GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. II. FILTERED RADIATIONS

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In treatments with filtered radiations the commercial arc described in the preceding paper was used in combination with various solution filters. In order to minimize heating effects and to increase the intensity of the radiation applied, a water cooling system was provided and the treating distance was reduced to 6.8 cm. The pollen, lying in a single layer on a glass slide, was placed in a brass "treatment cell" cooled by cold water circulating within its hollow walls. The treatment cell was covered by the "filter cell," a rectangular box of fused quartz, divided horizontally into two compartments each 1 cm. high. The lower compartment contained circulating distilled water for additional cooling and the upper contained the filter solution. The luminous portion of the arc was directly above the filter cell. Thus the radiation in all cases was filtered through 1 cm. of distilled water and about 3 mm. of fused quartz in addition to the solution filter used.

Preliminary trials with filtered radiations were made with a series of "long wave-pass" liquid filters, designed to detect gross differences in the effectiveness of broad spectral bands between 2500 and 3300 Å. Each of these filters absorbs all, or nearly all, radiation of wave-length shorter than a given value *a*, and passes with little absorption radiation of wave-length longer than a second value *b*, with a relatively narrow band of sharply decreasing absorption between *a* and *b*. The transmission characteristics of these filters and of the additional $HgCl_2$ filters mentioned below were determined by E. W. Landen, who will describe them in a separate report.

These trials were made with a multiple dominant stock and the "*a*-recessive" and "*c*-recessive" stocks described in the preceding paper. The results indicated that effectiveness in inducing endosperm deficiencies at all of the loci tested decreases sharply in the spectral region about 3000 Å. Numerous endosperm deficiencies occurred following treatment with radiation filtered through a solution of $HgCl_2$ which reduced sharply the intensity of λ 2804 and shorter wave-lengths but reduced only slightly the intensity of λ 2925 and longer wave-lengths. No endosperm deficiencies occurred (in populations of moderate size) following treatment with radiation filtered through a stronger solution of $HgCl_2$ which reduced sharply the intensity of λ 2967 and shorter wave-lengths while reducing only slightly that of λ 3130 and longer wave-lengths. This result showed also that the deficiencies induced by the effective treatments could not have been due to temperature effects, for the temperature of the pollen could not have differed materially in the treatments with these two filtered radiations.

More extensive trials were then made with three $HgCl_2$ solution filters, two similar to those used in the preliminary trial and the other intermediate in absorption. The filtered radiations are referred to below as radiations *A*, *B* and *C*. The spectral distribution of energy in the three radiations was determined by passing each radiation through a spectrograph and measuring the relative intensity line by line by means of a quartz-enclosed caesium oxide photocell of the type described by Young and Pierce.[1] The concentration of the filter solutions and the spectral distribution of energy in the filtered radiations are shown in table 1.

Treatments were made with the three filtered radiations with exposure periods of 1 to 32 minutes. The length of treatment tolerated by the pollen varies widely under different conditions of temperature and humidity, but under similar conditions there were consistent differences in tolerance of the three radiations. The maximum period of exposure tolerated with radiation *C* was ordinarily 4 to 8 times as long as that with radiation *A*, and was not consistently shorter than the period of delay in pollination tolerated by untreated pollen under similar conditions. Tolerance of radiation *B* was only slightly less than that of radiation *C*.

The endosperm constitution of the pollen parent in these trials was *A A Pr Pr;* that of the seed parent *a a Pr pr*. The results therefore show the frequency of loss of *A* and one-half the frequency of loss of *Pr*. The frequency of germless seeds under the three treatments was also determined. The results are presented in table 2.

The frequency of entire endosperm deficiencies for *A* and *Pr* is approximately equal, while the frequency of fractional deficiencies is very much higher for *A* than for *Pr*. Both entire and fractional endosperm deficiencies are materially increased in frequency by the treatment of the pollen. Radiation *A* is distinctly more effective than *C*, as measured by the frequency of entire or fractional endosperm deficiencies for either *A* or *Pr*. Radiation *B* is intermediate in effectiveness.

The frequency of germless seeds is increased slightly by treatment with radiation *A*, distinctly less by radiation *B* and not at all by radiation *C*. With x-ray treatment of pollen the frequency of germless seeds is much higher, and is usually considerably higher than the frequency of deficiencies marked by any one gene.

In comparing the three radiations, it is convenient to use a single figure expressing the effectiveness of the radiation in producing both entire and fractional deficiencies of both *A* and *Pr*. The measure used is computed as follows: From the numbers of entire and fractional deficiencies for *A* and *Pr* observed under each treatment are deducted the numbers expected in control populations of the same size. The remaining deficiencies, presumably those induced by the treatment, are combined by adding the total number of entire deficiencies and half the total number of fractionals. In this trial, as in that previously reported, the induced fractionals were chiefly of the "$^1/_2$" class, with about equal numbers of larger and smaller size. Thus calculated, the yield of induced deficiencies per minute of exposure for filtered radiations *A*, *B* and *C* is 1.99, 1.25 and 0.24%, respectively. These percentages represent only a small part of the deficiencies induced by the treatment, since only the deficiencies marked by one gene and half the deficiencies marked by another gene are included. They are, however, strictly comparable for the radiations compared.

TABLE 1

SPECTRAL DISTRIBUTION OF ENERGY IN FILTERED RADIATIONS
(Data of E. W. Landen)

WAVE LENGTH Å	UNFILTERED	FILTERED RADIATIONS[1] A	B	C
3654	105.5	74.7	74.4	74.1
3341	10.5	6.95	7.13	6.79
3130	100	61.9	58.6	53.5
3022	44.1	25.2	21.7	9.87
2967	22.7	11.4	7.88	0.772
2925	2.22	1.04	0.420	0.004
2894	6.90	2.48	0.470	..
2804	11.0	0.465	.0033	..
2752	3.08	0.017	..	..
2699	3.47	..	..	..
2652	15.72	..	..	..
2602	2.07	..	..	..
2576	2.06	..	..	..
2537	24.8	..	..	..
2482	3.90	..	..	..
2463	0.60	..	..	..
2447	0.54	..	..	..
2399	1.47	..	..	..
2378	1.28	..	..	..
2352	0.81	..	..	..
2323	0.43	..	..	..
2300	0.63	..	..	..

[1] Radiations were filtered through 1 cm. of distilled water and 3 mm. of fused quartz plus 1 cm. of filter solution as follows: (A) 30 g. $HgCl_2$ + 15.4 cc. HCl per liter, (B) 90 g. $HgCl_2$ + 46.2 cc. HCl per liter and (C) 270 g. $HgCl_2$ + 140 cc. HCl per liter. Energy measurements are in arbitrary units.

Since the filtered radiations differ so widely in effectiveness, it is clear that λ 3130 and longer wave-lengths are relatively ineffective, for the energy in this region of the spectrum was relatively high in radiation *C* as well as in radiations *A* and *B*. Such differences as are found must be ascribable in the main to differences in the intensity of the shorter wave-lengths. If all wave-lengths shorter than λ 3130 are equally effective, and λ 3130 and longer wave-lengths are wholly ineffective, the total effective energy in radiations *A*, *B* and *C* is approximately in the ratio 4:3:1. This is a considerable departure from the observed ratio of induced deficiencies, namely, 8:5:1. The fit may be materially improved by ascribing reduced effectiveness to λ 3022. For example, on the assumption that λ 3022 is only one-sixth as effective as shorter wave-lengths, the ratio is modified to 8:5:1. If, on the other hand, it is assumed that λ 3022 is entirely ineffective, the ratio of effective energy is approximately 20:11:1; and on the plausible assumption that λ 2804 and shorter wave-lengths are more effective than λ 2967, the fit becomes even poorer. It is therefore necessary to ascribe some effectiveness to λ 3022, although it is probable that its effectiveness is lower than that of shorter wave-lengths.

TABLE 2

EFFECT OF VARIOUS FILTERED ULTRA-VIOLET RADIATIONS ON FREQUENCY OF ENDOSPERM DEFICIENCIES

$HgCl_2$ FILTER	TIME, MIN.	NUMBER SEEDS EXAMINED	DEFICIENCIES OBSERVED								NUMBER GERMLESS SEEDS
			ENTIRE				FRACTIONAL				
			A	PR	TOTAL	%	A	PR	TOTAL	%	
A (30 g./l.)	1	797	6	2	8	1.0	22	1	23	2.9	11
	2	1247	18	13	31	2.5	73	9	81	6.5	9
	4	717	20	15	35	4.9	46	11	57	7.9	13
	8	218	10	1	11		18	3	21		8
	16	28	1	0	1		1	0	1		4
Mean yield per min. exposure:						1.03				2.20	
B (90 g./l.)	2	1614	29	15	44	2.7	50	7	57	3.5	7
	4	1100	24	10	34	3.1	50	11	61	5.5	2
	8	861	27	7	34	3.9	65	10	75	8.7	10
	16	65	1	0	1		7	1	8		2
	32	16	0	1	1		1	0	1		1
Mean yield per min. exposure:						0.71				1.26	
C (270 g./l.)	2	950	0	0	0	0.0	5	1	6	0.6	1
	4	1620	6	5	11	0.7	24	6	30	1.9	0
	8	903	6	3	9	1.0	15	2	17	1.9	5
	16	848	12	5	17	2.0	43	7	50	5.9	0
	32	128	2	2	4		6	2	8		4
Mean yield per min. exposure:						0.12				0.33	
Control	..	1941	1	2	3	0.15	7	2	9	0.46	5

The indicated differences in the effectiveness of various wave-lengths are not necessarily due to differences in the reaction of the cromosomes to the wave-lengths concerned. They are due in part, possibly in large part, to differences in the penetration of the different wave-lengths through the overlying non-nuclear material. The sperm nucleus, within which the chromosomal alterations are induced, is embedded in a mass of cytoplasm and cytoplasmic inclusions which consitutes the bulk of the pollen grain. The spectral distribution of energy in the radiations applied, as shown in table 1, represents the composition of the radiation incident at the surface of the pollen grain. The spectral constitution of the radiation reaching the sperm nucleus may be very different. Until the absorption characteristics of the various constituents of the pollen grain are more definitely known, no accurate comparison of the direct effects of different wave-lengths upon the chromosomes can be made.

The apparent effectiveness of λ 3022 and λ 2967 is of interest from an evolutionary viewpoint, since wave-lengths in this region are sometimes included in low intensity in summer sunlight. Under natural conditions the pollen grain may be exposed to sunlight for a brief period just preceding and just following pollination. Genetic changes induced by the ultra-violet constituent of sunlight would be included in the "natural" mutation rate. Data are not available for estimating the total intensity of radiations of genetically effective wave-length to which the pollen may be exposed under natural conditions of pollination; nor is the natural frequency of endosperm deficiencies, gametophytic lethals and mutations under conditions of open pollination accurately known. However, the controls in these experiments, which yielded several endosperm deficiencies, gametophytic lethals and mutations, were produced by the use of pollen shed indoors and shielded from sunlight before and after pollination. It is clear, therefore, that exposure to sunlight at this period is not the sole cause of naturally occurring deficiencies and mutations.

Summary.—1. Results with filtered radiations applied to maize pollen indicate that λ 3130 and longer wave-lengths are relatively ineffective in inducing deficiency, and that λ 3022 and shorter wave-lengths are effective. λ 3022 is probably less effective than shorter wave-lengths.

2. Although radiation of effective wave-length is sometimes present in low intensity in sunlight, the occurrence of various genetic alterations in the controls (shielded from sunlight) shows that exposure of the pollen to sunlight is not the sole cause of naturally occurring deficiencies and mutations.

3. The frequency of entire endosperm deficiencies of *A* and *Pr* induced by the ultra-violet radiations applied is approximately equal. The frequency of induced fractional endosperm deficiencies is much higher for *A* than for *Pr*.

4. The frequency of germless seeds induced by the ultra-violet radiations applied is low, and decreases with decreasing relative intensity of the shorter wave-lengths.

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[1] Young and Pierce, *Jour. Opt. Soc. Am.*, **21**, 497–501 (1931).