In trying to explain the differences between cultures such as those reported here, at least four main variables must be considered. They are:

1. Differences due to the effects of the cytoplasms themselves
2. Possible pleiotropic effects of the restorer genes on other functions of the plant in addition to the restoration of pollen fertility
3. Changes in the genotypic make-up of the counterpart inbred lines remaining after the conversion processes have been carried out
4. The effect of pollen shedding as such on the other functions of the plants.

The results reported above may be explained as being due to two or more of the factors listed above. One very important problem will be to devise matings and experimental methods which will determine, as far as possible, the effects of each of these variables with the others held constant. Such matings are being made in the Florida 1959-1960 nursery for testing in experiments in the Corn Belt in 1960.

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1. Characterization of sterile cytoplasms.

In addition to the original S and T sources of sterile cytoplasm, we are currently maintaining 12 other sources, each in process of being combined with the genotypes of inbreds WF9 and A158. The detailed origins of 8 of these sources are given in Bulletin 610 of The Connecticut Agricultural Experiment Station, but for convenient reference the origins of the 12 new sources are presented in the table below. It will be noted that three sources, C, D and E, stem from various Vg stocks. Source E was listed in Bull. 610 as having originated from a cross between two Pennsylvania lines, but records kindly furnished by J. E. Wright show clearly that the source of this sterile cytoplasm was Coop stock 49-10, which was segregating Vg. The records also state that earlier Coop notes made by Emerson mention the presence of male sterile plants in this Vg stock (some progenies consisted of all male sterile plants). It is entirely possible that the Vg material originally maintained by the Coop carried sterile cytoplasm and restorer genes. Since the anthers of Vg plants often blast, it is probable that such plants would be used as seed parents in crosses, and in this way the cytoplasm of the Vg stock would be transmitted along with the Vg gene. Continued backcrossing would eliminate the restorer genes. This could account for the origin of cytoplasmic male steriles from Vg stocks.

A preliminary genetic test has been conducted on nine of the sources to determine whether each is S, T or a different type. The A158 and WF9 series of steriles, with from two to seven generations of backcrosses, were crossed by the inbreds NY16 and Ky21, both of which restore S and T. However, Buchert's findings (MNL, 1959) on the behavior of S restorer genes makes it possible to distinguish between S and T type restored steriles, provided the restorer genes are in heterozygous condition. In heterozygous restored S steriles, pollen grains with the non-restoring allele abort; thus, restoration is only 50 per cent. Heterozygous restored T steriles, on the other hand, approach 100 per cent restoration, since T restorers behave as though sporophytic in action.

The last two columns in the table show the results of the crosses sterile x NY16, and sterile x Ky21. The degree of restoration was estimated on the basis of pollen examinations in the field with a pocket magnifier, and on microscopic examinations of pollen stained with IKI. Exact counts of normal and aborted pollen grains were not made, but the difference between approximately 50 per cent and approximately 100 per cent normal pollen grains is readily apparent. The nine sources A through I were 50 per cent restored by NY16 and Ky21 in both the A158 and WF9 series of steriles. This indicates that all nine sources of cytoplasm are S type.

One cannot, of course, be certain that the restoration in all cases is effected by the conventional S restorer genes known to be present in NY16 and Ky21, since these inbreds could possess other restorer genes. It is apparent, nevertheless, that the restoration of cytoplasms A - I is similar to that characteristic of S type restoration. If the A - I restored sterile plants do have S type cytoplasm and typical S restorer genes, these plants should give all fertile offspring when crossed as pollen parents to S steriles. These crosses plus additional tests involving known S and T restorer lines have been made with all sources of sterile cytoplasm, and the results should provide more critical evidence on the nature of these cytoplasms.

Cytoplasmic Designation	Origin of cytoplasm	Source of Seed	Restoration (100% or 50%) NY16	Ky21
T			100%	100%
S			50%	50%
A	Turkish Flint	PI 171 892	"	"
B	Brazilian Flint	F. G. Brieger	"	"
C	segregating progeny of Vg, sy, j, v_{16}	G. F. Sprague	"	"
D	B9 Vg su stock	W. C. Galinat	"	"
E	Coop 49-40: seg. Vg	J. E. Wright	"	"
F	iojap induced	M. M. Rhoades	"	"
G	3 way cross (Q63M^{x}C115)F5E	C. C. Wernham	"	"
H	Ind. 33-16	L. M. Josephson	"	"
I	New England Flint (Vt.)	R. M. Bailey	"	"
J	Bolivian variety	P. C. Mangelsdorf	-	-
K	Turkish Flint	PI 204 830 B	-	-
L	Turkish Flint	PI 204 830 A	-	-

Harry T. Stinson, Jr.

2. Comments on the comparison of sources of sterile cytoplasm.

If, as the evidence above indicates, all nine sources of sterile cytoplasm are S type, there are several interesting implications. First, this would mean that the sterile cytoplasms in our collection so far fall into either the S or T classification on the basis of the restorer tests. However, as reported in the 1959 MNL (p. 22), chromatographic analyses of mature anthers of nine sources of sterile cytoplasm (in various stages of backcrossing by WF9) revealed differences among them, especially in the presence of UV light fluorescent spots. On this basis of classification the nine sources of cytoplasm were tentatively put into 5 groups, as follows: 1) T; 2) S; 3) E; 4) B and F, 5) A, D, G, H. All steriles were chromatographically different from normal WF9. These results suggest that the chromatographic technique may disclose cytoplasmic differences not detected by the restorer tests. These findings may mean that while the sterile cytoplasms are restored by S restorer genes, perhaps even by the same S restorers, the cytoplasms are not absolutely identical in their action, that is, in the metabolic disturbances leading to pollen abortion. There is the possibility that subcategories of S cytoplasm