A STUDY OF THE EFFECTS OF X-RAY AND RADIOACTIVE PHOSPHORUS ON POTATOES

by

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ABSTRACT

A project to study the possibilities of inducing mutations in potatoes with X-ray and radiophosphorus was begun in 1954. Three varieties, Pontiac, Manota and Netted Gem were chosen. X-ray dosages ranging from 500 r (roentgen) to 7500 r were applied to potato tubers immediately before sprouting. The heavy isotope, P^{32}, was applied to potatoes in the following ways:

1. tuber injection: the dosages ranged from 5 uC (microcurie) to 50 uC per eyepiece.
2. root absorption: the dosages ranged from 1 uC to 50 uC per young sprout.
3. stem injection: the dosage was 50 uC per plant.
4. seriate treatment: above treatments given in successive stages.

Fifteen uC of P^{32} resulted in the same degree of sprout inhibition as 2000 r of X-rays. Several abnormal types were found after P^{32} treatment. Evidence was obtained to indicate that there was an auxin disturbance caused by P^{32}. Two smooth-skinned tubers were found from the russet-skinned variety, Netted Gem. One has proved stable while the other is still under test. Several other variants were found which are being tested to determine whether they are stable.

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INTRODUCTION

The potato is one of the most important food crop plants and is widely cultivated throughout the world. Selection and increase of mutations as a means of producing new varieties has been practiced for many years. The potato is an autotetraploid and highly heterozygous. Crossing and selfing commonly result in a very wide and elusive variation in seedlings and a severe breakdown in the gene balance and the overall performance present in the parental varieties. Mutations, however, furnish new variations without changing the original germ plasm to any great extent since usually only one gene changes at a time. The potato is propagated vegetatively and thus advantageous mutations can be utilized directly.

The main purpose of this investigation was to study methods and determine suitable dosage levels for applying mutagenic agents to potatoes. The work was limited to a study of X-ray and phosphorus 32. (See notes below for explanations of these terms).

X-ray treatment has been used as a mutagen since 1927 and its mutagenic ability has been proven by many workers. Phosphorus 32 (hereafter designated P32) was introduced into this field only after the Second World War. P32 when introduced into the plant should furnish a good source of internal prolonged irradiation as phosphorus is an important constituent of chromosomes.

The investigations were carried out during 1954 and 1955 using three varieties of potatoes, several methods of application, and a range of dosage levels. The effects of treatments on plant stand, vigour, physiological disturbances and mutations were observed and are recorded.

Note I. X-rays are similar to light, but the wave-length of X-rays are very small, being in the neighborhood of 1 A (1 A = 10^{-8} cm), whereas the wave-lengths of visible light are in the neighborhood of 5000 A (4000 A to 8000 A). When the atoms of an element are bombarded by very rapidly moving electrons, as in an X-ray tube, these atoms emit X-rays.

Note II. Phosphorus 32 ($_{15}P^{32}$) has one more unstable neutron than ordinary phosphorus ($_{15}P^{31}$). It emits one electron and is converted into sulfur ($_{16}S^{32}$). Phosphorus 32 has a half life of 14.3 days.

LITERATURE REVIEW

The work of Stadler (16, 17, 18, 19) on induced mutations of barley is fundamental to plant breeding. Yet Stadler himself took an almost negative position in regard to their practical value. He said "There is little chance of producing experimentally, variations which have not already occurred in nature". He is, however, more optimistic concerning their value in the breeding of vegetatively propagated crops.

Mutation studies with vegetatively propagated plants have been under way in Sweden (9, 10) since 1944. Scions of apples and pears were irradiated with X-rays and then grafted to trees. Six mutants were found, which included changes in fruit size, shape, color and time of ripening.

Ehrenberg *et al*. (5) studied the seedling lethality and chromosome disturbance caused by P^{32} in wheat, barley and onion. On the basis of their results they postulate that P^{32} must have a very high mutagenic effect. They found many cytological disturbances after treatment with 5 - 10 uC (microcurie*) of P^{32} per seed. These effects corresponded to the effects of 25,000 r** of X-rays.

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* A curie is a unit of radioactivity. One curie of any radioactive substance is an amount of the substance such that 3.7×10^{10} atoms of the substance undergo radioactive disintegration per second. One curie of $P^{32} = 3.5 \times 10^{-6}$ g, 1 uC = 10^{-6} curie.

** A roentgen (r) is roughly that quantity of X-rays that will produce 2 million ion pairs in a cc of dry air at standard pressure and temperature. The energy absorbed by plant tissue from one roentgen equals 90 ergs per gram of plant tissue (7, 13).

The mutagenic effects of P^{32} have been demonstrated by Giles and Lederberg (8) in Neurospora and by Bateman and Sinclair (3) in Drosophila.

In 1950 Thompson et al. (22) treated barley seeds with P^{32} and examined the X_2 population. They reported that P^{32} not only produced chlorophyll deficient mutants, but also mutants of types which had occurred only rarely with X-ray treatments.

In 1954 Shebeski and Lawrence (15) treated barley seeds and plants with different sources of radiation and counted the mutants in the X_2 generation. A large number of chlorophyll deficient mutants, which were non-vital, were found. A group of vital mutants which could be propagated were also found. They reported that P^{32} produced more "vital mutants" than other sources. Thirty per cent of the mutants were vital with the P^{32} treatments, while only 8% were vital with X-ray treatment, and 6% with radium beryllium treatments.

Arnason et al. (1, 2), placed dormant kernels of hexaploid wheat, one per test tube in 0.1 cc of Knop's solution with either 0.18 uC or 0.018 uC of P^{32} for thirteen days. Both concentrations were effective in causing chromosome breakage and rearrangements.

Spinks et al. (21), treated hexaploid wheat with P^{32} by sprouting the seeds in water for 48 hours, then placing them (one per container) in test tubes, with either 0.18 or 1.8 uC in 0.1 cc of solution for 2 - 4 days. Measurement showed that the plant absorbed 1.4 uC of the P^{32} in the case of the high dosage (1.8 uC), which proved lethal. Similar tests with other genera showed that 1.8 uC of P^{32} consistently killed or severely inhibited seedlings.

In 1955 Osborne and Elliot (14), counted the chromosome translocations induced in a Triticum x Agropyron hybrid by X-ray, P^{32} and S^{35} (sulphur 35). They reported that the number of translocations per cell induced by P32 was not significantly higher than that occurring spontaneously in the control.

Ehrenberg and Granhall (6) treated fruit trees with beta radiating isotopes (P^{32} and S^{35}). P^{32} produced no mutations but produced abnormalities (shoot bifurcation and leaf deformation) similar to that resulting from X-ray or neutron treatment.

In 1951 Stanton and Sinclair (20) studied the effect of P^{32} on the growth of potatoes. They placed the P^{32} in sand in which the potatoes were growing. Dosages of 10 uC and 100 uC per plant were administered. Twenty per cent of the applied P^{32} was taken by the plants. They found that P^{32} was concentrated in the growing point. The concentration of P^{32} in the growing point (meristematic tissue) was approximately six times greater than the average concentration of P^{32} in the whole plant.

In 1951 Howard (12) placed germinated broad bean (*Vicia faba*) seeds into P^{32} for 20 hours. Then by using the autoradiograph technique, he reported a heavy uptake of P^{32} in the meristematic tissue. Autoradiographs showed P^{32} in 92% of the cells which were in division, (in mitosis), in 80% of the meristematic cells not in division, in 61% of the round resting cells and in 13% of elongated resting cells.

In 1954 Hagberg and Nybom (11) reported on a comparative study of P^{32} and X-ray with potatoes. Two mutants were found as a result of X-ray treatment, but none with P^{32}. They concluded that such results indicated the possibilities of inducing hereditary changes by X-ray treatment.

MATERIALS AND METHODS

The studies were carried out during 1954 and 1955 using X-rays and P32 as sources of radiation. Three varieties of potatoes were used, viz, Pontiac, Manota and Netted Gem.

X-ray Treatments

Potato tubers of the three varieties were given X-ray treatment. The unfiltered radiation from a coolidge tube, run at 250 kilo volts and 15 milli ampere was used throughout the treatments. The dosage rate in air was 580 r units per minute. Five dosage levels of irradiation, 500 r, 1000 r, 2000 r, 5000 r, and 7500 r were applied separately to ten tubers of each of the three varieties. Each tuber was cut into three seed pieces; two of which were treated as described, with the third one kept as a non-treated check. The weight of the pieces ranged from 35 - 75 g for Pontiac, 20 - 60 g for Manota and 30 - 65 g for Netted Gem. In this way twenty pieces of each variety were exposed to each of the above X-ray dosages. At the time of treatment the new sprouts from the buds were commencing to show growth. Shortly after the treatments, both the treated pieces and the checks were planted in the test plot field at the University of Manitoba. One piece was planted per hill with a distance of 15 inches between plants and three feet between rows. The three pieces of each tuber were planted in succession in the row - two treated pieces followed by the check. After emergence, plant performance and growth were recorded.

Phosphorus 32 Treatments

The phosphorus isotope, phosphorus 32, in the form of sodium dihydrogen phosphate was used in all radiophosphorus treatments. A number of methods of application were carried out.

1. Tuber Injection

P^{32} was injected into tubers with a hypodermic needle of 1.5 mm diameter. By means of the needle a V-shaped tunnel was made into the tuber directly under a bud (Fig. 1) or a perpendicular hole was bored beside an eye (Fig. 2). One hour or more was permitted to elapse before the injection of the P^{32} solution to allow time for drying of free sap from the ruptured tissue in the needle hole. This was found necessary in order to have sufficient space in the openings for the P^{32} solution. Three dosage levels were used, i.e. 5 uC, 15 uC and 50 uC. All administered in 0.05 cc of injection solution (Plate 1). When no solution remained in the injection cavities the treated eyes were removed from the tubers by means of an eye baller. The average weight of eye pieces was about 11 g and they measured 27 mm wide and 20 mm thick.

Fig. 1

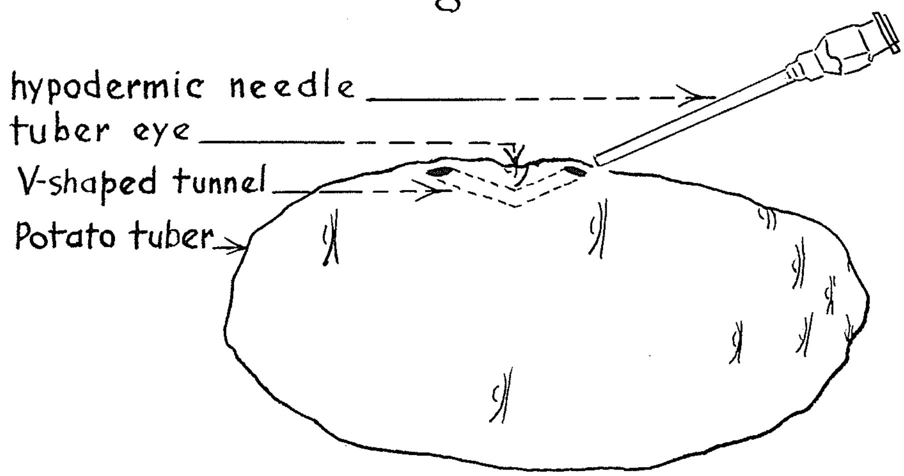
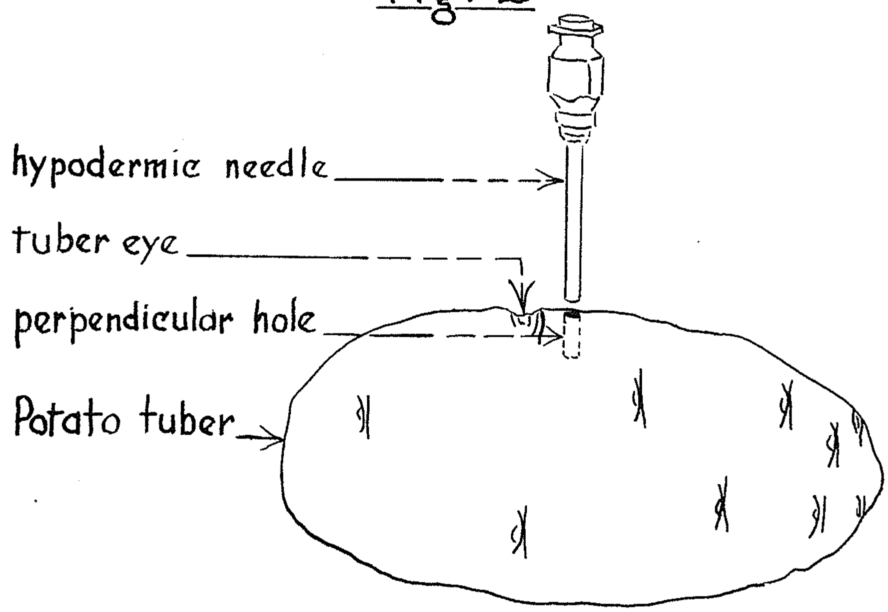

hypodermic needle
tuber eye
V-shaped tunnel
Potato tuber

Fig. 2


hypodermic needle
tuber eye
perpendicular hole
Potato tuber

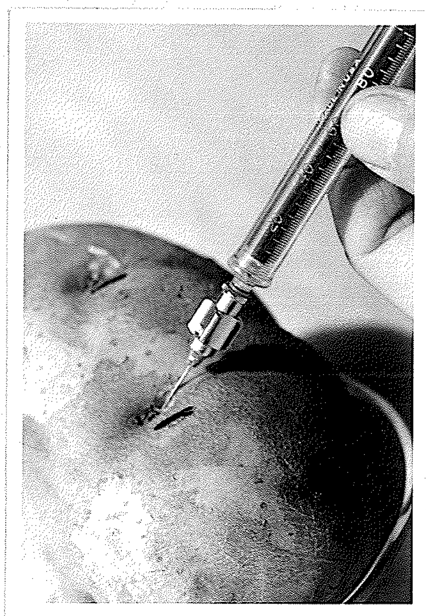


Plate 1. Injecting phosphorus 32 into tuber with a hypodermic needle

The same three varieties of potatoes were used as with the X-ray treatment. Five buds from each tuber were selected, four of them were treated while the fifth one was kept as a non-treated check. Ten tubers of each variety were treated with each of the above mentioned three dosages. Five tubers were treated by means of the V-shaped tunnel, the other five by means of the perpendicular hole beside the eyes. Altogether 90 tubers or 360 eye pieces were treated with P^{32} and 90 eye pieces (one from each tuber) were kept as checks. Eighty per cent of them were planted in succession in the field, four treated pieces followed by the check. The remainder were planted in pots in the greenhouse.

2. Treatment of Root Sprouts

Young rooted potato sprouts of the three varieties were treated with P^{32} in aqueous solution. Young sprouts under 5 cm in height and weighing approximately 2 g were selected. One hundred and forty sprouts of each variety were used. Ten sprouts were placed together in one petri dish thus fourteen petri dishes were required for each variety. Seven of them contained 8 g of peat moss and 75 cc of P^{32} solution. The other seven petri dishes contained the same amount of solution but were without peat moss. In the latter case, heavy paper with ten small round holes was used to hold the sprouts in position. Seven different strengths of P^{32}

solution were used, i.e. a water check, 10 uC, 20 uC, 50 uC, 100 uC, 200 uC, 500 uC per petri dish for each ten sprouts. As soon as the solution was completely absorbed by the sprouts, they were planted individually into pots using a prepared potting soil in the greenhouse. After two months they were transplanted to the field.

3. Stem Injections

Injections into the stems of growing plants were made in the following manner. The potato plants which were to be treated were not watered for one day previous to treatment. A leaf was cut at a point about 1 cm from the base of the petiole. Usually the largest leaf of the plant was chosen, since the stouter petiole made the injection easier. A longitudinal core was removed from the central portion of the petiole reaching into the pith of the stem with a hypodermic needle of 1.5 mm in diameter. Fifty uC of P^{32} solution in a volume of 0.05 cc was used for each injection. Four plants of the Pontiac variety were treated. The plants were in pots and they were approximately two months old and 25 cm tall. The plants were examined for radioactivity with a portable beta-particle counting geiger counter.

4. Seriate Treatments

Treatments which combined the three methods of administering P^{32} as described above were made in an additional series. These were begun in the winter of 1954. Fifty eyes from Pontiac tubers were selected for treatment. Just prior to active sprouting of the buds, a V-shaped tunnel was made under each bud as described under tuber injection treatments above. Each of forty eyes was treated with 20 uC of P^{32} in 0.05 cc of solution. The remaining ten (Group I) were injected with 0.05 cc of distilled water. This was done on December 14th. On January 18th, 1955 the young sprouts from treated buds were removed from the tubers. Each of thirty of the forty sprouts from treated buds was placed in a vial containing 50 uC of P^{32} in 3 cc of solution. Each of the remaining 20 (Groups I and II) was placed in 3 cc of distilled water in a separate vial to serve as checks. On February 24th, each of 20 plants from the thirty sprouts which had received the double treatments with P^{32} was injected with 50 uC of P^{32} in 0.05 cc solution. The technique used was as described above in section (3) dealing with plant injections. Each of the remaining thirty (Groups I, II, and III) was treated with 0.05 cc of distilled water. On March 25th, 10 of the 20 plants which had received injections were each reinjected with 100 uC of P^{32} in 0.05 cc of solution. The remaining 40 plants (Groups I, II, III, and IV) were treated with the same volume of distilled water. The following table summarizes these treatments.

TABLE I Summary Table of Seriate P^{32} Treatment with Pontiac Potatoes

Group	No. of plants or eye pieces	Date and Methods of Treatment			
		Dec. 14th	Jan. 18th	Feb. 24th	Mar. 25th
		Injection into the tuber	Immersing the young sprouts	Injection into the stems	Injection into the stems
I	10	water	water	water	water
II	10	20 uC	water	water	water
III	10	20 uC	50 uC	water	water
IV	10	20 uC	50 uC	50 uC	water
V	10	20 uC	50 uC	50 uC	100 uC

The distribution of P^{32} was checked by first thoroughly drying the plant part and then placing it on a sheet of X-ray film in the dark room. Drying between blotting papers in an oven at 60° C for twenty four hours was usually sufficient. The time of exposure to the X-ray film varied from several hours to two weeks depending on the amount of P^{32} present in the tissue.

Cuttings were taken from those plants which showed abnormal growth. They were planted in pots and placed near the mother plants.

All the treated plants were observed until the second clonal generation in order to check both injurious effects and possible heritable changes.

RESULTS AND DISCUSSION

X-ray Treatment

The plants from tubers treated with X-rays, when compared to the controls, showed retarded development. The degree of injury or retardation was directly proportional to the dosage level of X-rays applied. Tubers given a dosage below 100 r produced as many plants as the checks; with a dosage of 2000 r, only 80% of the tubers emerged; with a dosage of 5000 r only 15% emerged. No plant was produced from the tubers which had received the 7500 r treatment. The details covering plant emergence are presented in Table II. The plants from treated tubers were never as vigorous as the check plants but growth appeared normal. The yields were very low at the higher dosages, and several plants failed to set any tubers. The three varieties were about equal in their tolerance to the treatments although Netted Gem produced a few more plants at the higher dosage levels than did Pontiac and Manota. It is possible that Netted Gem, which breaks dormancy more slowly than the other two varieties, had less bud activity at the time of treatment and thus suffered less injury.

TABLE II Emergence of Potato Plants as Affected by Different Dosages of X-rays

Varieties	X-ray dosages	No. of seed pieces treated	No. of plants emerged
Pontiac	None	50	50
Manota	None	50	49
Netted Gem	None	50	50
Pontiac	500 r	20	20
Manota	500 r	20	20
Netted Gem	500 r	20	20
Pontiac	1000 r	20	20
Manota	1000 r	20	20
Netted Gem	1000 r	20	20
Pontiac	2000 r	20	16
Manota	2000 r	20	13
Netted Gem	2000 r	20	19
Pontiac	5000 r	20	2
Manota	5000 r	20	2
Netted Gem	5000 r	20	5
Pontiac	7500 r	20	0
Manota	7500 r	20	0
Netted Gem	7500 r	20	0

Phosphorus 32 Treatments

1. Tuber Injections

The injurious effects of P^{32} tuber injections on the development of the potato plants were similar to those of the X-ray treatments. The rate of development was inversely related to the dosage. The percentage of emergence decreased with increased dosage. Ninety nine per cent of the checks, which received no P^{32} emerged whereas the treated tubers receiving the highest dosage (50 uC per eye piece) emerged only 10.5%. The details of plant emergence are given in Table III.

Of interest is the fact that three Pontiac plants which had been treated with 50 uC of P^{32} per eye-piece did not emerge until the middle of September. (These three plants were not included in the emergence data given in Table III). Upon examination it was found that although the treated eye-pieces had been in the soil for a four month period, they were still in an apparently sound condition. A similar result was found with the non-emerged seed-pieces in the X-ray treated plots. Irradiation is being actively investigated at the present time as a possible means of lengthening the fresh storage period of potato tubers for food.

Nine plants were found to have "blind shoots" (20) or lack terminal growing apices. These are described in detail later (see page 18).

TABLE III Effect of P^{32} Solutions Injected into Potato Seed Tubers on Subsequent Emergence of Plants

Amount of P^{32} per eye-piece	Varieties	No. of eye-pieces A*	No. of eye-pieces B*	No. of emerged plants A*	No. of emerged plants B*
None	Pontiac	12	12	12	12
None	Manota	12	12	12	11
None	Netted Gem	12	12	12	12
5 uC	Pontiac	16	16	16	16
5 uC	Manota	16	16	16	15
5 uC	Netted Gem	16	16	16	14
15 uC	Pontiac	16	16	12	16
15 uC	Manota	16	16	14	15
15 uC	Netted Gem	16	16	16	15
50 uC	Pontiac	16	16	0	0
50 uC	Manota	16	16	2	2
50 uC	Netted Gem	16	16	3	3

A* Injected in V-shaped bore opening.
B* Injected in shallow perpendicular bore opening.

There was no difference between the two methods of injecting P^{32} into the tubers and little difference between varieties in reaction to P^{32} effects.

On fifth of the treated tubers were planted in pots in the greenhouse. Due to the suitable environment for growth in the greenhouse these tubers emerged much earlier than those of similar treatments planted in the field. A large number of the plants showed abnormal growth which was expressed in several morphological changes. A very common type was the development of "blind shoots". When the plants reached the 4 - 5 leaf stage, the stems were extremely short, and the stem apex or growing point ceased to make further new growth for a while, then recovered (see Plate 2). In other plants, the leaves were abnormally thick and succulent, and lacked secondary leaflets.

Table IV shows plant emergence and the number of abnormal plants resulting from the tuber treatments when the plants were grown in the greenhouse.

TABLE IV Plant Emergence and Number of Abnormal Plants Resulting from Different Dosages of Treatment, (Greenhouse Study)

Dosage of P^{32} per eye-piece	No.of treated eye-pieces	No.of plants emerged	No.of abnormal plants
0 uC	18	18	0
5 uC	24	23	16
15 uC	24	24	21
50 uC	24	7*	

* One died within a week; two remained very small (about 1 cm high) until September; growth of the remaining four plants was slow but normal.

The treated plants emerged at the same time as the checks. Several plants, which had been treated with 5 uC of P^{32} made faster growth at first than the checks. This might indicate that small amounts of P^{32} stimulate growth (see Plate 4, 5, 6).

In one experiment reported by Bould et al. (4) barley plants showed significant increases in dry weights of "tops" and "roots and tops" combined with certain levels of P32 treatment.

Table V shows the effect of different dosages of P32 on the size of the plants, four weeks after treatment.

Table V Effect of Dosage Levels of P32 Treatment on Size of Potato Plants Four Weeks After Treatment

Dosage	No. of treated eye-pieces	No. of plants emerged	Size of Plants*			
			Large	Medium	Small	Very small
0 uC	18	18	10	8		
5 uC	24	23	19	2	1	1
15 uC	24	24	2	14	2	6
50 uC	24	7			2	4

* Large - Spread of foliage over 12.5 cm
Medium - Spread of foliage 5.0 - 12.5 cm
Small - Spread of foliage 2.5 - 5.0 cm
Very small - Spread of foliage less than 2.5 cm

These results differ from those obtained in the field. The percentage of abnormal plants was much higher in the greenhouse than in the field. The time of emergence of the treated plants in comparison with the checks was the same regardless of treatment in the greenhouse. In the field the treated tubers emerged later than the checks, treatments of 5 uC, 15 uC and 50 uC, emerging about 2, 7 and 16 days respectively, later than the checks. The foliar and stem of the treated plants was reduced in proportion to dosage.

Plate 2. Blind shoot due to failure of the growing point

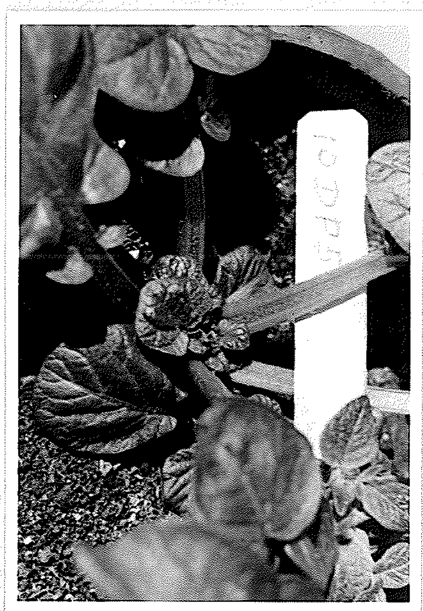


Plate 3. Later stage of blind shoot showing recovery

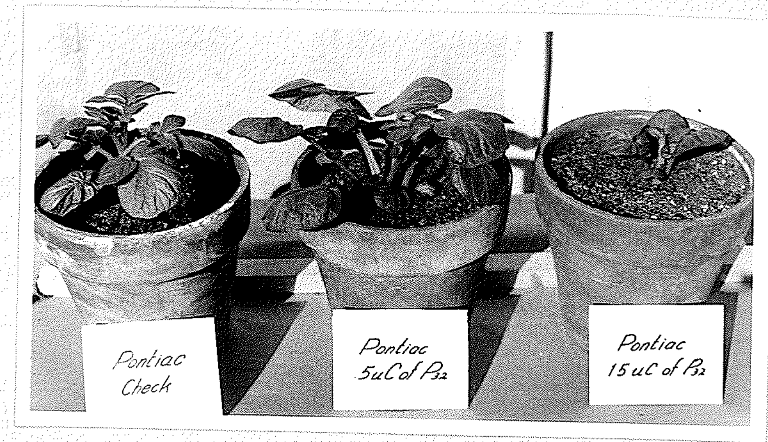


Plate 4. The visible dosage effect of phosphorus 32 on the variety Pontiac

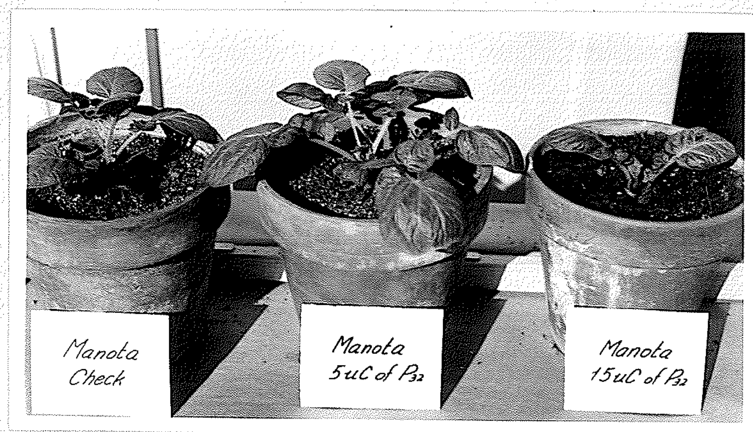


Plate 5. The visible dosage effect of phosphorus 32 on the variety Manota

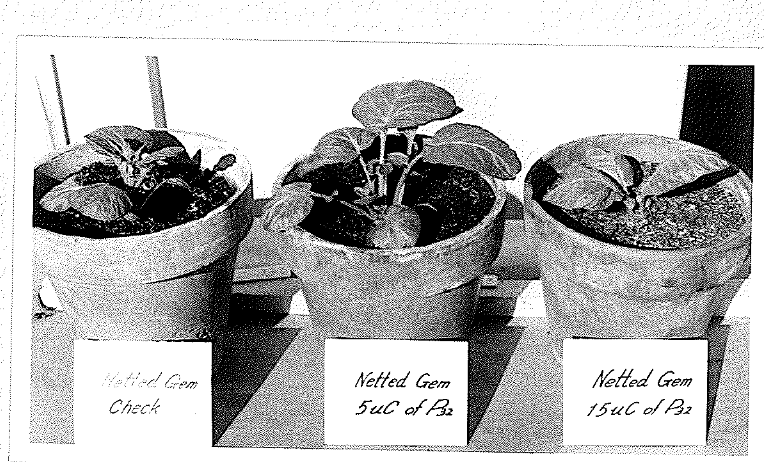


Plate 6. The visible dosage effect of phosphorus 32 on the variety Netted Gem

In the greenhouse population the small dosage did not reduce the plant size, but on the contrary, stimulated growth. This difference may have been due to environment. The plants emerged about three weeks earlier in the greenhouse than in the field. Thus they may have sprouted before P^{32} radiation seriously affected the growth process. The quick development of the plant after sprouting would result in a rapid dilution of the P^{32} thus reducing its effects.

2. Root Absorption Treatments

The rooted sprouts which had been treated with P^{32} solution on June 16th were planted in the greenhouse in pots and on August 5th transplanted to the field. The plants were normal at first but after one month in the greenhouse the young leaves of several treated plants were more narrow than leaves of the check plants. In the field several abnormal types appeared. One Pontiac plant which had been treated with 50 uC of P^{32} in water solution grew normally to a height of about 20 cm then produced eight blind branches (see Plate 8). In some leaves both primary and secondary leaflets were absent or fused together forming a long ovate simple leaf (see Plate 9). Only four abnormal plants developed as a result of 50 uC of P^{32} solution in peat, whereas 15 abnormal plants resulted from treatment with 20 uC and with 50 uC of P^{32} solution in water. This

Plate 7. Leaf shape of plant treated with 50 uC of phosphorus 32

Plate 8. The blind shoot of a plant treated with 50 uC of phosphorus 32

Plate 9. Abnormal leaf shape due to treatment with 50 uC of phosphorus 32

tends to indicate that soaking the sprouts in P^{32} solution without peat was more efficient than when peat was used.

3. Stem Injection

Four Pontiac plants which had been injected with 50 uC of P^{32} per plant were investigated for the distribution of P^{32} by means of a geiger counter. One plant was cut into pieces of less than 0.1 g weight two days after treatment. The radioactivity of each small piece was measured by the geiger counter in order to determine whether the P^{32} was located at the point of injection or whether it had been distributed to the different parts of the plant. Another plant was tested in the same manner one week after treatment. The third and the fourth plants were tested after two weeks and four weeks respectively. The geiger counter indicated that P^{32} was distributed into every part of the treated plants and also appeared in the tubers developed after injection. Autoradiographs also showed the presence of P^{32} in all plant parts. Plate 10 shows distribution by means of radioautograph in leaves of stem injected plants.

4. Seriate Radiophosphorus Treatments

The plants from the treatments which combined in series the three methods of application, namely, tuber injection, root absorption and plant injection were grown in the greenhouse. After the first treatment, i.e. tuber injection, about 80% of the plants showed "blind shoot" abnormalities. The abnormality disappeared with further

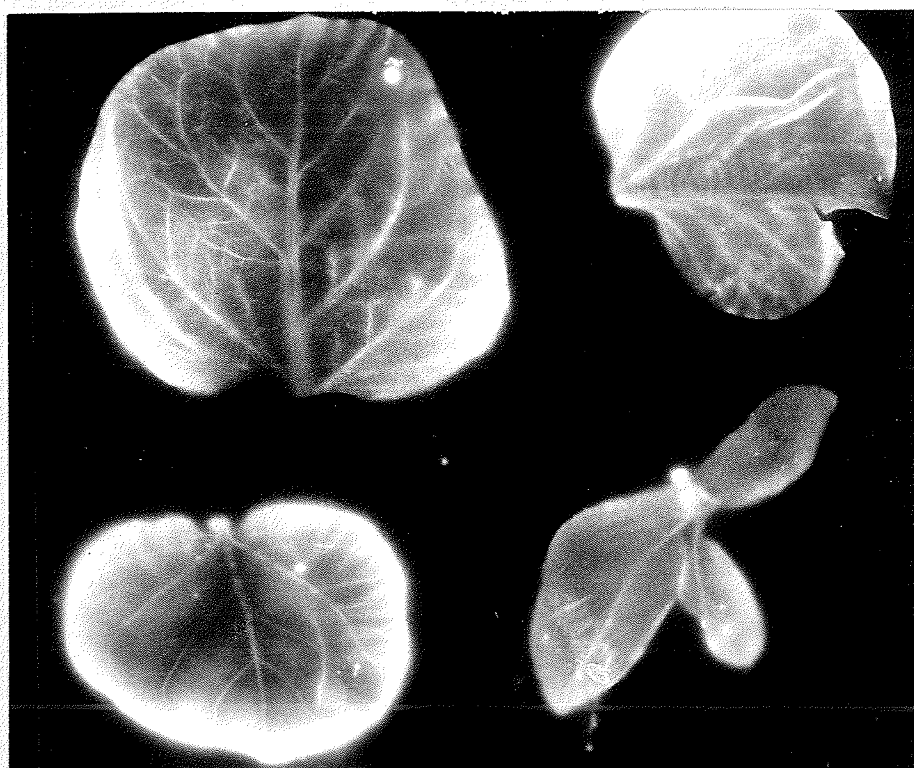


Plate 10. Radioautograph of treated leaves

growth. At the time after treatment about 55% of the treated plants started to show a new type of abnormality which was not found before. Most of them bore aerial stolons at the nodes above ground level, some of them bore aerial tubers; some had both. The development of abnormal stolons was progressive upward from the base of the plant. Some of the stolons grew to a length of more than 25 cm and branched several times. Some remained short, occasionally bearing tubers terminally. Growth of these aerial stolons was more conspicuous with the high dosage treatments than at low dosage (see Plates 11, 12).

Auxin was applied to test whether this abnormality was due to auxin disturbance. Two cc of 0.5 mg/L indole acetic acid was placed in a test tube hanging near the stem. One end of a small piece of cloth was dipped into the solution of indole acetic acid, while the other end was wrapped around a potato stem directly above a node from which abnormal stolons had developed. The solution was transported to the surface of the plant with the cloth acting as a wick. Two plants were treated with auxin, while two others were treated with water as checks. After one week no new abnormal stolons were formed above the region of treatment on auxin treated plants. Abnormal stolon growth continued in one of the water treated plants. The results here, although not definite, indicate the possibility of auxin disturbance being the cause of the abnormal stolon growth.

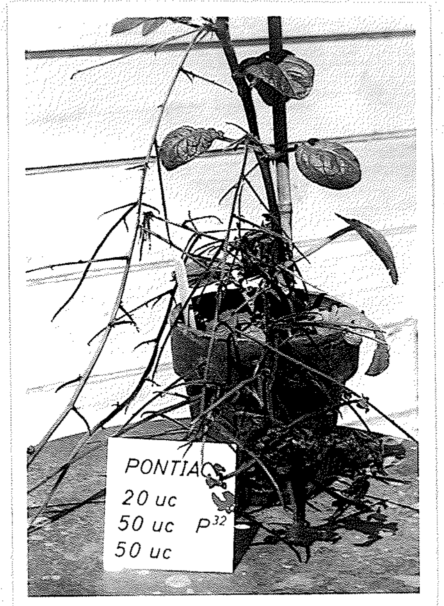


Plate 11. Aerial stolons due to seriate treatment

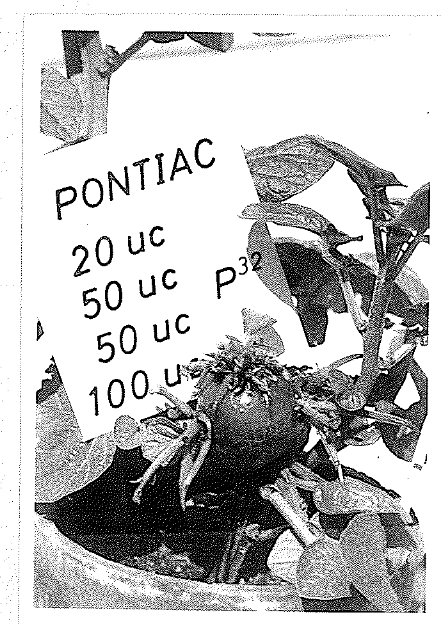


Plate 12. Aerial tuber due to seriate treatment

A similar series of combined treatments were made in the summer of 1955. The treated plants produced only "blind shoot" abnormalities and aerial tubers. No aerial stolons were produced. The production of aerial stolons in the greenhouse in 1954-1955 may have been due to an interaction effect of the P^{32} and the particular greenhouse environment which prevailed during the winter and spring of 1954-1955.

All cuttings from the abnormal growth plants grew normally and set tubers normally. This indicates that the abnormalities were due to temporary disturbance caused by the radiation rather than heritable changes.

Possible Mutations

Tubers produced by the plants resulting from the three treatment methods, (X-ray, P^{32} tuber injection and P^{32} root absorption) were harvested at the end of September 1954. Tubers were examined carefully for possible changes. From one Netted Gem plant which had been treated with 15 uC of P^{32} through tuber injection three tubers were obtained, one of which was russet like the clonal ancestor and two of which were smooth skinned, entirely free of russetting. These three tubers were planted in the greenhouse in separate pots. The russet one reproduced russet tubers. One of the smooth skinned tubers set smooth skinned tubers, while the other set russet skinned tubers. The one producing smooth skinned tubers might prove to be a stable variate (see Plate 14).

The tubers which had been harvested in 1954 from plots planted with treated seed pieces were stored and planted in 1955 to check further on possible mutations. These second clonal generation plants were carefully dug and examined at harvest time. Among the X-ray treated material, one smooth skinned hill of Netted Gem was obtained which resembled the one referred to above. This one resulted from the tubers treated with 500 r of X-ray. The stability of this variate has not yet been checked. Two round tubers were also found in a hill of Netted Gem, normally an elongated variety.

Plate 13. Netted Gem: russet skinned tuber and the smooth-skinned tuber variant resulting from treatment with 15 uC of phosphorus 32

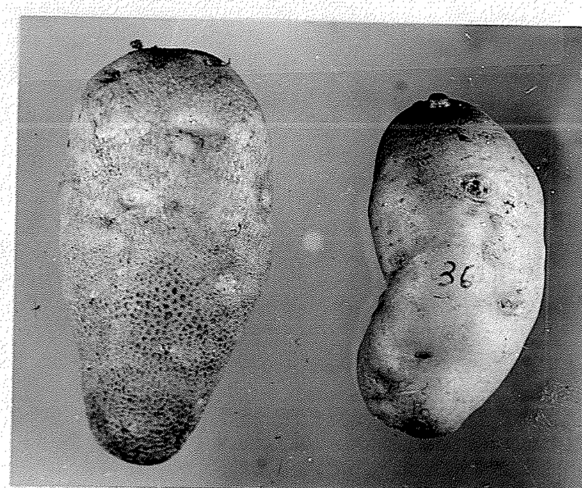


Plate 14. Netted Gem: russet-skinned tuber and the smooth-skinned tuber variant resulting from X-ray treatment

A Comparison of Radiophosphorus and X-rays

X-ray has the power of separating electrons from the atoms while P^{32} emits an electron. They both act through electrons and might be expected to have somewhat similar effects. Radiation from X-rays was administered to the potato tuber in this experiment over a short period of time from a powerful source (580 r/minute in the present experiment) while that from P^{32} whose half life is 14.3 days, represented a long exposure to a weak source. If 1 uC of P^{32} disintegrates in 1 g of plant tissue the maximum energy the plant tissue absorbs is equivalent to 0.18 r per minute (22). Both P^{32} and X-ray treatments retarded potato growth. However, the retardation effect became apparent earlier following X-ray treatments than following P^{32} treatments.

The weak and prolonged internal radiation of P32 on potatoes resulted in several striking morphological changes, while the X-ray treatments did not. The most common effect was development of "blind shoots" or the destruction of growing apices. The explanation might be that high P32 concentration in the meristematic tissue (20, 12) killed the young cells or suppressed the development completely in this region. The lack of development of the secondary leaflets might be a result of the same effect. Another kind of abnormality was the narrower leaves which resembled the auxin disturbances that result from 2, 4 dichlorophenoxy acetic acid treatment injury. The study suggests that the development of aerial stolons may be an auxin disturbance. The possible relationship between auxin and P^{32} effect is interesting but was not investigated in this study.

A comparison of X-ray and P^{32} treatment in relation to plant tolerance was attempted. Ehrenberg (6) pointed out that dosage calculation for P^{32} radiation is very complicated and based on the function of several factors, viz.: P^{32} uptake, distribution, decay, and growth of the tissue, causing a dilution of the radioactive substance. In the present experiment the lethal effect of 15 uC per eye-piece of about 11 g, given with the tuber injection method, corresponded to the effect of 2000 r of X-rays. Assuming that there was no loss of P^{32} through injection and that there was little growth during the first two weeks after treatment, and that P^{32} was distributed homogeneously throughout the tissue of the treated tuber, the maximum number of roentgen equivalents absorbed by the plant tissue during the first half life period (14.3 days) can be calculated. One uC of P^{32}, when completely disintegrated, releases 0.74×10^5 ergs of energy. The energy absorbed by plant tissue from 1 roentgen of X-ray is about 90 ergs per gram of plant tissue. With an application of 15 uC per eye piece of 11 g the energy equivalent in roentgen units is 560. According to Stanton and Sinclair (20) the distribution of P^{32} was concentrated in the meristematic tissue as in any other tissue of the treated plants. Assuming a distribution similar to this it may be estimated roughly that the potato growing point tolerated 3360 r (6 x 590) equivalents of P^{32}. Thus making allowances for unavoidable losses in administering P^{32} and for the growth of the plant tissue during the first two weeks, it may be concluded that the tolerance of potato to X-ray and to P^{32} on an energy basis is about the same. This information may prove useful in estimating treatment levels of P^{32} for use with plants for which tolerance levels to X-ray treatments are known.

SUMMARY

Potatoes were exposed to radiation by means of X-ray and phosphorus 32 to study radiation effects with respect to subsequent plant growth, tolerance and possibilities of inducing mutations. X-ray dosage levels ranged from 500 r to 7500 r. P^{32} was applied to potatoes by injection into the tubers and stems and by absorption through the roots of young rooted shoots. P^{32} dosage levels ranged from 5 uC per eye-piece or growing shoot to 50 uC per treatment. Three varieties of potatoes namely Pontiac, Manota and Netted Gem were used in the study.

X-ray dosage levels above 2000 r greatly reduced the plant stand and emergence. Stands were 80% of normal with 2000 r rate and only 15% with the 5000 r rate. The optimum dosage for mutation effects likely lies close to 2000 r. One possible mutation consisting of a change from russet skin to smooth skin character in the Netted Gem variety was found as a result of treatment at the 500 r dosage rate.

P^{32} treatments produced very striking morphological effects such as "blind shoots", leaf shape changes, aerial stolons, and aerial tubers. One smooth skinned variant was produced as a result of injecting 15 uC of P32 into a Netted Gem tuber. This variant reproduced smooth tubers and is a stable mutation. An attempt was made to determine whether the aerial stolon and tuber development was associated with

auxin disturbance. Although the study indicated such a relationship further evidence is required to establish it definitely.

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