3. Acetolysis: a positive test for the identification of normal and aborted pollen grains in grasses.

The importance of recognition of normal and aborted pollen grains in maize and other cereal crops is well known to plant breeders. Various staining procedures are used, such as cotton blue in lacto-phenol, aceto-carmine, various tetrazolium salts, iodine and potassium iodide in dilute alcohol. But most of these stains are either nuclear or protoplast dyes and often show fading when permanent mounts are made.

We have used the standard acetolysis technique following Erdtman (Sv. Bot. Tidskr., Vol. 54(4): 561 - 564; 1960), except we have increased the timing to 5 - 6 min. to give a differential staining of the pollen grain exine.

Permanent slides are made by mounting the acetolysed pollen grains in standard glycerine jelly, and the exine color is stable.

The normal pollen grains stain a dark-brown color, while the exine of aborted pollen grains is lighter and shows a pale yellow color. In addition, in glycerine jelly mounts the large majority of the aborted pollen grains collapse, and we consider this difference as a distinct character of the aborted pollen grain exine.

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4. Similarity of the ektexine pattern of normal and aborted pollen grains in maize and other grasses.

In an earlier study with the pollen grains of maize, Tsukada and Rowley (1964) postulated that the density of the spinules of the ektexine (outer sculptured layer of the exine) was different in normal and aborted (sterile) pollen grains, when spinules were counted per unit area. The spinule density in aborted pollen grains was always higher in comparison to the spinules of normal pollen grains.

Our observations, using an electron microscope, show that there is no such difference present among grasses when normal and aborted pollen grains are obtained from genetically similar lines. The only obvious difference we have noticed is that the pollen grains exhibit differential stainability when subjected to a prolonged acetolysis treatment. The

normal pollen grains are dark brown, while the exine of the aborted pollen grains becomes pale yellow. The acetolysed, aborted pollen grains also collapse when they are mounted in glycerine jelly. We suspect that this difference is due to a difference in chemistry of the pollen walls of the two types, or due to variation in wall thickness (not distinct with the light microscope). Immature pollen grains are not taken into consideration because they often stain like the aborted pollen grains.

We think that the basic exine pattern is not controlled entirely by the haploid protoplast, but is established by the proto-exine (primexine) during early stages of microsporogenesis, when the microspores are in the quartet stage, enclosed in a thick, callose wall. After the microspores are released into the anther locules, wall building substrates (sporopollenin) begin to deposit on the proto-exine framework, resulting in a similar ektexine pattern, irrespective of the presence of the pollen grain protoplast.

During the sporopollenin deposition the microspores increase in size and secondary spinules are added to the ektexine of the pollen grains by the Ubisch bodies (orbicules), regardless of their normal or aborted nature, and finally a characteristic, mature ektexine pattern of the species is formed.

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5. The structure of the Ubisch bodies (orbicules) and their control on mature ektexine pattern of grass pollen grains.

Zea mays and its related species were chosen for this investigation. The results reported here are based on electron microscopic observations. Among grasses, the Ubisch bodies (orbicules) are small, spinulate, spheroidal structures which are formed on the inner tangential, and partly on the radial surfaces, of the tapetal cells that are exposed to the thecal fluid (periplasmodium) of the anther locules. In fact, these minute structures are formed as a part of the tapetal membranes, as reported earlier by Banerjee (1967) and Banerjee and Barghoorn (1969). In the palynological literature, it has been often reported that either the function of these objects is unknown or they are a functionless,