

temperature the spore debris is removed mechanically, and spore impressions are then shadowed with 150 Å thick carbon and chromium. The carbon-chromium replica is then scored into small pieces with a sharp razor blade and suspended in a mixture of benzene and chloroform (1:1 by volume) to remove the plastic. Individual pieces of the replicas are then placed on 250 mesh copper grids for viewing. The electron microscopes Hitachi HS-7S and RCA EMU-3D are used for photography.

Under the electron microscope the spore shape appears spherical, the size varies from 7 - 12 μ , and the spores are covered with sparsely situated prominent spines (echinate). Occasionally some spines are slightly curved at the tips. Each spine is covered all over with very fine micro-spinules; in side view the spines appear finely serrated. At the base of each spine, the micro-spinules are found in rings. The spore membrane between the spines is smooth to slightly granular. We consider that the use of such micro-characters may be helpful in identification of various genetic races of corn smut, which so far has not been possible with the conventional light microscope.

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2. Electron microscopy of the pollen grains of maize, teosinte, and Tripsacum.

Precise identification of the pollen grains of maize, teosinte, and Tripsacum has great potential in tracing the past agricultural activities in the New World. Several distinguishing characters such as pollen grain size, pore-axis ratio, exine pattern, and spinule density per unit area were studied previously in this laboratory with conventional light and phase-contrast light microscopy (Barghoorn et al., 1954; Irwin and Barghoorn, 1962, 1965; and Bartlett et al., 1969).

The present report provides further observations on the ectexine (outer sculptured layer of exine) pattern at the ultrastructural level, using the scanning electron microscope (SEM) and the transmission electron microscope (TEM). The pollen grain samples of these genera

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were collected locally, or from herbarium sheets, and prepared for observation by using a standard acetolysis treatment (Erdtman, 1960). A portion of each sample was deposited on copper cylinders and thinly coated with a palladium-gold alloy by evaporating it under a very high vacuum, and finally the pollen grains were photographed at high magnifications with SEM. The remaining samples were used to prepare reference slides for light microscopy by mounting them in glycerine jelly, and to make carbon replicas for TEM by means of the methyl-methacrylate heat-pressure double-stage technique.

In maize (Zea mays) the ektexine shows the presence of evenly distributed spinules (except in a few pop-corn races where there is occasional clumping of 2 - 3 or rarely 4 spinules). The TEM pictures, at high magnification, show that the exine is perforated by minute holes. These holes are distributed between and around the spinules. We consider the patterning of these holes to be a very important, usable criterion for identification of grass pollen grains. In teosinte (Euchlaena mexicana) the ektexine spinules exhibit occasional clumping (2 - 4 spinules) as well as isolated spinules as in maize. The spinules are very similar to the maize spinules in their size and shape. The minute holes are also present and are distributed all over the ektexine, except between the clumped spinules. The diameter of the holes in the exine of maize and teosinte grains is quite similar.

Tripsacum dactyloides and T. floridanum show an entirely different ektexine pattern from maize and teosinte, which we consider negatively-reticuloid (a cluster of spinules present at the elevated lacuna (lumina) and the reticulum incised or depressed). The holes are not present between the spinules, but are restricted to the incised reticulum. The size of the spinules is also much smaller than those of maize and teosinte.

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