# 9. Time of the critical cytoplasmically-induced action causing pollen abortion.

The conclusions drawn in article 7 above help to establish the time when the critical step in the abortion mechanism occurs in S-steriles. Because more than one step must precede it, among them, the primary restorer gene action and the restoration process, and since the critical times for these processes occur after microsporogenesis, this critical stage determining the abortion must take place during the maturation of the pollen grain - only a short time before the deterioration can be seen.

Janson G. Buchert

#### 10. Effect of environment on pollen restoration of T (Texas) type cytoplasmic male sterility.

It has frequently been observed in T-sterile material where the restorers from Pll, P39, or NJll43y are being employed, that one part of a tassel may be more fertile than another. Occasionally plants will be found where the first day's shedding area will be fertile, the second day will be less-than-half fertile, the third day's will be better-than-half fertile, and the base perfectly normal. Sometimes the tassel on the main stem is almost sterile, while the tassels of the tillers will be normally fertile or nearly so. This behavior appeared attributable to the environ ment. To investigate this, samples of seed from the same ear, produced by the cross (Cl3T x NJ143y) Cl3, were planted at about 5 to 7 day intervals from May 15 to July 23, 1958. From the data of three backcrosses and one F2 progeny, it had been previously established that the restorati brought about by NJ11:3y segregated as a single dominant gene. Furthermore, seed from the same packet had been sampled the previous year and found to segregate in a 1:1 ratio; it had been planted May 31. Therefore on the basis of both the previous sampling and the previous behavior of this restorer, each planting in 1958 should have contained fertile and sterile plants in equal numbers. Those planted from May 15 through May 25 and from June 30 through July 23 had a significant (P <.01) excess of steriles, while the samples planted June 2 through June 16 had a significant (P=.02) excess of fertiles. Samples planted on May 28 and June 23 appeared to give a one gene ratio. There was no concious selection in removing seeds from the packet; also, some samples included various degrees of "partials" while others did not. The environmental factor(s) responsible is not known.

Janson G. Buchert

## 11. The effect of environment on pollen restoration of the S-type cytoplasmic male sterility.

When grown during the 1955 and 1956 seasons, all cultures having only plants with S cytoplasm, mainly the P39 residual genotype, and the

S-restorer from Ky21 exhibited various degrees of fertility. Never, however, were any plants completely fertile, nor were any completely sterile. The P39SF material grown in 1958, however, expressed itself differently. Conclusions are based on the following data.

Cross	Year Grown	Fertility *	Extrusion of anthers**
P39S8 x P39SF1	1955	all P	all M
P39S9 x P39SF2	1956	all P	all M
P39S9 x P39SF2	1956	all 13 P	all 13 M
P39S9 x P39SF2	1958	5P 32 S	all 37 M
P39S10 x P39SF3	1958	1P 37 S	36 M 2 F
P39SF1 x P39	1955	10 P 7 S	10 M 7 F or N
P39 <b>SF1</b> x P39	1955	9 P 3 S	9 M 3 F or N
P39SF2 x P39	1958	all 49 S	30 M
P39SF2 x P39	1958	all 32 S	
P39SF3 x P39	1958	1 P 45 S	
P39S10 x P39	1958	all S	all F or N

<sup>\*</sup> On the basis of pollen shed. P = partial fertile S = sterile

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Except for seven, all of the plants in 1958 were sterile, regardless of the type of cross they had resulted from. These seven, however, were the latest plants to flower; therefore, the critical environment may have been different for these plants. Although the expression of fertility was different in 1958, the degree of extrusion was not. In the 1955 and 1956 grown cultures all of the fertile plants had many anthers extruded, while the steriles had only a few or none. If this same relationship had existed in 1958 the same ratios of fertile:sterile as was expected (on the basis of male gametophytic selection - see article 6 above) would have resulted. That is, while the progenies of the crosses of the type S x SF were expected to have only fertile plants, almost all the plants were sterile, but, virtually all had many anthers just as the nonsegregating families in 1955 and 1956. SF x inbred families had almost all sterile plants, but they segregated in a 1:1 ratio for many: few or no anthers extruded, just as similar pedigreed families in 1955 and 1956. P39Sll was listed for comparison.

The environment responsible for this different expression in 1958 is not known. There was one major difference in the environment in

<sup>\*\*</sup> M = many (usually more than 150) anthers; F = few (usually less than 15) anthers; N = no anthers extruded.

Connecticut in 1958; the early part of the growing season (about 3 - 4 weeks) was unusually cool and wet.

Janson G. Buchert

## 12. Separation of cytoplasmic male sterility types by chromatography.

Chromatographic analysis applied to mature anthers of cytoplasmic male steriles from nine different sources in various stages of backcrossing to WF9 shows promise as a means for classifying these cytoplasms. The chromatograms were first inspected with short-wave ultra-violet light and later dipped in ninhydrin solution. Root tissues showed no marked differences in ultra-violet light fluorescence or absorption, or in their contex of ninhydrin-positive materials. Chromatograms of anthers in early stage of development were similar except for the T sterile (previously reported

Ultra-violet light fluorescence and absorption patterns of the normal WF9 and the cytoplasmic steriles E, T and S were distinctly differ from the other types examined (A, B, D, F, G and H) and from each other. The B and F sources appeared to be alike while the others fall into a separate group. Ninhydrin-positive patterns were less distinctly differe

It is hoped that with a refinement of techniques, a further separation and identification of the cytoplasms chromatographically will be possible.

Uheng Khoo Harry T. Stinson, Jr.

# CORNELL UNIVERSITY Ithaca, New York Department of Plant Breeding

### 1. Pollen viability studies.

Utilizing the bio-assay for corn pollen viability discussed previously, (MNL, Vol. 32, p. 18-19) additional experiments were undertaken in 1958. Some of the factors known to contribute to the pollen longevity viability problem were examined in greater detail. In some of the recent work, pollen kept viable for 8 days was not uncommon. The most favorable temperature for 8-day storage was +3°C., although temperatures from -8°C. to +10°C. will generally work nearly as well.

Attempts at suspending pollen in liquid diluents were entirely unsuccessful. 1.0M and 2.0M glycerol and mannitol and 100% glycerol failed to retain any viability in corn pollen for periods of time as short as 1 minute.