74 Micron Pollen		125 Micron Pollen	
Chrom. No.	No. of Individuals	Chrom. No.	No. of Individuals
20	1	23	1
21	1	30	2
22	1	33	3
23	4	34	2
24	2	36	5
25	5	37	4
26	2	38	4
27	1	39	1
28	1		
29	4		
30	1		
32	1		

It is evident that the screening procedure results in two nearly discrete populations. If one sibs among the 36-39 chromosome individuals, one obtains a new population having many individuals with 40 or more chromosomes. The chromatin of such newly constituted tetraploids should derive approximately 45% from the original diploid parent.

D. L. Shaver

3. A successful colchicine method for Zea.

In producing tetraploids of Zea, the "target" for colchicine action is the undifferentiated meristem of the shoot apex. Probably because of complications involved in the various techniques of getting colchicine through the several multicell-layered embryonic leaves which ensheath the apex, production of tetraploids by means of this mitosis inhibitor has with few exceptions been disappointingly difficult.

Faced with the necessity of deriving tetraploids from a 2N population directly, a method of applying colchicine proposed by Dr. L. F. Randolph in one of the early Maize Genetics Cooperation News Letters was used in conjunction with colchicine techniques suggested by Dr. J. Van't Hof at Brookhaven. Seeds of maize were germinated until the primary root reached about $1\frac{1}{2}$" in length. About $\frac{1}{4}$ to $\frac{1}{2}$ inch of the root tip was cut off under distilled water to expose the large, empty xylem vessels of the differentiated portion of the root. The seedling was then suspended on hardware cloth above an aqueous colchicine solution in such a way that only the decapitated root tip was immersed, and the young seedling therefore was forced to supply its entire transpiration stream through the decapitated root.

Effective colchicine concentrations were determined by dissecting out the shoot apex, smearing, and determining the proportion of colchicine metaphases present. In this manner, proper concentrations were determined for the specific material used. Possibly another strain or variety could have a different optimal level. In this work, one chose the lowest level at which all observed metaphases were "arrested." It was found that there is an accumulation of colchicine effects from one treatment

period to the next. Thus, 0.025% colchicine did not produce 100% arrested metaphases during the first 24-hour treatment, but during the second 24-hour treatment, all were arrested. The practice was eventually adopted of using a higher concentration for the first cycle of treatment than during succeeding ones.

In the first experiment, 500 maize seedlings were given three 24-hour treatments in 0.05% colchicine, interrupted by 24-hour recovery periods in distilled water. Of the 16 survivors of this treatment, 4 were confirmed tetraploids (their pollen produced full seed sets when tested upon an established tetraploid), and 2 were probably tetraploid (produced pollen, some of which "stayed" on a 125 micron screen). In the second experiment, 500 maize seedlings were treated for the first 24 hours in 0.05% colchicine, allowed to recover for 24 hours, and then treated for an additional 24 hours with 0.025% colchicine. Of 152 survivors of this treatment, 5 were confirmed tetraploids and 10 were probables. There appeared to be only one case of 2N-4N sectoring. The rest appeared to be cases where the entire shoot apex had become tetraploidized.

Dr. J. Van't Hof's "colchicine bible": Use colchicine from a source where the quality of the chemical is consistent, e.g., L. Light & Co., Ltd., Colnbrook, England, and store in the dark in a desiccator over $CaCl_2$. Always make up fresh solutions immediately before use. Always establish optimal levels for each new variety by experimental dissections and fixations.

D. L. Shaver

4. A mechanical method of classifying large numbers of seeds for waxy.

The work of O. E. Nelson of establishing the map characteristics of the wx cistron of maize has established an exceedingly useful system for the study of the nature of mutation in a higher organism. However, in the initial steps of producing and screening for mutations produced by radiations or chemical mutagens, one is faced with a Herculean task of physically classifying maize kernels for wx in sufficient number that one can realistically expect to recover a meaningful number of induced mutants.

In order to speed this task, a Red Devil paint shaker model 30 was obtained. The sides of two one-gallon paint cans were lined with floor sanding paper with a no. 2 grit (Behr-Manning Co., Troy, N.Y.). The machine was connected to a standard greenhouse timing switch so that runs could be made while the machine was unattended. It was found that this machine could easily scarify 2,000 grams or ca. 11,000 kernels at one time. Doubtless it could be adapted to larger containers and this could be greatly increased. It was necessary to perforate the ends and sides of the cans with numerous small holes to allow the dust to sift out, and thus avoid "filling" of the sandpaper. Frictional heating of the can and its contents could be avoided by setting the timing switch to 15 minutes on, 15 minutes off, etc. Two and one half to three hours running time was sufficient to scarify the seeds for wx classification.