4. The effect of X-rays and fast neutrons on maize pollen.

An experiment was conducted in the last two years to compare the effects of X-rays and fast neutrons on maize pollen. Pollen from Plants 65-27 and 65-26 was irradiated with X-rays and fast neutrons of the same dose (1500 rads), and was used in self-pollinations. Five plants for each of these two types of radiation were employed. At harvesting, it was found that about 70 per cent of the ovules pollinated with X-rayed pollen set well-filled kernels, while about 95 per cent of the ovules pollinated with fast neutron treated pollen set well-filled kernels. Last summer, 345 bulked kernels from neutron treatment and 330 bulked kernels from X-ray treatment were planted in the field. Only four per cent of the former emerged while over 90 per cent of the latter gave viable seedlings.

It is postulated that acute irradiation with fast neutrons at a dose of 1500 rads on the pollen is adequate in inducing dominant embryo lethal mutation in maize of the immediate generation while X-rays of the same dose are less effective. However, at the same dose rate, X-rays are more effective than fast neutrons in reducing the percentage of seedset.

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1. Chemical mutagens on maize: Ethyl methanesulfonate.

Many geneticists have been interested in the production of mutations, to be used mostly in fundamental studies. The chemical mutagen ethyl methanesulfonate (EMS) has been shown to produce a high frequency of mutations accompanied by a relatively low frequency of chromosomal aberrations in plants.

The following studies have been conducted to study the feasibility of modifying the effects of EMS on maize. If the effect of this chemical mutagen can be modified it may also be possible to alter its effectiveness and efficiency. These terms have been defined by Nilan et al (1965). They state that effectiveness of a mutagenic agent usually means the rate of "point" mutations as related to dose. Efficiency usually refers to the "point" mutation rate in relation to other biological effects induced, usually a measure of damage. Biological effectiveness is used in this paper as the amount of damage as related to dose.

Post-treatments being investigated to influence the effectiveness and efficiency of the mutagen treatment are soaking of the seeds in water and drying them. Other modifying factors being investigated are temperature and duration of treatment and concentration of mutagen. If seeds

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Table 1

Plant height at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

| Molarity | Planted wet % of control | Post-soak time (hr.) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 6 | | 12 | | 18 | | 24 | | 36 | | 48 | |
| | | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry |
| 0.00125 | 100.0 | 100.0 | 100.0 | 104.8 | 104.8 | 99.1 | 99.1 | 112.7 | 112.7 | 97.1 | 97.1 | 87.0 | 86.9 | 104.0 | 104.0 |
| 0.0025 | 88.5 | 55.4 | 62.5 | 100.0 | 113.0 | 91.5 | 103.3 | 104.4 | 117.9 | 96.2 | 108.7 | 83.3 | 94.1 | 96.5 | 109.0 |
| 0.005 | 99.5 | 75.8 | 76.2 | 102.0 | 102.4 | 80.7 | 81.1 | 87.3 | 87.8 | 84.6 | 85.0 | 100.9 | 101.4 | 92.0 | 92.4 |
| 0.01 | 107.6 | 38.6 | 35.8 | 74.0 | 68.8 | 57.0 | 53.0 | 70.2 | 65.2 | 80.3 | 74.6 | 78.9 | 73.2 | 81.4 | 75.6 |
| 0.02 | 78.5 | 5.6 | 7.1 | 14.9 | 19.0 | 22.9 | 29.1 | 36.1 | 46.0 | 40.0 | 50.9 | 63.5 | 81.0 | 75.4 | 96.1 |
| 0.04 | 61.7 | -- | -- | 0.7 | 1.2 | -- | -- | -- | -- | 11.1 | 18.0 | 25.2 | 40.9 | 36.7 | 59.4 |
| 0.08 | 50.7 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.6 | 3.1 | 5.0 | 9.9 |

are dried immediately after being treated with EMS, many dose levels will prove fatal to the seeds. This is probably because EMS and its hydrolysis products, which are apparently detrimental, remain in the seed and accumulate or are more harmful when the seeds are dried. There is also the possibility that if seeds are post-soaked, some of the unhydrolyzed EMS may be removed from the seed, and the mutation frequency will be decreased.

If a satisfactory treatment regime can be established by post-soaking, drying and storing seeds after EMS treatment, it should give the investigator considerably more control over conditions at planting time such as inclement weather and will allow the shipment of treated seeds, that would not be possible if wet seeds are used. Of equal or greater importance is the possibility that the efficiency of the mutagen may be increased.

A genetic stock dominant for many endosperm and plant genes was used in this study. The seeds were treated with EMS (Eastman Organic Co.) for 10 hrs. at 25°C. $\pm$ 0.02°C. in a 0.1 M aqueous phosphate buffer (pH 7.5). The seeds were treated with 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08 M EMS. After treatment the seeds were rinsed in distilled water and soaked at 3°C. $\pm$ 0.02°C. in distilled-deionized water for 0, 6, 12, 18, 24, 36, 48 hrs. and shaken at 75 cycles/min. After post-soaking, the seeds were dried for 72 hrs. after the last post-soaking collection in a room maintained at 22°C. and 60% relative humidity. A fan was used to circulate the air. A portion of the seeds were planted in flats containing soil, peat and sand in a growth chamber maintained at 22°C., 2600 foot candles light intensity and 18 hr. photoperiod. Seeds were also stored at 25°C. and -20°C. for 4 and 8 weeks and then planted. For the 4 and 8 week storage periods the seeds were stored in evacuated desiccators which had a relative humidity of approximately 35%. For a further comparison, when the 0, 4 and 8 week experiments were planted, seeds were treated as described above but were planted immediately after the EMS treatment, without being post-soaked or dried. Two replicates of 15 seeds each were used. The criteria used to evaluate biological effects of the treatments were plant height and survival at 14 days and survival at 30 days.

As indicated in Table 1 and Figure 1 there is an increase in plant height by post-soaking the EMS treated seeds in water before drying. This is also noted for survival at 14 and 30 days (Tables 2 and 3). The advantage of post-soaking is more pronounced with the higher concentrations of EMS. In fact there is no survival of the 0.08 M treatment unless the seed is post-soaked for at least 36 hrs. before drying. As indicated in Tables 1, 2 and 3 and Figure 1, 0.02 M is the heaviest dose that can be used and still obtain surviving plants, if the material is dried immediately after the EMS treatment.

The data in the second column in the tables were obtained from seed treated with EMS planted wet without being post-soaked. This material was planted with the material that had been treated with EMS, post-soaked and dried. This is displayed as per cent of control also. In the columns designated "wet compared to dry" the material planted wet

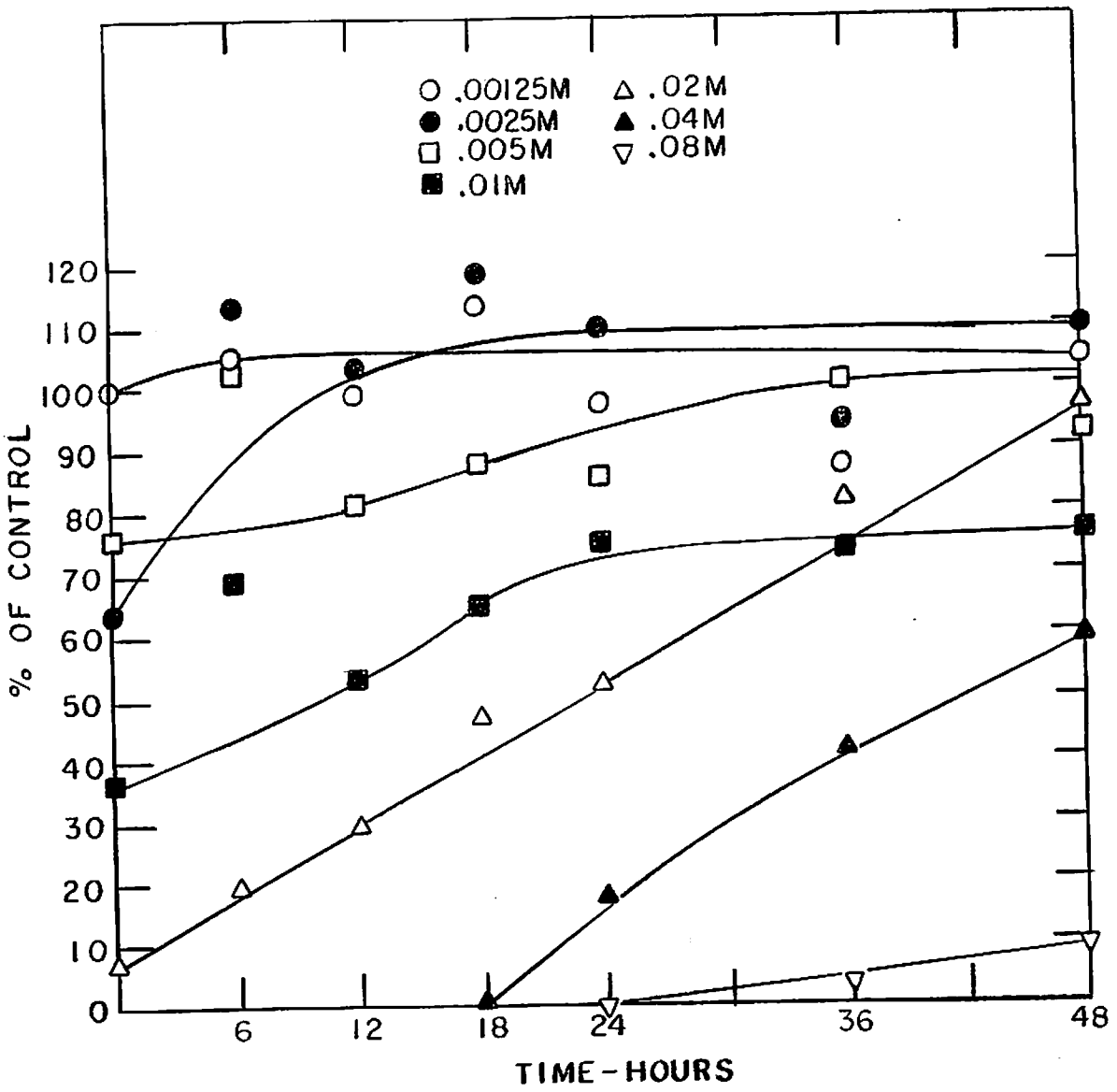


Figure 1. Plant height at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment, compared to an EMS treatment that was not post-soaked and planted wet.

was compared to the material that had been treated with EMS, post-soaked, dried, then planted. In addition to this comparison, in which the dried seed treatment was compared to the material planted wet, the treatment post-soaked and dried was evaluated alone. Although a similar response was obtained from both comparisons, the one involving the drying of seed generally gave lower values, especially for survival.

Experiments using diethyl sulfate, post-soaking and drying barley seed, were done by Konzak et al (1961). Related studies by Froese-Gertzen et al (1964) and Gaul et al (in press) have also been done. Barley seed can be soaked in water for rather short periods of time and still maintain germination (Froese-Gertzen et al, 1964). Maize seed is apparently much more resistant than barley to soaking in water and drying (Briggs, in press and unpublished).

The biological effectiveness of the chemical is increased considerably if the seed is dried without post-soaking. That is, plant height and survival are considerably reduced. This can be seen if, for example, column 2 is compared to column 3 etc. in the tables.

The treatment levels chosen in this experiment range from those that produce very little to no effect compared to those that produce a severe effect and sometimes lethality. Based on observations made at this laboratory, an EMS treatment of 10 hrs. and 0.05 M is the heaviest treatment that will produce an adequate seed set if treated seeds are planted wet.

It is quite apparent that when the seeds were dried, post-soaking improved plant height and survival. However, the effects of drying the seeds after being treated with high concentrations of EMS were rather severe, as determined by reduced plant height and survival, compared to the EMS treatment in which the seeds were planted wet. Even the 48 hr. post-soaking conditions did not increase plant height and survival above the material that was treated with EMS and planted wet. When seeds treated with low concentrations of EMS were dried back they did not differ greatly from the material planted wet. However, post-soaking did improve this material somewhat.

From general observations, variability seems to be increased by the 4 and 8 week storage times at -20°C. and 25°C. Storing treated material at the higher temperature lowered plant height and survival more than the lower temperature. Also the -20°C. temperature permits survival whereas in many cases the 25°C. storage temperature does not, especially at the higher concentrations of EMS. The 8 week storage at both temperatures seemed to have reduced plant height and survival more than the 4 week storage condition. Therefore, if storage of treated material for any length of time before planting is contemplated a low temperature such as -20°C. should probably be used.

When seeds are treated with the above concentrations of EMS and post-soaked up to 48 hrs and not dried but planted immediately after collection little advantage is obtained. The values (actual data not reported) remain nearly the same, within concentrations, from one

Table 2

Survival at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

| Molarity | Planted wet % of control | Post-soak time (hr.) 0 | | 6 | | 12 | | 18 | | 24 | | 36 | | 48 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry |
| 0.00125 | 115.4 | 86.7 | 75.1 | 111.5 | 96.7 | 96.6 | 83.7 | 103.4 | 89.7 | 96.6 | 83.7 | 90.0 | 78.0 | 108.0 | 93.6 |
| 0.0025 | 107.7 | 96.7 | 89.8 | 115.4 | 107.1 | 89.7 | 83.3 | 96.6 | 89.7 | 100.0 | 92.9 | 90.0 | 83.6 | 112.0 | 104.0 |
| 0.005 | 100.0 | 100.0 | 100.0 | 115.4 | 115.4 | 96.6 | 96.6 | 89.7 | 89.7 | 100.0 | 100.0 | 93.3 | 93.3 | 116.0 | 116.0 |
| 0.01 | 111.5 | 90.0 | 80.7 | 115.4 | 103.4 | 103.4 | 92.8 | 82.8 | 74.2 | 89.7 | 80.4 | 96.7 | 86.7 | 116.0 | 104.0 |
| 0.02 | 103.8 | 40.0 | 38.5 | 100.0 | 96.3 | 93.1 | 89.6 | 93.1 | 89.6 | 100.0 | 96.3 | 90.0 | 86.7 | 120.0 | 115.6 |
| 0.04 | 92.3 | -- | -- | 7.7 | 8.3 | -- | -- | 10.3 | 11.2 | 48.3 | 52.3 | 93.3 | 101.1 | 100.0 | 108.3 |
| 0.08 | 84.6 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 3.3 | 3.9 | 24.0 | 28.4 |

Table 3

Survival at 30 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

| Molarity | Planted wet % of control | Post-soak time (hr.) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 6 | | 12 | | 18 | | 24 | | 36 | | 48 | |
| | | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry | % cont | wet cf dry |
| 0.00125 | 115.4 | 100.0 | 86.7 | 103.6 | 89.8 | 100.0 | 86.7 | 103.4 | 89.7 | 100.0 | 86.7 | 93.3 | 81.0 | 103.8 | 90.0 |
| 0.0025 | 111.5 | 100.0 | 89.6 | 107.1 | 96.1 | 100.0 | 89.6 | 96.6 | 86.6 | 96.6 | 86.6 | 90.0 | 80.7 | 107.7 | 96.6 |
| 0.005 | 103.8 | 103.4 | 99.6 | 107.1 | 103.2 | 93.1 | 89.6 | 86.2 | 83.0 | 96.6 | 93.0 | 93.3 | 90.0 | 111.5 | 107.4 |
| 0.01 | 107.7 | 75.9 | 70.4 | 92.9 | 86.2 | 100.0 | 92.9 | 79.3 | 73.6 | 86.2 | 80.0 | 96.7 | 89.8 | 115.4 | 107.1 |
| 0.02 | 100.0 | -- | -- | 32.1 | 32.1 | 62.1 | 62.1 | 79.3 | 79.3 | 86.2 | 86.2 | 86.7 | 86.7 | 115.4 | 115.4 |
| 0.04 | 92.3 | -- | -- | -- | -- | -- | -- | -- | -- | 13.8 | 14.9 | 53.3 | 57.8 | 69.2 | 75.0 |
| 0.08 | 80.8 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 7.7 | 9.5 |

collection time to the next, i.e., there is very little if any increase in plant height or survival by the post-soaking. However, the heavier concentrations do give a greater effect.

The hydrolysis products and EMS are probably removed by post-soaking but little advantage is noted from their removal if seeds are planted wet. Apparently the effect produced by the EMS treatment is irreversible in this case. Therefore, it appears that post-soaking is of little value in influencing the biological effectiveness if the seeds are planted wet. Genetic studies are in progress to determine if any of these post-treatment conditions will increase the effectiveness of EMS.

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References:

Nilan, R. A., C. F. Konzak, J. Wagner and R. R. Legault. 1965. In The Use of Induced Mutations in Plant Breeding, Pergamon Press, Oxford, pp. 71-88.

Konzak, C. F., R. A. Nilan, J. R. Harle and R. E. Heiner. 1961. Brookhaven Symp. Biol. 14: 128-157.

Froese-Gertzen, E. E., C. F. Konzak, R. A. Nilan and R. E. Heiner. 1964. Radiation Botany 4: 61-69.

Gaul, H., K. Bender, E. Ulonska and M. Sato. (in press). Intern. Atomic Energy Tech. Rep. Ser., Vienna.

Briggs, R. W. (in press). Intern. Atomic Energy Tech. Rep. Ser., Vienna.