A COMPARISON OF THE EFFECTS OF HEAT AND X-RAYS ON DORMANT SEEDS OF CEREALS, WITH SPECIAL REFERENCE TO POLYPLOIDY[1]

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INTRODUCTION

The extent to which genetic effects of heat and X-rays are comparable has been the subject of considerable discussion. Studies of X-ray effects have shown that tolerance and frequency of chromosomal aberrations increase with polyploidy. The present investigation was undertaken to verify and extend these observations with X-rays and to compare the results with those obtained with heat treatment. The first part of the paper deals with the effects of heat and X-rays on the viability of seeds of diploid and polyploid lines of the same or related species of cereals and with the effects of combinations of heat and X-ray treatments on seeds of diploid wheat and barley. The rest of the paper is concerned with the effects of heat and X-rays on certain genetic aberrations, namely frequencies of chromatinic bridges in mitotic divisions of root tips, translocations, and mutations.

Some of the results of these and other experiments on the relation of number of chromosomes in pollen to the frequency of X-ray-induced chromosomal aberrations have been briefly presented by Smith (*28*).[3]

REVIEW OF LITERATURE

Age, heat, and X-rays have been reported to produce a number of similar genetic, cytologic, or physiologic effects in seeds and in plants grown from treated seeds. After appropriate action of these agents, germination has been reported to be lower and slower, viability of the seedlings reduced, chromosomal irregularities in root tips more numerous, meiotic disturbances more frequent, sterility increased, and mutation frequency stepped up. Furthermore, the activity of each of

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[1] Received for publication December 9, 1944. This work was carried out in cooperation with the Field Crops Department, Missouri Agricultural Experiment Station. Contribution from the Field Crops Department, Missouri Agricultural Experiment Station, Journal Series Paper No. 908.

[2] Grateful thanks are extended to all who provided the seed stocks used in this study and also to Miss Ruth E. Ray, who prepared many of the slides for cytological examination and made most of the counts on bridges in root-tip smears.

[3] Italic numbers in parentheses refer to Literature Cited, p. 157.

Journal of Agricultural Research, Washington, D. C.

Vol. 73, No. 4
Aug. 15, 1946
Key No. G-1360

these three agents is intensified by increasing the moisture content of the seeds. The reader is referred to Shkvarnikov (*24*) and Crocker (*4*) for reviews of literature on the effects of age; to Plough (*20*) and Randolph (*21*) for literature on the effects of heat; and to Goodspeed and Uber (*10*) for that on the effects of X-rays.

Although age, heat, and X-rays produce a number of similar effects, there is disagreement as to how these three agents reduce viability in seeds. Crocker (*4, p. 249*), from his analysis of the literature on the life span of seeds, concluded that "the age degeneration of seeds is probably due to a gradual dislocation in the delicate chromosome apparatus of cells of the embryo." On the other hand, Navashin et al. (*16, p. 951*) stated that "dying out [of old seed] cannot depend merely on accumulation of mutations, but should be caused by some other factors, too." Avery and Blakeslee (*2*) found that the mutation rate of *Datura* seed buried 39 years in soil was higher than in the controls but (*p. 70*) "much lower than from seed 'aged' under laboratory conditions." This indicates that the conditions under which aging takes place may be more important in producing genetic changes than age itself.

Groves (*12*), from his experiments on the relation of temperature to the life duration of seeds of common wheat, concluded that the killing effect of high temperatures followed the pattern of coagulation of proteins by heat. Peto (*18, 19*), Shkvarnikov (*24*), and Shkvarnikov and Navashin (*25*) reported that heat induced high frequencies of chromosomal irregularities, which could result in loss of viability.

As for the killing effects of X-rays, Fröier and Gustafsson (*8, p. 44*), from their experiments on barley and wheat, concluded that "seed mortality induced by X-radiation is most probably an expression of chromosomal and genic disturbances." However, Kempton and Maxwell (*14*) doubted that chromosomal derangements were the cause of the death of seedlings grown from irradiated seeds of maize. This latter view is given some support by the evidence of Smith (*27*) on a mutant type of wheat that was killed by smaller doses of X-rays than the normal, although unpublished data indicate that the frequencies of chromosomal aberrations in the mutant and normal given the same X-ray dose are comparable. Sax (*23*) concluded that X-ray-induced chromosomal alterations are the chief cause of injury to individual cells, but (*p. 537*) "in the reaction of tissues to X-rays the physiological effects may play a more important part."

Little information on the relation of polyploidy to the effect of age on viability can be obtained from the literature on longevity of seeds, because few comparisons have included polyploid and nonpolyploid species of the same genus. From a small number of such comparisons in the experiments of Ewart (*5*), Carruthers (*3*), Goss (*11*), and Robertson and Lute (*22*) there is no indication that polyploidy has any effect on the longevity of seeds.

The relation of polyploidy to tolerance of seeds to heat has received little attention, although some observations that bear on the problem have been made. In the experiments of Atanasoff and Johnson (*1*), testing the efficacy of dry heat as a sterilizing agent for certain disease organisms borne on cereal seeds, there was no significant difference in the tolerance of durum wheat ($2n=28$), varieties of common wheat

($2n=42$), and varieties of other farm crops, polyploid and nonpolyploid, though not of the same genus. Data of other writers reveal similar results.

The relation of polyploidy to the effect of X-rays has been studied to a greater extent. Stadler (*30*), Fröier (*6*), and others have shown that seeds of polyploid species are able to survive heavier treatments of X-rays than diploid related species. Fröier, Gelin, and Gustafsson (*7*) irradiated dormant seeds of *Triticum monococcum*, *T. durum*, *T. vulgare*, and *Avena sativa* and observed that after treatment with 5,000 roentgens (r.) the frequency of telophases with bridges and fragments in root-tip cells was directly proportional to the number of genoms present. This relation was not so apparent at higher doses. Germination and sprouting ability of the tetraploid and hexaploid species were unimpaired even when 50 percent of the mitoses had bridges or fragments or both, but the growth of the diploid was impaired with low disturbance frequencies and low dosages.

MATERIAL AND METHODS

Seeds of diploid, tetraploid, and hexaploid species of wheat and oats; diploid and autotetraploid barley, rye, and maize; and an amphidiploid were included in the heat and X-ray comparisons. Table 1 gives a list of the species and varieties used in these studies, their chromosome numbers, and the percentage of moisture in the dormant seeds. In addition to the varieties, several lines of einkorn derived from crosses between mutants were used. Descriptions of most of these mutants were given by Smith (*26*). The diploid line of maize was derived from the autotetraploid.

No attempt was made to determine the percentage of moisture in the seeds at the time the various treatments were made. For this study it was considered more important and sufficient that the conditions of storage be comparable for the various stocks. For that reason the seeds were stored in the same room and same type of container and for the most part in the same metal storage box. Near the conclusion of the experiments in March 1942, a determination of the moisture content was made by drying seeds of the various stocks at about 110° C. for 2 to 3 days. The percentages are presumably less than they would have been if the determinations had been made at another time of year when the humidity in the building in which they were stored was higher.

Various tests made are not reported here, because they were of a preliminary nature or because the results from other tests were more significant.

The X-ray treatments were applied to the dormant seeds as they lay in a single layer on a piece of cardboard. The seeds of maize were turned germ up; the others lay at random. In order to keep the temperature down, the cardboard was supported on a tray containing ice cubes. Under these conditions and with a fan stirring the air, the temperature of the atmosphere surrounding the seeds remained below 33° C. during the treatments. The X-ray apparatus was operated at about 130 kilovolts (peak), 4-milliampere tube current. The cardboard on which the seeds lay was 7¾ inches from the target of the

X-ray tube. Under these conditions the seeds received approximately 325 r. per minute.

TABLE 1.—*Species and varieties of cereals, chromosome groups and numbers, and percentage of moisture in dormant seeds*

Species and variety	Chromosome group	Somatic chromosomes	Moisture
		Number	*Percent*
Triticum monococcum L. var. *flavescens* Koern	Diploid wheat	14	9.3
Triticum monococcum var. *hornemanni* (Clem. y Rubio) Koern. and Wern.	do	14	9.0
Triticum monococcum (g e–2)	do	14	9.2
Triticum monococcum (ga xs)	do	14	9.0
Triticum durum Desf. (purple-seeded)	Tetraploid wheat	28	--------
Triticum durum var. Acme	do	28	8.9
Triticum durum var. Kubanka	do	28	--------
Triticum persicum (Boiss.) Aitch and Hemsl	do	28	9.4
Triticum timopheevi Zhuk	do	28	9.2
Triticum aestivum [1] L. var. Marquis	Hexaploid wheat	42	8.7
Triticum aestivum var. Chinese Spring	do	42	9.9
Avena strigosa Schreb	Diploid oats	14	7.8
Avena abyssinica Hochst	Tetraploid oats	28	9.4
Avena sativa L. var Columbia	Hexaploid oats	42	9.9
Avena byzantina C. Koch var. Bond	do	42	10.2
Hordeum vulgare L. var. Everest	Diploid barley	14	8.9
Hordeum vulgare var. autotetraploid [2] Everest	Tetraploid barley	28	9.7
Secale cereale L. var. Rosen	Diploid rye	14	11.6
Secale cereale var. autotetraploid [3] Rosen	Tetraploid rye	28	11.6
Zea mays L	Diploid maize	20	9.4
Zea mays (autotetraploid [4] maize)	Tetraploid maize	40	8.8
Aegilops uniaristata Vis	Diploid goatgrass	14	--------
Triticum monococcum × *A. uniaristata* [5]	Amphidiploid	28	9.1

[1] *Triticum aestivum,* originally applied by Linnaeus to the bearded spring wheats only, under the International Rules of Botanical Nomenclature becomes the specific name for all common bread wheats, since it was the earliest name applied to any of the varieties of this group. The concept of this species as emended by Host under the name of *T. vulgare* Vill. is that now most generally accepted by wheat specialists. Wheat specialists likewise have preferred the name *T. vulgare* because it is more truly descriptive of the species. The name *T. vulgare,* however, is untenable under present rules of nomenclature, since it was originally proposed by Villars merely to replace the earlier name, *T. aestivum.* It is hoped, however, that at an appropriate time, the rules can be amended so that the name *T. vulgare* can be conserved for this species.

[2] Obtained from Harland Stevens.

[3] Obtained from Ernest Dorsey.

[4] Obtained from L. F. Randolph.

[5] Obtained from E. R. Sears.

For the heat treatments the seeds were placed in vials (30-cc. for maize, 10-cc. for other seeds), which were then tightly corked. The vials were placed in thermostatically regulated electric ovens in which, for most tests, the temperature did not vary more than 1.5° C. There was considerable air space in the vials, but since the relative spaces were comparable this is considered to be of no importance in the comparisons between stocks.

Conditions for germination and growth are given in the presentation of results. When grown for seedling data, the seeds were planted in soil flats in a greenhouse, where both germination and growth were less variable than in petri dishes.

In order to determine whether the treatments had any delaying effect, the date when germination began in petri dishes or when the seedlings began to emerge from the soil was recorded. Later the height of the seedlings, as well as their number, was noted for the plantings in soil. Measurements were made by placing a rule beside the clump of seedlings and estimating their average height.

Certain of the seeds were planted in metal bands filled with soil, and the plants were transferred to pots or to the field, grown to maturity, and examined cytologically for meiotic disturbances, particularly translocations. The acetocarmine smear technique was used for cytological observations. The plants from some of these experiments were tested for mutations.

For determining the frequency of chromatinic bridges in somatic divisions, root tips were taken from seeds germinating in petri dishes, fixed in Carnoy's fluid for a week or more, and smeared in acetocarmine.

EXPERIMENTAL RESULTS

HEAT TOLERANCE AS AFFECTED BY POLYPLOIDY

In some tests the relation of polyploidy to the tolerance of dormant seeds to heat treatment was determined by germination and seedling growth and in others by germination only. Table 2 summarizes the results of three tests in which germination was determined. In two tests the seeds were sterilized in bichloride of mercury (2 parts in 500 parts of 50-percent ethyl alcohol) for 1 minute, after which they were germinated on 2-percent sterile agar at 26° C. In the third test the seeds were germinated on moist blotters in petri dishes. The stocks tested included seven strains of diploid, four strains of tetraploid, and two strains of hexaploid wheat; diploid and autotetraploid barley and rye; and the amphidiploid.

The results were irregular and not ideal, mainly because of practical difficulties in sterilizing and germinating the seeds, and because germination of seeds of some of the stocks was low. Nevertheless, it is apparent that, with the possible exception of the amphidiploid, polyploidy did not appreciably improve the survival. The amphidiploid survived the treatments better than *Aegilops uniaristata* and better than some stocks of diploid wheat.

Seedlings from the controls and from some of the more severe treatments were planted in soil to be grown to maturity for cytological study and harvested for a study of mutation rate. Observations on these seedlings indicated that those from the controls and from seeds treated at 60° and 65° C. for 5 days differed little. Most of the seedlings from the seeds treated at 70° for 5 days were distinctly injured. There were differences in the injury to the various stocks of diploid wheat, some being injured less, but none more than the tetraploid or hexaploid. Seedlings of the diploid barley and rye were at least as vigorous as the autotetraploids. Seedlings of the amphidiploid were injured as much or more than seedlings of the diploid parents.

Table 3 presents the combined results of two tests in which data on both germination and growth of seedlings were recorded. In these tests the treatments were at a higher temperature and of shorter duration than in the previous experiments. After treatment the seeds were planted in soil flats. When the seedlings began to emerge, their number and estimated average height were recorded at intervals of 2 to 5 days for about 20 days.

The stocks tested included diploid, tetraploid, and hexaploid wheat and oats; diploid and autotetraploid rye and maize; and the amphidiploid. Two varieties of tetraploid and hexaploid wheat and hexaploid oats were used, and one stock of each of the others. From table 3

TABLE 2.—*Relation of polyploidy to tolerance of dormant seeds to heat treatments, as measured by germination*

Stock	Somatic chromosomes	Treatment		Total seeds	Seeds germinating
		Temperature	Duration		
	Number	°*C.*	*Days*	*Number*	*Percent* [1]
Diploid wheat	14	(2)	----------	423	86
Tetraploid wheat	28	(2)	----------	131	56
Hexaploid wheat	42	(2)	----------	129	96
Diploid wheat	14	50	5	212	77
Tetraploid wheat	28	50	5	114	38
Hexaploid wheat	42	50	5	98	22
Diploid wheat	14	60	5	664	66
Tetraploid wheat	28	60	5	200	39
Hexaploid wheat	42	60	5	210	48
Diploid wheat	14	70	5	375	50
Tetraploid wheat	28	70	5	84	32
Hexaploid wheat	42	70	5	80	26
Diploid wheat	14	50	10	160	43
Tetraploid wheat	28	50	10	63	45
Hexaploid wheat	42	50	10	48	102
Diploid wheat	14	60	10	570	66
Tetraploid wheat	28	60	10	103	23
Hexaploid wheat	42	60	10	109	33
Diploid wheat	14	70	10	161	0
Tetraploid wheat	28	70	10	59	0
Hexaploid wheat	42	70	10	50	0
Diploid barley	14	(2)	----------	98	92
Autotetraploid barley	28	(2)	----------	79	87
Diploid barley	14	55	5	50	42
Autotetraploid barley	28	55	5	50	14
Diploid barley	14	60	5	201	50
Autotetraploid barley	28	60	5	158	37
Diploid barley	14	70	5	49	0
Autotetraploid barley	28	70	5	29	0
Diploid barley	14	60	10	49	71
Autotetraploid barley	28	60	10	27	68
Diploid barley	14	65	10	53	0
Autotetraploid barley	28	65	10	27	0
Diploid rye	14	(2)	----------	50	34
Autotetraploid rye	28	(2)	----------	49	55
Diploid rye	14	60	5	50	71
Autotetraploid rye	28	60	5	52	4
Diploid rye	14	65	5	52	12
Autotetraploid rye	28	65	5	45	0
Diploid rye	14	70	5	50	18
Autotetraploid rye	28	70	5	49	0
Diploid rye	14	60	10	50	18
Autotetraploid rye	28	60	10	49	0
Diploid rye	14	65	10	49	0
Autotetraploid rye	28	65	10	49	0
Aegilops uniaristata	14	(2)	----------	28	68
Diploid wheat-*A. uniaristata* amphidiploid	28	(2)	----------	50	92
Aegilops uniaristata	14	60	5	27	44
Diploid wheat-*A. uniaristata* amphidiploid	28	60	5	49	104
Aegilops uniaristata	14	65	5	24	43
Diploid wheat-*A. uniaristata* amphidiploid	28	65	5	47	104

See footnotes at end of table.

TABLE 2.—*Relation of polyploidy to tolerance of dormant seeds to heat treatments, as measured by germination*—Continued

Stock	Somatic chromosomes	Treatment		Total seeds	Seeds germinating
		Temperature	Duration		
	Number	*°C.*	*Days*	*Number*	*Percent* [1]
Aegilops uniaristata	14	70	5	26	68
Diploid wheat-*A. uniaristata* amphidiploid	28	70	5	47	90
Aegilops uniaristata	14	60	10	21	35
Diploid wheat-*A. uniaristata* amphidiploid	28	60	10	48	71
Aegilops uniaristata	14	65	10	23	32
Diploid wheat-*A. uniaristata* amphidiploid	28	65	10	51	73

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[1] After 5 days; for untreated seeds, value indicates actual germination percentage; for treated seeds, percentage of the germination of the control.

[2] Control.

it is evident that seeds with the higher numbers of chromosomes had no greater tolerance for heat treatment as measured either by survival or by growth of the seedlings. Actually in several cases the polyploids were injured more.

The species of oats survived considerably longer treatments than the other stocks; it is of interest to note in this connection that, according to Crocker (*4*), seeds of oats usually remain viable longer than those of wheat, rye, barley, or maize. This may indicate a similarity in the effects of age and heat on viability.

In these and other tests it was apparent that, though in some cases germination was reduced or delayed by heat treatment, once the seedlings emerged they had a tendency to recover quickly and approach the height of seedlings from the untreated seeds.

Several plants of hexaploid oats and the amphidiploid were more or less devoid of chlorophyll in the early stages of growth. These seedlings resembled the "ghostlike" mutants in wheat, barley, and maize. Such peculiar individuals were observed among seedlings of several stocks from a number of the more severe treatments in various experiments. These "ghostlike" seedlings emphasize the relation inheritance bears to the control of sensitivity of biological responses (probably biochemical reactions) to environmental factors, including temperature. Thus many mutants, such as virescents, can be distinguished from normal plants only when grown at low temperatures, but other mutant characters have a different response and are exaggerated by high temperatures and become distinguishable.

X-RAY TOLERANCE AS AFFECTED BY POLYPLOIDY

Germination alone is not a critical test of the tolerance of seeds to X-rays, since there is a delayed killing effect. Therefore, after treatment with X-rays all seeds were planted in greenhouse flats for data on emergence and height of seedlings.

Table 4 summarizes the results of four tests. Two of these were run in conjunction with two of the heat-treatment tests included in table 3. The seeds were divided into two groups that were handled in the same way except that one group was given heat treatments

TABLE 3.—*Relation of polyploidy to tolerance of dormant seeds to heat treatments, as measured by germination and seedling height*

Stock	Somatic chromosomes	Treatment		Total seeds	Seeds germinating	Height of seedlings
		Temperature	Duration			
	Number	° *C.*	*Minutes*	*Number*	*Percent* [1]	*Percent* [2]
Diploid wheat	14	[3]	----------	50	96	100
Tetraploid wheat	28	[3]	----------	50	96	100
Hexaploid wheat	42	[3]	----------	50	100	100
Diploid wheat	14	80	45	50	94	91
Tetraploid wheat	28	80	45	50	90	99
Hexaploid wheat	42	80	45	50	100	97
Diploid wheat	14	80	90	50	50	86
Tetraploid wheat	28	80	90	50	48	73
Hexaploid wheat	42	80	90	50	76	75
Diploid wheat	14	80	135	50	29	78
Tetraploid wheat	28	80	135	50	40	61
Hexaploid wheat	42	80	135	50	4	6
Diploid oats	14	[3]	----------	50	100	100
Tetraploid oats	28	[3]	----------	50	92	100
Hexaploid oats	42	[3]	----------	50	94	100
Diploid oats	14	80	45	50	98	95
Tetraploid oats	28	80	45	50	104	100
Hexaploid oats	42	80	45	50	100	93
Diploid oats	14	80	90	50	98	90
Tetraploid oats	28	80	90	50	100	83
Hexaploid oats	42	80	90	50	96	78
Diploid oats	14	80	135	50	90	82
Tetraploid oats	28	80	135	50	81	76
Hexaploid oats	42	80	135	50	76	68
Diploid oats	14	80	180	25	100	90
Tetraploid oats	28	80	180	25	104	86
Hexaploid oats	42	80	180	25	67	67
Diploid rye	14	[3]	----------	50	72	100
Autotetraploid rye	28	[3]	----------	50	58	100
Diploid rye	14	80	45	50	89	100
Autotetraploid rye	28	80	45	50	110	100
Diploid rye	14	80	90	50	36	88
Autotetraploid rye	28	80	90	50	3	73
Diploid rye	14	80	135	50	6	58
Autotetraploid rye	28	80	135	50	0	0
Diploid maize	20	[3]	----------	50	100	100
Autotetraploid maize	40	[3]	----------	50	76	100
Diploid maize	20	80	45	50	96	96
Autotetraploid maize	40	80	45	50	111	97
Diploid maize	20	80	90	50	18	26
Autotetraploid maize	40	80	90	50	21	37
Diploid maize	20	80	135	50	2	43
Autotetraploid maize	40	80	135	50	0	0
Diploid wheat-*Aegilops uniaristata* amphidiploid	28	[3]	----------	25	92	100
		80	45	25	96	93
		80	90	25	48	71
		80	135	25	17	43

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[1] After 16 days; for untreated seeds, value indicates actual germination percentage; for treated seeds, percentage of the germination of the control.

[2] Percentage of control after 18 days.

[3] Control.

and the other irradiation. The heat- and X-ray-treated seeds of the same stock were planted side by side for direct comparison. The seeds tested included two stocks each of diploid, tetraploid, and hexaploid wheat and of hexaploid oats; one stock each of diploid and tetraploid oats, of diploid and autotetraploid barley, rye, and maize, and of the amphidiploid.

TABLE 4.—*Relation of polyploidy to tolerance of dormant seeds to X-ray treatments, as measured by germination and seedling height*

Stock	Somatic chromosomes	Treatment	Total seeds	Seeds germinating	Height of seedlings
	Number	*Roentgens*	*Number*	*Percent* [1]	*Percent* [2]
Diploid wheat	14	(3)	75	88	100
Tetraploid wheat	28	(3)	75	96	100
Hexaploid wheat	42	(3)	75	100	100
Diploid wheat	14	10,000	50	98	38
Tetraploid wheat	28	10,000	50	92	39
Hexaploid wheat	42	10,000	50	98	82
Diploid wheat	14	20,000	100	96	43
Tetraploid wheat	28	20,000	100	83	50
Hexaploid wheat	42	20,000	100	99	59
Diploid wheat	14	30,000	100	92	12
Tetraploid wheat	28	30,000	100	80	37
Hexaploid wheat	42	30,000	100	97	32
Diploid wheat	14	40,000	50	96	6
Tetraploid wheat	28	40,000	50	98	13
Hexaploid wheat	42	40,000	50	94	12
Diploid oats	14	(3)	50	96	100
Tetraploid oats	28	(3)	50	100	100
Hexaploid oats	42	(3)	50	96	100
Diploid oats	14	10,000	25	104	84
Tetraploid oats	28	10,000	25	100	93
Hexaploid oats	42	10,000	25	88	100
Diploid oats	14	20,000	50	88	51
Tetraploid oats	28	20,000	50	58	58
Hexaploid oats	42	20,000	50	81	57
Diploid oats	14	30,000	50	52	20
Tetraploid oats	28	30,000	50	66	37
Hexaploid oats	42	30,000	50	60	41
Diploid oats	14	40,000	25	0	0
Tetraploid oats	28	40,000	25	0	0
Hexaploid oats	42	40,000	25	54	18
Diploid rye	14	(3)	50	86	100
Autotetraploid rye	28	(3)	50	84	100
Diploid rye	14	10,000	50	102	45
Autotetraploid rye	28	10,000	50	93	45
Diploid rye	14	20,000	50	84	8
Autotetraploid rye	28	20,000	50	79	13
Diploid rye	14	30,000	50	84	8
Autotetraploid rye	28	30,000	50	69	9
Diploid maize	20	(3)	50	96	100
Autotetraploid maize	40	(3)	50	76	100
Diploid maize	20	10,000	50	92	42
Autotetraploid maize	40	10,000	50	95	62
Diploid maize	20	20,000	75	83	31
Autotetraploid maize	40	20,000	75	89	43
Diploid maize	20	30,000	75	70	16
Autotetraploid maize	40	30,000	75	75	28

See footnotes at end of table.

TABLE 4.—*Relation of polyploidy to tolerance of dormant seeds to X-ray treatments, as measured by germination and seedling height*—Continued

Stock	Somatic chromosomes	Treatment	Total seeds	Seeds germinating	Height of seedlings
	Number	*Roentgens*	*Number*	*Percent* [1]	*Percent* [2]
Diploid maize	20	40,000	25	79	19
Autotetraploid maize	40	40,000	25	76	42
Diploid barley	14	(3)	25	64	100
Autotetraploid barley	28	(3)	25	88	100
Diploid barley	14	16,000	50	56	30
Autotetraploid barley	28	16,000	50	41	63
Diploid barley	14	27,000	50	31	8
Autotetraploid barley	28	27,000	50	41	36
Diploid barley	14	38,000	50	3	7
Autotetraploid barley	28	38,000	50	32	19
Diploid wheat-*Aegilops uniaristata* amphidiploid	28	(3)	25	92	100
		10,000	25	100	67
		20,000	25	57	8
		30,000	25	61	4

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[1] After about 14 days; for untreated seeds, value indicates actual germination percentage; for treated seeds, percentage of the germination of the control.

[2] Percentage of control after 19 days.

[3] Control.

Figures 1 and 2 show some of the seedlings from the heat and X-ray treatments. From these figures and from tables 2, 3, and 4 it is clear that tolerance to X-rays did and tolerance to heat did not increase with chromosome number. Also, as already noted, severe heat delayed or prevented germination, but if the seeds germinated they usually grew vigorously; whereas X-rays did not delay or reduce germination so much, but after the more severe treatments all the seedlings died at various stages after emergence. Growth of seeds given the heaviest doses of X-rays ceased with the elongation of the coleoptile. After lighter treatments, some seedlings died when 5 cm. or more high. In addition to a slower onset of X-ray-injury symptoms, recovery of the seedlings, if it occurred, was more gradual than that following heat treatments.

TOLERANCE FOR COMBINATIONS OF HEAT AND X-RAY TREATMENTS

If heat and X-rays reduce viability of dormant seeds by effecting similar changes in the seeds, then treatments by both these agents on the same seeds should have more or less additive effects. Kempton and Maxwell (*14*) and Maxwell, Kempton, and Mosley (*15*) reported some observations on this problem. They found that seeds of maize heated to 60° C. for 4½ hours after irradiation were little if at all affected in their development by the heat treatment. They observed, however, that seeds oven-dried at 70° to 2 percent of moisture and subsequently irradiated (*14, p. 617*) "were clearly more, rather than less, sensitive to X-rays." They also reported (*15*) that seeds treated with heat for 4½ to 5½ hours at 50° and then irradiated gave smaller seedlings than similar lots given the same heat and X-ray treatments simultaneously or in the reverse order.

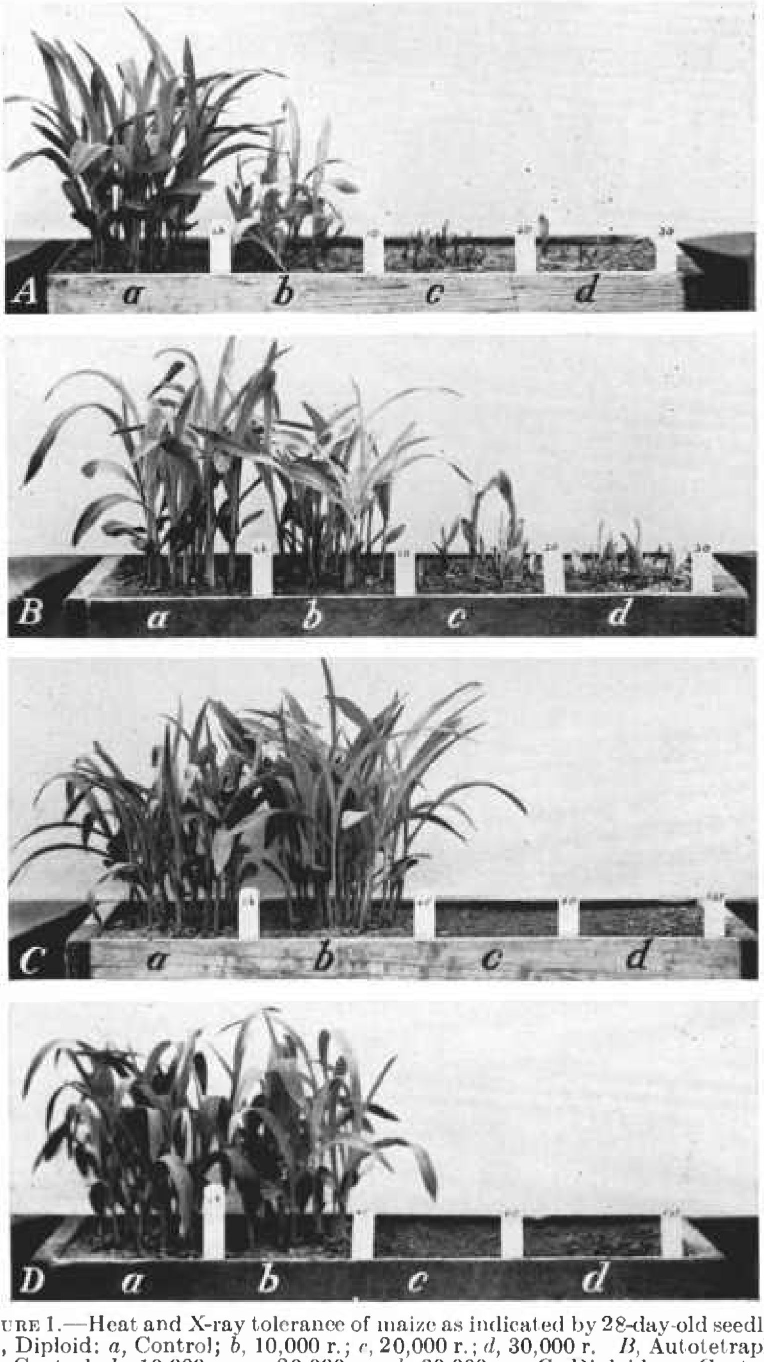

FIGURE 1.—Heat and X-ray tolerance of maize as indicated by 28-day-old seedlings. *A*, Diploid: *a*, Control; *b*, 10,000 r.; *c*, 20,000 r.; *d*, 30,000 r. *B*, Autotetraploid, *a*, Control: *b*, 10,000 r.; *c*, 20,000 r.; *d*, 30,000 r. *C*, Diploid: *a*, Control; *b*, 80° C. for 45 minutes; *c*, for 90 minutes; *d*, for 135 minutes. *D*, Autotetraploid: *a*, Control; *b*, 80° for 45 minutes; *c*, for 90 minutes; *d*, for 135 minutes.

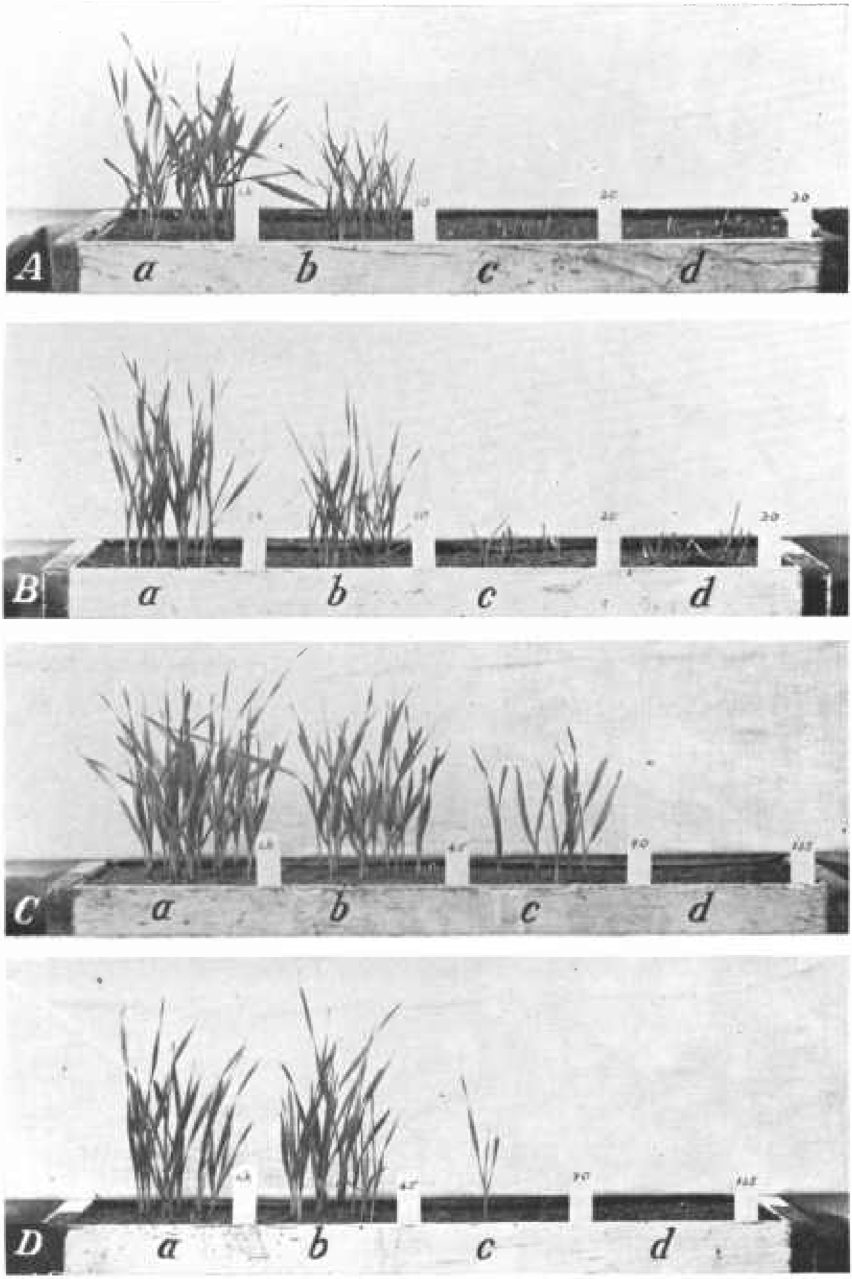

FIGURE 2.—Heat and X-ray tolerance of Rosen rye as indicated by 17-day-old seedlings. *A*, Diploid: *a*, Control; *b*, 10,000 r.; *c*, 20,000 r.; *d*, 30,000 r. *B*, Autotetraploid: *a*, Control; *b*, 10,000 r.; *c*, 20,000 r.; *d*, 30,000 r. *C*, Diploid: *a*, Control; *b*, 80° C. for 45 minutes; *c*, for 90 minutes; *d*, for 135 minutes. *D*, Autotetraploid: *a*, Control; *b*, 80° for 45 minutes; *c*, for 90 minutes; *d*, for 135 minutes.

For a study of this question, some seeds of diploid wheat (g c-2) and barley (Everest) were subjected to heat and X-ray treatments alone, while others were irradiated and then subjected to high temperatures and still others were given heat treatment and then irradiated. The second treatment followed the first immediately. The X-ray apparatus was operated only once for each experiment; so the irradiation was a constant.

The results of two experiments on wheat and one on barley are presented in table 5. It is apparent from the record of tests 1 and 2 shown in this table and from figure 3 that when the heat treatment was applied to seeds of the diploid wheat first, there was less injury from irradiation; whereas if the irradiation was applied first, the seeds were injured slightly more. This result was obtained in each of the treatments except the combinations with 18,000 r. (test 1). With this dose the seeds were so badly damaged that pretreatment with heat did not improve their growth.

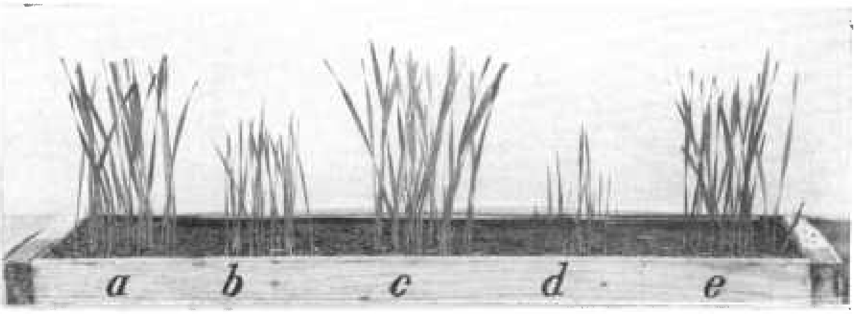

FIGURE 3.—Effect of combinations of heat and X-ray treatments on dormant seeds of diploid wheat as indicated by 11-day-old seedlings. *a,* Control; *b,* 8,000 r.; *c,* 80° C. for 60 minutes; *d,* 8,000 r. and 80° for 60 minutes; *e,* 80° for 60 minutes and 8,000 r.

Similar results from combination treatments were obtained when seeds of diploid barley were used (table 5, test 3), except that the benefit from pretreatment with heat was not apparent. This lack of benefit probably can be accounted for by the low survival. In this experiment an effort was made also to determine the relation of moisture and the time of the heat treatment to the degree of injury. For this purpose, in addition to the combinations tried on diploid wheat in the previous experiments, one lot of seed was treated with heat 2 days before the irradiation. Another lot was given the heat treatment in a saturated atmosphere, and still another lot was desiccated over calcium chloride for 2 days before irradiation. The seeds treated with heat 2 days before irradiation were injured slightly more than those treated immediately before irradiation, whereas those desiccated for 2 days survived about as well as those pretreated with heat. The seeds treated with heat in a saturated atmosphere did not survive.

An experiment similar to the one with barley was run with diploid wheat. For some reason the heat treatments (80° C. for 1 hour), including the treatment in a saturated atmosphere, killed all the

TABLE 5.—*Tolerance of dormant seeds of diploid wheat (g e–2) and diploid barley (Everest) to combinations of heat and X-ray treatments, as measured by germination and seedling height*

Test and treatment	Total seeds	Seeds germinating	Height of seedlings
	Number	*Percent* [1]	*Percent* [2]
No. 1 (diploid wheat, g e-2):			
Control	10	100	100
6,000 r	10	90	100
80° C. for 45 min	10	100	108
6,000 r. and 80° C. for 45 min	10	100	75
80° C. for 45 min. and 6,000 r	10	90	108
Control	10	100	100
12,000 r	10	70	4
80° C. for 45 min	10	100	100
12,000 r. and 80° C. for 45 min	10	70	2
80° C. for 45 min. and 12,000 r	10	100	46
Control	10	90	100
18,000 r	10	100	1
80° C. for 45 min	10	89	100
18,000 r. and 80° C. for 45 min	10	100	1
80° C. for 45 min. and 18,000 r	10	67	1
Control	10	100	100
12,000 r	10	60	4
80° C. for 60 min	10	100	93
12,000 r. and 80° C. for 60 min	10	20	1
80° C. for 60 min. and 12,000 r	10	100	43
No. 2 (diploid wheat, g e-2):			
Control	25	96	100
8,000 r	25	96	71
80° C. for 60 min	25	104	100
8,000 r. and 80° C. for 60 min	25	87	50
80° C. for 60 min. and 8,000 r	25	104	86
No. 3 (diploid barley, Everest):			
Control	50	58	100
10,000 r	50	45	36
80° C. for 60 min	25	88	73
10,000 r. and 80° C. for 60 min	50	34	23
80° C. for 60 min. and 10,000 r	25	34	36
80° C. for 60 min. 2 days before and 10,000 r	25	28	18
80° C. in saturated air for 60 min. and 10,000 r	25	0	0
Desiccated for 2 days over $CaCl_2$ and 10,000 r	25	21	36

[1] After 13 days for tests Nos. 1 and 2 and after 16 days for test No. 3; for untreated seeds, value indicates actual germination percentage; for treated seeds, percentage of the germination of the control.

[2] Percentage of control, after 20 days for tests Nos. 1 and 2 and after 16 days for test No. 3.

seeds, except the 25 given the heat treatment 3 days before irradiation, and only 10 of these survived, as compared with 24 for the control and the lots receiving X-rays only. Of a lot desiccated for 3 days and X-rayed, 14 survived. This lot was injured distinctly more than the seeds given X-rays only and less than the seeds given heat 3 days before irradiation.

These combination treatments were limited, but it is probable that (1) pretreatment with heat decreased the injury resulting from X-ray treatments; (2) when the order of the treatments was reversed (i. e., X-rays followed by heat), the seeds were injured more than by either treatment alone; and (3) seeds desiccated 2 or 3 days over calcium chloride before irradiation survived about as well as those pretreated with heat.

The effect of the treatments on the weight of the seeds was as follows: Everest barley kept in the desiccator for 2 days lost 0.7 percent of weight. Himalaya barley kept in the desiccator for 3 days lost 1.0 percent of weight. Everest barley and g e–2 wheat, given the usual corked-vial treatment for 1 hour at 80° C., lost 1.5 percent and 0.9 percent of weight, respectively.

This loss, presumably of moisture, from pretreatment with heat, may account for the greater tolerance of preheated seeds to irradiation. Stadler (*29*), Gelin (*9*), and others have shown that the amount of moisture in seeds has a profound effect on the degree of X-ray injury. However, that does not account for the greater injury when the heat treatment followed irradiation. Additional information is needed before a complete interpretation of the results can be made. This information should come from observations on the frequencies of chromosomal irregularities in root tips from seeds given heat or X-ray treatments and combinations of the two and from a more thorough study of the relation of time and moisture to the effects of combination treatments.

CHROMOSOMAL ABERRATIONS AFTER HEAT AND X-RAY TREATMENTS

FREQUENCY OF BRIDGES AS AFFECTED BY POLYPLOIDY

An effort was made to compare the effects of heat and X-rays on the frequency of chromatinic bridges at late anaphase and early telophase in root-tip cells. These bridges appear to be one of the most reliable indexes for comparison of the effects of treatments in relation to chromosome number.

For this study seeds of diploid, tetraploid, and hexaploid wheat and diploid and autotetraploid barley were used. The seeds were treated as in previous experiments, germinated on blotters in petri dishes at 12° C. for 36 hours, and then removed to a germinator kept at 26°. The root tips were fixed in Carnoy's fluid after 1 to 3 days. The length of the roots varied, but averaged 8 mm.

TABLE 6.—*Frequencies of chromatinic bridges after heat and X-ray treatments of dormant seeds*

Stock	Somatic chromosomes	Treatment	Total cells examined	Cells with indicated number of bridges							Bridges per cell
				0	1	2	3	4	5	6	
	Number		*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Diploid wheat	14	Control	30	28	1	1					0.10
		80° C. for 1 hour.	104	103		1					.02
		10,000 r	731	466	80	146	22	13	3	1	.70
Tetraploid wheat	28	Control	66	62	2	2					.09
		80° C. for 1 hour.	174	169	2	3					.05
		10,000 r	170	54	24	43	24	13	6	6	1.76
Hexaploid wheat	42	Control	79	76	2	1					.05
		80° C. for 1 hour.	175	168	1	4	2				.09
		10,000 r	121	40	13	23	14	11	9	11	2.12
Diploid barley	14	Control	119	114		3	1		1		.12
		80° C. for 1 hour.	422	408	3	7	4				.07
		10,000 r	84	49	6	19	4	4	2		.98
Autotetraploid barley.	28	Control	73	71	1		1				.05
		80° C. for 1 hour.	137	127	2	6	2				.15
		10,000 r	178	52	16	50	25	20	7	8	1.99

Here, again, there was a pronounced difference in the effects of heat and X-rays (table 6). X-rays produced a marked increase over the controls in the number of bridges. Heat, on the other hand, seemed to have little if any effect on the number of bridges. In the X-rayed material there was also a positive correlation between the number of bridges and the number of chromosomes. The ratios of the number of bridges per cell in the diploid, tetraploid, and hexaploid wheat were 1:2.5:3, and the ratio in the diploid barley as compared with the autotetraploid was 1:2 per cell. The data are not directly comparable with those of Fröier, Gelin, and Gustafsson (*7*), but they do indicate a similar relation.

FREQUENCY OF RECIPROCAL TRANSLOCATIONS AS AFFECTED BY POLYPLOIDY

Unfortunately, observations on the frequencies of reciprocal translocations resulting from a complete series of heat and X-ray treatments are not available. However, some observations were made on diploid and tetraploid wheat; these may indicate the probable results of such tests.

The effect of heat on the frequency of translocations in the diploid was determined from specimens of plants grown in the field from certain untreated and treated seeds mentioned in table 2. Data for the X-rayed stocks and for the untreated and heat-treated tetraploid were obtained from plants grown in the greenhouse. Seeds of 3 stocks (2 diploid, 1 tetraploid) were X-rayed simultaneously and planted immediately in soil. Because of limitations of space in the greenhouse, only 55 plants per stock of each treatment were grown. From these 55 plants, 2 or 3 of the first spikes were taken for cytological study. Cytological observations were limited to the 3 anthers from a single floret of any one spike. It was assumed (an assumption based on results of mutation studies from similar treatments) that in most cases a translocation in a given spike would not occur in the sector examined in another spike. That is, the 2 or 3 specimens from a plant would be practically as independent of each other, for purposes of determining translocation frequency, as if they had been taken from different plants. In a number of instances different configurations were noted in the pollen mother cells from the same floret. For example, a ring of 4 chromosomes appeared in some pollen mother cells and was absent in others in other parts of the same slide. This would further indicate that the sector containing any particular rearrangement induced by a treatment of dormant seeds is small.

From table 7 it is apparent that, unlike X-rays, heat had little effect on the production of translocations. Only 5 of 199 spikes from the heat-treated seeds of both diploid and tetraploid had translocations, as compared with none of 72 spikes from the controls. On the other hand, a relatively light dose of X-rays (5,000 r.) produced the equivalent of 0.2 ring of 4 chromosomes per spike in the diploid and about twice this frequency in the tetraploid. A dose of 10,000 r. increased the frequency of translocations in the diploid to 1.7 and in the tetraploid to 1.6 times that induced by 5,000 r.

There were chromosomal and meiotic disturbances other than translocations in some spikes grown from X-rayed seeds. In one spike of a diploid plant, meiosis was partially desynaptic (fig. 4, *A–C*), resembling gene-controlled irregularities in einkorn previously described by Smith (*26*) and by others in a number of other plants. Meiosis in another spike from the same plant was normal. Thus, the partial desynapsis was presumably due to a deficiency or dominant mutation.

TABLE 7.—*Frequencies of reciprocal translocations after heat and X-ray treatments of dormant seeds of diploid and tetraploid wheat*

Species and variety	Somatic chromosomes	Treatment	Spikes with indicated arrangement of chromosomes							
			Normal	R_4	$2R_4$	$3R_4$	R_6	$R_4 + R_6$	Total spikes	R_4 per spike [1]
	Number		*Number*	*Number* [2]	*Number* [2]	*Number*	*Number* [2]	*Number*	*Number*	*Number*
Triticum monococcum.	14	Control	46						46	0
		60° C. for 5 days	46						46	0
		60° C. for 10 days	30	3 (2)					33	.09
		65° C. for 15 days	40						40	0
		70° C. for 5 days	38	2					40	.05
		5,000 r	141	29 (26)	2		4		176	.22
		10,000 r	58	21 (16)	4		2		85	.38
Triticum durum var. Acme.	28	Control	26						26	0
		80° C. for 3 hours	40						40	0
		5,000 r	50	20 (19)	3		2	1	76	.41
		10,000 r	44	23 (22)	9 (8)	1	4 (3)	1	82	.64

[1] In calculating the rings of 4 per spike, a spike with a ring of 6 was considered as having 1.5 rings of 4, etc.

[2] The first number is the number of spikes examined which had the indicated rearrangement. The number in parentheses indicates the number of plants with the rearrangement. That is, in a number of instances more than 1 specimen from a plant had a particular rearrangement, but, as indicated in the text, the occurrence of a ring of 4, e. g., in different spikes from a plant, can probably be assumed to be unrelated.

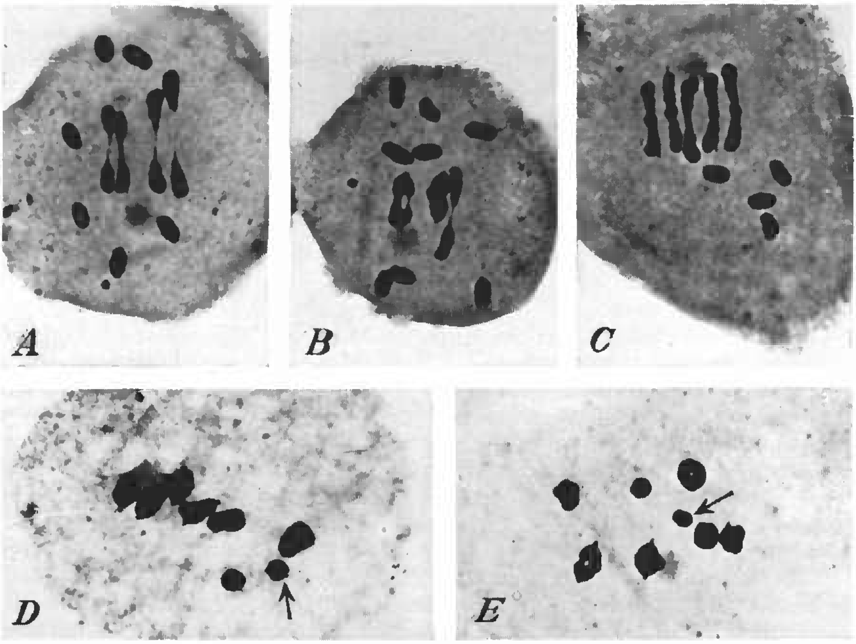

FIGURE 4.—*A–C*, Partial desynapsis at first meiotic metaphase in pollen mother cells of a spike on a diploid wheat plant grown from an X-rayed seed. *D* and *E*, Fragments (see arrows) at first meiotic metaphase in pollen mother cells from spikes of two different diploid wheat plants grown from X-rayed seeds. The fragments are not paired with their normal mates, which appear as univalents

Examination of meiosis in spikes from two other diploid plants revealed fragments (fig. 4, *D* and *E*). A deficiency of sufficient size to be visible in a condensed metaphase chromosome is rare in einkorn.

Since einkorn has only seven pairs of chromosomes, large deficiencies would be expected to be lethal, though large deficiencies are commonly observed in the polyploid species of wheat after irradiation.

In the tetraploid (*Triticum durum*) two slides revealed deficiencies for a whole chromosome and two for a large part of a chromosome.

MUTATION FREQUENCY AFTER HEAT AND X-RAY TREATMENTS

The comparison of frequencies of mutation was limited to diploid wheat. Data on the frequency of mutations after X-ray treatment of dormant seeds were accumulated during the 4 years from 1939 to 1942, inclusive. For comparison, head progenies of plants from some of the heat treatments included in table 2 were grown and examined for seedling mutants. From table 8 it is apparent that X-rays increased mutation frequency, although the treatment was not particularly heavy. Heat treatments, on the other hand, had little if any effect on the occurrence of mutants. The high natural mutation rate itself is noteworthy.

TABLE 8.—*Mutation frequency after heat and X-ray treatments of dormant seeds of diploid wheat*

Treatment	Head progenies	Seedlings per progeny	Mutants	Heads with mutants
	Number	*Number*	*Number*	*Percent*
Control	407	15.2	5	1.2
60° C. for 5 days	318	15.7	0	0
70° C. for 5 days	239	15.5	4	1.7
60° C. for 10 days	200	12.3	2	1.0
65° C. for 15 days	258	16.2	0	0
5,000 r	2,382	13.9	84	3.5
7,500 r	981	16.7	37	3.8

DISCUSSION

There were rather marked differences in the tolerance of a given stock in different experiments. Part of this variability may have been due to unavoidable changes in X-ray or heat apparatus, environmental conditions, and moisture content. These variations are not considered to be serious in comparisons between stocks, since in each experiment the seeds were handled together in order to make the tests as accurate and reliable as possible. That this was accomplished is shown by the fact that, though a stock varied in its response to a given treatment from test to test, its tolerance with relation to those of other stocks remained practically constant.

There was a tendency for the tolerance to heat to increase from fall through the winter. This was presumably associated with a decrease in moisture content of the seeds under the conditions of storage. Since the seeds had been harvested for several months prior to the treatments, the change in tolerance supposedly had little to do with postharvest dormancy. Kempton and Maxwell (*14*) and others have pointed out that there are important, uncontrolled factors that affect the degree of injury caused by X-rays. Two of these probably are moisture and temperature imposed after irradiation. In the present experiments environmental conditions during and after irradiation were made as nearly uniform as possible, and as the comparisons between stocks were repeated several and in some cases many times it is felt that the conclusions regarding the relative tolerance of the various stocks are entirely reliable and justified, though the tolerance in absolute values of time and intensity varied from experiment to experiment.

If age, heat, and X-rays produce similar effects in seeds and reduce viability by the same processes, then, other factors being equal, seeds of polyploid species should live longer and withstand more severe heat treatments than related diploids. There is ample evidence that they withstand heavier doses of X-rays. This greater tolerance of polyploids for X-rays has been attributed to the fact that a chromosomal derangement would not be as likely to be lethal in a polyploid as in a diploid.

In the cereals studied in these experiments, polyploidy was associated with greater tolerance to X-rays but with no greater tolerance to heat. Thus, heat and X-rays must have some injurious effects that are different. This was borne out by cytological observations on bridges in root-tip cells, which indicated that killing by heat was not due to chromosomal derangements, since the frequency of bridges from heat treatments was very low and not appreciably different from the frequency in root tips from untreated seeds. Also, from observations on meiosis in plants grown from treated seeds it was apparent that X-rays induced many more translocations than did heat treatments, which may have had no effect. Furthermore, rather severe heat treatments of seeds had little if any effect on mutation frequency, whereas after moderate X-ray treatments the frequency was markedly increased.

That certain effects produced in seeds by heat are different from those produced by X-rays was further indicated by the results from treating seeds first with X-rays and then with heat, and in the reverse order. If these treatments affected viability by disturbing the same processes, their injurious effects should have been more or less additive, regardless of the order in which the treatments were given. This was not the case. In this connection Nichols (*17*) found no interrelation between age and radiosensitivity. He stated (*17, p. 756*) "while both age and irradiation cause increases in the frequency of chromosomal aberrations, the causal mechanisms may be different."

There might be some question whether it is legitimate to compare the tetraploid and hexaploid species of wheat and oats with the diploid for the frequency of chromosomal effects, since in such a comparison it is assumed that the tetraploid has twice and the hexaploid three times as much chromatin as the diploid. However, from the measurements of Kagawa (*13*) and others on the length of the chromosomes in species of wheat, it would appear that for the purposes of this study the assumption is justified. Presumably the autotetraploids do have twice as much chromatin as the diploids.

Results obtained by others from heat treatments are somewhat different from those reported here. Heat treatments of germinating seeds have been observed by Peto (*18, 19*) and others to have pronounced effects on the number of chromosomal irregularities in root-tip cells. Similar increases in chromosomal irregularities from heat treatments on dry seeds of *Crepis tectorum* L. were reported by Shkvarnikov (*24*) and Shkvarnikov and Navashin (*25*). Also, Atanasoff and Johnson (*1*) noted that, after 30 hours at 100° C. in open containers, seeds of various cereals gave rise to plants that showed severe effects of treatment until maturity, whereas in the experiments reported here the plants recovered more or less completely from the initial effects of treatment.

The variance of the results obtained by different observers may indicate that there are critical conditions under which heat produces effects comparable in type and frequency with those of X-rays. Even if this were so it would still be true that there are significant differences between the mechanisms by which heat and X-rays produce their effects, because X-rays are not so limited as to conditions under which they are effective.

Thus, in the final analysis, the injurious effects of heat (and probably of age as well) seem to be mainly physiological. On the other hand, it appears that X-ray injury may be due at least in part to induced chromosomal aberrations, though in some instances physiological effects may assume the dominant role. That physiological effects of X-rays may be important was further indicated by preliminary experiments, which showed that considerably heavier doses of X-rays are tolerated by dormant seeds of diploid wheat and barley if the seeds are in a vacuum or in a nitrogen atmosphere during irradiation.[4] Presumably the physical effects of X-rays in producing genetic changes should be independent of the atmosphere, but it is evident that certain physiological effects are not.

The results of experiments reported herein have some bearing on evolutionary processes and plant-breeding problems. Apparently, high temperature readily affects viability but is a relatively weak agent for producing variations among which natural and artificial selection could operate. Short-wave radiation would be much more effective in producing genetic variations. X-radiation produces a higher frequency of genetic alterations in polyploid than in related diploid species, and polyploid species are able to survive more extreme changes, probably accounting in part for the greater variability observed in many polyploid species.

SUMMARY

Comparisons between the effects of heat and X-rays on dormant seeds of cereals gave the following results.

Diploids were as tolerant of high temperatures as polyploids, whether the duration of treatment was a few hours or several days. Polyploids showed greater tolerance to X-radiation. The stocks tested included diploid, tetraploid, and hexaploid wheat and oats; diploid and autotetraploid barley, maize, and rye; *Aegilops uniaristata* and an amphidiploid of *A. uniaristata* and diploid wheat.

Seeds of diploid wheat given heat treatments after irradiation were injured slightly more than those given irradiation only. Comparable seeds given heat treatments before irradiation were injured less than those given irradiation only. Data from such combination treat-

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[4] Unpublished data of the writer.

ments on diploid barley were not conclusive, but indicated similar results.

Heat had little if any effect on the frequency of chromatinic bridges in root-tip cells of diploid, tetraploid, and hexaploid wheat and diploid and autotetraploid barley. On the other hand, X-ray treatment (10,000 r.) of the same stocks resulted in a marked increase in the frequency of bridges in root-tip cells. In the diploid wheat there was 0.7 of a bridge per cell, as compared with 1.8 and 2.1 bridges in the tetraploid and hexaploid, respectively. In the diploid and autotetraploid barley there were 1.0 and 2.0 bridges per cell, respectively.

Heat treatment of seeds of diploid and tetraploid wheat had little if any effect on the frequency of translocations. On the other hand, X-ray treatments produced a marked increase in the frequency of translocations. After a treatment of 5,000 r. applied to dormant seeds of tetraploid wheat, there was 0.4 ring of four per spike, as compared with 0.2 per spike in the diploid given the same treatment. For 10,000 r. the corresponding values were 0.6 and 0.4.

Heat treatments of seeds of diploid wheat did not appreciably affect the mutation rate, whereas X-rays distinctly increased it.

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U. S. GOVERNMENT PRINTING OFFICE: 1946

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