MICROSCOPY RESEARCH AND TECHNIQUE 26:180–181 (1993)

Rapid Communication

Use of Dental Wax for the Study of Insect Behavior by Scanning Electron Microscopy

KENNETH M. BART AND ERNEST H. WILLIAMS
Department of Biology, Hamilton College, Clinton, New York 13323

While using scanning electron microscopy (SEM) to investigate the impact of ovipositing butterflies on the leaf surface, it became clear to us that the complex topography of natural leaf surfaces, including the crystalline structure of surface waxes (as shown in Hallam & Juniper, 1971), makes it difficult to find and recognize marks made by insects. In consequence, we devised a new technique, using an artificial leaf surface, for viewing such marks.

Dental wax (Hygenic Extra Tough Base Plate Wax, Pelco Inc. #109) was cut into 1 sq. cm pieces, and one surface of each wax substrate was heated for <2 sec with a propane torch to remove surface imperfections. We then attached the wax substrates to the upper surface of leaflets of potted hostplants, *Pastinaca sativa* L., by placing a coil of cellophane tape under each wax square. Recently eclosed and mated *Papilio polyxenes* Stoll females were then allowed to fly freely in a well-lit cage with a potted hostplant. The butterflies began to drum the leaves (tap their forelegs alternately on the leaf surfaces; Ilse, 1937) soon after contacting the plant, occasionally drumming directly on the wax squares. This behavior exposes sensors on the legs of the butterfly (Ma and Schoonhoven, 1973) to phytochemicals at the leaf surface.

The wax was removed immediately and stored at $4^{o}C$ as a precaution against softening. The wax squares were then mounted on aluminum specimen stubs with colloidal graphite. Stubs were sputter-coated with Au/Pd using an EmScope SC500 sputter coater operated at 110V at 10mA. We viewed the specimens with an AMRAY 1000B scanning electron microscope operated at an accelerating potential of 1 - 7 KeV.

The value of using dental wax as a viewing substrate is demonstrated in the figures. The dorsal surface topography of a leaf of *Pastinacea sativa* (Fig. 1), a common host of *P. polyxenes*, reveals complex features that make it difficult to recognize marks made by insects. The control substrate after heating (Fig. 2), on the other hand, presents a relatively smooth surface. In consequence, the impact of drumming on the leaf surface (Figs. 3 & 4) is readily apparent on the wax substrate. The typical drumming pattern consists of one or two puncture marks from the impact of the tarsal claws, followed by a more shallow scraping of the wax surface. These scratches are typically about 10μm wide and up to 1mm long.

The technique described here has proven to be a very rapid and reproducible method to study aspects of insect behavior. The wax substrate is dimensionally stable in the SEM and does not require normal fixation and dehydration procedures. Specimens are prepared typically in a matter of minutes rather than hours. Minimized handling of the samples also reduces the likelihood of loss or change in the marks made by the insects.

The technique is limited by technical considerations, however. The electron beam may cause thermal damage if operated at high accelerating voltages. We have observed good results operating our instrument at 1-7 KeV accelerating potential and 5-35 μA beam currents; these operative conditions provide high-resolution, dimensionally-stable images free of charging artifacts. Operation at higher (10-15) KeV resulted in images which exhibited edge effect charging; higher KeV may also cause melting. If charging persists or operation at low KeV is not feasible, imaging with a backscatter detector may provide acceptable results. Also, inappropriate coating procedures may damage the wax surface thermally. Sputter-coating at low voltage produces negligible heat at the specimen surface and so ensures the dimensional integrity of the specimen. Furthermore, we have found that the wax substrate does not produce excessive contamination of the optical column, and specimens stored in a dessicator for more than 2 years show no deterioration.

We thank Paul P. Feeny, Cornell University, in whose lab we did the behavioral studies.

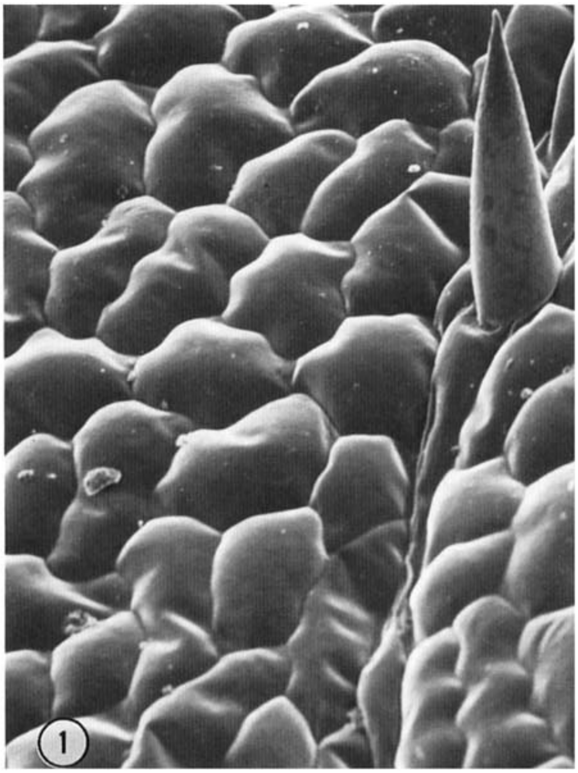
REFERENCES

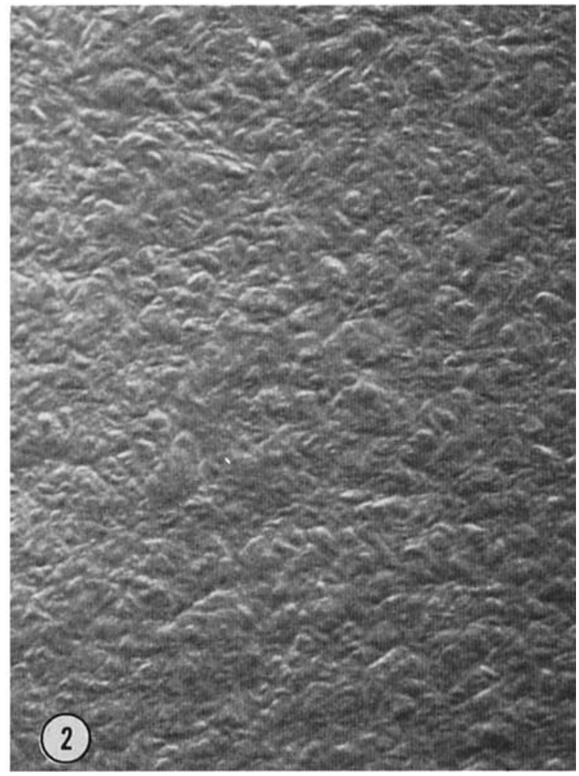
Hallam, N.D., and Juniper, B.E. (1971) The anatomy of the leaf surface. pp. 3-37 In: Ecology of Leaf Surface Micro-organisms. T.F. Preece and C.H. Dickinson, eds. Academic Press, New York, pp. 3-37.

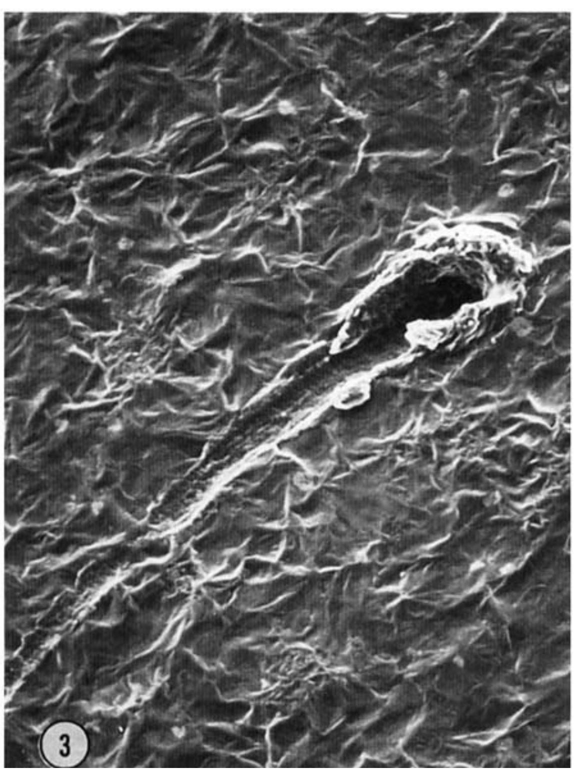
Ilse, D. (1937) New observations on responses to colours in egg-laying butterflies. Nature, 140:544.

Ma, W.C., and Schoonhoven, L.M. (1973) Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behavior. Ent. exp. & appl., 16:343-357.

Received March 21, 1993; accepted in revised form June 15, 1993.







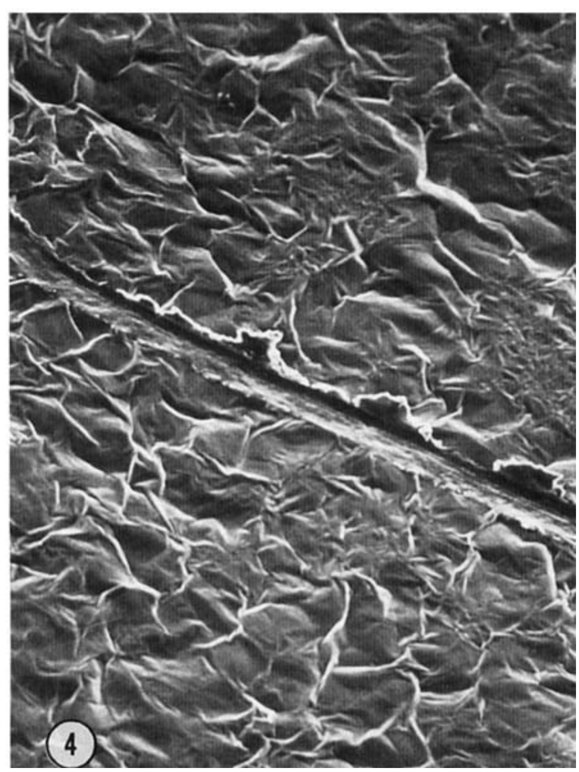


Fig. 1. Dorsal leaf surface of *Pastinacea sativa*, illustrating complex topography, X400. Fig. 2. The smooth topography of the wax substrate control, X400. Fig. 3. Typical drumming pattern, consisting of an initial tarsal impact point followed by a scratch mark, X750. Fig. 4. Long, shallow scratches resulting from drumming, X750.