Combining Sputter Coating with OTOTO Treatment to Eliminate Charging Artifacts in Pollen Preparations

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Pollen grains made conductive for SEM by coating in a series of osmium and thiocarbohydrazide solutions (OTOTO) show charging artifacts when nonconductive detritus (sand grains, plant fragments, etc.) is present in the immediate sample area of the SEM scanning probe. Standard sputter coating of the sample makes the detritus electrically conductive and eliminates all electrical instabilities.

When acetolysis (*1*) is used to prepare pollen for scanning electron microscopy (SEM), some residue such as undigested plant parts, sand grains and other inorganic particles, collectively referred to as detritus, may remain, even following procedures designed to remove it (*2, 3*). Some of the detrital material does not incorporate osmium metal when the osmium – thiocarbohydrazide – osmium – thiocarbohydrazide - osmium (OTOTO) method is used to make the pollen grains electrically conductive (*4*). When struck by the electron beam this detritus becomes highly charged and can cause artifacts when it resides within the overscan portion of the raster, yet is outside the frame of the electron micrograph. The escape of secondary electrons from the surface of nearby electrically conductive pollen is essentially blocked giving rise to "shaded images" (Fig. 1) and "fuzzy" pollen surfaces (Fig. 3). Other effects of charging can be seen as scan lines (Fig. 3) and "glowing" pollen grains (Fig. 5).

The above artifacts can be easily removed by pulse sputter coating, usually for 3 minutes, using a gold or gold/palladium (60/40) target. This renders the detritus conductive and allows the secondary electrons from the neighboring pollen grains to be emitted and detected unimpeded to form crisp, artifact-free SEM images (Figs. 2, 4, 6).

Sputter coating of OTOTO-prepared pollen does not diminish the value of the OTOTO technique for pollen SEM or obviate it in favor of traditional sputter coating or vacuum evaporation methods. The consistently high quality of SEM images obtained after OTOTO, in contrast to these other problematic metal deposition methods, and the general paucity of detritus in pollen samples, greatly favor OTOTO as the most efficient way of preparing pollen for SEM. Detrital contamination was not considered in our earlier study (*4*), and it is included here as a supplement to it.

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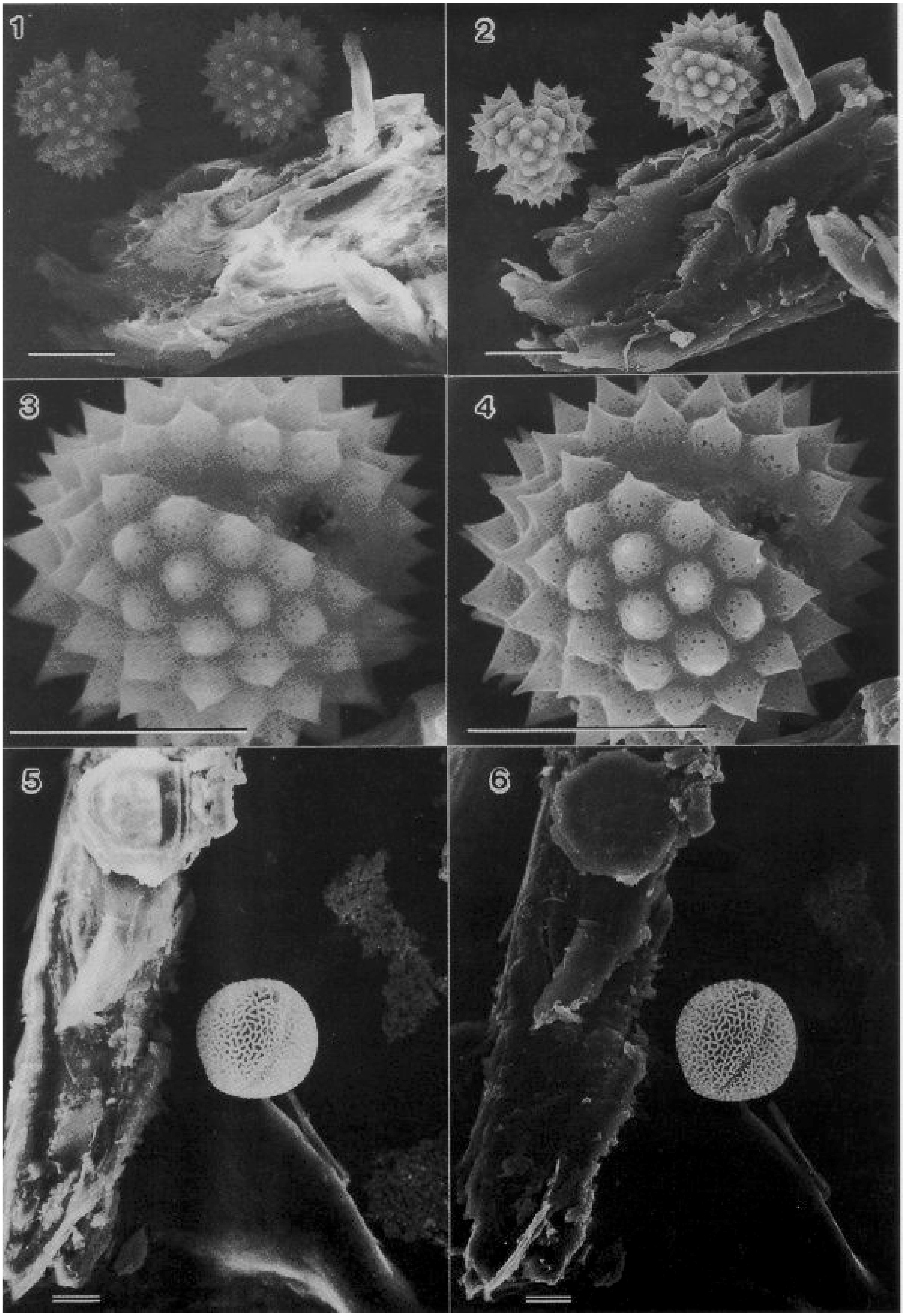
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Figures 1-6: Pollen grain charging from detritus and sputter coating remediation. Figs. 1-4. *Felicia smoragdina* (S. Moore) Neux. (South Africa; L. C. Leach & R. D. Bayliss 12951; K). Figs. 5-6. *Leavenworthia aurea* Torr. (Oklahoma, Choctaw Co.; B. Amos & S. Barber 741 , BEBB). Treatment: all pollen coated with OTOTO (*4*) and mounted on JEOL TEMSCAN 100-CX rectangular copper specimen boats (2.4×5.6 mm with 4 mm high ends and 3 × 9 mm specimen framing marks) using carbon tape mounting adhesive. In Figs. 2, 4, and 6 pollen was pulse sputter coated for 3 minutes with gold/palladium (60/40) target in a Hummer VI Sputter Coating System. Pollen examined with a JEOL 880 scanning electron microscope equipped with a lanthanum hexaboride gun operating at 15 KeV accelerating voltage. Printing conditions (print paper grades and developing times) identical for each comparative set of micrographs. Scale bars equal 10 μm.

Fig. 1 Two pollen grains in direct association with a highly charged detrital fragment showing darkened pollen images.

Fig. 2 Same area as Fig. 1 but after sputter coating. The pollen grains are clearly shown and the detrital fragment no longer is charged.

Fig. 3 Enlargement of pollen grain nearest to detrital fragment. Note overall "fuzzy" resolution and scan lines (lower portion of micrograph).

Fig. 4 Same pollen grain as Fig. 3, after sputter coating. Image quality is improved to resolve fine holes in the bases of the pollen spines.

Fig. 5 Pollen grain and neighboring detrital elements are highly charged.

Fig. 6 Same pollen grain as in Fig. 5, after sputter coating.