Some of this work and the details of analysis have been reported by Briggs and Smith in MNL 38 (pp. 25-27). The $wx^C$ x $wx^{90}$ heteroallelic cross received 200 r of x-rays in meiosis. An effect on intragenic recombination is noted in that x-rays increase and decrease genetic recombination as compared to unirradiated plants (Table 3). A rough extrapolation of Stadler's data provides an estimate that he used doses of x irradiation up to approximately twenty times higher than those used here. His doses ranged from about 250 to 4000 r.

Maize and other organisms may have two mechanisms of genetic recombination, i.e., one for intergenic and one for intragenic recombination. If there are two mechanisms of genetic recombination in maize, the mechanism involved in intragenic recombination appears to be affected by irradiation, whereas the mechanism involved in intergenic recombination does not appear to be affected. Therefore, on the basis of an indirect comparison, there is some indication that there may be two mechanisms of genetic recombination in maize.

R. W. Briggs

3. Chemical mutagens on maize: Myleran.

In an experiment designed to investigate the mutagenicity of chemical mutagens on several endosperm genes on the short arm of chromosome 9, the agent Myleran (di-methane-sulphonyloxy-butane) was used. Myleran (m. w. 246.31) was investigated because its molecular relationship to ethyl methanesulfonate (m. w. 124.16) is essentially that of two EMS molecules joined together, and it is bifunctional. Effects of this agent have been reported in the literature, particularly by the Moutschen-Dahmens and Michaelis and Rieger. This experiment was performed to determine the most efficient treatment procedures for using this agent.

The Myleran used was obtained from Burroughs Wellcome and Co., Tuckahoe, New York. Its solubility presents a problem which, under current experimental procedures, seems to be even more difficult than has been reported. Methods for increasing solubility have been investigated by varying temperature, time and organic solvents. To date the maximum treatment time has been 24 hours and

the maximum temperature 35° C. A 7% ethyl alcohol solution has been used in attempting to increase solubility. The various combinations of treatments are shown in Table 4.

Seed treatments were used with this agent. In all cases the seeds were presoaked for 24 hours at 25° C. The treatments were administered in an aqueous phosphate buffer (0.02 M, pH 7.5). The genotype of the treated stock was $C^I$ *Sh Bz Wx*. The tester stock, which was generously supplied by A. L. Caspar of Blandy Farm, had the genotype of $A_1$ $A_2$ *R C sh bz* $wx^C$ $v_1$. Two field replications were used and each plot consisted of 50 seeds except that the controls C and D had 25. The treated stock was detasseled and used as the female parent, with the tester stock as the male. The field planting was arranged so that there was a male, then two females, then a male. This procedure gave a tester male alongside each row of treated females. With this arrangement an ample amount of pollen and good seed set was produced. An abundance of material was available for observing mutants, so that 1363 ears were examined in the entire experiment, an average of 3.04 ears per plant (tillering stock). Also, the treated stock, except for some treatments, produced 200-250 kernels per ear based on a conservative estimate. This material was grown in an isolated field which allowed the use of the open-pollinated crossing procedure.

From a nonmutated event the triploid genotype of the aleurone tissue should be $C^I$ $C^I$ *C*. If the $C^I$ mutated to *C* the cross should be *C C C* or produce "fully colored" aleurone tissue. If $C^I$ mutated to *c*, the cross *c c C* should be "pale with frequent colorless patches." The phenotypes and genotypes of the crosses are quoted from the work of E. H. Coe (Genetics 47: 779, 1962). All except one of the aleurone color mutants may be mutations of $C^I$ to *c*, since they were not fully colored and fitted quite closely to the above description of the *c c C* genotype. However, other explanations of some of these mutants may be possible since waxy sectors were detected in some kernels. These kernels may have been caused by a phenomenon related to the breakage-fusion-bridge cycle. Also, several of these kernels occurred in the controls, and may, therefore, have little bearing on evaluating the effectiveness of the treatments. For purposes of classification in the table they were designated as *c* mutants. One full color kernel was produced; this may possibly represent a mutation of $C^I$ to *C*. This mutant occurred in a control; therefore, it represents a spontaneous mutant.

During the growing season and before anthesis (ca. 10 leaf stage) the leaves were scored in the field for chlorophyll sectors. The classes were arbitrarily called "yellow-green" and "other." The "yellow-green" class is merely descriptive and may or may not be associated with the known locus on chromosome 9. All other chlorophyll abnormalities were included in the "other" class, except albino sectors. These have been noted following ethyl methanesulfonate treatments (Briggs, unpubl.), but not after Myleran.

The sector data were not taken completely quantitatively in that: 1) each leaf was not scored separately, and 2) if there were sectors on several leaves which appeared to come from one event, they were considered as one. The data as presented can be used to assess the relative effect of the treatments. Twenty plants per replication were used whenever available.

The treatments with .003 and .005 molarity for 12 hours at 35° C, seem to be quite effective in producing leaf sectors. However, very few plants were available for analysis. Also, treatments with .003 M for 24 hours at 25° C seemed rather effective in producing sectors.

The .002, .003 and .004 M treatments for 10 hours at 25° C and .003 M for 24 hours at 25° C appeared to be the most effective for affecting the aleurone and endosperm. It cannot be concluded that Myleran was responsible for the production of the waxy mutant, since one also occurred in a control containing alcohol, and the other occurred with Myleran and alcohol. Therefore, there is some indication that the alcohol was mutagenic.

Plant height, from ground level to tip of top leaf, was measured during the season, before and after anthesis. All treatments seemed to reduce plant height compared to the control. The most reduction was with the .003 and .005 M treatments for 12 hours at 35° C. However, the numbers of plants were also reduced with these treatments.

Silking date was taken as the time when 50% of the plants in a plot were 50% silked out and was recorded as the number of days after July 1. Little difference was noted in silking date except with the .003 and .005 M treatments for 12 hours at 35° C, but again there were few plants in these treatments.

The number of plants was also reduced with the higher molarity treatments for 12 hours at 35° C and no plants survived longer treatment periods. Also the .003 M treatment for 24 hours at 25° C considerably reduced the stand.

Insolubility, is defined here, as when Myleran was observed in the buffer at the end of the treatment time. Some Myleran was seen in all of the treatment containers after treatment. Since full solubility had apparently not been attained, the concentrations or molarity noted may not be too meaningful. That is, "higher molarity" treatments may give the same effect as lower ones. However, this apparently is not entirely true as far as physiological effects are concerned, cf. Table 4. Ostensibly, the Myleran was soluble enough to reduce stands when .003 and .005 M treatments for 12 hours at 35° C were used, and caused essentially complete killing when .002, .003 and .004 M treatments for 24 hours at 35° C were used.

The .003 and .005 M treatment for 12 hours at 35° C had a drastic physiological effect. However, the treatments with .002, .003 and .004 M for 10 hours at 25° C with alcohol had essentially no physiological effect. Therefore, it appears that heat is more effective than alcohol in causing a physiological effect with Myleran. However, based on mutations produced, the alcohol seemed to give better results.

The treatments for 24 hours at 35°C were too drastic and left essentially no plants for analysis. However, treatment at 24 hours and 25° C with alcohol appeared to be effective in sector production, but did not cause such a drastic physiological effect. It was also rather effective in producing aleurone color mutations. This treatment seemed to be the best used in this experiment.

The maximum treatment temperature which will permit some viability of seeds has not yet been determined in this laboratory. However, 24 hours at 35° C (no Myleran) reduced survival to about half, but essentially nothing else was affected. Therefore, higher temperatures and longer treatments may be used and still permit a reasonable survival rate. However, the data indicate that exceeding 12 hours at 35° C with the Myleran doses used here is not feasible.

Table 4
Treatment specifications, Plant Data and Mutations with Myleran

| Control designation | Molarity | Time hours | Temperature °C | Alcohol* | No. of plants ($\bar{X}$) | Silking date | Plant height (cm) Early | Plant height (cm) Late | Leaf sectors "yellow green" | Leaf sectors "other" | Mutants |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Treatment--Myleran** | | | | | |
| | .002 | 24 | 35 | | | | | | | | |
| | .003 | 24 | 35 | | | | | | | | |
| | .004 | 24 | 35 | | 0.5† | | 38.0‡ | -§ | | | |
| | .003 | 12 | 35 | | 2.0 | 32.0 | 31.3 | 119.3 | 200.0 | 75.0 | |
| | .005 | 12 | 35 | | 0.5† | 37.0 | 20.5‡ | 90.0‡ | 100.0 | 0.0 | |
| | .002 | 10 | 25 | * | 32.5 | 24.5 | 75.2 | 171.7 | 7.5 | 5.0 | 3 c |
| | .003 | 10 | 25 | * | 28.5 | 25.5 | 72.2 | 166.8 | 10.0 | 7.5 | 2 c |
| | .004 | 10 | 25 | * | 32.0 | 26.0 | 70.7 | 167.1 | 5.0 | 5.0 | 4 c, 1 wx |
| | .005 | 10 | 25 | * | 26.0 | 27.5 | 74.8 | 161.7 | 10.0 | 15.0 | |
| | .003 | 24 | 25 | * | 9.5 | 28.0 | 59.7 | 143.5 | 26.6 | 37.8 | 5 c |
| | | | | | | **Control--Buffer** | | | | | |
| A | | 10 | 25 | | 35.0 | 25.0 | 81.8 | 175.6 | 20.0 | 7.5 | 3 c, 1 C |
| B | | 10 | 25 | * | 39.5 | 25.0 | 76.1 | 176.4 | 10.0 | 20.0 | 4 c |
| C□ | | 24 | 35 | | 14.0 | 25.0 | 78.4 | 174.6 | 7.0 | 21.4 | |
| D□ | | 24 | 25 | * | 22.0 | 29.0 | 67.6 | 157.3 | 0.0 | 20.0 | 1 wx |

* 7% ethyl alcohol added to Myleran and buffer.

† Only one plant in one replication survived this treatment.

‡ Actual value for one plant, no average.

§ The plant did not survive to postanthesis.

□ One replication, see text for details.

This experiment was performed to determine efficient treatment procedures with Myleran based on physiological and genetic effects (detected from leaf sector analysis and mutants obtained). Apparent mutations have been obtained; however they have not met the criteria of Stadler (1946), nor have they been checked for correspondence or contamination. However, contamination should not be a problem, since the field was isolated from other maize and the treated stock was dominant for the genes that were analyzed.

Apparently Myleran is not nearly as efficient as ethyl methanesulfonate in producing mutations (Amano and Smith, in manuscript). Solubility may be a factor affecting its mutagenic efficiency. However, certain treatments are rather effective in producing leaf sectors and aleurone color mutations. Also, these data indicate that still more effective treatments can be devised.

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1. Further progress in perennialism of Zea.

A. Diploids. A continuation of the work of selective breeding in the Clone A family of perennial clones (MNL 38: 17-21) has resulted in the production of several 20 chromosome derivatives which can be cloned and apparently maintained indefinitely with careful handling. While they do not breed true for perennialism upon selfing, they are much more fertile than the parental Clone A. Moreover, it is the first time that factors needed for a minimal expression of perennialism have been shown to be transmitted by near diploids (though this transmission of course occurs rather readily by triploids). This indicates

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