The single cross, Oh51A^T x IoB8, and the assay techniques were described earlier (Walden and Everett, Crop Science 1, 1961). Limited pollen was applied to a number of previously prepared 7-day old silk-brushes, a different set every two hours between 9 AM and 9 PM. Starting one hour after pollination and continuing at two hour intervals for 30 hours (excluding 1-6 AM) silks of different treatments were cut to the ear tip. The length of cut silks averaged 2 cm for all treatments. Presumably sperm nuclei which had passed the region of cut-off completed fertilization; thus kernel counts reflected the events measurable after silk cutting.

Such an assumption seems justified as there were silk-cut treatments which scored a kernel value equal to the control, the uncut treatment. Pollinations made prior to and including 2 PM showed the same effect:

About 85% of the sperm nuclei passed the point of cut-off in the interval between 5 and 7 hours post-pollination. About 10% were "precocious germinators", passing through prior to 5 hours and the residual were delayed.

Pollinations made after 2 PM demonstrated an increasing lag phase and a reduced slope to the growth curve, such that pollinations made at 8 PM failed to pass the cut-off point for 12 hours or more. Yet we repeatedly obtain maximum kernel counts in other experiments from pollinations made in late afternoon and early evening, indicating that fertilization is finally accomplished.

This study suggests:

1. On a typical summer day, during which pollination in the field is accomplished by noon, it is followed by rapid pollen germination and pollen tube growth; fertilization is completed within 24 hours, probably during the cool, damp hours of early morning.
2. Pollination delayed until late afternoon or evening results in good fertilization, but only after germination and/or initial pollen tube growth are delayed until the following late morning-afternoon growth period.

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2. Pollen longevity.

Storage between -5° and +5°C at a high humidity in aseptic cultures will retain a satisfactory number of pollen grains in a viable condition as measured by syngamy for 14 days. Such cultures will show a reduced

viability but more than adequate numbers of pollen grains survive for genetic or plant breeding studies. Pre-treatment of pollen will augment the longevity. Pollen collections before 10AM should be cleaned and spread to dry for 1 - 2 hours before introduction into the storage environment.

Our studies of pollen storage in liquid nitrogen and via centrifugal freeze drying are being continued.

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3. Soluble-sugars and free amino acids of corn pollen.

Soluble sugars and free amino acids from the pollen of many genotypes have been studied by means of one and two dimensional paper chromatography. Pollen that has been shed, or whole anthers, have been squashed onto Whatman 3MM filter paper and developed by an ascending technique.

For the separation of amino acids the papers were run in the first direction in 3 parts methyl ethyl ketone, 5 parts butanol, 1 part ammonia and 1 part water and in the second direction in 12 parts n-butanol, 3 parts glacial acetic acid and 5 parts water. After drying they were dipped in 0.2% ninhydrin in acetone and heated. Soluble sugars were separated in one dimension using the butanol-acetic acid solvent described above, dipped in an aniline-diphenylamine-phosphoric acid reagent and heated.

Glucose, fructose and sucrose are present in corn pollen in large amounts and in addition trace amounts of other soluble sugars are recovered. The relative amounts of the above named sugars are dependent upon the immediate post-harvest treatment. For example, the amounts of sucrose increase if the pollen remains unrefrigerated for more than 30 minutes between dehiscence and analysis.

We have tentatively identified 9 amino acids in most corn pollen samples examined, including, proline, α-alanine, glutamic acid, aspartic acid, γ-amino butyric acid, serine, glycine, valine, threonine and 2 acid amides, glutamine and asparagine. Some additional unidentified spots have also appeared. Proline shows up in very large amounts relative to the other spots. Quantitative determinations on a dry weight basis are not yet completed.