5. The use of restoring inbreds in commercial double crosses.

One question needs answering as soon as possible. How much pollen restoration is necessary or desirable in the production of hybrid seed corn? In the last News Letter it was stated that WF9T restored by I153 and related lines and used as a pollinator on standard sterile seed parents restored at least 50 percent of the plants to full fertility. Several of these combinations were grown again in 1956 in Connecticut and throughout the corn belt and again produced about 50 percent of the normal amount of pollen. Since they were grown in trial plantings no reliable test of their pollen production was possible, but in time and amount of pollen shedding they were considered to have sufficient pollen for normal grain production. Other hybrids restored by various combinations of Oh29, Oh41, and Ml4 were also grown in many locations and produced 50 percent or more of the normal amount of pollen. Many of these restored steriles were outstanding in yield of grain and stalk quality.

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6. Free amino acid differences between cytoplasmic sterile and normal fertile anthers.

The free amino acid content of cytoplasmic sterile and fertile anthers was investigated in the hybrids C106T8 x A158, C106 x A158, and C106TF5(Ky21) x A158 by the application of paper chromatographic techniques. The C106 parent used in the last mentioned hybrid was derived from a C106 T sterile line which was restored to normal fertility by crossing with Ky21. Restored fertile plants were backcrossed to a C106T line, which had been converted to C106 type, for five generations.

Chromatograms of anthers in stages beyond meiosis showed distinct differences between sterile and fertile anthers. The first difference seen was in the alanine content, this amino acid being accumulated precociously in sterile anthers. A detailed and quantitative study of the pattern of alanine accumulation in the development of anthers revealed little or no differences in the amount of the substance between sterile and fertile anthers in the premeiotic or meiotic stages of development. Occasionally, diads from sterile plants had noticeably larger quantities of alanine, but in all cases, quartets from sterile plants had considerably more alanine (at least a two-fold increase). This disparity became still more pronounced as the age of the anther advanced, although at maturity, sterile anthers had somewhat less alanine than fertile anthers per anther. However, if the alanine content of anthers was compared on an equivalent dry weight basis, it was found that sterile anthers continued to have a large excess of the substance over the fertile counterpart throughout development. This precocious accumulation of alanine in the spore quartets of sterile anthers is of particular interest in

view of the fact that it precedes any detectable morphological differences. Young microspores from sterile and fertile anthers also are indistinguishable, but soon after, it is possible to tell them apart. Fertile microspores increase rapidly in size, develop heavy walls and a definite pore whereas sterile microspores do not enlarge greatly and wall thickening and pore-development are limited.

At later stages of anther development, further differences became apparent. Chromatograms of sterile anthers with old microspores had two other ninhydrin-positive spots absent or less intense in chromatograms of normal anthers. One spot, designated as Y, was distal to alanine and has not been identified, while the other was asparagine. In mature anthers (4-5 days prior to anthesis in the normal plant and later), large quantities of proline are characteristic of normal anthers but not present in sterile ones. This accumulation of asparagine in the mature sterile anther, together with the lack of proline, has been reported by Fukasawa (1954).

Since the restored C106 parent used in the hybrid (C106TF5(Ky21) x A158 was heterozygous for the restorer gene(s), segregation for fertile and sterile plants occurred in the $F_1$ hybrid families. Anthers from sterile plants followed the chromatographic pattern of the sterile C106T8 x A158. Anthers from restored fertile plants were chromatographically identical in appearance to the normal C106 x A158. Thus, the deviation from the normal free amino acid content associated with T cytoplasm does not take place when the T cytoplasm is combined with restorer genes of Ky21.

These chromatographic investigations were extended to several other lines of corn which had been sterilized by male sterile cytoplasm from different sources, namely, the A, B, and S steriles. It was found that in all lines tested (C106, A158, WF9, WF9-4, W22) T cytoplasmic sterile anthers invariably developed alanine precociously, and always before morphological differences became apparent. A, B, and S types of sterility did not, at all stages of development, affect the ninhydrin-positive patterns of the anthers.

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