three plants of each family by the A-B translocation set, planting 100 kernels from each ear in a sand bench, and noting the hypoploid seedlings that expressed the mutant phenotype.

The set of A-B translocations used covered 16 chromosome arms to some degree. They consisted of a group of 17 obtained from various sources as indicated below.

1b Roman	6a Roman
1c Beckett	6b Beckett
2S, $3L_{6270}$ Robertson	7b Roman
2L, $1S_{4464}$ Robertson	8a Roman
2L, $3L_{7285}$ Robertson	9b Roman
3b Beckett	9c Beckett
3a Roman	10S Beckett translocation not verified
4a Roman	
5a Beckett	10a Roman

The relationship of these translocations to the current linkage map and the frequency and types of mutants uncovered by each is shown in figure 1.

Fifty-two of the 116 mutants tested were tentatively located to chromosome arm. This proportion is about what one would expect, considering that the translocations covered roughly 66% (our estimate) of the known linkage map and that some of the crosses were missed, many female plants were homozygous normal and not all male parents carried the translocation.

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4. Chemical mutagens and in vitro germination of pollen.

An earlier report (MNL 42:124) on the use of paraffin oil as a carrier for chemical mutagens in treating corn pollen showed the method was promising. Subsequent experiments using this method have given mixed results because of difficulties in standardizing dosages. Variations occur because of purity and potency of chemicals and because of differences in mixing technics. These variations often result in complete killing as one extreme, or ineffective treatment as the other. A quick method for reducing these extremes has been developed.

Corn pollen can be germinated easily on an agar medium (Cooke and Walden, Canadian Journal of Botany 43:779-786). We found that pollen in paraffin oil, when placed on Cooke and Walden's media in a petri dish, germinated very well. The oil spread rapidly over the surface, leaving the pollen grains scattered and the whole surface covered with a film that sealed in the moisture but did not interfere with germination. This provides a convenient way to check the effect of various concentrations of chemical mutagens in paraffin oil on pollen germination and tube growth which should be correlated with seed set and mutagen effectiveness.

A stock solution of 1% ethylmethanesulfonate in paraffin oil was prepared. From this, dilutions of 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, and 1:8 with oil were used to treat pollen. The pollen was left in the oil for 16 minutes before placing on agar media. Pollen tube growth, which was prohibited by the stock solution, increased with dilution to 1:4 when growth appeared to be normal.

Pollinations were made with similar pollen (RR) held for sequential intervals of 3 to 20 minutes in 1:4 or 1:8 dilutions and crossed on Rr ears. Seed set was very poor for the 1:4 dilution while that of the 1:8 dilution was excellent. Harvested ears from the 1:8 dilution had no whole kernel and only 6 fractional losses for R among 4188 kernels in the treatments lasting 3 to 15 minutes, but had 5 whole and 15 fractional losses among 1076 kernels on the last 10 ears representing 16 to 20 minutes of treatment.

From these results we have concluded that the best concentration is a 1:7 dilution (.13% EMS) with a treatment time before pollination beginning at 15 minutes.

A solution of nitrosoguanidine (NG) was made as follows: pulverize 1 g of NG crystals to a granular consistency and mix in 100 ml of paraffin oil, stir vigorously for 2 hours, allow to sit undisturbed for 18 hours and pour off the cloudy suspension and use it as a stock solution. All these procedures must be carried out in reduced light as NG is inactivated in 3 minutes of sunlight in the greenhouse.

Using a series of dilutions as described above, it was found that a 1:19 dilution was the strongest that gave no visible effect on germination or tube growth. Pollination made from similar pollen, held 3 to 20

Table 1

Comparison of seed set and frequency of induced changes of the $\underline{\alpha}$ $\underline{\beta}$ $\underline{Sh_2}$ segment of chromosome #3 in timed treatments of $\underline{A_1^b}$-$\underline{Sh_2}$,$\underline{Dt_1}$ pollen with nitrosoguanidine in paraffin oil. The ear parent was $\underline{a^m\text{-}1}$ $\underline{sh_2}$ $\underline{dt}$.

Mean treatment time in minutes	Number of ears	Seed set in average # kernels	Induced changes per 1000 kernels*	
			Whole kernel	Fractional 1/8+ kernel
NG oil				
2	1	160	0	263
3	5	213	8.5	151
4	10	107	7.5	122
5	5	139	2.9	96
6	6	187	8.9	117
7	5	190	6.3	128
8	4	177	4.2	155
9	5	172	4.6	109
10	5	150	14.6	146
15	18	157	6.4	129
20	21	180	4.5	121
25	16	161	6.6	117
30	18	191	6.1	119
75	3	252	6.6	184
90	12	108	3.8	142
Control oil				
25	5	132	0	5
30	6	125	0	4
40	9	155	0	7
50	9	135	0	7
75	10	124	0	10
200	15	109	0	4
Control no oil				
-	9	194	0	6

*All observable types of changes of $\underline{\alpha}$ $\underline{\beta}$ $\underline{Sh_2}$

minutes in a 1:19 dilution gave fair seed set with no whole kernel and 5 fractional losses for R among 238 kernels for the 3 to 4 minute time but a rapid reduction in seed set for progressively longer treatment times.

These results contradict an earlier report (MNL 42:125) where it was stated that no reduction in seed set occurred after the first 3 minutes of treatment time. The reason for this is that in the first experiment a glass vial was used allowing the sunlight to inactivate the nitrosoguanidine, while in the second experiment a plastic vial covered with masking tape was used, thus protecting the solution which retained its activity. The effect of sunlight inactivation is demonstrated by the data in Table 1 where seed set and frequency of whole and fractional kernel changes for the $\underline{A}^{b}$-Sh segment of chromosome #3 are compared. Note that there is no significant change in seed set or frequency of changes after 3 minutes.

From these tests we have concluded that the best results can be obtained with a 1:19 dilution of our stock solution which is kept in the dark but mixed with pollen in daylight and used within a 50 minute period following initiation of treatment.

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1. The endosperm mutant described as "similar to $\underline{sh}_2$" in News Letter 44 proved to be allelic to brittle-1. It was induced in the field corn inbred B14, and is Y Su. Aleurone constitution is AAccrrPrPr. Seed is available.

2. An induced B14 plant mutant has proved to be allelic to brachytic-2. Vigor of mutant is good. Seed is available.

3. Seed is available of most of the seedling mutants described in the Maize News Letter 44. I will not be growing any of these in 1971.

W. Ralph Singleton