a standard pollination technique, experimental design and subsequent statistical analysis of the data (K), the contribution of the number of mature egg cells per ear to the variability in the numbers of kernels per ear per treatment can be discounted. Thus a bio-assay for viable corn pollen can be developed that possesses only a few restrictions and provides several advantages over existing assays: e.g. germination of pollen on agar. Foremost among the advantages is the production of the succeeding generation. An obvious disadvantage for the application of this bio-assay in some studies is the time required to obtain the data and the infrequency with which the assay can be used. However, in contrast with the other assays available for testing pollen viability, this bio-assay provides a continuity to the research program.

Using this bio-assay, the study of pollen longevity was continued in 1957. The summarized results from these experiments will not be available until a later date. Of interest, however, was an experiment involving the cooperation of Dr. Patterson at Urbana, Illinois, and Drs. Jones and Stinson at New Haven, Connecticut. Pollen, some of which was previously held at -10°C., was shipped to these cooperators and was used successfully in effecting pollinations on the female Oh51A$^{T}$ x B8. Similar pollinations were made in Ithaca. The seed yield from these three locations will be tested in a yield trial in New York in 1958.

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D. B. Walden

2. Diepoxybutane as a chemical mutagen in maize.

Experiments were conducted for three seasons to determine the possible mutagenic effects of diepoxybutane, $CH_2 - CH - CH - CH_2$ (with O bridges between the CH and CH$_2$ groups), on pollen of Zea mays. (The term "mutation" unless otherwise specified includes both chromosome breaks and so-called "point" mutations.) Two methods of treatment were used. In one method the cut ends of newly shedding tassels were taken from homozygous multiple dominant stocks and were placed in 0.2 per cent solutions of the chemical for 18 hours. Pollinations were made on homozygous multiple recessive stocks immediately following the treatment. The other method of treatment involved boring a hole in the corn stalk about four inches below the lowest tassel branch. A one dram vial containing 4.5 cc. of 0.2 per cent diepoxybutane (DEB) solution was attached to the stalk and a piece of woven glass wicking was used to introduce the solution into the plants. Pollinations were made for five successive days from each treated and control tassel.

Losses of dominant marker genes in the endosperm of the resulting kernels were used to evaluate the mutagenicity of the DEB. All of the experiments utilized multiple recessive stocks having the chromosome

nine markers c sh wx or C sh bz wx, all of which affect the endosperm. These linked genes were used as material well-suited to investigate the problem of whether the appearance of the recessive characters involved chromosomal deficiency or gene mutation. Since the relative order of marked loci distal to the centromere in chromosome nine is known to be Wx, Bz, Sh, and I, the position and proportionate number of breaks within marked regions of the chromosome arm can be determined from the phenotypic appearance of the endosperm. Breakage-fusion-bridge cycles as well as interstitial deletions and end losses were induced frequently by the treatment. The type and frequency of endosperm deficiencies observed are presented in Tables 1 and 2.

The extent of dominant marker loss varied from a tiny spot to the entire endosperm, with all intermediate types occurring. Losses occupying less than 1/8 of the kernel were not scored since they are both difficult to classify and are not markedly increased by the treatment.

A summary of the results and conclusions follows:

1. Diepoxybutane is a powerful inducer of mutant endosperm sectors in maize. The number of kernels showing single or multiple gene losses was either 10 or 14 per cent in the $F_1$ kernels, depending on the method of treatment. This frequency is approximately equivalent to that obtained from treatment of maize pollen with 1500 r of X-rays in similar stocks. The frequency of mutation induced by DEB is not influenced by the year of treatment or stock used. However, the cut tassel method of treatment resulted in a significantly higher rate of mutant kernels than the wick method. The wick method has the advantage that a greater number of mutant kernels is obtained per ear owing to increased seed set.

2. There appears to be no differential sensitivity of maize pollen to DEB treatment on any of the five days preceding pollen shedding.

3. The two stocks differ significantly in the number of breaks observed distal to C or I as well as the number observed between Wx and the centromere. It is suggested that this may be due either to sampling error or to differential sensitivity of some chromosomal regions to breakage.

4. The distance between marked loci in the short arm of chromosome nine as determined by the relative frequency of breaks occurring between these loci is very similar to the cytological distances as determined at pachytene. This is considered evidence that breakage resulting from DEB treatment is induced approximately at random along the chromosome arm.

5. About twice as many kernels had sectors of mutant tissue as had exclusively mutant tissue in the endosperm. This frequency of

Table 1. Type and frequency of mutant endosperms induced by 0.2 per cent DEB treatment of maize pollen in C sh bz wx x I Sh Bz Wx by the cut tassel method.

| Treat. | Year | B.-F.-B. cycles starting in | | | | Interstitial Losses | | End Losses | | | | More Complex | All Affected | Total Examined | Per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | E-I | I-Sh | Sh-Bz | Bz-Wx | ShWx | Wx | I | ISh | IShBz | IShBzWx | | | | |
| 0.2% DEB | 1956 | 117 | 4 | 4 | 28 | 3 | 6 | 15 | 2 | 8 | 163 | 7 | 357 | 3314 | 10.8 |
| | 1957 | 44 | 1 | 1 | 10 | - | 1 | 3 | 1 | 2 | 57 | 3 | 123 | 920 | 13.4 |
| | Total | 161 | 5 | 5 | 38 | 3 | 7 | 18 | 3 | 10 | 220 | 10 | 480 | 4234 | 11.3 |
| Control | 1956 | 2 | 2 | - | - | - | | 1 | | - | 9 | 1 | 15 | 6377 | 0.2 |
| | 1957 | 1 | - | - | - | - | | - | | - | - | - | 1 | 874 | 0.1 |
| | Total | 3 | 2 | - | - | - | | 1 | | - | 9 | 1 | 16 | 7251 | 0.2 |

Table 2. Type and frequency of mutant endosperms induced by 0.2 per cent DEB treatment of maize pollen in c sh wx x C Sh Wx.

| Treat. | Method | Year | B.-F.-B. cycles starting in | | | Interstitial Losses | | End Losses | | | More Complex | All Affected | Total Examined | Per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | E-C | C-Sh | Sh-Wx | ShWx | Wx | C | CSh | CShWx | | | | |
| 0.2% DEB | Cut | 1956 | 74 | 7 | 26 | - | 3 | 6 | 17 | 153 | 3 | 289 | 2436 | 11.9 |
| | Tassel | 1957 | 44 | 0 | 23 | 1 | 1 | 4 | 3 | 85 | 1 | 162 | 1145 | 14.2 |
| | | Total | 118 | 7 | 49 | 1 | 4 | 10 | 20 | 238 | 4 | 451 | 3581 | 12.6 |
| | Wick | 1957 | 273 | 20 | 85 | 0 | 16 | 26 | 34 | 606 | 9 | 1069 | 10862 | 9.8 |
| | Grand | Total | 391 | 27 | 134 | 1 | 20 | 36 | 54 | 844 | 13 | 1520 | 14443 | 10.5 |
| Control | Cut | 1956 | 5 | - | 1 | - | - | 1 | - | 2 | - | 9 | 4338 | 0.2 |
| | Tassel | 1957 | - | - | - | - | - | 2 | - | - | - | 2 | 1548 | 0.1 |
| | Wick | 1957 | 5 | 1 | - | - | - | 5 | - | 2 | - | 13 | 5711 | 0.2 |
| | | Total | 10 | 1 | 1 | - | - | 8 | - | 4 | - | 24 | 11597 | 0.2 |

sectoring is similar to that obtained following ultraviolet treatment of pollen and is in contrast to the results following X-ray treatment of pollen in which sectoring is rarely observed. The ratio of fractional to entire endosperm effects following DEB treatment was not influenced by the year of experimentation, the stock used, or the method of treatment. The size of mutant sectors forms a nearly normal frequency distribution about the center value of one-half the endosperm. The following factors may be involved in sectoring: (a) If the chromosome has effectively two strands at the time of treatment, breaks in chromatids rather than whole chromosomes would result in sectoring. (b) If breaks are entirely chromosomal but the centric and acentric portions are held together by the matrix until division occurs, a sector would result if following division, one acentric chromatid restitutes while the other is lost. (c) Chromosomal instability induced by the treatment may be involved in sectoring.

6. There is no evidence that DEB markedly increases the frequency of gene mutation in maize, although the loss of single loci may include some gene mutations. Furthermore, the treatment of maize pollen with DEB has little or no effect on the $F_1$ plant generation. This is similar to results following ultraviolet treatment of maize pollen and is in contrast to the results following X-ray treatment in which there is a close correlation between the frequency of mutation in the embryo and endosperm. The absence of any effect on the $F_1$ plant generation suggests that DEB will be of little value in the production of mutations in maize for plant breeding purposes using the above methods of treatment. However, since DEB is extremely effective as an inducer of gene mutation in the Neurospora back mutation test and of chromosomal deficiencies in Drosophila, there is the possibility that it may be effective in increasing the mutation rate in other organisms or in maize embryos under different conditions.

Jean D. Kreizinger

CROW'S HYBRID CORN COMPANY
Milford, Illinois

1. Dwarf prolific corn.

We have been doing some preliminary work since 1950 on a new type of corn that could be harvested with a combine. In order to get a heavy set of ears, we have crossed our inbred lines with teosinte and have backcrossed once to corn, then started a selfing program. We crossed about a hundred lines with teosinte this year to broaden the program. We have also crossed it with all our dwarf types, and with male sterile and male restorer. We believe that this project has merit, and we are going to make every effort to produce an acceptable