

compared with the usual 25%, when they are in repulsion with respect to the lethals. Further tests are, of course, needed to verify the linkage which is indicated by an excess of mutants.

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1. Estimating pollen restoration.

In the process of converting inbreds to the cytoplasmic pollen sterile condition and the conversion of these sterile inbreds to restorers it is necessary to have some reliable measure of normal pollen production. The examination of anthers from small pieces of tassel under a low power microscope gives a fairly accurate measure of the amount and degree of pollen abortion. The staminate flowers are collected shortly before the time of normal dehiscence and usually preserved in acetic-alcohol until they can be examined. The proportion of normal, well filled pollen grains can be determined approximately but this method is slow and tedious. It also gives no information about dehiscence and ability of the pollen to function.

Pollen examinations over a period of years indicated that there was a close correlation between the amount of normal pollen produced and the time and pattern of pollen shedding. Tassels with anthers well filled with normal pollen grains begin shedding at the time of first silk emergence or before. Anthers that had any unusual amount of partially filled or completely aborted pollen were usually delayed in appearance until after the first silks appeared, and the anthers did not follow the usual pattern of emergence. The normal pattern of pollen shedding is for the anthers to be extruded first below the tip of the central spike. Extrusion then extends evenly to the tip and the base of the central spike, followed or accompanied by the appearance of anthers on the lateral branches near the tips of the upper branches extending evenly to the tips and the bases of all the branches. Any delay in the appearance of anthers beyond the appearance of the first silk on any part of the tassel, or the first appearance of anthers on the lateral branches or at the tip or base of the main spike, or gaps without anthers is usually an indication of some degree of pollen abortion. In some partially or completely sterile plants the anthers may be well filled with normal appearing pollen grains but these anthers are not extruded, or if extruded the pollen may not be released.

By using these manifestations plants can be easily and quickly classified in the field at the time of silking. We usually put the plants in a segregating population in four arbitrary categories:

(1) normally fertile, (2) partially fertile with about 50 percent or more of normally released pollen, (3) partially fertile with about 50 percent or less of normally released pollen, (4) completely sterile. In the first category the normal functioning of the pollen has been checked many times by self and cross pollinations made by hand. The last category shows no anthers as long as the silks are receptive, and the anthers are devoid of any normally appearing pollen grains.

For convenience in classification the first three categories are lumped together to compare the effectiveness of this method of testing segregation. In 1956 six progenies were grown in replicated plantings, about 100 plants in each replication. Five of the lots were F<sub>2</sub> selfed progenies of crosses of S and T sterile inbreds by the pollen restoring inbreds NC77, Tx127 and Ky21. One lot was the F<sub>1</sub> cross of a T sterile inbred by the single cross (Ky21 x Tx127). The differences in the percent of plants with or without anthers appearing ranged from 0 to 12 percent. None of these differences is significant.

## 2. Seasonal differences in pollen restoration.

Using this method of field examination and the same arbitrary classification of plants with and without anthers the differences shown by the same F<sub>2</sub> segregating progenies were determined for the two growing seasons of 1955 and 1956. The same lots of seed were planted each year and the results averaged for the three pollen restoring inbreds given above. In 1955 the growing season up to the time of flowering was unusually dry and above normal in temperature. The leaves were wilted and rolled on many days. The 1956 season was quite adequate in moisture before flowering and temperatures were normal. The results combined from the three inbreds used as pollinators in 18 different selfed F<sub>2</sub> progenies are as follows:

	Number of Plants		Percent	
	<u>With Anthers</u>	<u>Without Anthers</u>	<u>With Anthers</u>	<u>Without Anthers</u>
1955 Observed	480	356	57	43
Calculated 9:7	470	366	56	44
1956 Observed	1014	331	75	25
Calculated 3:1	1009	336	75	25

The agreement in 1955 with a 9:7 calculated ratio and in 1956 with a 3:1 calculated ratio is remarkably close. This indicates that in the relatively unfavorable season of 1955 two restoring genes were needed for the plants to show any anther. In the more favorable season of 1956 only one restoring gene was necessary.