# GENETIC EFFECTS OF ULTRAVIOLET RADIATION IN MAIZE. IV. COMPARISON OF MONOCHROMATIC RADIATIONS[1]

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## INTRODUCTION

EARLIER investigations with various heterochromatic radiations (STADLER and SPRAGUE 1936b, c) indicated that λ3131 Å and longer wave lengths were genetically ineffective or nearly so, while λ3022 and shorter wave lengths were effective but with pronounced differences in efficiency.

The measure of genetic effectiveness in these experiments was the frequency of endosperm deficiencies. Since these deficiencies may be identified in the seeds which develop directly from the treated pollen, they provide a convenient measure of genetic effectiveness and make it possible to compare wave lengths and doses on the extensive scale required for significant quantitative comparisons.

The high frequency of coincidence of genetically independent mutations (STADLER and SPRAGUE 1936a) suggested that, although the pollen grains were in a single layer during irradiation, there were considerable differences in the dose applied to the gametic nuclei in the different pollen grains treated. Such differences would be expected if the radiation is highly absorbed in the overlying cytoplasm, since the gametic nuclei are eccentrically located and casual orientation would place them at different levels in different pollen grains.

This heterogeneity of the treated sample, owing to variation in internal filtration among the pollen grains, could result in a serious error in the determination of relative effects of different wave lengths or of different doses at the same wave length. In an investigation of the frequency of occurrence of deficiency for a specific region, for example, it would tend to reduce the relative frequency of effects of larger doses, for the probability of affecting pollen grains with the more favorably oriented nuclei would be greatest for the first unit of dosage and, as these individuals were affected, the probability of successful hits in later increments of dosage would become steadily smaller. The resulting "levelling" of the dosage curve would be expected to vary in amount at different wave lengths, depending upon the rate at which the radiation is absorbed in the overlying material. A

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TABLE 1

*Preliminary comparisons of embryo abortion and endosperm deficiency produced by monochromatic ultraviolet radiation. (Stadler and Uber 1938)*

| WAVE LENGTH Å | DOSE IN $10^3$ ERGS/MM$^2$ | EMBRYO ABORTION | | ENDOSPERM DEFICIENCY (TOTAL FOR *A*, *Pr*, *Su*, *Wx*) | | |
|---|---|---|---|---|---|---|
| | | NUMBER TESTED | NUMBER ABORTED | NUMBER TESTED | DEFICIENT ENDOSPERMS | |
| | | | | | NUMBER | %±S.E. |
| 2352 | 1.0 | 530 | 5 | 468 | 11 | 2.4±0.7 |
| | 2.1 | 429 | 0 | 377 | 22 | 5.8±1.2 |
| 2483 | 0.6 | 584 | 2 | 417 | 14 | 3.4±0.9 |
| | 4.0 | 165 | 3 | 144 | 16 | 11.1±2.6 |
| 2652 | 0.9 | 646 | 12 | 625 | 49 | 7.8±1.1 |
| | 1.9 | 255 | 7 | 223 | 27 | 12.1±2.2 |
| | 2.8 | 264 | 8 | 231 | 32 | 13.9±2.3 |
| | 6.2 | 255 | 19 | 223 | 42 | 18.8±2.6 |
| | 12 | 81 | 15 | 71 | 13 | 18.3±4.6 |
| 2804 | 0.7 | 791 | 3 | 663 | 40 | 6.0±0.9 |
| | 1.5 | 293 | 3 | 257 | 27 | 10.5±1.9 |
| | 2.1 | 315 | 9 | 262 | 33 | 12.6±2.1 |
| | 4.5 | 332 | 31 | 252 | 54 | 21.4±2.6 |
| | 9.0 | 168 | 7 | 147 | 49 | 33.3±3.9 |
| 2967 | 0.8 | 105 | 0 | 92 | 1 | 1.1±1.1 |
| | 1.7 | 290 | 0 | 272 | 10 | 3.7±1.1 |
| | 2.5 | 253 | 1 | 222 | 22 | 9.9±2.0 |
| | 5.5 | 622 | 8 | 500 | 119 | 23.8±1.9 |
| | 11 | 158 | 2 | 138 | 47 | 34.1±4.0 |
| 3022 | 2.6 | 321 | 2 | 281 | 3 | 1.1±0.6 |
| | 5.9 | 92 | 2 | 88 | 5 | 5.7±2.5 |
| | 57 | 266 | 8 | 228 | 56 | 24.6±2.9 |
| 3131 | 2.7 | 331 | 3 | 290 | 2 | 0.7±0.5 |
| | 5.9 | 100 | 0 | 100 | 0 | 0 |
| | 60 | 490 | 3 | 399 | 3 | 0.8±0.4 |
| | 600 | 440 | 2 | 392 | 45 | 11.5±1.6 |
| Control | | 614 | 2 | 536 | 6 | 1.1±0.5 |

comparison of the relative effectiveness of different wave lengths, at doses equal in the energy incident at the surface of the pollen grain, therefore might yield different results when different doses were used as the basis of comparison.

Preliminary comparisons of monochromatic radiations were made in 1937 (STADLER and UBER 1938). Since the experimental data were not included in the published abstract, they are now presented in table 1.

These trials, while showing large differences in the effect of different wave lengths upon the frequency of endosperm deficiency, indicated also that the dosage curves at the different wave lengths were widely different. A comparison of the deficiency rates at the smallest doses used shows that the most effective wave lengths were λ2652 and λ2804, with lowered effectiveness on the short wave length side of this region and much lowered effectiveness on the long wave length side. λ3131 was ineffective at doses comparable to those used for the other wave lengths, but by using a very much larger dose tolerated at this wave length, it was possible to produce appreciable effects.

It was evident from these trials that the determination of wave length effectiveness would require closer study of the dosage relation and of internal filtration in the pollen grain. Spectrophotometric studies of absorption in the pollen grain wall and contents were made (UBER 1939), and a more extensive determination of genetic effects was undertaken. These experiments were carried on in 1938–1940 and are the subject of this report.

## REVIEW OF LITERATURE

This brief review of relevant papers is limited to researches dealing with genetic effects of monochromatic radiations.

NOETHLING and STUBBE (1934, 1936; see also STUBBE and NOETHLING 1936) determined the frequency of mutation in *Antirrhinum majus* following exposure of the pollen to various monochromatic radiations. Since the occurrence of mutation in each treated pollen grain must be determined by segregation of the mutant in an $F_2$ culture, the numbers involved in individual comparisons were necessarily limited. An extensive study was made, involving mutation tests for more than 3000 treated pollen grains, and the identification of more than 100 mutations. The spontaneous mutation rate in the standard line used was 1.6 percent. Significant increases in mutation frequency were obtained by treatment with λ2652, λ2967, λ3022, and λ3131, the highest rates observed being about four times the control frequency.

Several doses were compared at λ2652 and λ2967. At λ2652 doses of 2 M (where M represents $10^3$ ergs/mm$^2$) failed to increase the mutation rate significantly. Larger doses resulted in significant increases in mutation frequency, but the mutation rate increased very slowly with increase in dose. For example, at a dose of 2.7 M the mutation rate was approximately twice that of the control; at doses of 14.6 and 50.3 M it was about three times the control rate. At λ2967 all four doses applied yielded significant increases over the spontaneous mutation rate, but though the doses covered a wide range (1.8 M–65.0 M), there were no significant differences in the mutation

rates observed. λ3131 at a dose of 120 M approximately quadrupled the spontaneous rate, as did λ3022 at a dose of 65 M.

From these results NOETHLING and STUBBE conclude that λ2967 is more effective than λ2652 or λ3131 and that the maximum of genetic effectiveness is at approximately 3000 Å. The transmission of ultraviolet radiation through a pollen suspension in 30 percent alcohol showed a minimum at 3000 Å and was considered to be in good agreement with the data on genetic effectiveness.

KNAPP, REUSS, RISSE, and SCHREIBER (1939) compared the effectiveness of six ultraviolet wave lengths, ranging from λ2536 to λ3131, in inducing mutation in *Sphaerocarpus Donnellii*. This material is especially favorable since the radiation may be applied to the spermatozoid, which is an almost naked nucleus about 0.5μ in thickness. The spermatozoids were irradiated in water suspension, and the mutations were identified by tetrad analysis of the sporogonia of $F_1$ individuals. The technic has been described by KNAPP (1937). By the analysis of 50 to 75 individuals from each treatment, it was possible to show sharp differences in genetic effectiveness of the wave lengths applied. A uniform dose of $2 \times 10^3$ ergs/mm$^2$ was used. No mutations were found in the control or in the series irradiated at 3131 Å; a few occurred at 3022 Å and 2967 Å; but mutations were frequent at 2804 Å, 2652 Å; and 2536 Å. The highest frequency occurred at 2652 Å, although the significance of the differences between the mutation frequencies observed at the shorter wave lengths is perhaps questionable.

EMMONS and HOLLAENDER (1939) have investigated the effectiveness of monochromatic radiation in inducing cultural variations in the fungus *Trichophyton mentagrophytes*. Such variations occur frequently without treatment in old cultures but are not found in young cultures. By the application of ultraviolet radiation or various other treatments, variants may be induced in young cultures. The variants, whether spontaneous or induced, are permanent through numerous transfers. Unfortunately the fungus has no known sexual stage, and it is therefore impossible to investigate the genetic nature of the variants. The treatments were applied to spores in suspension. Wave lengths differed in lethal effect and in the frequency of induced variants. With increasing dose, the percentage of variants among the surviving individuals rose to a maximum and then declined. This is a phenomenon not previously found in genetic experiments with ultraviolet radiation and one which suggests the possibility that the induced changes involved in the production of variants and in the production of lethal effects are directly related. In general, λ2536 and λ2652 were most effective in killing and in the production of variants. λ2950 was distinctly less effective than shorter wave lengths.

## EXPERIMENTAL TECHNIC

### *Apparatus*

The mercury discharge tube, to which several references are made later, was a low-pressure type (Hanovia Sc-2537) operating at 5000 volts and 100 milliamperes. It emitted primarily the 2536 Å line of mercury (STADLER and SPRAGUE 1936c) and was used without any filter except a quartz microscope slide which covered the pollen.

For the greater part of the pollen treatments, monochromatic radiation was obtained with the aid of a large crystal quartz monochromator, described in detail elsewhere by UBER and JACOBSOHN (1938), which was designed specifically for the irradiation of pollen. To this end, it had a vertical disposition so that the pollen to be treated would lie on the horizontal surfaces provided by trays (2 mm. × 50 mm. in area) at each ultraviolet emission line of the mercury arc. The arrangement of the individual trays along the spectrum was such that any tray could be inserted or removed independently of the others.

The mercury arc source for the monochromator was of the water-cooled capillary type and operated at atmospheric pressure. Its design and characteristic features have been discussed elsewhere (UBER 1940). Since the ultraviolet energy output of an arc would usually decrease to about one-half of its initial value after three hours of continuous operation, it was customary to replace it with a new or reconditioned one at that point. An experimental check on the relative intensity of λ2536 compared to λ3131 did not reveal any appreciable variation due to the decline in output of the arc over the time interval indicated.

The surface-type vacuum thermopile used to measure the radiation was calibrated with a standard carbon-filament lamp (C-241) obtained from the U. S. Bureau of Standards. The radiation has been measured in ergs per square millimeter of the treatment surface, but for convenience in presentation, the dose is usually stated in M units where 1 M represents $10^3$ ergs/mm$^2$.

### *Physical Measurements*

For the purpose of measuring the dosage rate during treatment, a slot beneath the λ3131 tray permitted the radiation to pass through to the thermopile. All doses were based on energy measurements at λ3131, the values for other lines being computed from relative intensity data. Table 2 gives the wave lengths employed, their relative intensities with respect to λ3131, and average values for the dosage rates in ergs/mm$^2$ per minute. The lengths of time required for administering the various doses varied from around 15 seconds up to 30 minutes. In order to make the exposure times susceptible to accurate measurement for the smallest doses at some

of the lines, an iris diaphragm was placed in the collimating tube of the monochromator; in that case the dosage rate was reduced to about 12 percent of the values shown in Table 2.

Thermopile readings were taken at ten to 15 minute intervals as a basis for calculation of the doses actually applied. In making the treatments, the time required for the desired dosage was estimated from the previous intensity readings, and the dose actually applied in each treatment was calculated later from the completed intensity curve. The treatments aver-

TABLE 2

*Characteristics of the monochromator radiation.*

| λ | INTENSITY RELATIVE TO λ3131 | AVERAGE DOSAGE RATE | IMPURITY OF OTHER LINES AT λ3131 | |
|---|---|---|---|---|
| | | | WITHOUT FILTER | WITH FILTER* |
| Å | | ergs/mm²/minute | Percent | Percent |
| 3131 | 1.000 | 30,000 | | |
| 3022 | .506 | 15,000 | 0.100 | .0210 |
| 2967 | .256 | 7,500 | .050 | <.0074 |
| 2925 | .033 | 990 | .003 | trace |
| 2894 | .086 | 2,580 | .020 | |
| 2804 | .167 | 5,010 | .030 | |
| 2752 | .071 | 2,130 | .015 | |
| 2699 | .065 | 1,950 | .008 | |
| 2652 | .351 | 10,530 | .050 | |
| 2536 | .274 | 8,220 | .040 | |
| 2483 | .103 | 3,090 | .010 | |
| 2399 | | | .003 | |
| 2378 | .0487 | 1,460 | .003 | |
| 2352 | | | .001 | |
| | | | 0.333 | <0.025 |

* Corning #014, clear blue fluorescing glass filter, one mm thick.

aged in a single figure therefore may include doses varying as much as ten percent from the average. The doses as stated in the tables are the unweighted mean of the actual doses applied in the treatments averaged.

In practice, the uniformity of the ultraviolet illumination over the area of a spectral line covered by the tray was checked visually with a fluorescent screen while aligning the arc source. Tests of the uniformity made with a Geiger-Mueller photoelectric counter showed that in going 1.75 cm on either side of the center along the five cm trays, there was a reduction in intensity of three percent compared to the intensity at the center; at the ends of the trays, the corresponding reduction in intensity was ten percent.

Purity of the radiation is an important factor, especially at the longer

wave lengths which are relatively ineffective genetically. By analyzing the radiation photographically with the aid of a medium Hilger quartz spectrograph and suitable filters, approximate estimates of spectral impurity have been obtained. These values for λ3131 are given in the fourth column of table 2 and include only the ultraviolet impurities of wave lengths less than 3131 Å, longer radiation not being appreciably effective genetically. It will be noted that the measured impurity at λ3131 owing to the radiation of all other important mercury lines in the ultraviolet region is around one-third of one percent. To a rough approximation, the percentage of impurity at the other wave lengths is inversely proportional to their relative intensity. At λ2652, for example, which is about one-third as intense as λ3131, the total impurity would be about one percent. For very weak lines, the impurity may become a serious factor.

The total impurity at λ3131 could be reduced to less than one part in 4,000 by the use of a short wave length cut-off filter consisting of a one mm thick Corning #014 glass plate, whose transmission at λ3131 was 58 percent.

### *Treatment Routine*

The pollen was irradiated in an insulated field house provided with mechanical refrigeration. Thus the treatment room could be air-conditioned to the extent that the temperature remained within the range 20–23°C, and the relative humidity varied from 45–60 percent. These conditions contrasted with outside temperatures as high as 37°C and with much lower relative humidity readings.

The treatments were made in the morning beginning as early as pollen-shedding conditions would permit (6:30 to 8:30 a.m.) and continued until about noon. Tassel branches bearing mature anthers were removed from the parent plant when the anthers began to dehisce, wrapped in moist paper towelling, and held in the treatment room for use as needed. The pollen was poured directly from the anther to the slide for treatment, the spheroidal grains readily rolling into place in a single layer.

No accurate comparisons to determine killing effects of treatments have been made, but large differences in tolerance for the wave lengths compared are obvious. In general the longer wave lengths were tolerated at much larger doses than the shorter. In spite of the uniform conditions of temperature and humidity during treatment, there was a pronounced loss of tolerance when the weather became hot and dry. This might have been due to changes in the condition of the pollen at shedding or in the conditions of pollen germination or pollen tube growth. The most favorable period for treatment at Columbia is the early part of the pollen-shedding season (about June 20 to July 5). Under optimum weather conditions, the maxi-

mum tolerated dose at the shortest wave lengths (2352–2399 Å) was about 2 M. At λ2483–λ2804 much larger doses were tolerated, and a small set of seed was sometimes obtained with treatments of 16–32 M. At λ2967 under similar conditions a dose of 64 M could be applied, and at λ3022 a dose of 256 M. At λ3131 doses of more than 1,000 M have been applied without apparent injury to the pollen. With less favorable weather, tolerance was reduced, but relative tolerance at the different wave lengths was not greatly changed. Under the hot dry conditions characteristic of late July and early August in this region, tolerance is so greatly reduced that experiments with ultraviolet treatment are not practicable.

As in previous experiments, the general method followed was the pollination of a multiple recessive stock by a multiple dominant. Since fractional endosperm deficiencies for the genes *Y* and *Wx* cannot be detected with certainty in all cases when the aleurone is colored, the comparisons in this study were based entirely on deficiencies for the genes *A* (colored vs. colorless aleurone), *Pr* (purple vs. red aleurone), and *Su* (smooth vs. wrinkled endosperm).

The seed parent stock (*R*$^{g}$ *C a pr su y wx b pl*) was grown from several closely related ears, with successive plantings so as to provide a supply of ears through the period of favorable weather. The pollen parent stock was an inbred strain dominant for the genes mentioned. The pollen used for each day's treatment was obtained from a single plant, and the same plants were used so far as possible for treatment on successive days.

### *Classification of Effects*

The effects recorded for the treatments compared in the study are "endosperm deficiencies" and "embryo abortions." The term endosperm deficiency is applied to all losses of effect of the dominant marker genes *A*, *Pr*, and *Su*. It is possible that some of these losses result from mutation rather than deficiency, but comparable trials with the linked genes *C*. *Sh*, and *Wx* indicate that the great majority if not all of the cases represent the loss of a chromosome segment.

In earlier experiments each fractional deficiency was classified as to the area involved. Since the proportion of fractionals and the distribution of fractionals in the various size classes in the earlier experiments were found to be independent of dosage and wave length, the deficiencies in the present experiment have been classed merely as "entire" and "fractional." As previously reported, the most frequent type of fractional is that in which the deficient sector includes about one-half of the endosperm.

The subjective element in the determination of the frequency of genetic alterations is at a minimum in the case of these endosperm deficiencies, since the three dominant markers used are usually quite clear in effect.

The loss of *A* is always unmistakable, and in the stocks used *Su*-loss is almost equally distinct. In occasional ears the difference due to *Pr*-loss is faint in some seeds. A few such ears occurred in the present experiment, and in these the *Pr* data are excluded and the number of seeds examined is taken as $\frac{2}{3}$ of the actual number. Since the average frequency of *Pr* loss is lower than that of *A* loss, this introduces a slight error in comparisons. Only 15 of the 369 ears examined in the experiment were affected in this way. Since these were distributed at random among the treatments compared, the error is negligible.

Loss of *Pr* cannot be detected in a seed or sector which is also deficient for *A*. A fractional deficiency for *A* would be recognizable in a seed entirely deficient for *Pr*, but the resulting endosperm would have one sector colorless and the other red, a type which could be produced also by a fractional deficiency for *A* with a complementary fractional deficiency for *Pr*. It was noted that in the majority of endosperms with complementary colorless and red sectors, the aleurone cells along the margin separating the sectors were purple, while in other endosperms of this class there was no appearance of purple pigmentation in the marginal cells. The ratio of the two classes was approximately the same as the ratio of fractional to entire *Pr* deficiencies among the seeds without deficiency of *A*. It is therefore assumed that the seeds of the first class described represent fractional deficiencies for both *Pr* and *A*, while those of the second class represent entire deficiencies for *Pr* with fractional deficiency for *A*. Presumably the development of the purple phenotype (*A Pr*) occurs in the marginal cells as a result of the interaction of adjacent cells of *A pr* and *a Pr*.

Coincident losses of *A* and *Su* or of *Pr* and *Su* are detectable in all combinations. The coincidences observed include cases of entire deficiency for both markers concerned, cases of entire deficiency for one with fractional deficiency for the other, and cases of fractional deficiency for both, which may involve the same sector or different sectors. In almost all cases, when two deficient sectors occur in the same seed, the two together cover the entire endosperm, even though the size of the two sectors may be very different. This supports the view that the fractionals of the size classes larger or smaller than "$\frac{1}{2}$" are in the main the result of unequal development of sectors, and that the fractional deficiencies in general represent endosperms which at a two-cell stage had one deficient and one non-deficient cell.

Coincident loss of *A* and *Su*, or of all three markers, involving the entire endosperm, results in a colorless, sugary endosperm which is phenotypically indistinguishable from an endosperm produced by pollen contamination from the multiple recessive stock. Although the seed parent cultures were regularly detasseled before pollination, there is always a possibility of occasional contamination from neighboring plots or from tassels

or florets missed in detasseling. Genetic tests of several seeds of this type were made, and some were found to be the result of pollen contamination. All seeds of this type have therefore been excluded from the data. This may result in the exclusion of some cases of induced deficiency, and the error might affect disproportionately the more effective treatments. However the total number of seeds excluded as possible contaminations was only 17, among a total of 35,086 seeds examined and 2,775 deficiencies identified.

The number of coincident deficiencies included in any of the deficiency counts may be determined from the tables by comparison of the data given for "number of deficient seeds" and "number of deficiencies."

The term "embryo abortion" is applied to those seeds in which a normally developed embryo is not produced. In external appearance the seed seems to lack an embryo, although usually the endosperm is fully developed. On dissection it is often possible to find dead rudiments of the embryo. This is the type of seed usually designated as "germless."

A small proportion of germless seeds is found in many varieties of maize, and though they are more common in some strains than in others, there is no strain known to us in which a germless seed is not occasionally found. Among inbred strains distinct differences in the frequency of germless seeds appear, but according to WENTZ (1930) the inheritance of these differences is usually complex. In a few cases simple recessive factors for germless seed have been identified (DEMEREC 1923; WENTZ 1930; see also EMERSON, BEADLE and FRASER 1935). The frequency of occurrence of germless seeds is markedly increased by X-ray or ultraviolet treatment of pollen.

## EXPERIMENTAL RESULTS

### *Control*

Control pollinations were included among the treatments made each day and were distributed at random among the cultures of the female parent stock. The control pollen was handled like the treated pollen except for the application of ultraviolet radiation, some samples being held 15 seconds and others three minutes to correspond to the approximate length of time required for the treatments used. In addition a special contro was included for comparison with λ3131 treatments, since these treatments required an exposure of 20 to 30 minutes. Each treatment at λ3131 was paralleled by a control held on a pollen slide for an equal period of time.

The frequency of endosperm deficiency in the two control populations was not significantly different. The results are shown in table 3.

There was no indication of heterogeneity among the ears included. Two large ears had three deficiencies each, three ears had two deficiencies each,

and the remaining 20 deficiencies occurred singly. The distribution of deficiencies among the multiple recessive cultures was random.

The total control population included 68 seeds with aborted embryos, or 0.93 ±0.11 percent. It was obvious, however, that the population was not homogeneous, for six of the 57 ears had four or more aborted embryos each, and one of these had 13 aborted embryos in a total of only 88 seeds. Four of these six ears occurred in one culture (23-221) of the female parent stock. The control pollinations were distributed at random among the cultures of the female parent, and there were only three cultures in which eight or more control ears were included. In culture 23-221 each of nine control ears had one or more aborted embryos, and the total frequency for the culture was 36/1251 (2.88 percent). In culture 23-201 there was only one aborted embryo among the eight ears tested. In culture 23-203 there were ten control ears, among which six had one or more embryo abortions each, the total frequency for the culture being 13/1741, or 0.75 percent.

TABLE 3

*Frequency of endosperm deficiency in control pollinations.*

| CONTROL STOCK | NUMBER EARS | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | TOTAL FREQUENCIES | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A* | | *Su* | | *Pr* | | | |
| | | | E* | F* | E | F | E | F | NUMBER | %±S.E. |
| General control | 45 | 27 | 0 | 13 | 4 | 0 | 0 | 10 | 27/5949 | 0.45±0.09 |
| Special control for λ3131 | 12 | 5 | 0 | 2 | 0 | 0 | 0 | 3 | 5/1459 | 0.34±0.14 |
| Total | 57 | 32 | 0 | 15 | 4 | 0 | 0 | 13 | 32/7408 | 0.43±0.07 |

* E=entire; F=fractional

This comparison shows that embryo abortion, independent of the pollen treatment, is distinctly more likely to occur in some of the female stock cultures than in others. In plants with the tendency toward frequent embryo abortion, the average frequency was less than three percent, far below the frequency induced by heavy treatment of pollen. But among such plants wide fluctuations occur in frequency of abortion. Obviously in comparisons based on a small number of ears, the standard errors calculated from the totalled populations would not accurately measure the true sampling error

It is desirable therefore to limit the comparisons of treatments in their effect on the frequency of embryo abortion to the ears of cultures free from this tendency. The number of control ears included in each culture was not sufficient in all cases to determine the presence or absence of the abortion tendency. A further comparison of the female stock cultures was there-

fore made in pollinations with very small doses or doses with little genetic effect. For this purpose treatments of $\frac{1}{2}$ M or less at λ2894, λ2925, λ2967, treatments of 2 M or less at λ3022, and a treatment of 500 M at λ3131 were used. On the basis of this comparison several cultures of the seed parent stock were found to include plants with the tendency toward embryo abortion. Comparison of the effects of the treatments upon the frequency of embryo abortion has therefore been made only from the pollinations on ears of the remaining cultures. In these cultures the spontaneous frequency of germless seeds was 0.31 percent. It is possible, however, that some of these cultures may have included individuals with the tendency toward embryo abortion.

The foregoing description applies to the control of the extensive trial of monochromatic radiations made in June and July, 1938. Additional comparisons made in 1939 and 1940, chiefly with the discharge tube radiation, were accompanied by separate controls, which are reported with the data of the experiments concerned.

### *The Dosage Relation*

Extensive dosage comparisons were made at λ2652 and λ2967, since the preliminary trials made in 1937 had indicated extreme levelling of the dosage curve at the former wave length and much less levelling at the latter. At λ2652 the doses applied ranged from approximately $\frac{1}{4}$ M to 16 M; at λ2967, from approximately 2 M to 64 M. At each wave length, larger doses also were applied, but the yield of seeds was too small to permit comparison.

The results are shown in tables 4 and 5. The significance of the comparisons in frequency of embryo abortion is limited because of possible fluctuation in spontaneous frequency, as has been mentioned. There is, however, a distinct effect of the treatment upon the frequency of embryo abortion,

TABLE 4

*Relation of dosage at λ2652 to frequency of embryo abortion and endosperm deficiency.*

| DOSE (M) | NUMBER EARS | FREQUENCIES OF EMBRYO ABORTION | | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *A* | | *Su* | | *Pr* | | TOTAL FREQUENCIES | |
| | | NUMBERS | %±S.E. | | E* | F* | E | F | E | F | NUMBERS | %±S.E. |
| .24 | 16 | 18/1880 | 1.0±0.2 | 26 | 3 | 16 | 0 | 3 | 2 | 2 | 26/2047 | 1.3±0.3 |
| .49 | 10 | 6/1006 | 0.6±0.2 | 16 | 2 | 4 | 2 | 4 | 1 | 3 | 16/1311 | 1.2±0.3 |
| .99 | 7 | 8/932 | 0.9±0.3 | 40 | 4 | 19 | 4 | 5 | 0 | 12 | 44/989 | 4.4±0.7 |
| 1.99 | 9 | 23/1107 | 2.1±0.4 | 102 | 9 | 47 | 8 | 25 | 6 | 28 | 123/1063 | 11.6±1.0 |
| 3.87 | 4 | 7/262 | 2.7±1.0 | 29 | 1 | 18 | 1 | 4 | 2 | 6 | 32/307 | 10.4±1.7 |
| 7.85 | 9 | 70/927 | 7.6±0.9 | 124 | 12 | 62 | 7 | 29 | 4 | 27 | 141/927 | 15.2±1.2 |
| 15.6 | 5 | 20/106 | 18.9±3.8 | 16 | 4 | 6 | 2 | 2 | 2 | 3 | 19/106 | 17.9±3.7 |

* E = entire; F = fractional

TABLE 5

*Relation of dosage at λ2967 to frequency of embryo abortion and endosperm deficiency.*

| DOSE (M) | NUMBER EARS | FREQUENCIES OF EMBRYO ABORTION | | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *A* | | *Su* | | *Pr* | | TOTAL FREQUENCIES | |
| | | NUMBERS | %± S.E. | | E* | F* | E | F | E | F | NUMBERS | %± S.E. |
| 1.99 | 11 | 5/1006 | 0.5±0.2 | 67 | 3 | 33 | 4 | 12 | 3 | 15 | 70/1498 | 4.7±0.5 |
| 3.98 | 6 | 8/658 | 1.2±0.4 | 67 | 4 | 31 | 1 | 13 | 2 | 20 | 71/1160 | 6.1±0.7 |
| 7.69 | 6 | 16/684 | 2.3±0.6 | 74 | 6 | 34 | 3 | 12 | 7 | 16 | 78/684 | 11.4±1.2 |
| 15.6 | 6 | 4/131 | 3.1±1.5 | 170 | 15 | 83 | 7 | 39 | 9 | 33 | 186/671 | 27.7±1.7 |
| 32.9 | 6 | 22/283 | 7.8±1.6 | 76 | 9 | 37 | 3 | 19 | 3 | 15 | 86/283 | 30.4±2.7 |
| 38.6 | 5 | 14/118 | 11.9±3.0 | 32 | 7 | 15 | 4 | 4 | 2 | 5 | 37/120 | 30.8±4.2 |
| 46.4 | 5 | 7/160 | 4.4±1.6 | 59 | 10 | 33 | 5 | 4 | 1 | 8 | 61/198 | 30.8±3.3 |
| 53.7 | 3 | 2/46 | 4.4±3.0 | 32 | 6 | 16 | 0 | 8 | 2 | 6 | 38/98 | 38.8±4.9 |
| 65.6 | 5 | 14/134 | 10.5±2.6 | 56 | 4 | 21 | 6 | 22 | 5 | 12 | 70/142 | 49.3±4.2 |

* E = Entire; F = fractional

which results, at least at the larger doses, in frequencies clearly beyond the maximum spontaneous frequency. At λ2652 the increased frequency of embryo abortion is evident at doses of 2 M and higher, and the frequency rises steadily with the dose. At λ2967, at the smaller doses used, the frequency of embryo abortion is much less than at equal doses of λ2652. Even at a dose of 16 M, λ2967, although it yields many more deficiencies than λ2652, yields only about one-sixth as many aborted embryos. Nevertheless it is clear that λ2967, at the largest doses tolerated, has a significant effect upon the frequency of embryo abortion.

The course of the dosage curve for endosperm deficiency is distinctly different for the two wave lengths compared. At λ2652 with the smallest doses used, the yield of deficiencies in excess of the control frequency is very slight. With increasing dosage, the deficiency rate rapidly increases to about ten percent at 2 M, but increases of dose beyond this point produce relatively little increase in the frequency of deficiency, an eight-fold increase of dose resulting in less than a doubling of the deficiency rate. At λ2967 a dose of 2 M has much less effect than a similar dose at λ2652, but with increased dosage the deficiency rate rises almost proportionately up to a dose of approximately 16 M. Beyond this point there is little increase in the yield of deficiencies with increasing dosage. A comparison of the two wave lengths at a dosage of 2 M indicates that λ2652 is the more effective, whereas at 16 M the reverse is true.

### *Effect of Size of Pollen Sample*

Some deviation from a linear dosage relation might be expected to result from different conditions of competition at the different doses. The number of pollen grains applied is considerably in excess of the number of silks pollinated, but with increasing dosage the number of pollen grains capable

of accomplishing fertilization declines until with the largest practical doses only a few seeds are produced on each ear. There is therefore an opportunity for competition among equally treated but differentially affected pollen grains at the smaller doses, but because of the limited pollen specimens applied it would be minimized at the larger doses.

If injury affecting functional efficiency is correlated with genetic effect, the grains most affected genetically would be those at the greatest disadvantage in survival and in competition. Their lowered survival would reduce the frequency of deficiencies detected below the frequency actually induced, and among the surviving pollen grains competition would further modify the result to a degree dependent upon the quantity of functional pollen remaining. *A priori* it seems improbable that there would be much

TABLE 6

*Relation of the quantity of pollen applied to the silks to the frequency of deficiencies. (Unfiltered radiation from mercury discharge tube, 7.5 minutes' exposure.)*

| POLLEN QUANTITY | NUMBER EARS | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | TOTAL FREQUENCIES | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A* | | *Su* | | *Pr* | | | |
| | | | E* | F* | E | F | E | F | NUMBERS | %±S.E. |
| 1 anther | 15 | 193 | 28 | 74 | 8 | 17 | 19 | 60 | 206/997 | 20.7±1.3 |
| ½ anther | 16 | 121 | 13 | 62 | 3 | 16 | 10 | 32 | 136/687 | 19.8±1.5 |
| ¼ anther | 15 | 86 | 9 | 37 | 7 | 8 | 8 | 25 | 94/434 | 21.7±2.0 |
| Total | 46 | 400 | 50 | 173 | 18 | 41 | 37 | 117 | 436/2118 | 20.6±0.9 |

* E=entire; F=fractional

correlation between genetic effect and injury to pollen function, for genetic effects are the result of alterations within the gametic nucleus while the effects on functioning are presumably due in the main to changes produced in other parts of the pollen grain.

If competition is a significant factor, the effect should be detectable in comparisons of treated pollen applied in varying quantities. A treatment dose with a distinct effect in reducing seed set was taken as a basis, and further reduction of seed set was obtained by reducing the size of the pollen sample.

In order to obtain adequate replication of the treatments, the discharge tube radiation was used. The pollen was irradiated for 7.5 minutes, a dose approximately equivalent to 8 M of λ2536. Eight samples were irradiated simultaneously in each treatment. In one treatment these consisted of approximately one anther each; in another treatment of one-half anther each; and in a third of one-quarter anther each. The three treatments were made consecutively, and on the following morning the experiment was

repeated. Two of the 48 ears were lost through earworm injury. The data obtained from the remaining ears are shown in table 6. Although the number of seeds per ear was considerably reduced by the reduction in quantity of pollen applied, no effect upon the deficiency rate is evident.

## *"Threshold Effect"*

In the data for λ2652 (table 4) the course of the dosage curve suggests the possibility of a "threshold effect." Doses of $\frac{1}{4}$ M and $\frac{1}{2}$ M had little if any effect upon the frequency of endosperm deficiencies, while there was a disproportionate increase at 1 M and 2 M. If the frequency of deficiencies in excess of the control at 1 M and 2 M is fairly representative of the effect of the treatment, the number of deficiencies found at $\frac{1}{2}$ M is well below that expected on the basis of a linear dosage relation. While the deviation from linearity in this region of the curve is not certainly significant, there was additional support of the possibility in the results of comparisons of doses of $\frac{1}{2}$ M and 2 M at many other wave lengths. The data available are shown in table 7.

TABLE 7

*Relative effect of large and small dosages on the frequency of endosperm deficiencies at various wave lengths.*

| WAVE LENGTH Å | "2 M" | | | | "½ M" | | | | EXPECTED DEFICIENCIES ½ M |
|---|---|---|---|---|---|---|---|---|---|
| | DOSE | NUMBER EARS | FREQUENCY OF DEFICIENCIES | | DOSE | NUMBER EARS | FREQUENCY OF DEFICIENCIES | | |
| | | | NUMBERS | %± S.E. | | | NUMBERS | %± S.E. | |
| 2925 | 2.00 | 3 | 46/510 | 9.0±1.3 | 0.47 | 6 | 5/619 | 0.8±0.4 | 15 |
| 2894 | 1.91 | 8 | 63/1010 | 6.2±0.8 | 0.50 | 11 | 22/1275 | 1.7±0.4 | 29 |
| 2804 | 1.97 | 7 | 94/1016 | 9.3±0.9 | 0.48 | 10 | 20/1749 | 1.1±0.2 | 45 |
| 2752 | 2.03 | 3 | 39/399 | 9.8±1.5 | 0.50 | 4 | 8/471 | 1.7±0.6 | 13 |
| 2699 | 1.96 | 2 | 21/268 | 7.8±1.6 | 0.48 | 5 | 10/569 | 1.8±0.6 | 13 |
| 2652 | 1.99 | 9 | 123/1063 | 11.6±1.0 | 0.49 | 10 | 16/1311 | 1.2±0.3 | 42 |
| 2536 | 1.96 | 7 | 124/802 | 15.5±1.3 | 0.49 | 12 | 34/1973 | 1.7±0.3 | 83 |
| 2483 | 1.96 | 9 | 98/987 | 9.9±1.0 | 0.49 | 12 | 41/2178 | 1.9±0.3 | 61 |
| 2378 | 2.04 | 2 | 16/231 | 6.9±1.7 | 0.48 | 4 | 8/635 | 1.3±0.5 | 12 |
| Total | | | 670/6852 | | | | 164/10780 | | 313 |

The numbers given under "Expected Deficiencies" are those which should occur if the effect of the lower treatment were directly proportional to that of the higher treatment, due allowance being made for the control frequency. The numbers observed at the lower doses are in general somewhat lower than the expected, and the total yield in excess of the control is less than half of that expected on the basis of a linear dosage relation.

A disproportionately low yield of deficiencies from small doses would be expected if deficiencies are produced by a multiple-hit mechanism, or if for any other reason a small dose may increase the probability of occur-

rence of deficiencies which it alone is not able to induce. At the larger doses, the levelling of the dosage curve expected as a result of variation in orientation of the pollen grains treated (cf. Introduction) might obscure the effect.

However, there is reason to suspect that, for differences of such small magnitude as those with which we are here concerned, the treatments at 2 M and $\frac{1}{2}$ M are not strictly comparable. There is at present no evidence that the frequency of deficiencies induced by a given dose of ultraviolet radiation is wholly independent of environmental conditions. In order to minimize the effects of such possible variations, an attempt was made in these experiments to seriate the treatments in such a way as to ensure the maximum comparability of treatments to be directly compared. For example, in the comparison of different wave lengths at a given dose, the treatments at the different wave lengths were made in rotation, the entire series being repeated as often as necessary to obtain the number of ears required. So far as possible dosage comparisons at the same wave length were seriated similarly, although the greater part of the available data on dosage effect comes from treatments seriated for wave length at constant dose rather than for dose at constant wave length.

In comparing doses of $\frac{1}{2}$ M with 2 M and larger doses, seriation for dosage was not feasible, since the very low dose of $\frac{1}{2}$ M required the use of the monochromator diaphragm to reduce the intensity of the radiation. The use of the diaphragm was not in itself the cause of the discrepancy, since comparisons of treatments with $\lambda 2652$ at 2 M, made with and without the use of the diaphragm, showed no significant difference in frequency of induced deficiencies. The use of the diaphragm, however, made it necessary to apply the small doses in separate series of treatments. The data of table 7 for $\frac{1}{2}$ M represent the results of several series of treatments (with diaphragm) chiefly from June 23, 24, and 28, those for 2 M the result of separate series of treatments (without diaphragm) chiefly from June 22, 24, 25, and 27. The treatments with various wave lengths at $\frac{1}{2}$ M therefore are directly comparable with one another, but they are comparable with the treatments at 2 M only to the extent that the results are independent of the varying conditions at the different times of treatment.

Examination of the results summarized separately for each day of treatment indicated the possible occurrence of significant daily variation (see next section). A further trial of small doses of $\lambda 2652$ was therefore made in 1940, with special precautions to avoid these and certain other possible sources of error. The plants pollinated were grown from a single selfed ear of an inbred *a C R* stock related to that used in the previous studies. The pollen parent in all pollinations was a single plant of an inbred dominant stock. This cross shows deficiencies only for *A*. The data therefore are free

from the possible slight errors due to variation of the sub-cultures of the parent stocks and to fluctuations in the phenotypic expression of *Pr* and *Su*. By the use of a commercial mercury arc with activated electrodes as a source, the variation of the radiation intensity with time was avoided, and better uniformity over the irradiated area was obtained. The treatments were made on July 2, 3, and 4; each day's run consisted of a continuous series with regular seriation of all doses and the control. The results are shown in table 8.

TABLE 8

*Frequency of endosperm deficiencies (A) in relation to dosage (λ2652).*

| DOSAGE IN "M" UNITS | NUMBER EARS | FREQUENCY OF DEFICIENCIES | |
|---|---|---|---|
| | | NUMBERS | %±S.E. |
| 0.25 | 21 | 10/1524 | 0.7±0.2 |
| 0.50 | 20 | 27/2044 | 1.3±0.3 |
| 1.0 | 16 | 45/1907 | 2.4±0.4 |
| 2.0 | 10 | 37/690 | 5.4±0.9 |
| Control | 23 | 17/3172 | 0.5±0.1 |

Although the increase over the control frequency per unit of dose is again somewhat smaller at the smallest doses, the deviation from linearity is not statistically significant.

### *Daily Fluctuation*

The extent of daily fluctuation is best determined from the results of treatments made with the discharge tube, since in these treatments usually four samples were treated simultaneously, and the data are therefore subject to a smaller sampling error. Treatments were made with a discharge tube in 1939 in several experiments in which the same male and female parent stocks were used as in the monochromator treatments. These experiments were concerned chiefly with the effect of size of the pollen sample and of irradiation from opposite directions, and their results are discussed in subsequent sections. They were not designed with the purpose of detecting daily variation, but since the comparisons made on different days included comparable treatments in many cases, the results will serve to indicate the amount of daily fluctuation. The unit of dosage was an exposure of seven and one-half minutes to radiation from the discharge tube at a distance of 21 centimeters above the pollen, with no filtration except that of a microscope slide of fused quartz covering the pollen (similar to that on which the pollen was placed). This is approximately equivalent to a dose of 8 M at λ2536. These treatments are comparable with the monochromator treatments except for the difference in the source of the radia-

tion and in the quantity of pollen treated. In the discharge tube treatments the pollen sample consisted of the pollen from three anthers of average size, instead of one as in the monochromator treatments.

During the period June 21 to July 1, treatments with the dose described above were included in trials made on six different days. On three other

TABLE 9

*Daily variation in the frequencies of induced endosperm deficiencies.*
*(Discharge tube radiation, 7½ minutes, 21 cm.)*

| DATE (1939) | DIRECTION OF EXPOSURE* | POLLEN QUANTITY (ANTHERS) | NUMBER OF EARS | FREQUENCY OF DEFICIENCIES | |
|---|---|---|---|---|---|
| | | | | NUMBERS | %±S.E. |
| June 21 | A | 3 | 2 | 38/169 | 22.5±3.2 |
| June 22 | B | 3 | 2 | 35/154 | 22.7±3.4 |
| June 23 | A | 3 | 4 | 136/531 | |
| | B | 3 | 4 | 82/309 | |
| Total | | | 8 | 218/840 | 26.0±1.5 |
| June 24 | A | 3 | 4 | 102/411 | |
| | B | 3 | 4 | 80/332 | |
| | A | 1 | 4 | 36/152 | |
| Total | | | 12 | 218/895 | 24.4±1.4 |
| June 26 | A | 3 | 4 | 98/581 | |
| | B | 3 | 3 | 95/392 | |
| | B | 1 | 4 | 54/251 | |
| Total | | | 11 | 247/1224 | 20.2±1.1 |
| June 27 | A | 3 | 4 | 54/332 | |
| | B | 3 | 4 | 41/316 | |
| | A | 1 | 4 | 42/336 | |
| Total | | | 12 | 137/984 | 13.9±1.1 |
| June 29 | A | 3 | 8 | 130/1004 | 12.9±1.1 |
| June 30 | A | 1 | 8 | 115/496 | 23.2±1.9 |
| July 1 | A | 1 | 7 | 91/501 | 18.2±1.7 |

* A=from above; B=from below

days of this period, though an identical treatment was not made, a treatment was included which was the same except that the radiation was applied from below instead of above the pollen, or that the treated specimen was reduced to the pollen from one anther. Since comparisons of consecutive treatments showed no significant difference due to the direction of exposure or to the pollen quantity, the results of these treatments also are included. The data are shown in table 9.

It is evident that the frequency of induced deficiencies at a given dose (or at any rate of surviving deficiencies) may differ very widely under the varying conditions of the different days of treatment. The extent of these differences among random comparisons within a rather brief period indicates that the effect of the unknown secondary factor or factors must be large. Since environmental conditions during irradiation of the pollen were substantially constant, and since they were necessarily variable during the ensuing period of pollen tube growth and early endosperm development, it seems probable that the environmental influence may be effective during some period subsequent to treatment. It is not impossible that conditions previous to treatment may be a factor, through their effect upon the final development of the pollen grain and consequently upon the absorption and internal filtration of the radiation. Correlation of the results with detailed weather records for the period under consideration showed no clear relation to specific changes of temperature or other weather conditions. The identification of the environmental conditions affecting the frequency of deficiencies must be left for further investigation.

### *Dosage Curves for Endosperm Deficiency*

The dosage data for λ2652 and λ2967, presented in tables 4 and 5, include all treatments made at these doses during June and July 1938, the period in which the comparison of monochromatic radiations was made. Although an effort was made to seriate doses for comparison, it was not possible within the short period of high tolerance (June 21 to July 1) to obtain adequate numbers for the dosage comparison as well as the wave length comparison. The data on dosage obtained during this period were inadequate in number, especially for the larger doses, and were supplemented by the results of additional treatments made whenever the weather would permit during July. These data therefore are subject to error from daily fluctuation. The comparison of equal doses applied during the first period with those applied later, in the few cases in which significant numbers are available, indicates that the frequency of deficiency was lowered in the later supplementary treatments. Since the data for the higher doses are largely from these later pollinations, it is probable that the lower relative yield of large doses is due in part at least to this cause.

A special trial of the effect of dosage was therefore made, for λ2536, λ2967, and λ3022, during a period of four consecutive days (August 29 to September 1, 1938) with regular seriation of the doses used at each wave length. The results are shown in table 10.

The declining effectiveness of successive increments of dose is clearly evident in the results for λ2536. It is much less pronounced, if indeed it occurs at all, at λ2967 and λ3022. It is perhaps worthy of note that at both

TABLE 10

*Relation of dosage to frequency of endosperm deficiency.*
*(Pollinations of August 29–September 1, 1938.)*

| DOSE (M) | NUMBER EARS | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A* | | *Su* | | *Pr* | | TOTAL FREQUENCIES | | |
| | | | E* | F* | E | F | E | F | NUMBERS | %±S.E. | $\alpha$ |
| | | | | | λ2536 | | | | | | |
| 1.0 | 10 | 24 | 1 | 12 | 1 | 4 | 4 | 5 | 27/892 | 3.0±0.6 | .0032 |
| 2.0 | 5 | 23 | 3 | 5 | 4 | 1 | 1 | 10 | 24/419 | 5.7±1.1 | .0044 |
| 4.1 | 4 | 24 | 1 | 5 | 1 | 5 | 1 | 11 | 24/293 | 8.2±1.6 | .0034 |
| 8.1 | 4 | 54 | 6 | 17 | 4 | 10 | 3 | 21 | 61/571 | 10.7±1.3 | .0022 |
| 17.1 | 4 | 33 | 3 | 13 | 1 | 3 | 1 | 14 | 35/208 | 16.8±2.6 | .0025 |
| | | | | | λ2967 | | | | | | |
| 4.0 | 5 | 13 | 0 | 5 | 1 | 2 | 1 | 5 | 14/543 | 2.6±0.7 | .0003 |
| 7.9 | 8 | 77 | 8 | 34 | 8 | 11 | 4 | 19 | 84/786 | 10.7±1.1 | .0009 |
| 15.7 | 4 | 46 | 3 | 23 | 2 | 9 | 5 | 13 | 55/305 | 18.0±2.2 | .0008 |
| 33.3 | 8 | 121 | 7 | 55 | 4 | 23 | 6 | 42 | 137/420 | 32.6±2.3 | .0010 |
| | | | | | λ3022 | | | | | | |
| 15.6 | 9 | 18 | 2 | 9 | 0 | 3 | 1 | 3 | 18/625 | 2.9±0.7 | .0001 |
| 32.0 | 6 | 68 | 2 | 36 | 0 | 15 | 6 | 16 | 75/802 | 9.4±1.0 | .0002 |
| 63.8 | 5 | 122 | 13 | 58 | 4 | 14 | 8 | 44 | 141/568 | 24.8±1.8 | .0003 |
| 132.0 | 9 | 99 | 15 | 39 | 11 | 13 | 6 | 28 | 112/310 | 36.1±2.7 | .0003 |

* E=entire; F=fractional

of these wave lengths there is again a suggestion of the disproportionately small effect of very small doses.

In general the frequency of deficiencies in this series of late-summer treatments is materially lower than that from equal doses in the early-summer treatments previously reported, another indication of the effect of environmental factors upon the frequency of induced deficiency.

### *Irradiation from Opposite Directions*

If the diminishing return from successive equal increments of radiation is due to the varying depth dose at the position of the gametic nuclei, it should be possible to reduce this filtration loss by irradiating the pollen from different directions.

Let us first suppose that a sample of pollen is irradiated uniformly from above with a given dose of radiation and that each pollen grain is then tested for the occurrence of a specific effect, say deficiency for *A*. The pollen grains affected will tend to be those in which the nucleus was so placed as to be least shielded by overlying material, for the relative probability of effect in different nuclei will be dependent upon the relative intensity

of radiation to which they are exposed. Assume that ten percent of the pollen grains are affected. If now the sample is exposed to a second dose identical with the first, the increase in the frequency of affected pollen grains will tend to be less than the nine percent (ten percent of the remaining 90 percent) which would be expected if the pollen grains were uniformly susceptible to the treatment. The second treatment must produce its effects among those pollen grains which were unaffected by the first, and to the extent that these represent the grains with deeper-lying nuclei the second treatment will be less effective.

Consider next a pollen sample irradiated with the same total dose, but with the first treatment applied from above and the second from below. The pollen grains most favorably oriented for the first treatment are those least favorably oriented for the second, and *vice versa*. If differences in internal filtration are extreme, the yield of effects from the second treatment may be almost as large as that from the first, and the deficiency rate therefore may be nearly doubled.

Irradiation of pollen from opposite sides without disturbing orientation of individual grains was not feasible with the monochromator, but it could be performed conveniently with the mercury discharge tube which operates readily in any position. The discharge tube was mounted on a swinging arm which could be rotated so as to hold the tube in place either 21 cm above the pollen sample or an equal distance below. The pollen was exposed between quartz slides of equal thickness. Four pollen samples were irradiated simultaneously in each treatment, each on a separate slide, and one ear was pollinated from each sample. One treatment of 7.5 minutes' duration was made each day with radiation from above and a treatment of equal duration with radiation from below. The eight ears produced were taken together to represent the single dose. The double dose similarly represents four samples irradiated from above and four from below, all being irradiated for 15 minutes. The divided dose represents two treatments, in each of which the pollen was irradiated 7.5 minutes from above and 7.5 minutes from below. To obviate any day-to-day fluctuations in treating conditions or discharge tube output, the experiment was conducted so as to carry through the complete comparison in consecutive treatments, and the entire experiment was repeated on different days to accumulate sufficiently large numbers.

A single trial made in 1938 gave results in agreement with expectation as outlined above, but the numbers involved, particularly in the divided treatment, were inadequate. The control for this trial is the general control already described. Three additional trials were made in 1939, each with a separate control. The conditions were similar to those of the 1938 trials, except that in the 1938 trial the radiant surface of the source was

limited by covering a portion of the tube with adhesive tape, while in the 1939 trials the radiant surface was limited instead by a baffle of aluminum.

These four trials are reported separately in table 11.

TABLE 11

*Enhanced frequency of endosperm deficiencies produced by a given dose when pollen is irradiated on opposite sides instead of on one side only.*

| EXPERIMENT NUMBER | TREATMENT | NUMBER EARS | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | TOTAL FREQUENCIES | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *A* | | *Su* | | *Pr* | | | |
| | | | | E* | F* | E | F | E | F | NUMBERS | %±S.E. |
| 1 | Half Dose (From One Side) | 4 | 82 | 10 | 42 | 8 | 12 | 6 | 13 | 91/701 | 13.0±1.3 |
| | Full Dose (From One Side) | 6 | 68 | 8 | 42 | 4 | 17 | 2 | 9 | 82/374 | 21.9±2.1 |
| | Full Dose (Half from Each Side) | 4 | 36 | 2 | 9 | 1 | 13 | 2 | 12 | 39/137 | 28.5±3.9 |
| 2 | Half Dose (From One Side) | 8 | 191 | 15 | 69 | 14 | 22 | 15 | 83 | 218/840 | 26.0±1.5 |
| | Full Dose (From One Side) | 8 | 78 | 9 | 28 | 1 | 7 | 8 | 35 | 88/265 | 33.2±2.9 |
| | Full Dose (Half from Each Side) | 8 | 93 | 9 | 34 | 6 | 10 | 10 | 35 | 104/265 | 39.3±3.0 |
| | Control | 2 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3/494 | 0.6±0.3 |
| 3 | Half Dose (From One Side) | 7 | 178 | 15 | 81 | 13 | 19 | 8 | 57 | 193/973 | 19.8±1.3 |
| | Full Dose (From One Side) | 8 | 116 | 13 | 53 | 13 | 11 | 6 | 35 | 131/472 | 27.8±2.1 |
| | Full Dose (Half from Each Side) | 8 | 61 | 10 | 24 | 4 | 6 | 8 | 18 | 70/187 | 37.4±3.5 |
| | Control | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3/284 | 1.1±0.6 |
| 4 | Half Dose (From One Side) | 8 | 86 | 8 | 38 | 7 | 10 | 6 | 26 | 95/648 | 14.7±1.4 |
| | Full Dose (From One Side) | 6 | 87 | 7 | 38 | 1 | 15 | 8 | 23 | 92/465 | 19.8±1.8 |
| | Full Dose (Half from Each Side) | 8 | 87 | 9 | 32 | 5 | 12 | 8 | 31 | 97/287 | 33.8±2.8 |
| | Control | 3 | 4 | 0 | 1 | 0 | 2 | 1 | 0 | 4/389 | 1.0±0.5 |

* E=entire; F=fractional

In each comparison the yield of deficiencies from the double dose applied from opposite directions is larger than that from the double dose applied from the same direction. The former treatment on the average produces almost twice as many deficiencies as the single dose, while the latter treatment produces only a small increase in the frequency. For the totalled data, the percentages of deficiencies for the single dose, the double dose, and the divided dose are 18.9, 24.9, and 35.4, respectively. The results thus indicate that the leveling of the dosage curve in this region is largely the result of selective effect of the radiation upon the pollen grains most favorably oriented.

### *Internal Filtration in Pollen*

Maize pollen is approximately spherical with an average diameter of about 93$\mu$. The surface of the pollen grain is smooth, and the thin wall is transparent in visible light. Since the reserve carbohydrate present is starch, the entire grains are somewhat translucent but not transparent in visible light. The pollen grain contains three nuclei, a large vegetative nucleus and two small, highly condensed sperm nuclei which are produced

by the division of the generative nucleus four or five days before maturity of the pollen grain. The structure of the pollen grain has been studied by WEATHERWAX (1919) and RHOADES (1934) who describe the sperms as thin crescent-shaped cells consisting almost entirely of nucleus but showing cytoplasm at their fine-pointed ends. The sperm nuclei are eccentrically located in the pollen grain, as may be seen readily in sectioned material or in specimens stained entire.

In order to obtain evidence on the approximate location of the sperm nuclei within the pollen grain, measurements were made upon mature pollen grains which had been stained and sectioned. We are indebted to MR. JAMES W. CAMERON for these measurements. Mature anthers, in which the pore had opened but the pollen grains had not been shed, were used in making the preparations. The material was sectioned at a thickness of 9–10 microns. The positions of the three nuclei were plotted in three dimensions for each of the 67 pollen grains examined, the position of each nucleus being recorded in one of five concentric shells. Although the two sperm nuclei sometimes differ somewhat in appearance, there is no way of discriminating between the one which will function in fertilization and the one which will function in endosperm fusion. The locations of the three nuclei were tabulated separately for each pollen grain, and the data thus constitute a sample of 67 vegetative nuclei and 134 sperm nuclei. The frequency distribution of their locations in the five concentric shells is shown in table 12. Both the vegetative nucleus and the sperm nuclei are eccentric in location, but in general the sperm nuclei are closer to the periphery of the pollen grain. Only about six percent of the sperm nuclei are located in the two innermost shells, while more than 30 percent of the vegetative nuclei occupy this position. In general the two sperm nuclei in the same pollen grain were about equally eccentric, the two nuclei being in the same or adjacent shells in more than half of the pollen grains examined.

TABLE 12

*Location of nuclei within the pollen grain.*

| CONCENTRIC SHELL | VEGETATIVE NUCLEUS | | SPERM NUCLEI | |
|---|---|---|---|---|
| | NUMBER | % | NUMBER | % |
| 1 (Innermost) | 1 | 1.5 | 2 | 1.5 |
| 2 | 20 | 29.9 | 6 | 4.5 |
| 3 | 27 | 40.3 | 35 | 26.1 |
| 4 | 18 | 26.9 | 68 | 50.7 |
| 5 (Outermost) | 1 | 1.5 | 23 | 17.2 |
| Total | 67 | 100 | 134 | 100 |

Assuming random orientation of pollen grains lying in a single layer, the vertical depth distribution of sperm nuclei beneath the upper hemispherical surface of the pollen grains may be calculated. The distribution represented in figure 1 indicates the relative numbers of nuclei per unit depth in a large sample of pollen, where each successive layer of unit depth is bounded by *hemispherical* shells of constant radius, with all points on any one shell lying at equal distances vertically from the upper surface of the pollen grain.

Ultraviolet transmission data on the walls and contents of the pollen grains have been published by UBER (1939). Transmission measurements were made with a quartz microscope for various wave lengths from 2378 to 3655 Å. Fragments of individual walls were obtained by squeezing the contents out of the pollen grains. Transmission data for the pollen contents were obtained from layers eight microns thick pressed between quartz slides. The transmission data for the wave lengths used in the present experiment are shown in table 13.

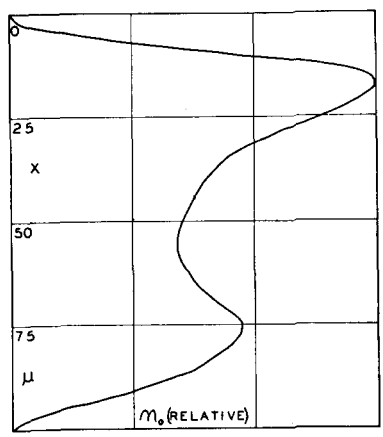
TABLE 13

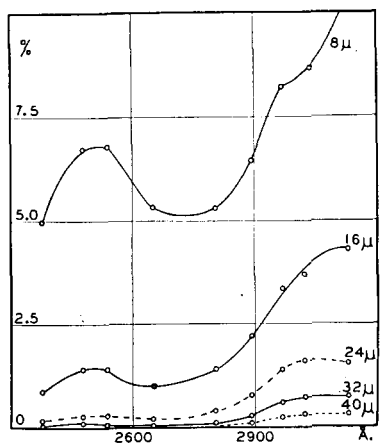
*Ultraviolet transmission by pollen grain wall and contents.*

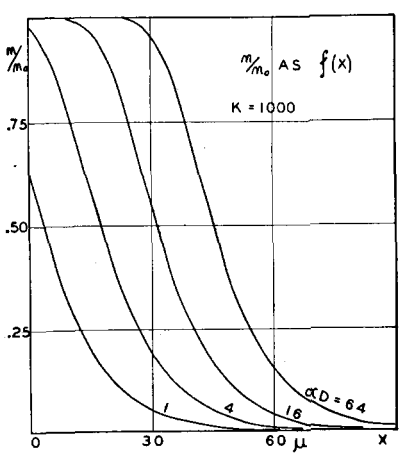
| λ | WALL % | CONTENTS (PER 8μ LAYER) % | $k\left(=\frac{\ln(I_0/I)}{0.0008}\right)$ |
|---|---|---|---|
| 3131 | 25 | 41.5 | 1100 |
| 3022 | 20 | 43 | 1055 |
| 2967 | 20 | 41 | 1115 |
| 2894 | 19 | 34 | 1350 |
| 2804 | 20 | 26.5 | 1660 |
| 2752 | 23 | 23 | 1835 |
| 2652 | 28 | 19 | 2075 |
| 2536 | 33 | 20.5 | 1980 |
| 2483 | 32 | 21 | 1950 |
| 2378 | 28 | 18 | 2145 |

Transmission through the pollen wall is at a minimum of about 20 percent at 2800–3000 Å and reaches a maximum of about 33 percent at 2536 Å. When equal doses of λ2536 and λ2804 are applied, therefore, the dose which reaches a point immediately under the pollen wall is almost twice as great for the shorter wave length as for the longer. Transmission through an eight μ layer of pollen contents is only 19 percent at λ2378 and at λ2652, but amounts to about 42 percent at λ3022. Corresponding differences in intensity would occur at a point eight microns below the wall and would be increased exponentially at greater depths.

The relative proportion of incident energy which penetrates to various depths within the pollen grain, neglecting corrections for reflection and refraction, is shown in figure 2. Thus doses equal in incident energy are far from equal in the energy which reaches the sperm nuclei. With equal incident energy at λ2967 and λ2652, the dose applied at a point only 16 microns



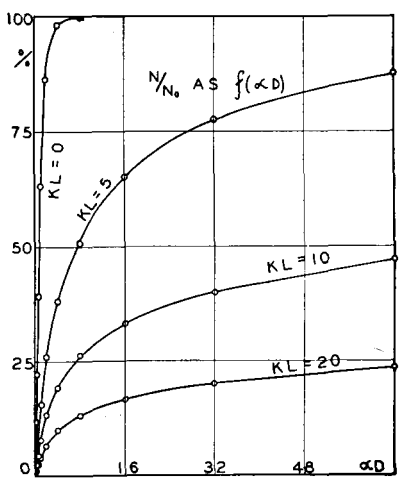




FIGURE 1 (upper left).—Relative distribution of nuclei (abscissa) in a pollen sample at depths ($x$) of equal energy penetration for radiation incident vertically from above.

FIGURE 2 (upper right).—The percent of incident energy (ordinate) which penetrates to the indicated depths within a pollen grain as a function of the wave length (abscissa). Absorption by the pollen grain wall has been taken into account.

FIGURE 3 (lower left).—The fraction of nuclei affected ($n/n_0$) by radiation at various depths within a pollen grain for several values of $\alpha D$ and for $k = 1000$.

FIGURE 4 (lower right).—The percentage of nuclei affected ($N/N_0$) in a large sample of pollen as a function of αD for several values of kL, where L is the diameter of a pollen grain.

beneath the wall is three times as large for the longer wave length as for the shorter. At a depth of 32 microns it is about fifteen times as large.

*Relation of Internal Filtration to the Dosage Curve*

If the nuclei in a pollen sample (or more strictly the absorbing entities within them which are responsible for the occurrence of deficiencies) were so disposed as to be equally shielded from the radiation, internal filtration would have no effect upon the form of the dosage curve. Each unit of radiation incident at the surface of the pollen grain would have the effect of only that fraction of a unit which penetrates to the site of nuclear absorption. If this fraction were constant, the dosage curve stated in terms of incident energy would be correct in form; it would be in error only as to the absolute value of the dosage unit.

Variation in the thickness of the shielding layer, however, may result in a distortion of the dosage curve. Due to this variation, the nuclei in a sample of pollen grains exposed to a uniform beam of radiation may constitute in fact a population of individuals receiving widely varying doses. The incident dose on the pollen is uniform for all grains, but it is reduced in amount within each individual grain depending upon the depth of the gametic nucleus below the exposed surface (figure 1, table 12) and on the ultraviolet transmission characteristics of the pollen grain wall and contents (fig. 2, table 13).

The effect of this variation in shielding upon the dosage curves for incident radiation is to reduce the relative effectiveness of the later dose increments. With equal shielding, the probability of affecting a nucleus not previously altered by the radiation would be the same for successive dose increments. With unequal shielding, this probability declines with each increment of dose applied, since each dose increment tends to affect most the least shielded pollen grains and to leave unaffected a residual population with fewer nuclei in the less shielded positions. The extent of this tendency will vary with different wave lengths, depending upon (1) the rate at which the energy is lost in penetrating the over-lying material and (2) the genetic effectiveness of the energy which reaches the nucleus.

The amount of modification expected in the dosage curve for deficiencies at each wave length may be estimated in terms of two variables, k and $\alpha$. The first variable, k, is defined as an absorption coefficient for the pollen contents by the equation, $I = I_0 e^{-kx}$, where x is the thickness of the absorbing layer, and I and $I_0$ are the transmitted and incident intensities, respectively. The value of k is known, to a sufficient approximation, from data on absorption of the pollen contents and is tabulated in table 13. Since much of the radiation is scattered by the starch granules in pollen,

k is not strictly an absorption coefficient, but includes also the radiation loss by scattering.

The second variable, $\alpha$, is a proportionality constant in the following fundamental equation (cf. CLAUS 1933): $dn/dt = \alpha(n_0 - n)\ I_0\ e^{-kx}$. This equation assumes that the time rate of production of deficiencies (dn/dt) depends only on the intensity of the radiation reaching the germinal material ($I_0e^{-kx}$) and on the number of unaffected centers in the population ($n_0 - n$) at the time (t). Since dn/dt is independent of the total dose ($D = I_0t$), this case is usually referred to as one requiring only a single absorption act to bring about a deficiency.

Intrinsically the proportionality constant, $\alpha$, depends on two factors: (1) the probability of absorption of the energy incident on a potentially effective center or region, and (2) the probability of production of a detectable genetic alteration by the energy which is absorbed. The second factor is analogous to the quantum yield of a photochemical reaction and concerns us at this point only as regards its possible variation with wave length. In the absence of available information as to wave length dependence, it has been customary in studies of this nature to assume constancy over the ultraviolet region investigated. This assumption is probably not entirely justified but will serve as a first approximation.

The fraction of the incident energy which is absorbed by a substance occupying a small volume is equal to $1 - e^{-\xi cd}$, where $\xi$, c, and d represent its molecular extinction coefficient, its concentration, and its depth, respectively. In case $\xi$ c d is small compared to unity, which would presumably be true for minute absorbing structures, this fraction becomes equal to $\xi$ c d, and hence the energy absorbed would be proportional to the coefficient $\xi$. In other words, to a first approximation, $\alpha$ should be proportional to the absorption coefficient of the substance responsible for the production of the deficiencies.

Our problem, then, is to determine a relationship between the several variables so that the value of $\alpha$ may be obtained from the curves relating deficiencies to the dose incident upon the pollen at each wave length.

For a population which is uniformly shielded by a substance having an extinction coefficient, k, such as the population ($n_0$) of a layer dx thick at a depth x, the number of deficiencies (n) produced in the layer is given by $n = n_0(1 - e^{-\alpha De^{-kx}})$. Values of $n/n_0$ as a function of x for various values of $\alpha D$ are given for the case where $k = 1000$ in figure 3. It will be noted that when $\alpha D$ is large practically all of the nuclei near the surface are affected. Since additional increments of dose cannot increase $n/n_0$ in these uppermost layers, the dosage curve becomes proportionately less steep at the larger doses.

Inasmuch as the experimental data on deficiencies represent the effects on the population as a whole ($N_0$), we must sum up the effects expected from the different "layers" of the pollen grain. The relative distribution of nuclei among the various layers is shown in figure 1, where x represents the vertical depth of a layer below the upper wall of the pollen. If we multiply the actual number of nuclei at any depth x by the above value of $n/n_0$ for the same depth, the product is the number of nuclei affected at that depth. By plotting the values thus obtained against the depth x and integrating graphically from O to the diameter L, the total number of deficiencies, N, is found. A series of values for $N/N_0$ as a function of $\alpha D$ may then be calculated. The resulting dosage curves for several values of kL, shown in figure 4, indicate how marked the levelling of the dosage curve may be, for a kL value of 10 corresponds to $\lambda 3022$ and a kL value of 20 to $\lambda 2652$.

The experimental values of $N/N_0$ for endosperm deficiencies as a function of dose for three different wave lengths have been given in table 10. Taking any particular value of $N/N_0$ for which D is known, it is possible to determine $\alpha$ from the appropriate curve of the type shown in figure 4. This has been done, using as a basis the dose just inside the pollen wall, and the values of $\alpha$ are given in table 10.

According to this analysis, the values of $\alpha$ for different doses of the same wave length should be equal, except for sampling fluctuations. The calculated values show considerable variation, and in the case of $\lambda 3022$ and $\lambda 2967$ they tend to increase somewhat with increasing dosage. The extent to which these variations may be accounted for by sampling errors may conveniently be tested by constructing a predicted dosage curve at each wave length, against which the experimental values may be plotted with their standard errors. Predicted dosage curves for the wave lengths appearing in table 10 are presented in figure 5; these curves have been constructed on the basis of the $\alpha$ values yielding the best fit.

At all wave lengths except $\lambda 3022$ the observed deficiency rates are in reasonably good agreement with prediction. At $\lambda 3022$ the data clearly depart from expectation, the deficiency frequency being distinctly too low at the smaller doses and too high at the larger doses. Such behavior suggests a multiple-hit curve for the dosage relation.

In the light of this indication, and in view of earlier remarks on the shape of the dosage curve for endosperm deficiencies, it seemed profitable to calculate values of $\alpha$ on the assumption that the cumulative effect of two quanta would be required to give an effect. In this case the equation for the number affected ($n_2$) in a thin layer becomes

$$n_2 = n_0[1 - e^{-\alpha De^{-kx}}(1 + \alpha De^{-kx})].$$

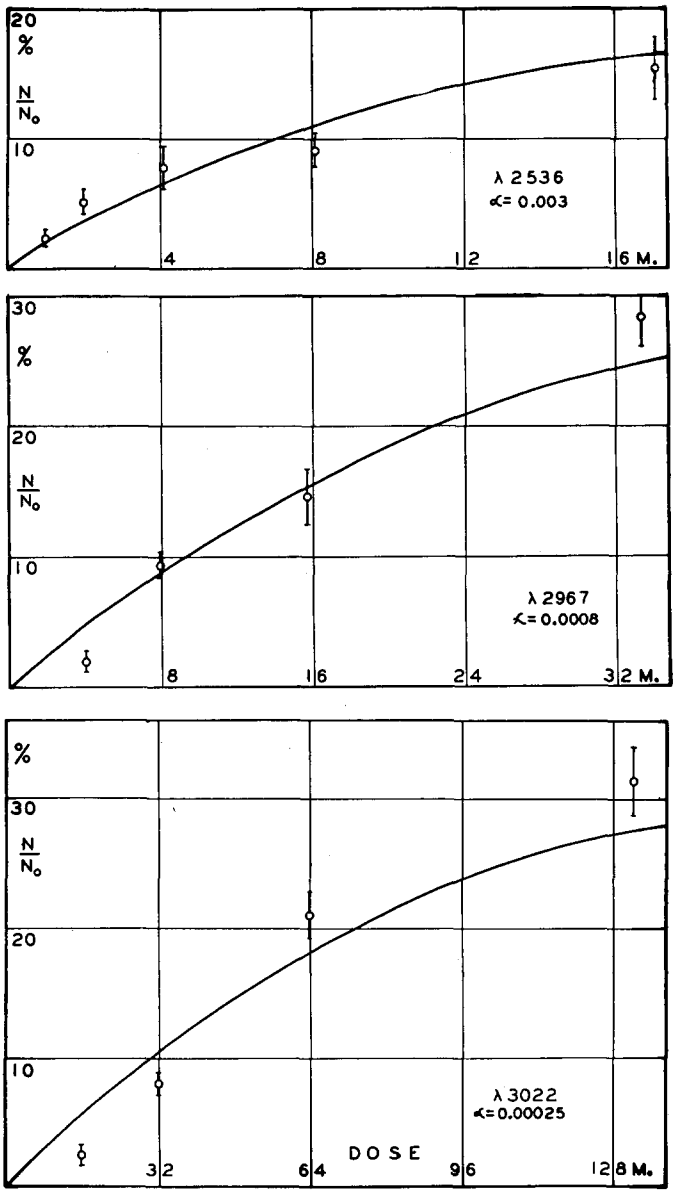



FIGURE 5.—The experimental values of the frequency of deficiencies ($N/N_0$) as a function of the incident dose in comparison with the theoretical curves of the expected variation of $N/N_0$ with $D$. The curves are based on the arbitrary values of $\alpha$ which are indicated on each of the curves.

Integration over the total depth of the pollen grain results in slightly S-shaped curves for $N/N_0$ as a function of $\alpha D$, from which values of $\alpha$ may be computed as previously described. When this was done, the constancy of $\alpha$ was found to be somewhat better at λ2967 and λ3022 for the 2-quanta

case than for the single quantum treatment, while at λ2536 the reverse was true.

While the improvement noted in the fitting of these curves is not necessarily significant, the possibility of a multiple-hit type of relationship between deficiency rate and dosage certainly merits further investigation.

### *Comparative Effectiveness of Different Wave Lengths*

Comparisons of treatment at different wave lengths were made on a rather large scale during June and July, 1938. The most extensive data for this purpose were obtained at a dose of 2 M, but fairly extensive comparisons were made also at 8 M. The treatments at ½ M, which have been summarized in an earlier table, resulted in such small increases over the control frequency that the differences between wave lengths have little statistical significance.

The totalled data for wave length comparisons at 2 M and 8 M are shown in table 14.

TABLE 14

*Relation of wave length to frequency of endosperm deficiency.*

| WAVE LENGTH (Å) | DOSE (M) | NUMBER EARS | FREQUENCIES OF EMBRYO ABORTION | | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | TOTAL FREQUENCIES | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *A* | | *Su* | | *Pr* | | | |
| | | | NUMBERS | %±S.E. | | E* | F* | F | E | E | F | NUMBERS | %±S.E. |
| 3022 | 1.98 | 14 | 3/820 | 0.4±0.2 | 15 | 0 | 6 | 2 | 1 | 2 | 4 | 15/1696 | 0.9±0.2 |
| 2967 | 1.99 | 11 | 5/1006 | 0.5±0.2 | 67 | 3 | 33 | 4 | 12 | 3 | 15 | 70/1498 | 4.7±0.5 |
| 2925 | 2.00 | 3 | 0/270 | 0 | 43 | 1 | 20 | 2 | 8 | 1 | 14 | 46/510 | 9.0±1.3 |
| 2894 | 1.91 | 8 | 7/946 | 0.7±0.3 | 55 | 1 | 31 | 2 | 6 | 5 | 18 | 63/1010 | 6.2±0.8 |
| 2804 | 1.97 | 7 | 8/1016 | 0.8±0.3 | 86 | 6 | 44 | 7 | 17 | 5 | 15 | 94/1016 | 9.3±0.9 |
| 2752 | 2.03 | 3 | 0/91 | 0 | 32 | 2 | 10 | 3 | 13 | 5 | 6 | 39/399 | 9.8±1.5 |
| 2699 | 1.96 | 2 | 0/0 | | 19 | 5 | 4 | 1 | 6 | 0 | 5 | 21/268 | 7.8±1.6 |
| 2652 | 1.99 | 9 | 23/1107 | 2.1±0.4 | 102 | 9 | 47 | 8 | 25 | 6 | 28 | 123/1063 | 11.6±1.0 |
| 2536 | 1.96 | 7 | 17/736 | 2.3±0.6 | 108 | 11 | 50 | 8 | 19 | 5 | 31 | 124/802 | 15.5±1.3 |
| 2483 | 1.96 | 9 | 13/762 | 1.7±0.5 | 89 | 12 | 44 | 9 | 17 | 5 | 11 | 98/987 | 9.9±1.0 |
| 2378 | 2.04 | 2 | 0/150 | 0 | 15 | 0 | 8 | 1 | 1 | 2 | 4 | 16/231 | 6.9±1.7 |
| 2967 | 7.69 | 6 | 16/684 | 2.3±0.6 | 74 | 6 | 34 | 3 | 12 | 7 | 16 | 78/684 | 11.4±1.2 |
| 2894 | 7.82 | 6 | 34/582 | 5.8±1.0 | 109 | 11 | 60 | 5 | 16 | 9 | 24 | 125/675 | 18.5±1.5 |
| 2804 | 7.97 | 6 | 48/648 | 7.4±1.0 | 180 | 16 | 76 | 11 | 45 | 7 | 47 | 202/851 | 23.7±1.5 |
| 2699 | 8.05 | 5 | 45/450 | 10.0±1.4 | 81 | 6 | 40 | 5 | 12 | 6 | 25 | 94/450 | 20.9±1.9 |
| 2652 | 7.85 | 9 | 70/927 | 7.6±0.9 | 124 | 12 | 62 | 7 | 29 | 4 | 27 | 141/927 | 15.2±1.2 |
| 2536 | 7.82 | 6 | 44/383 | 11.5±1.6 | 81 | 4 | 28 | 9 | 19 | 8 | 21 | 89/383 | 23.2±2.2 |
| 2483 | 7.94 | 7 | 20/184 | 10.9±2.3 | 67 | 4 | 36 | 5 | 18 | 5 | 11 | 79/346 | 22.9±2.3 |

* E = entire; F = fractional

These data involve the same error from daily fluctuation which has been discussed in connection with the totalled dosage data. They will serve, however, for the consideration of questions involving embryo abortion and the relative frequency of fractional and entire deficiencies.

Embryo abortion is distinctly more frequent at the shorter wave lengths. The increased frequency is appreciable at the 2 M dose at λ2652–λ2483 but

not at longer wave lengths. With a dose of 8 M, appreciable embryo abortion occurs at all wave lengths, but it is more frequent at the shorter wave lengths than at the longer.

The relative frequency of fractionals as a function of wave length is of interest in connection with the problem of their origin. Fractionals are much more frequent with ultraviolet treatment than with X-ray treatment, and in the case of ultraviolet fractionals the sizes of the deficient and non-deficient sectors are usually approximately equal. The ultraviolet

TABLE 15

*Relation of Frequency of Deficient Seeds to Wavelength*

*(Dose 2 M)*

| λ | FREQUENCIES ON THE DATE INDICATED | | | MEAN | $(N/N_0)$ CALC. | $\alpha$ |
|---|---|---|---|---|---|---|
| | 6/24 | 6/25 | 6/27 | | | |
| 3022 | 4/462 | 6/621 | 5/613 | 0.9±0.2 | 1.0 | 0.0001 |
| 2967 | 9/266 | 35/459 | 17/600 | 4.6±0.6 | 5.1 | 0.0013 |
| 2894 | 5/89 | 8/95 | 37/512 | 7.2±1.0 | 6.8 | 0.0043 |
| 2804 | 9/111 | 24/180 | 15/249 | 8.9±1.2 | 9.4 | 0.0082 |
| 2752 | 14/265 | 5/43 | 13/91 | 8.0±1.4 | 10.2 | 0.0082 |
| 2652 | 16/183 | 14/129 | 43/266 | 12.6±1.4 | 11.8 | 0.0166 |
| 2536 | 14/140 | 33/205 | 41/289 | 13.9±1.4 | 13.0 | 0.0169 |
| 2483 | 10/140 | 25/168 | 37/353 | 10.9±1.2 | 11.7 | 0.0115 |
| 2378 | | 5/81 | 10/150 | 6.5±1.6 | 7.3 | 0.0061 |
| Control | | 15/2075 | 1/585 | 0.6±0.2 | | |

fractionals may represent the result of breakage of the half-chromosome, in contrast to the entire deficiencies resulting from breakage of the undivided chromosome or of the two half chromosomes. If fractionals result from absorption of the radiation in different parts of the chromosome and if these parts differ in absorption characteristics, we might expect a different ratio of entire and fractional deficiencies at different wave lengths. The data however give no indication of any such difference.

For calculation of relative genetic effectiveness of the wave lengths it is desirable to limit the data to those which are the result of experiments seriated for the control of daily variation. In the case of the treatments at a dose of 2 M, fairly extensive data are available from seriated treatments, and they are presented in table 15.

The relative effectiveness of the wave lengths, corrected for loss due to internal filtration, may be compared by calculating the value of $\alpha$ for each wave length in the manner which has been described. These values are also listed in table 15. The calculations have been made on the basis of

the dose expressed in terms of total energy rather than total number of quanta.

These values of $\alpha$ are plotted against the absorption spectrum of thymonucleic acid (CASPERSSON 1936) in figure 6. Considering the various approximations involved, the agreement is surprisingly good and lends further support to the frequently advanced hypothesis that nucleic acid is intimately associated with the functioning of the germinal material.

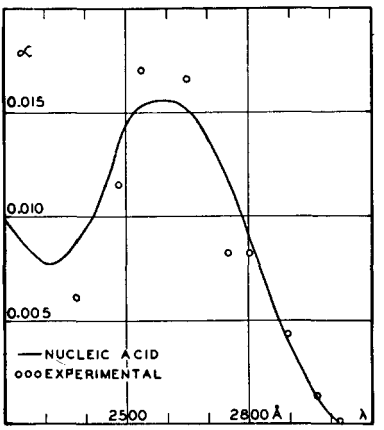



FIGURE 6.—Experimental values of $\alpha$ (ordinate) are plotted against the wave length (abscissa) for comparison with the absorption spectrum of thymonucleic acid (CASPERSSON 1936) the latter being shown as a full curve.

The significance of the deviations with relation to sampling error may be determined by comparison of the observed deficiency rates with values given in table 15 under "$N/N_0$ calculated." These are calculated on the assumption that the genetic effectiveness of the energy that reaches the gametic nucleus at any wave length is proportional to the absorption of that wave length by nucleic acid. The experimental values do not deviate significantly from the calculated values.

### *The Genetic Effectiveness of λ3131*

The earlier comparison (STADLER and UBER 1938) showed no appreciable effect of λ3131 on endosperm deficiency rate. At a dose of 600 M there was a distinct increase in the frequency of deficiencies (see table 1), but since impurities of shorter wave length radiation were present in sufficient strength to constitute a considerable dose in this very long exposure, the genetic effects could not be ascribed definitely to λ3131. The impurity normally present, totalling all wave lengths shorter than λ3131, amounted to less than one percent. It was not feasible to use a double monochromator to increase the purity of the radiation, since the dose required for genetic

effectiveness is so large that the energy available would be inadequate within the time limits.

It was found, however, that the impurity could be very materially reduced without serious reduction in the intensity of the desired radiation by the use of a Corning #014 glass filter (see table 2). By the use of this filter, radiation impurities of wave lengths shorter than 2900 Å are reduced to an entirely negligible value, and that due to λ3022 and λ2967 is seen to be very small. In order to ascertain the deficiency rate which could result from such slight percentages of impurity, special treatments were given at λ3022 and λ2967 for doses of approximately $\frac{1}{2}$ M and $\frac{1}{4}$ M, respectively. Even these low doses are greatly in excess of the impurity from these lines

TABLE 16

*Data relative to the genetic effectiveness of λ3131.*

| TREATMENT | | NUMBER EARS | NUMBER DEFICIENT SEEDS | ENDOSPERM DEFICIENCIES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *A* | | *Su* | | *Pr* | | TOTAL FREQUENCIES | |
| λ Å | DOSE M | | | E* | F* | E | F | E | F | NUMBERS | %±S.E. |
| 3131† | 505.2 | 16 | 27 | 1 | 12 | 0 | 8 | 2 | 4 | 27/1641 | 1.78±0.23 |
| 3022 | 0.46 | 17 | 15 | 0 | 10 | 0 | 1 | 2 | 2 | 15/2092 | 0.72±0.12 |
| 2967 | 0.25 | 18 | 9 | 1 | 3 | 0 | 1 | 0 | 4 | 9/1618 | 0.58±0.13 |
| Control | | 12 | 5 | 0 | 2 | 0 | 0 | 0 | 3 | 5/1500 | 0.33±0.08 |

* E=entire; F=fractional

† Filtered (See text and Table 2.)

found to be present in a dose of 512,000 ergs/mm² at 3131 Å. The data on the control frequency of endosperm deficiencies were obtained from simultaneous pollinations, the pollen being exposed in trays near the monochromator for the same period of time as the λ3131 samples. The exposure time varied from 20 to 30 minutes, depending on the intensity of the radiation. The data are presented in table 16.

The deficiency rates for the low doses at λ3022 and λ2967, from which the control frequency has not been subtracted, will be seen to be too low to account for the effect at λ3131. Apparently this wave length has a real though very slight effect on the frequency of endosperm deficiency.

## SUMMARY

The results of comparisons of the effectiveness of different wave lengths differ rather widely in trials made at different levels of dosage. The problem of comparing wave length effectiveness is therefore chiefly that of analyzing the dosage relation.

In general the dosage curve for ultraviolet radiation tends to level at the larger doses; the return from large doses is proportionately less than

from small. This tendency is more pronounced at λ2536 and λ2652 than at λ2967 and λ3022.

The disproportionately small return from an increment of dose may be avoided if the increment is applied from the opposite side of the pollen grain, indicating that variations in the susceptibility of the pollen grains treated are related to their orientation.

The gametic nuclei within the pollen grains are eccentrically located, so that in a layer of pollen grains oriented at random there are large differences in the depth of the nuclei below the irradiated surface.

The absorption of ultraviolet radiation in the pollen wall and contents is great enough to cause large differences in the intensity of the radiation penetrating to the site of the nucleus in different pollen grains. Transmission losses are greatest for the shorter wave lengths, at which the levelling of the dosage curve is most pronounced.

Using observed values for the position of the nuclei within the pollen grain and for the transmission losses in pollen wall and contents, and assuming that the effectiveness of each wave length per unit of energy is constant, it is possible to determine the form of the dosage curve expected at each wave length. The amount of levelling in the calculated dosage curves was in fairly good agreement with that observed.

The value of a constant representing the effectiveness per unit of energy reaching the nucleus was determined for each of nine wave lengths in the range λ2378–λ3022, from data representing the effect of doses of 2000 ergs/mm$^2$ of energy incident at the surface of the pollen grain. These values agreed within the limits of sampling error with the relative absorption coefficients of nucleic acid for the same wave lengths.

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