X-RAYS AFFECTING FARM CROPS

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By

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INTRODUCTION

Plants exposed to x-radiation or those produced from x-rayed seed may show any of the following: no beneficial or harmful effects (10)(22), stimulation in growth (9)(14) (23), mutations and morphological variations (7)(11)(15) (16)(25), and malformations never reaching maturity (4) (11)(19)(25)(27).

The use of x-rays for inducing variation in plants is relatively new, most work dating back less than two decades. Experiences during this period have been quite variable and expectations following exposure of plant tissue to x-rays are very indefinite. As expressed by Hanson (5):

> "Mutations by x-rays are fortuitous or chance occurrences at the present time. The operator may be likened to a hunter shooting birdshot into a flock of ducks. As the hunter accepts with natural piety what comes down, so the investigator shooting x-rays into a flock of genes accepts what is given. For it is impossible to aim at any particular gene at the present time."

Were it possible to direct treatment at a particular gene, difficulties would still be encountered in trying to get specified results.

The infrequent occurrence of mutations in nature limits their importance from the standpoint of introducing new and possibly superior germ plasm which is needed so much for present day plant improvement. This limitation has created considerable interest from the standpoint of induced variation by artificial means of which the use of short wave length rays, and particularly x-rays, is receiving much attention.

To determine possible stimulation of plant growth and to observe any morphological changes that might occur as a result of exposing seed to x-radiation a series of seed treatments have been made. These have been followed for two generations.

REVIEW OF LITERATURE

Johnson (12) irradiated dry and soaked seed of Marquis wheat with doses of 1,000, 5,000, 10,000, 20,000, 40,000, and 60,000 r-units.[1] An exposure of 5,000 r-units reduced total survival of plants to approximately 5 per cent while plants from irradiated dry seed showed approximately 50 per cent survival with an exposure of 20,000 r-units. In a few instances dry seed receiving light doses excelled the controls in percentage of plants surviving and in average height of plants.

Goodspeed (4) working with tobacco found that dry seeds were affected very little when exposed to x-rays. Some lethals and a few plants with thicker leaves resulted from treated germinating seed. Dry seeds of sunflower are likewise much less susceptible to harmful effects of x-rays than are seed containing 50 per cent or more of water (8). Stadler (23) found that dormant seed were much more resistant to injury by irradiation and will withstand 15 to 20 times as heavy dosage as germinating seed. The rate of mutation in the plants treated as germinating seed was about four times as high as that in plants treated as dormant seed. In both cases mutations occurred at a significantly higher rate than in the control plants.

Lemans (13) discusses differential effects on plants by

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[1] r-units, roentgen units

treating seed with different types of x-rays. The soft or shortest wave length rays, with a minimum length of 0.000,000,000,38 inch or 0.1 Angström unit, are much less destructive to plant tissue than the hard or long rays which have a maximum length of 0.000,000,17 inch or 45 Angström units. Duggar (2) attributes the mild effect of soft x-rays to their absorption by surface layers of tissue exposed. The degree of absorption varies directly with the atomic weight of the material treated (13).

Soaked wheat seeds were exposed to x-rays with a dosage of 18 kv.[2], 10 ma.[3], and 3 cm.[4] target distance for 5 seconds to 2 minutes by Benedict and Kersten (1). It was found that as the time of irradiation was increased there was a decided and progressive decrease in diastatic activity, sugar content, and rate of respiration of the wheat seedlings.

Variable affects on sorghums were obtained by Wheldon and Haskins (27) when dry seed received a treatment of 200 kv., 30 ma., at a target distance of 50 cm. and exposures of 4, 8, 16, 32, 64, and 128 minutes. Progeny of plants produced from seed exposed up to 32 minutes behaved perfectly normal. The F_1 plants of the 64 minute group matured

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[2] kv., kilovolt

[3] ma., milliampere

[4] cm., centimeter

seed but their progeny or F_2 generation was malformed and defective with no plants reaching maturity. Seedlings of the group of longest exposure were uniformly malformed, feeble, and stunted, and perished early.

Moore and Haskins (19) exposed dry Sea Island cotton seed to 200 kv., 30 ma., with a target distance of 50 cm. for 0.5, 2, 4, 8, 16, 32, and 64 minutes. No plants from seed exposed for 32 minutes and longer attained more than one pair of true leaves. Extreme fasciation was evident in a large percentage of the 16-minute group with development proceeding from lateral buds, and terminal buds developing only to limited degrees.

Startex cotton, used by Horlacher and Killough (7), was given an x-ray dosage of 100 kv., 10 ma., with 17 cm. target distance and exposed for 60 minutes. One-year old seed was not affected while two-year old seed showed some normal, intermediate, and dwarfed plants.

McKay and Goodspeed (16) treated pollen grains of Half and Half cotton prior to pollination for 4, 8, 12, 16, and 25 minutes with an exposure of 50 kv., 5 ma., and a target distance of 6 cm. with an aluminum filter between the tube and material exposed. Seventeen mature fruits, yielding 311 seeds or about one-half the usual number, were obtained from x-rayed pollen and untreated eggs. These seed were produced on 21 mature plants which showed twisted and deformed stigmas, anastomosing leaf veins, peculiarities in leaf shape, fasciated and enlarged stems, incomplete flowers, and dwarfed plants. Nine plants were sterile. Two plants produced empty seed. The remaining fertile plants produced perfect seed, with two plants having naked seed and three plants showing the naked character to a noticable extent which did not occur in the controls.

Stadler (25) treated barley, corn, wheat, and oats with hard rays, exposing germinating seed for 30 minutes to 78 kv., 5 ma., with a target distance of 22.7 cm. Mutations occurred in the barley to the extent of 1.9 per cent. The mutants reaching maturity proved to be homozygous recessive. Common wheat and oats showed no evidence of mutation probably due to higher chromosome number as explained by the investigator. Variations occurred in corn but were of a heterozygous nature. When corn seed were x-rayed as early as six days following pollination some chimeras were observed in the resulting plants.

Shull and Mitchell (23) report stimulation in growth of wheat, corn, and oats when germinating seed received a dosage of 100 peak kv., 5 ma., through an aluminum screen 1 mm. thick, and with a target distance of 30 cm. Wheat exposed up to one or two minutes, depending upon variety, was decidedly more vigorous; the greatest difference being shown in the degree of tillering. Corn treated up to three minutes emerged from the soil more rapidly, had thicker stems, was more succulent, and showed a darker green color than either the controls or plants resulting from seed receiving longer exposures. These investigators concluded that the total dosage for stimulation does not much exceed 100 r-units. This is borne out by Russell (22) who observed delayed germination, reduced height of plants, chlorotic disturbance and malformation of leaves, delayed lateral root growth, and death of corn plants as a result of irradiating germinating seed with a dosage varying from 60 to 10,000 r-units.

Johnson (10) reports no increased growth of tomato, sunberry, sunflower, vetch, tulips or Colorado wild potatoes when the seeds, bulbs and tubers were irradiated with light x-ray dosages. Seed pieces of certified Early Ohio potato were irradiated and the resulting plants produced 27 per cent more tubers than the controls with no increase in yield as based on weight (9).

Extreme leaf abnormalities, affecting external form, cell arrangement, and cell structure, were observed in soybeans by Long and Kersten (15). Affected plants were produced from seed exposed, while dry, to 20 peak kv., 10 ma., with a target distance of 8 cm. for 10 to 80 minutes.

Goodspeed (4) x-rayed sex cells of tobacco which gave rise to a considerable number of new monosomic and trisomic races because of continual occurrence of chromosome fragmentation. This fragmentation, the investigator explains, may be due to a degree of molecular instability as a result of x-ray bombardment.

Johnson (11), working with 70 species of flowering plants distributed in 35 families, exposed germinating seed to a dosage of 44 to 105 kv., 5 ma., with 30 cm. target distance for 10 to 37 minutes. Fifteen species were apparently unaffected, 15 species were slightly affected particularly during early stages of growth, and 40 species were noticably affected to show dichotomous branching, dwarfing, terminal buds killed, leaf deformities, color variations, delayed fruiting, reduced blossoming to no blossoming, and lethals.

MATERIALS AND METHODS

Spencer's White June corn, White darso, Sudan grass, Blackeye cowpeas, Acala 8 cotton, Turkey wheat, Michigan Winter barley, and Winter Turf oats, obtained from the Oklahoma A. and M. College Agricultural Experiment station, were used to study the effects of x-rays upon farm crops.

Preliminary treatments given all seeds prior to x-radiation were as follows. Sound seeds for each crop were sorted and duplicate samples of each variety placed into petri dishes between moist blotting paper, keeping them sufficiently moist for germination. Following the period of soaking for 24 hours at 85 degrees F., one set of the duplicate was exposed to x-radiation while the other set was used as a check. Both sets of seeds were planted either in the field on the Agronomy farm or in the greenhouse.

X-rays used were of two types, soft rays ranging in wave length from 0.1 to 2 Angström units, and hard rays ranging in wave length from 20 to 45 Angström units. Seed exposed to soft rays received a dosage of 50 kv., 6 ma., with a target distance of 3 cm. for 5 seconds with the exception of cotton seed which received an exposure of 10 seconds. The tube was of a Coolidge type being enclosed in a lead-glass case equivalent to a 1/16 inch lead filter. Exposure of seeds to hard rays consisted of a dosage of 85 kv., 10 ma., the seed being located 30 cm. from the target and being exposed six minutes without a filter. The six-minute exposure was divided into twelve 30-second periods with 30-second to 5-minute intervals to permit the tube to cool.

On June 2, 1938, immediately following exposure to soft x-rays, both treated and untreated seed of corn, darso, Sudan grass, cowpeas, and cotton were planted on the Agronomy farm in replicated plots consisting of two 30-foot rows each. Loss of stand following seedling emergence made it necessary to repeat treatment and to replant the darso and Sudan grass on June 15.

Observations were made to determine any possible differences in seedling emergence and the occurrence of abnormal plants. Pollen mother cell smears were made of abnormal plants to detect any possible chromosome irregularities. Pollen grains were mounted in glycerine and examined with the microscope for differences in size. Earliness of flowering was studied in corn, darso, and Sudan grass by making counts on emergence of tassels, heads, and panicles respectively.

Yields of corn were determined by weighing the mature ears, while darso, Sudan grass, and cowpea yields were determined by taking the green weight of plants harvested when the earliest fruits were in the hard dough stage. Cotton yields were not comparable because of irregular stands.

Corn and darso seeds from the F_1 or first generation plants of x-rayed seed were planted in the greenhouse in the fall of 1938 in order to produce third generation seed to be used in field tests the following year.

Plantings of the same crops receiving the same treatment were again made on the Agronomy farm in April and May of 1939. Wherever possible F_2 and F_3 generation seed of x-rayed plants were included. An additional planting consisted of F_2 seed of corn receiving an x-ray dosage of 85 kv., 10 ma., with 30 cm. target distance and exposed for 6 minutes. Plantings were replicated as follows: corn six times, darso four times, cowpeas three times, while cotton and Sudan grass were planted in single plots. Studies this time were concentrated on abnormalities and yields.

In September of 1938 seeds of corn, darso, Sudan grass, cowpeas, wheat, barley, and oats were treated with hard x-rays. The summer annuals and a few seeds of wheat, oats, and barley were planted in the greenhouse while the remaining treated seeds of wheat, oats, and barley together with their checks were planted in the open on the Agronomy farm. The following February soaked seeds of wheat, oats, and barley were exposed to the same treatment and planted in the field beside the fall seedings. In all cases abnormal plant growths were the major consideration. Length of exposure and difficulty in obtaining use of the x-ray machine made it impossible to treat seed with hard rays in sufficient quantity to check yields.

Several amino acid determinations were made from extracts of treated and untreated seedlings. Amino nitrogen was determined in corn and darso according to Van Slyke's methods (6).

A photelometer, consisting of a photo-electric cell with a standard light source used to determine turbidity of solutions, was used to read the candle power of light absorbed by various solutions of plant sap. These readings are comparative determinations of indoleacetic acid (17), xantho proteins (18), and available proteins (6).

The pH values of water extracts of plant sap from seedling plants were determined by using a glass electrode, reading on an electrometer.

RESULTS AND DISCUSSION

To determine whether or not stimulation of growth was induced by x-radiation comparative studies were made of treated and untreated plants at the time of seedling emergence, at the time of flowering, and at maturity when yields were obtained.

Since both the radiated seed and seed used for checks were soaked for the same period of time prior to planting, rate of germination counts were easily comparable. These counts indicated no difference in seedling emergence between treated and non-treated seed of the crops studied.

The percentage of germination of x-rayed seed, whether treated with soft or hard rays, was not affected.

Figure 1 shows a slight increase in the rate of flowering of corn, darso, and Sudan grass plants produced from seed treated with soft x-rays as compared to the check plants. It will be noted that the treated plants of darso showed a gradual increase in rate of flowering until the fifty-first day when the peak was reached, after which the rate showed a decline. The treated plants of Sudan grass continued at an increased rate throughout the period. It is doubtful whether the difference in rate of flowering is significant in any case.

Differences in corn yields are given in table 1. The higher yields in 1938 for both check and treated plots were due to more desirable distribution of rainfall during

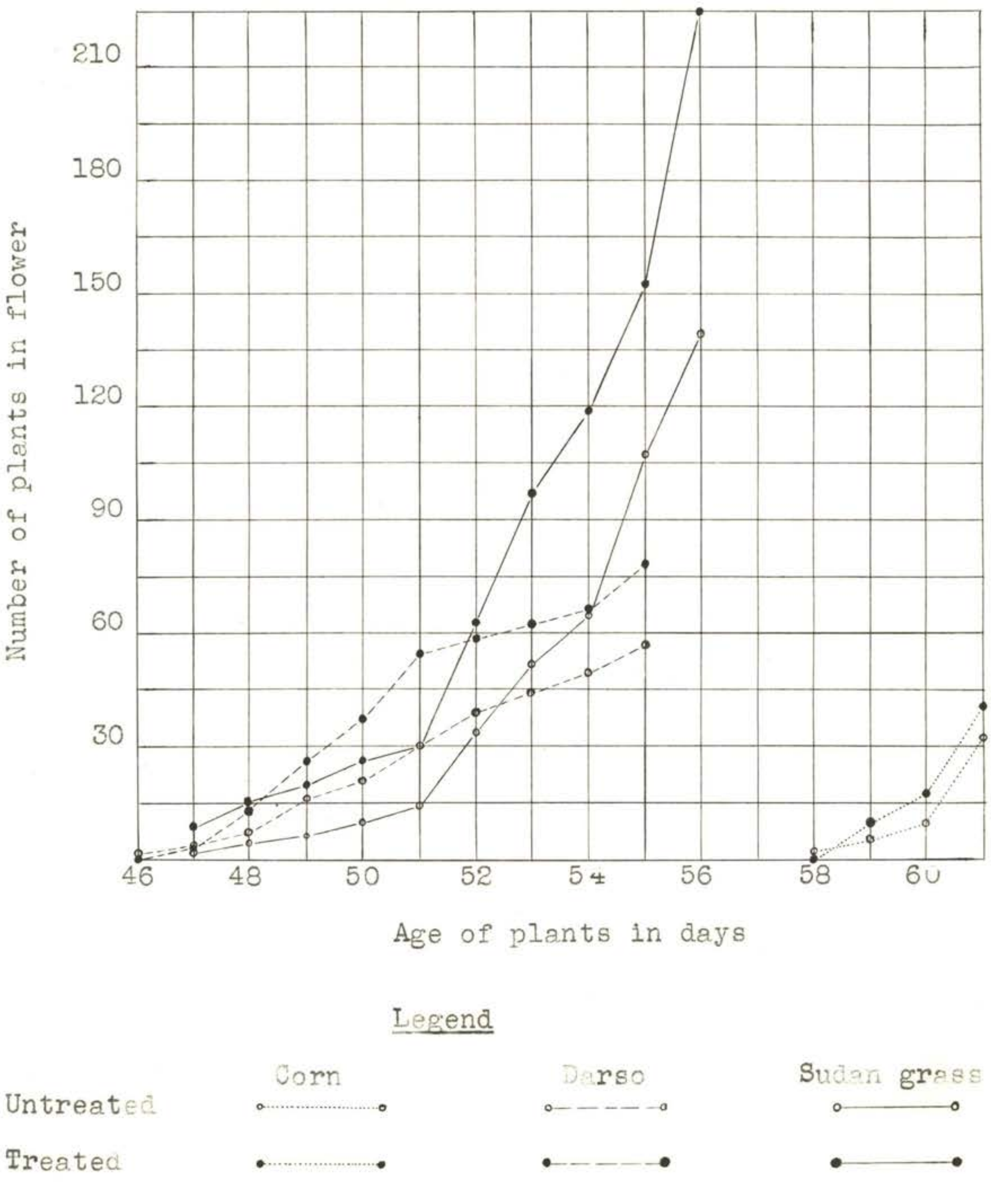

Figure I. Flowering rate of untreated and treated corn, darso, and Sudan grass during the early part of the flowering season.

the growing season. The average yield shows an increase in yield of approximately 34% for the F_1 or immediate plants produced from x-rayed seed. The 1939 results indicate that possibly the stimulation by x-rays which resulted in increased yields of the F_1 plants was carried over into the F_2 generation. Individual plot yields for 1939 show that F_1 plants outyielded the check plants in 5 of the 6 replications while the F_2 plants produced greatest yields in 4 of these tests. The lower yield of the F_3 plants suggests that the induced stimulation had run its course, and the plants had returned to near normal production. It must be borne in mind that no effort was made to control pollination except for a few plants. This made it possible for F_1 and F_2 treated plants to be pollinated by normal pollen from the check plants.

Table 1 Corn yields as affected by x-radiation

Treatment	Acre yield in bushels		
	1938	1939	Average
Check (Untreated)	41.49	21.80	31.64
X-rayed with soft-rays			
F_1	60.75	24.01	42.38
F_2		29.81	
F_3		19.35	
F_2 (self fertilized seed from F_1)		16.20	
X-rayed with hard-rays			
F_2		5.58	

The F_2 plants, produced from inbred seed of the treated F_1 plants, produced considerable less than the controls. The second generation plants produced from seed exposed to hard x-rays produced only about 25% as much as the check plants. The plants themselves showed normal stature but produced very few ears. Apparently most of the cells which normally develop into ears were destroyed by the rays.

First and second generation darso plants, produced from seed exposed to soft x-rays, showed increases in yield over the check plants as shown in table 2. The slight decrease in yield of the F_2 plants as compared to the F_1 plants suggests that the stimulation induced by x-rays is lost in later generations.

Table 2 White darso yields as affected by x-radiation

Treatment	Pounds of green forage per acre		
	1938	1939	Average
Check (Untreated)	12,867	8297	10582
X-rayed with soft rays			
F_1	16,179	9695	12937
F_2		9542	

In table 3 variable differences are shown in cowpea yields. The 1938 yields show that soft x-rays had a stimulative effect while in 1939 the same rays apparently caused slight injury which resulted in decreased yields of the F_1 plants. The F_2 plants, produced from seed obtained from treated plants showing stimulation, yielded slightly less than the check plants.

Table 3 Cowpea yields as affected by x-radiation

Treatment	Pounds of green forage per acre		
	1938	1939	Average
Check (Untreated)	7,882	4,513	6197
X-rayed with soft rays			
F_1	9,334	4,210	6772
F_2		4,455	

According to table 4 Sudan grass treated with soft x-rays resulted in decreased yields. The increased rate of flowering as shown in figure 1 did not result in increased yield as one would expect. These results are also contrary to those found in darso showing that like species are affected differently. Yields of Sudan grass were not taken in 1939 because of poor stand.

Table 4 Sudan grass yields as affected by x-radiation

Treatment	Pounds of Green Forage per acre
	1938 yields
Check (Untreated)	19,083
X-rayed with soft rays	
F_1	17,217

The stand of cotton plants in plots comparing check plants with plants produced from seed treated with soft x-ray was too poor to check yields in both 1938 and 1939. Comparative studies showed there was no significant difference in the staple length or lint per cent of the normal and treated plants.

Several laboratory tests were made to correlate a possible cause with yield increases of some x-rayed plants. These results are listed in table 5.

The milligrams of amino nitrogen present in one gram of seedling plant material show that there is an increase of available proteins in the treated seed. More amino nitrogen in the F_1 and later generations of x-rayed seed indicated that this material may be responsible for increased yields.

Photelometer readings of water extracts of plant material having potassium nitrite and nitric acid added showed that extracts from x-rayed plants in general absorbed less light which indicated less indoleacetic acid according to Mitchell and Brunstetter (17). The decrease in amount of this growth promoting substance does not necessarily hamper growth according to Sugiura (26) who found that the destruction of the major portion of Vitamin B in wheat embryos by x-radiation did not prevent growth of the wheat seedlings.

Table 5 Amino acids in corn, darso, and cowpea seedlings as affected by x-radiation

Treatment	Mgs. of amino nitrogen in one gram of plant material		Photolometer readings in candle power					
			Indolescetic acid (Water extract + KNO_2 and HNO_3)			Xanthoprotein		Available protein (Chloroform extract)
	corn	darso	corn	darso	cowpea	corn	darso	corn
Check	8.8	4.3	69.0	61.0	71.0	74.0	94.6	58.0
X-rayed (soft rays)								
F_1	9.0	8.4	73.5	73.0	77.0	80.0	90.0	47.0
F_2	9.5	10.1	80.5	45.0	71.0	84.0	89.0	27.0
F_3	9.3		80.5			65.0		38.0

The xantho protein determinations showed photolometer readings, in the case of corn, that are in negative correlation with yield. In this case there is a progressive increase in amounts of light absorbed by solutions from plants showing decreasing yields. The readings of darso were very irregular showing no correlation.

Chloroform extracts from corn seedlings contained available proteins and showed lower readings or larger amounts of light absorbed for the radiated plants than for the check plants.

There were no significant differences in pH values of water extracts from normal and treated seedlings as shown in table 6.

Table 6 pH values of water extract from seedling plants

Treatment	Corn	Sorghums	Cowpeas
Check	5.73	5.40	6.3
X-rayed with soft rays			
F_1	5.69	5.45	6.16
F_2	5.79	4.99	6.10
F_3	5.74		

Morphological studies made of both treated and check plants to detect possible variations resulted in the finding of several fasciations and a few other minor variations.

A fasciated corn plant showing twice the usual number of anthers per flower occurred in the group of plants produced from seed exposed to hard x-rays. Figure 2 illustrates the entire tassel while figure 3 shows a close-up of individual male flowers from the fasciated and normal plants. Progeny from this abnormal plant showed no fasciations.

Figure 4 shows variations in radiated oats, two and three panicles growing from upper nodes of single tillers.

Soaked wheat seed treated with hard x-rays resulted in an abnormal compound spike as shown in figure 5. The rachis of this spike was divided with each division bearing seed at the nodes.

Spring plantings of wheat, oats, and barley exposed to hard rays showed no differences from the normal check plants. No plant produced fruit. All plants showed a winter habit of growth until they were overcome by rust in late June.

Second generation plants, from small grains exposed to hard rays, showed no chimeras.

Of all the plants produced from crop seed exposed to soft x-rays only two corn plants showed visible variations. One plant bore a whorl of four leaves just below the tassel. Each leaf was borne upon a separate node, but no seed was produced. Another plant bore a small ear on the first node below the tassel as illustrated in figure 6. Progeny from this plant were normal.

Figure 2. Corn tassel at left is from a treated plant and shows twice the number of anthers per flower as is found in the normal tassel shown at the right.

Figure 3. Individual flowers from corn tassels of treated plant (left) and check plant (right) shown in figure 2.

Figure 4. Three panicles growing from a single tiller of an oat plant produced from seed exposed to hard x-rays.

Figure 5. Compound spike from a treated wheat plant.

Figure 6. Corn plant produced from radiated seed showing ear developing from the first node below the tassel.

Pollen grains from treated and check plants of all crops were mounted in glycerine and examined with a microscope. No differences were found.

Smears of pollen mother cells were made of all abnormal plants to determine possible chromosome irregularities, but there were no visible differences.

SUMMARY AND CONCLUSIONS

1. Exposure of soaked seeds to soft x-rays resulted in increased yields which is in accordance with work of Long and Kersten (14).

2. X-radiation of seed increased the available nitrogen in the resulting seedling plants.

3. Water extracts of plant sap from treated and check plants showed comparatively little difference in hydrogen-ion concentration.

4. Slight structural variations appear more frequently in plants after radiation.

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