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 content of ninhydrin-positive materials. Chromatograms of anthers in early stages 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 different 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.

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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.

Fresh corn pollen was successfully "diluted" with previously killed corn pollen. Dilutions of from 1:1 to 100:1 were used effectively in viability studies. The mechanical mixing did not appear to have any deleterious effects on the fresh pollen longevity.

Additional experiments were conducted to determine optimal pollen collection, optimal storage, and the changes that take place in the pollen during storage. These will be reported in detail in a thesis in preparation by the junior author.

H. L. Everett
D. B. Walden

2. Oxygen utilization by fresh pollen.

Some of the pollen treatments observed in the longevity-viability studies have been subjected to elementary physiological analyses. The results can be summarized as follows:

a) The $O_2$ uptake of fresh pollen can be measured with appropriate manometric techniques. Thus $O_2$ uptake as a function of time of storage, storage conditions, etc. can be determined. In our work, 0.2 - 0.3 gm. fresh wt. of pollen was inserted into 15 ml. "Warburg" vessels and attached to manometers.

b) The $O_2$ uptake of fresh pollen suspended in 0.05M phosphate buffer, pH 7.3 can also be measured. In such a system, the pollen homogenate can be shown to oxidize some of the organic acids of the "Krebs" cycle. It can also be shown in the case of succinate oxidation that the respiration pathway is at least partially sensitive to cyanide and azide. A general interpretation of these pollen "respiration" studies indicates that pollen respiration is not unlike the classical respiration of yeast.

c) The preparation of an active "mitochondrial" suspension from corn pollen has not been successful with classical methods.

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3. Respiration studies with preparations from corn seedlings.

A preparation with oxygen uptake activity can be prepared from corn seedlings: Six-day old mesocotyls and cotyledons are ground for 3 minutes in a 0.05M $NaHCO_3$ buffer in 0.25M mannitol solution at 0°C. Differential centrifugation allows sedimentation of a pellet at 15,000 g. This pellet is washed and re-suspended in 0.25M mannitol.

Utilization of some organic acids, inhibitor studies, determination of P:O ratios, have aided in the characterization of the respiration path-