2 produced by those tubers receiving dosages of 5, 15, and 25 kilorep was less than that of the controls and all except those receiving the 200 kilorep were respiring less than at the preceding analysis and then continued to respire less. In general, the observation was made that after the first rise in respiration the rate of respiration coincided with the dosage given. Those given the lowest dosage respired the least and those having received the highest dosage used (200 kilorep) respired the most. There may be slight individual variations but in general this is true.

A few days after the last CO_2 determination was made the potatoes that had been used for the duration of the experiment were examined externally and internally. Both control lots had a few small sprouts, never over 5 mm long, and they were somewhat wilted, but the flesh was white, with no blemishes. None of the irradiated tubers had any sprouts. The 5-, 15-, and 25-kilorep dosages caused internal browning in one tuber in each lot; otherwise they were as good as the controls. Fifty-kilorep irradiation caused more browning and 100 and 200 kilorep caused still more browning.

The oxygen consumption is given in Fig. 3 and Table II for the Sebago, and in Fig. 4 and Table III for the Pontiac. The day after irradiation there was an increase in oxygen consumption over that in the controls, but by the end of the week these had developed a decrease, except in those that had received the 200-kilorep irradiation. Those receiving the two lowest dosages were actually using less oxygen than the untreated samples. The assay made during the third week indicated a second peak, but from then on there was a decrease in consumption by the tubers having received the lower irradiation doses, and the rate varied little from that of the control, though generally it was a little lower. The tubers having received the higher dosage treatment continued to respire at a rate considerably higher than their control.

Three weeks after irradiation controls began to sprout, but only nonsprouting eyes were used. On the fourteenth week tubers having received 25 kilorep were wilted and showed pits caused by fungus infection. However, there were areas not infected, and these were used. The 50-kilorep treatment was causing considerable wilting. All others used were in good condition. By the eighteenth week all treated tubers showed considerable wilting. The 50-kilorep-treated tubers were the poorest and they showed black areas in the flesh, but these areas were not used. Twenty-two weeks after irradiation the 5- and 15-kilorep-treated tubers were essentially like the unirradiated controls, except that they had no sprouts. All were a little wilted.

The variety Pontiac was studied for only 6 weeks. The results are given in Fig. 4 and Table III. The oxygen consumption on the second day after irradiation shows no relation to the dosage applied, but by the fourth week there was a positive relationship. By the sixth week there was a leveling off, but the tubers having received the 100- and 200- kilorep dosages were still consuming more oxygen than any of the others. As was to be expected there was no sprouting of any of these tubers, and all were in excellent condition.

IV. DISCUSSION AND CONCLUSION

It has been demonstrated that both CO_2 production, with whole potatoes, and oxygen consumption, with slices, increase within a few hours after irradiation. The oxygen consumption decreased during the first week following the initial rise and then increased again, whereas the CO_2 production remained high for several weeks. The remarkable observation made during this study is that after several weeks of a high rate of respiration there was a drop of both CO_2 production and oxygen consumption by those tubers that had received dosages of 5, 15, and 25 kilorep. From the seventh week on these tubers respired at approximately the same rate as the controls. On the other hand, the tubers that had received dosages of 50, 100, and 200 kilorep respired throughout the experiment, at a rate higher than the controls.

The early rise may be associated with a greater utilization of energy, thus using up more ATP and producing more ADP, which could increase the respiration. The drop in oxygen consumption associated with a high CO_2 production may be due to a temporary aerobic fermentation, which has been frequently mentioned in the literature.[1] The continued high rate could be associated with a rise in the sugar content, which lasts for several weeks.[2] When this surplus sugar is used up, those tubers that received the lower dosages could be thought of as going back to their normal rate; no permanent physiological changes had been induced. Those tubers that had received dosages of 50, 100, and 200 kilorep continued to respire at a rate greater than that of the controls. This could be associated with injury as a result of the irradiation. There could be a shift in the path of respiration, whereby phosphorylation might be avoided. Millerd, Bonner, and Biale[3] have suggested that the increased respiration in ripening avocado fruits is due to an uncoupling of the phosphorylation in respiration.

From this study one can conclude that gamma irradiation of potatoes with dosages of 5-15 kilorep are most likely to prove satisfactory. These dosages inhibit sprouting over a storage period of 22 weeks, produce little or no alteration in the physical appearance of the tubers and after an early spurt in respiration settle down to a rate very nearly that of the nonirradiated tubers. Higher dosages caused disturbances as browning, blackening, fungus infection, and an increase in respiration, which continued throughout the investigation.

V. REFERENCES

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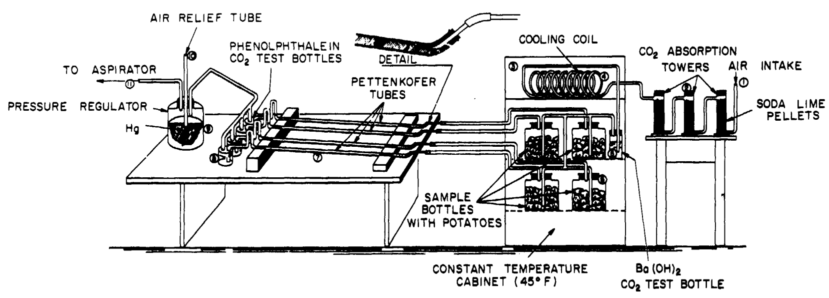

Fig. 1. Schematic view of gas train used in whole-tuber respiration studies.

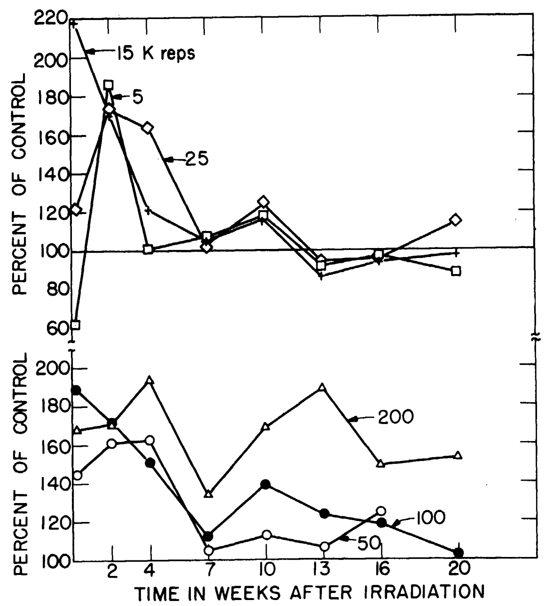

Fig. 2. Carbon dioxide production by whole tubers (Sebago), presented as percent of control tubers. Irradiation dosages given in kilorep.

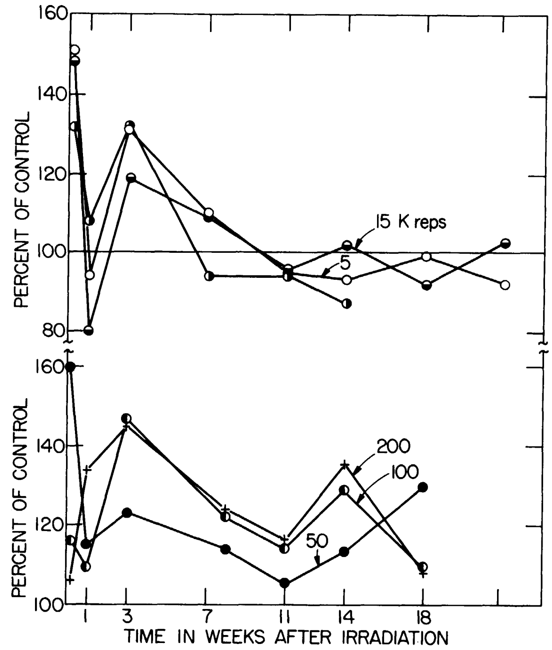

Fig. 3. Oxygen consumption by potato slices (Sebago), presented as percent of the control tubers. Irradiation dosages given in kilorep.

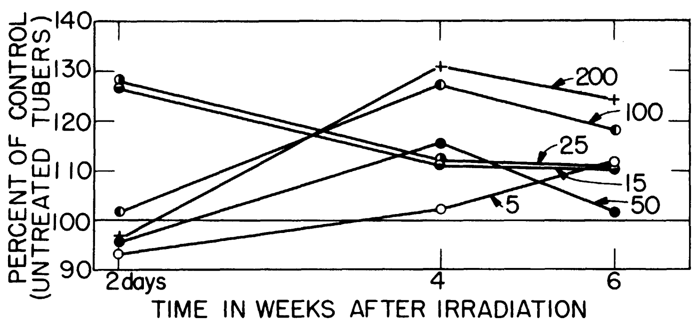

Fig. 4. Oxygen consumption by slices (Pontiac), presented as percent of the control tubers. Irradiation dosages given in kilorep.

TABLE I

CARBON DIOXIDE PRODUCTION BY WHOLE POTATO TUBERS. THE UPPER FIGURE IN EACH SQUARE DENOTES MILLIGRAMS OF CO_2 PRODUCED PER KILOGRAM OF FRESH POTATO PER HOUR, AND THE FIGURES WITHIN PARENTHESES THE PERCENT OF THE CONTROL. DOSAGES ARE GIVEN IN KILOREP.

Dosage \ Time After Irradiation	2 Days	2 Weeks	4 Weeks	7 Weeks	10 Weeks	13 Weeks	16 Weeks	20 Weeks
0 Control	1.40	1.35	1.94	1.48	1.22	1.97	2.06	1.79
5	0.87 (62%)	2.49 (184%)	1.96 (101%)	1.57 (106%)	1.45 (119%)	1.80 (91%)	2.00 (97%)	1.59 (89%)
15	3.06 (219%)	2.30 (170%)	2.34 (121%)	1.56 (105%)	1.42 (116%)	1.69 (86%)	1.94 (94%)	1.75 (98%)
25	1.70 (121%)	2.32 (172%)	2.79 (164%)	1.55 (105%)	1.53 (125%)	1.85 (94%)	1.95 (95%)	2.06 (115%)
0 Control	2.35	1.84	1.34	1.82	1.94	1.56	1.87	2.05
50	3.41 (145%)	2.96 (161%)	2.17 (162%)	1.91 (105%)	2.19 (113%)	1.47 (106%)	2.32 (124%)	1.24 (60%)
100	4.44 (189%)	3.17 (172%)	2.02 (151%)	2.04 (112%)	2.79 (144%)	1.92 (123%)	2.20 (118%)	2.08 (102%)
200	3.95 (168%)	3.15 (171%)	2.59 (193%)	2.44 (134%)	3.24 (168%)	2.97 (190%)	2.80 (150%)	3.14 (153%)

TABLE II

OXYGEN CONSUMPTION BY SLICES OF POTATO TUBERS. THE UPPER FIGURE IN EACH SQUARE DENOTES MICROLITERS OF OXYGEN CONSUMED PER MILLIGRAM OF DRY WEIGHT PER HOUR, AND THE FIGURES IN PARENTHESES THE PERCENT OF CONTROL. DOSAGES ARE GIVEN IN KILOREP.

Dosage \ Time After Irradiation	27 Hours	1 Week	3 Weeks	7 Weeks	8 Weeks	11 Weeks	14 Weeks	18 Weeks	22 Weeks
0 Control	.730	.60	.651	.64		.71	.725	.710	.605
5	1.10 (151%)	.56 (93%)	.851 (131%)	.705 (110%)		.675 (95%)	.675 (93%)	.705 (99%)	.555 (92%)
15	1.08 (148%)	.48 (80%)	.778 (119%)	.70 (109%)		.68 (96%)	.74 (102%)	.66 (93%)	.62 (102%)
25	.993 (132%)	.65 (108%)	.858 (132%)	.60 (94%)		.665 (94%)	.625 (86%)		
0 Control	.752	.73	.558		.705	.755	.660	.745	
50	1.204 (160%)	.84 (115%)	.689 (124%)		.805 (114%)	.795 (105%)	.75 (114%)	.965 (130%)	
100	1.124 (116%)	.80 (110%)	.821 (147%)		.86 (122%)	.860 (114%)	.85 (129%)	.815 (109%)	
200	1.029 (106%)	.98 (134%)	.813 (146%)		.875 (124%)	.880 (117%)	.895 (136%)	.80 (107%)	

TABLE III

OXYGEN CONSUMPTION BY SLICES OF PONTIAC POTATO TUBERS. THE UPPER FIGURES IN EACH SQUARE DENOTE MICROLITERS OF OXYGEN CONSUMED PER MILLIGRAM OF DRY MATERIAL PER HOUR, AND THE FIGURES IN PARENTHESES THE PERCENT OF CONTROL. DOSAGES ARE GIVEN IN KILOREP.

Dosage \ Time after Irradiation	2 Days	4 Weeks	6 Weeks
0 Control	0.865	0.840	0.660
5	0.805 (93%)	0.860 (102%)	0.737 (112%)
15	1.095 (127%)	0.930 (111%)	0.728 (110%)
25	1.110 (128%)	0.940 (112%)	0.733 (111%)
0 Control	0.930	0.865	0.781
50	0.885 (95%)	1.000 (116%)	0.791 (101%)
100	0.945 (102%)	1.100 (127%)	0.918 (118%)
200	0.890 (96%)	1.130 (131%)	0.968 (124%)