SCANNING ELECTRON MICROSCOPY OF INSECTS: TECHNIQUES FOR THE NOVICE

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Scanning Electron Microscopy of Insects: Techniques for the Novice.

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INTRODUCTION

The scanning electron microscope (SEM) has been used to detail the morphology of small insects (Cornford et al., 1973, Howden and Ling 1973, Pease et al., 1966, and Reese and Carlson 1974). However, it is often difficult for the novice to obtain acceptable results based on the information presented in these papers without time-consuming and costly experimentation. This brief paper outlines some of the techniques and problems encountered in an examination of the balsam bark beetle Pityokteines sparsus (Le Conte) and the hymenopterous parasitoid Brachymeria intermedia (Nees). The information presented herein is mainly for the benefit of individuals wishing to use the SEM but having little or no experience concerning the special problems associated with the photography of insect specimens, and may form a base from which reasonable results can be obtained after limited experimentation.

The SEM is a valuable tool for the examination of the morphological structures of insects because of its great depth of field. Reese and Carlson (1974) examined the larval mouthparts of the black cutworm, Agrotis ipsilon (Hufnagel). The versatility of the SEM was clearly demonstrated relating form and function in the feeding habits of the insect. Antennal sensilla of the European corn borer, Ostrinia nubilalis (Hübner) were investigated by Cornford et al. (1973) in relation to sex pheromone detection. Serial photographs were presented in that paper to produce complete pictures of structures too large to be photographed in their entirety. Howden and Ling (1973) and Pease et al. (1966) photographed dry uncoated specimens at low magnification using a short exposure time. However, this technique was only recommended for valuable specimens where coating was undesirable, as some loss in resolution was unavoidable.

MATERIALS AND METHODS

All specimens were mounted on standard studs using clear Duco (DuPont) cement which was trimmed from around the specimen be-

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fore coating with gold-palladium. Coating was carried out in a Denton DV-515 automatic high vacuum evaporator at a vacuum of $2 \times 10^{-5}$ torr. The table was rotated at maximum speed while using the tilting mechanism. All specimens were coated to 100-400A. The SEM was a standard Cambridge Stereoscan S4. All micrographs were taken on Polaroid Type 55 P/N (4 x 5-in.) Land film.

RESULTS
The standard studs were found to be perfectly adequate for mounting small insect specimens and silver conducting paint between specimen and stud was not used as it almost always obscured parts of the specimen.

All specimens of *Pityokteines sparsus* were mounted directly from 95% alcohol and those of *Brachymeria intermedia* were freeze-dried. The freeze-drying technique was found to be particularly suitable for soft-bodied larvae and adults, with no evidence of structural collapse in the specimens (Fig. 1). The most suitable coating thickness was found to be between 150 and 200A.

The single most serious problem in photography of insect specimens in the SEM is "charging." This is the uneven build-up of electrons on the

FIGURE 1. Sensory hairs on the terminal segment of a freeze-dried female *Brachymeria intermedia*, 1,150X, 3.0kV.
surface of the specimen causing an uneven discharge of secondary electrons which are collected to produce the final picture. Charging lines, or streaks, usually originating from specimen projections, may appear on the micrograph (Fig. 2). The charging evident in Figure 2 is slight and does not obscure details of the specimen. Usually the streaks were more pronounced and seriously affected the final image.

![FIGURE 2. Tarsi of second leg of female *Pityokeines sparsus*, 300X, 2.0kV.](image)

Insect specimens with hairs, spines, and other projections were particularly prone to charging even at low accelerating voltages. Charging could be reduced or eliminated, and the quality of the final micrograph improved, by following a few basic rules.

1. **Accelerating voltage.** The use of a low accelerating voltage, sometimes as low as 2kV, was essential on hairy structures at magnifications above 200X (Figs. 2 and 3). More solid structures accepted up to 10kV with no noticeable charging. Six to 7kV, used only for short periods, gave good resolution without serious charging, even on quite hairy structures (Fig. 4). At voltages below 2kV the signal-to-noise ratio became unacceptable and the resolution in the final micrographs suffered accordingly.

2. **Beam exposure time.** The longer the period of time that the specimen was exposed to the electron beam, the greater the charging
problem. Voltages as high as 6 to 7kV could be used if the micrograph was taken less than 10 minutes after opening the isolation valve (Figs. 4 and 5). At low magnification, with the specimen in close contact with the stud, prolonged exposure at 6 or 7kV was also found to present no problems (Figs. 6 and 7). However, specimens with a restricted conducting surface showed severe charging under the same conditions.

3. Filament and beam currents. Careful peaking of the filament current considerably improved the signal and final micrograph. This was particularly true when the instrument had been in operation for more than half an hour. It was worthwhile to repeak the filament current after this period. Following peaking, the bias was set to maintain a beam current of about 200µA. This gave the best signal-to-noise ratio, particularly when working in the low kV range.

Some attention to the primary and secondary condensers was effective in decreasing the noise in the final image. Low condenser settings were most satisfactory, 0.54 on both primary and secondary.

4. Specimen angle. The angle of the specimen to the electron beam had a profound effect on the final resolution obtained. The generally accepted 45° viewing angle for flat specimens did not always apply to very irregular insect specimens. It was often important to have a suitable background to highlight small projections. Increasing the speci-
FIGURE 4. Mouthparts of male *Pityokteines sparsus*, 200X, 7.0kV.

FIGURE 5. Elytral hair of female *Pityokteines sparsus*, 1,900X, 6.0kV.
FIGURE 6. Ventral view of female *Pityokteines sparsus*, 30X, 6.0kV.

FIGURE 7. Elytral declivity of male *Pityokteines sparsus*, 90X, 6.7kV.
men angle to almost 90° was found to be the best solution. Incorrect specimen angle led to bright highlights and deep shadows resulting in very high contrast negatives. The black level was a valuable aid in varying contrast where a change in specimen angle was undesirable.

5. Photography. A uniform signal reading between 1 and 2 units was found to give best results, with high and low readings during the scan within 0.5 and 2.5. These levels produced acceptable contrast in the negative without serious highspots. Camera contrast settings were best between 6 and 7 and brightness between 4 and 6.

CONCLUSION

Some of the settings recommended in this paper will vary from one instrument to another and should be regarded only as starting points about which experimentation should be carried out. The most important factor is the accelerating voltage and its effect on specimen charging. However, careful attention to the other factors discussed can significantly improve the quality of the final micrographs.

LITERATURE CITED


