Theor. Appl. Genet. 49 (1977) 253-258



# Ultraviolet Irradiation of Maize (*Zea Mays* L.) Pollen Grains
## I. Pollen Genotype Effects on Kernel Development

P.L. Pfahler and H.F. Linskens
University of Florida, Gainesville, Florida (USA) and University of Nijmegen, Nijmegen (the Netherlands)

Summary. Mature pollen grains from two single cross hybrids, Wf9 × H55 and K64 × K55, were exposed to eleven levels (0 to 6.80 erg/cm$^2$ × 10$^5$ at 0.68 intervals) of ultraviolet irradiation and then were used to pollinate their genetic source. The number and weight of the normal and shrunken (partially aborted) kernels on each ear were tabulated. In general, the number of normal kernels decreased and the number and percentage of shrunken kernels increased with increasing exposure. However, significant exposure × hybrid interactions were present indicating that the amount of change depended on the hybrid. No consistent relationship between exposure and either normal or shrunken kernel weight was apparent, but pollen source hybrid was a contributing factor. The embryo weight and coleoptile length after germination were also determined for the normal kernels. Changes in these characters by irradiation were also strongly influenced by the hybrid. These results indicate that the direction and magnitude of the changes in kernel development produced by ultraviolet are modified considerably by the genetic source of the pollen grains. Presumably, genetic variation for ultraviolet response is present and selection would be successful.

## Introduction

Pollination with pollen grains exposed to ultraviolet irradiation is known to result in a decrease in the number of normally developed viable seeds and an increase in the number of shrunken inviable seeds (Brewbaker and Emery 1962). Apparently, ultraviolet induces nuclear changes in the pollen grain which disrupt embryo and endosperm development. No detailed information regarding the complex relationships between exposure level, kernel development and pollen source is available. Therefore, the purpose of this study was to determine the number and weight of normal and shrunken (partially aborted) kernels produced after pollination with ultraviolet-irradiated pollen grains from two genetic sources of maize.

## Materials and Methods

Large quantities of mature pollen grains from two single cross hybrids, Wf9 × H55(W) and K64 × K55(K), were collected on each of two dates, June 1 (Date 1) and June 9 (Date 2) in 1972 by the method of Pfahler (1965). On each date the pollen grains from each hybrid were exposed to eleven levels (0 to 6.80 erg/cm$^2$ × 10$^5$ at 0.68 intervals) of ultraviolet using an apparatus which exposes grains to irradiation from all directions (Pfahler 1973). After irradiation, the pollen grains from each level were used to pollinate six ears of their source. All pollinations were made with equal quantities of pollen grains in amounts adequete to fully pollinate the ears. Thus, for each level and hybrid, 12 ears (six from each date) were obtained.

On each pollinated ear, the numbers of the normal and shrunken (partially aborted) kernels were determined. The normal classification included those kernels whose embryo and endosperm had a normal size and appearance and were viable. The shrunken classification contained kernels whose embryo and/or endosperm were reduced in size and defective in appearance and also were generally inviable. The differences between the two kernel types were well-defined and, in most cases, obvious. A percent shrunken classification was computed based on the total number.

An equal number of normal kernels from each of the 12 ears from each level and hybrid was mixed, eliminating the ear and date variable. The same procedure was done with the shrunken kernels. From each of the resulting 22 groups of both normal and shrunken kernels, 100 kernels were weighed, with some exceptions noted in the tables. The embryos from the normal kernels in each group were removed after soaking the kernels in water for 24 hours at 60°C. After removal, the embryos were dried at 60°C for 72 hours. The weight of 100 embryos in each group was determined after drying. Coleoptile measurements were taken on 100 normal kernels in each group after germination for 7 days at 23°C.

Appropriate analyses of variance were performed on each character measured. Square root transformations were applied to the shrunken kernel number data before analysis to reduce variance heterogeneity. Inverse sine transformations were applied to all percentage data before analysis. The minimum differences for significance in the tables were obtained by means of the revised Duncan's ranges using for $p$ only the maximum number of means to be compared (Harter 1960).

Table 1. Mean squares from the variance analyses involving kernel number and their relationships

| Source of variation | Degrees of freedom | Normal kernel number | Shrunken kernel number[a] | Percent shrunken kernels[b] |
|---|---|---|---|---|
| Hybrid (H) | 1 | 80152** | 21.03** | 121.58* |
| Exposure (E) | 10 | 441835** | 204.35** | 4045.46** |
| E × H | 10 | 15019** | 5.28** | 77.31** |
| Date (D) | 1 | 1700 | 1.63 | 107.75* |
| D × H | 1 | 15781 | 12.87** | 53.21 |
| D × E | 10 | 10477* | 6.17** | 24.10 |
| D × E × H | 10 | 34292** | 9.46** | 29.43 |
| Error | 220 | 4452 | 1.76 | 20.37 |

[a] Square root transformation
[b] Inverse sine transformation
*, ** F values significant at the 5 and 1% level respectively

## Results

### Number

Mean squares from the analyses of variance are presented in Table 1. For all characters measured, the main effects, hybrid and exposure, and the interaction, exposure × hybrid, were significant at least at the 5% level and, in the majority of cases, at the 1% level. It is apparent that in both hybrids kernel number and percent shrunken kernels were greatly influenced by ultraviolet exposure and that the magnitude of the changes depended on the hybrid involved. The influence of the main effect, date, and its interactions was quite inconsistent and erratic.

The means associated with the analyses of variance are presented in Table 2. In both hybrids, increasing exposures decreased the normal kernel number so that the number at the highest exposure level was about 20% of the 0 level. The significant exposure × hybrid interaction was associated with the lower and intermediate exposure levels, with W showing

Table 2. Means associated with kernel number and their relationship. Each value represents the mean of 6 ears

| Character | Hybrid | Date | Exposure ($erg/cm^2 \times 10^5$) 0 | 0.68 | 1.36 | 2.04 | 2.72 | 3.40 | 4.08 | 4.76 | 5.44 | 6.12 | 6.80 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal kernel number[a] | W | 1 | 474 | 471 | 486 | 389 | 418 | 307 | 275 | 231 | 189 | 136 | 118 |
| | | 2 | 534 | 525 | 493 | 334 | 419 | 318 | 253 | 135 | 136 | 100 | 82 |
| | K | 1 | 428 | 420 | 261 | 351 | 371 | 292 | 254 | 200 | 182 | 139 | 104 |
| | | 2 | 465 | 441 | 405 | 329 | 336 | 286 | 249 | 199 | 133 | 131 | 83 |
| Shrunken kernel number[b] | W | 1 | 1.46 | 2.11 | 3.55 | 4.61 | 7.13 | 7.58 | 9.93 | 9.75 | 10.19 | 9.54 | 9.14 |
| | | 2 | 0.93 | 2.01 | 4.08 | 4.73 | 7.26 | 9.62 | 8.62 | 7.27 | 8.59 | 10.03 | 8.71 |
| | K | 1 | 1.36 | 1.28 | 2.19 | 4.38 | 5.97 | 7.75 | 8.35 | 8.37 | 7.99 | 7.89 | 8.38 |
| | | 2 | 1.94 | 1.75 | 4.25 | 6.63 | 7.92 | 9.04 | 9.00 | 8.95 | 7.47 | 7.14 | 6.40 |
| Percent shrunken kernels[c] | W | 1 | 4.64 | 5.59 | 9.09 | 13.30 | 19.40 | 23.67 | 30.88 | 32.85 | 36.58 | 41.08 | 40.81 |
| | | 2 | 2.31 | 4.98 | 10.63 | 14.70 | 19.70 | 28.41 | 28.40 | 28.72 | 36.62 | 45.12 | 43.19 |
| | K | 1 | 4.10 | 3.54 | 8.08 | 13.11 | 17.41 | 24.52 | 28.12 | 30.59 | 30.78 | 34.17 | 39.38 |
| | | 2 | 5.06 | 4.79 | 11.95 | 20.02 | 23.40 | 28.21 | 29.80 | 32.85 | 33.93 | 31.93 | 35.79 |

[a] Minimum differences between any two normal kernel means for significance are 98 and 127 at the 5 and 1% level respectively
[b] Transformed (square root) means. Minimum differences between any two transformed shrunken kernel means for significance are 1.94 and 2.53 at the 5 and 1% level respectively
[c] Transformed (inverse sine) means. Minimum differences between any two transformed percent shrunken kernel means for significance are 6.61 and 8.61 at the 5 and 1% level respectively

Table 3. Mean squares from the variance analyses involving kernel weight, embryo weight and coleoptile length

| Source of variation | Degrees of freedom | Normal kernel weight | Shrunken kernel weight | Embryo weight | Coleoptile length |
|---|---|---|---|---|---|
| Hybrid (H) | 1 | 9871.2 | 71252.65** | 277.57** | 162 |
| Exposure (E) | 10 | 23970.7** | 26853.86** | 505.10** | 9450** |
| E × H | 10 | 65990.9** | 17878.68** | 312.80** | 9784** |
| Error | 2178[a] | 2589.6 | 2504.71 | 27.83 | 1516 |

[a] Error degrees of freedom for shrunken kernel weight were 1935
** F values significant at the 1% level

less decrease in this range than K. A somewhat different pattern was found with shrunken number and percentage. In both hybrids, increasing exposure sharply increased both number and percentage. However, the significant exposure × hybrid interaction was primarily associated with the higher exposure levels. This response is obvious in Fig. 1, which is plotted in actual rather than transformed means. The rate of increase of W and K was almost identical at the lower and intermediate exposure levels. Above 4.76 erg/cm$^2$ × 10$^5$, the rate of increase in K was considerably lower than that in W.

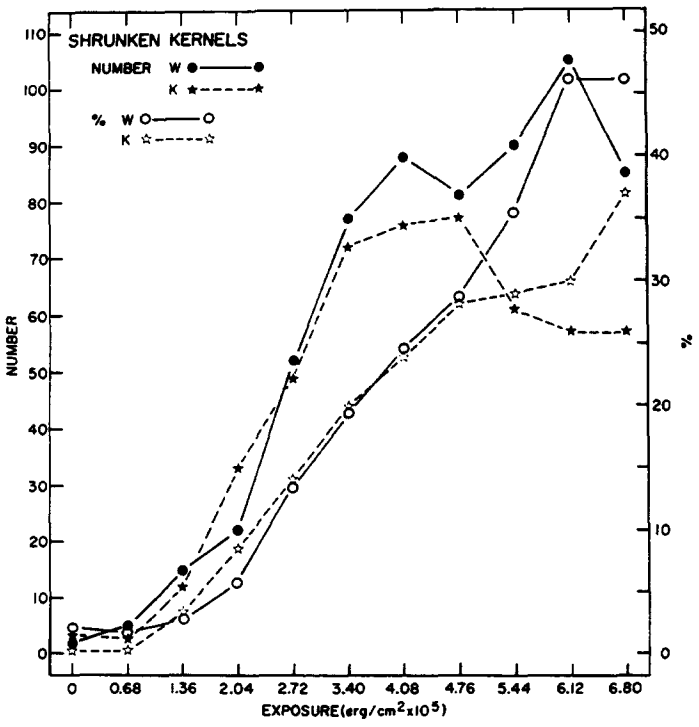


Fig. 1. The effect of various ultraviolet exposure levels on the number and percent of shrunken kernels obtained on the two hybrids

## Weight and Length

Mean squares from the analyses of variance are presented in Table 3. For all characters measured, the main effect, exposure, and the interaction, exposure × hybrid, were significant at the 1% level. These results indicated that in both hybrids exposure to ultraviolet altered kernel weight and coleoptile length and the degree of change was related to the hybrid involved.

The means of each hybrid at the various exposure levels are shown in Table 4 and are plotted as a % of O exposure in Fig. 2. For normal kernel weight, shrunken kernel weight and embryo weight, increasing exposure increased the means in W and produced little or no change in K. The means in W significantly exceeded the O exposure level at all exposure levels, whereas the means in K at most levels were not significantly different from the O exposure level. An entirely different relationship emerged with coleoptile length. With this character, a reduction was found with increasing exposure. Significant differences from the O exposure level were found at the intermediate and higher exposures in W while the mean of K was significantly different from the O exposure level only at 6.80 erg/cm$^2$ × 10$^5$.

Table 4. Mean associated with kernel weight, embryo weight and coleoptile length. Each value represents the mean of 100 measurements with exceptions noted

| Character | Hybrid | Exposure ($erg/cm^2 \times 10^5$) 0 | 0.68 | 1.36 | 2.04 | 2.72 | 3.40 | 4.08 | 4.76 | 5.44 | 6.12 | 6.80 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal kernel weight (mg)[a] | W | 286.14 | 313.33 | 301.68 | 328.33 | 331.06 | 346.40 | 335.46 | 371.34 | 368.15 | 339.05 | 336.35 |
| | K | 341.31 | 337.22 | 354.41 | 353.33 | 359.37 | 337.50 | 320.57 | 313.26 | 330.19 | 341.68 | 315.06 |
| Shrunken kernel weight (mg)[b] | W | 38.88[e] | 67.07[f] | 83.52 | 94.29 | 97.87 | 119.19 | 71.62 | 83.45 | 86.73 | 71.31 | 80.33 |
| | K | 60.69[g] | 74.95[g] | 104.78 | 75.57 | 78.00 | 68.68 | 58.53 | 79.12 | 59.18 | 73.77 | 59.75 |
| Embryo weight (mg)[c] | W | 25.13 | 28.36 | 27.12 | 30.23 | 31.01 | 32.01 | 30.03 | 33.47 | 32.39 | 29.71 | 28.99 |
| | K | 28.22 | 28.46 | 29.66 | 29.34 | 32.22 | 30.81 | 29.61 | 27.87 | 29.76 | 26.68 | 28.02 |
| Coleoptile length (mm)[d] | W | 118 | 125 | 111 | 111 | 117 | 94 | 105 | 98 | 100 | 99 | 99 |
| | K | 111 | 104 | 111 | 102 | 102 | 104 | 113 | 119 | 112 | 111 | 83 |

[a] Minimum differences between any two normal kernel weight means for significance are 17.54 and 22.75 at the 5 and 1% level respectively
[b] Minimum differences between any two shrunken kernel weight means for significance are 17.25 and 22.37 at the 5 and 1% level respectively
[c] Minimum differences between any two embryo weight means for significance are 1.82 and 2.36 at the 5 and 1% level respectively
[d] Minimum differences between any two coleoptile length means for significance are 13 and 17 at the 5 and 1% level respectively
[e, f, g, h] The mean of 35, 57, 37, and 28 measurements respectively

## Discussion

In maize, kernel abortion resulting from pollination with ultraviolet - irradiated pollen grains has been recognized (Brewbaker and Emery 1962, Stadler and Sprague 1936, Stadler and Sprague 1937, Stadler and Uber 1942). However, no experimental results relating abortion to exposure level have been reported, since the main thrust of most studies dealt with the mutagenic activities of this agent on specific loci or chromosomes. Also, ultraviolet irradiation studies with maize pollen grains are difficult because of the limited penetration of ultraviolet and the large diameter and eccentric nuclear orientation within maize pollen grains (Brewbaker and Emery 1962, Uber 1939). The apparatus used in this study reduces these problems to a minimum (Pfahler 1973). Our present knowledge, gained largely from research with other species, indicates that seed abortion results from cytogenetic damage or dominant lethality induced in the mature pollen grain at the time of exposure. To fertilize the ovule and produce abortion, the pollen grains containing irradiation-induced damage must germinate, produce a pollen tube and compete with undamaged pollen grains. It is obvious from this study that induced damage does not completely inhibit the fertilization ability of the damaged pollen grains. However, the relationship between irradiation damage and fertilization ability may not be completely independent since a pollen source effect was evident in this study. This independence of damage and fertilization ability was observed in a study involving gamma irradiation of maize pollen grains, but differences between pollen sources were not significant (Pfahler 1967). An *in vitro* study (Pfahler 1973) indicated that the germination percent and pollen tube growth of maize pollen grains from one genetic source decreased only slightly when the grains were exposed to the levels used in this study. Our limited knowledge in this area suggests that the relationship between irradiation damage and fertilization ability is independent. However, the differences between pollen sources found in the present study indicate that this observation may have to be modified, at least in regard to ultraviolet.

Another important consideration that should be included in interpreting these results is the trinucleate condition of the mature maize pollen grain and its re-

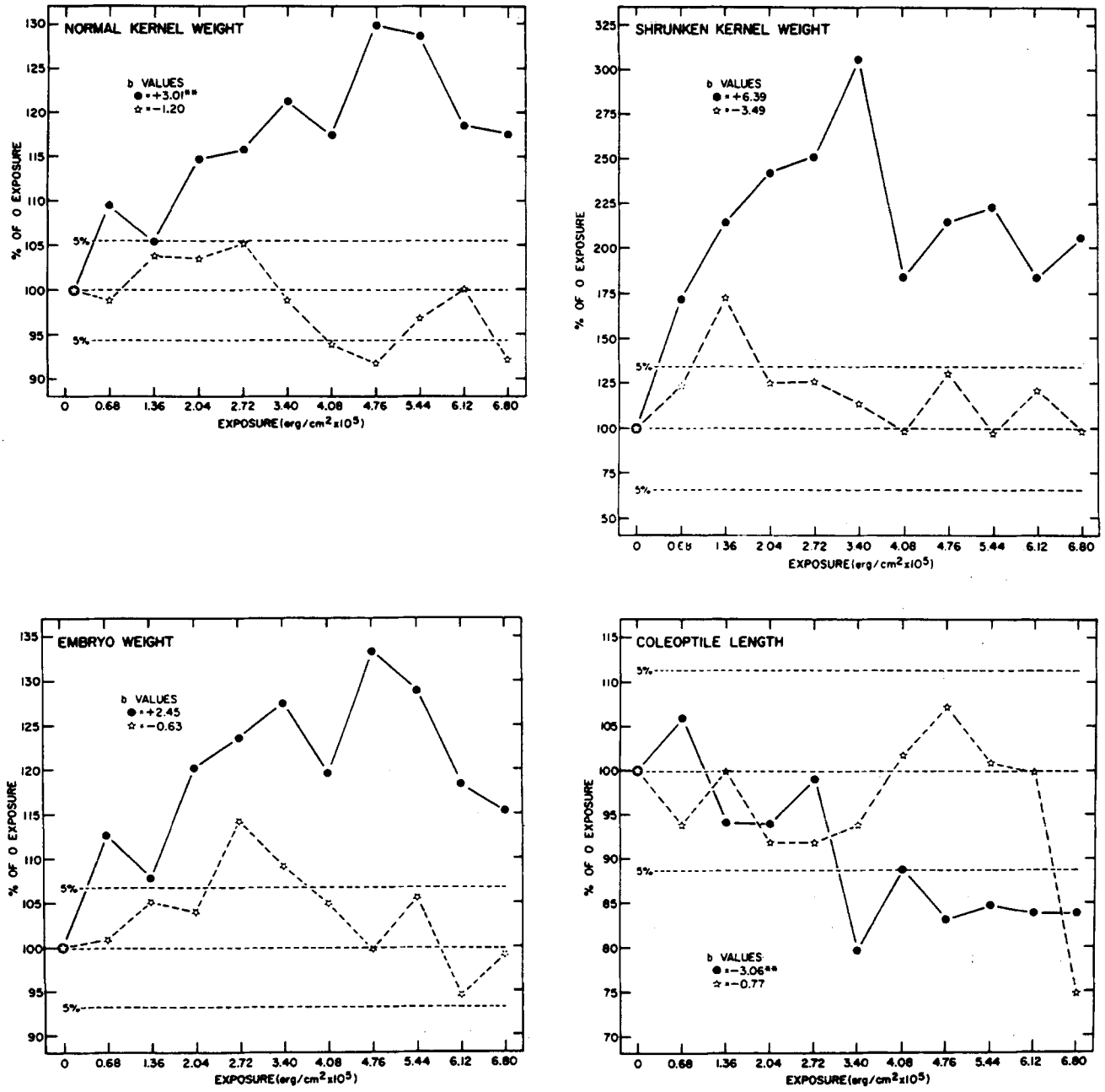


Fig.2. The effect (in % of O exposure level) of various exposure levels on the weight of the various kernel types and the coleoptile length of the normal kernels obtained on the two hybrids. The 5 % significance range from O exposure level is indicated by the horizontal lines equidistant above and below 100 %. Circles = W; stars = K

lationship to the double fertilization process and kernel abortion. Three nuclei, two sperm and one pollen tube, are present at the time of irradiation. Irradiation induces changes in these nuclei independently and at random so that in the resulting kernel the embryo and endosperm contain male genomes that probably have different degrees of damage. The diploid embryo, which is highly organized and differentiated, can tolerate little damage introduced by the male genome before abortion occurs. On the other hand, the triploid endosperm, which is less organized, can tolerate relatively large amounts of damage introduced by the sperm nucleus before abortion occurs. Therefore, kernel abortion is more commonly as-

sociated with the embryo rather than the endosperm (Brewbaker and Emery 1962). In the study reported here, the normal kernel weight and embryo weight in both hybrids followed the same pattern with increasing exposure, indicating that the effects of ultraviolet irradiation on the embryo and endosperm were essentially the same.

The mechanisms responsible for the pronounced differences between pollen sources are unknown at present. Apparently, the differences were present or occurred at the time of irradiation and resulted in less damage to the pollen grains from one source than the other. Many factors and their interactions may be involved. Firstly, the nuclei in the pollen grains from one source may be inherently more radiosensitive, and thus, more extensive damage from irradiation occurs. Secondly, the repair or reactivation mechanism (Fujui 1969, Ikenaga and Mabuchi 1966), in which the effects of ultraviolet irradiation revert to normal, may be more active or effective in pollen grains from one source than another. Thirdly, morphological and physiological differences in pollen grains would be very important because of the low penetrance of ultraviolet. In this case, differences in size, shape, opacity, density, nuclear orientation or even extine structure or thickness would be critical. Substantial diameter differences between pollen grains from various sources have been reported (Pfahler 1965). If all or any of these factors contribute to radiosensitivity differences, then genetic variation for these factors is probably present. Therefore, selection for pollen radiosensitivity would be possible.

Acknowledgement

Journal Series No. 6142, Florida Agricultural Experiment Station. Thanks are extended to H.S. Anspach, J.C. Harper and W.T. Mixon for their excellent technical assistance.

Literature

Brewbaker, J.L.; Emery, G.C.: Pollen radiobotany. Radiat. Bot. 1, 101-154 (1962)
Fujui, T.: Photoreactivation of mutations induced by ultraviolet radiation of maize pollen. Radiat. Bot. 9, 115-123 (1969)
Harter, H.L.: Critical values for Duncan's multiple range test. Biometrics 16, 671-685 (1960)
Ikenaga, M.; Mabuchi, T.: Photoreactivation of endosperm mutations induced by ultraviolet light in maize. Radiat. Bot. 6, 165-169 (1966)
Pfahler, P.L.: Fertilization ability of maize pollen grains. I. Pollen sources. Genetics 52, 513-520 (1965)
Pfahler, P.L.: Fertilization ability of maize pollen grains. III. Gamma irradiation of mature pollen. Genetics 57, 523-530 (1967)
Pfahler, P.L.: *In vitro* germination and pollen tube growth of maize (*Zea mays* L.) pollen VII. Effects of ultraviolet irradiation. Radiat. Bot. 13, 13-18 (1973)
Stadler, L.J.; Sprague, G.F.: Genetic effects of ultraviolet radiation in maize. Proc. Natl. Acad. Sci. U.S. 22, 572-591 (1936)
Stadler, L.J.; Sprague, G.F.: Contrasts in the genetic effects of ultraviolet radiation and X-rays. Science 85, 57-58 (1937)
Stadler, L.J.; Uber, F.M.: Genetic effects of ultraviolet radiation in maize. IV. Comparison of mochromatic radiations. Genetics 27, 84-118 (1942)
Uber, F.M.: Ultraviolet spectrophotometry of *Zea mays* pollen with the quartz microscope. Amer. J. Bot. 26, 799-807 (1939)

Received September 30, 1976
Communicated by H.F. Linskens

Prof. Dr. P.L. Pfahler
Agronomy Department
University of Florida
Gainesville, Florida 32611 (USA)

Prof. Dr. H.F. Linskens
Department of Botany
University of Nijmegen
Toernooiveld
Nijmegen (the Netherlands)