

## LILIACEAE

### A METHOD FOR THE NON-DESTRUCTIVE EXAMINATION OF LEAVES OF *ALOE* SPECIES BY SEM

Cutler and Brandham (1977; see also Cutler 1969, 1972 and Brandham & Cutler 1978) have shown that the leaf surface structure of members of *Aloineae* is not only under precise genetic control with little if

any environmental influence, but is also species (or infra-specific taxon) specific. This means that it can be used to identify otherwise unidentifiable specimens, and to assist in the identification of others.

Techniques are known for preparing surface-anatomical material of dried herbarium specimens without disturbing them and causing minimal distortion. The making of replicas is a well known method of examining otherwise difficult material by both scanning and transmission electron microscopy. The application of replicas to scanning electron microscopy (SEM) is reviewed by Hearle *et al.* (1974). As part of a study of the southern African species of *Aloe* for the *Flora of southern Africa*, we experimented with a number of replica techniques, notably those of Chapman (1967) using polystyrene foam dissolved in toluene, of De Winter (pers. comm.) using clear nail varnish, and of Watkins (pers. comm.). We found Watkins' technique of using a small piece of cellulose acetate film soaked in acetone was ideal for application to valuable specimens, because the dry acetate replica lifts off the specimen without damaging it, and removes at most some of the surface wax and dust. A piece of cellulose acetate film c.  $10 \times 5$  mm (the dimensions are not critical) is softened for a few seconds in acetone and laid on a suitable piece of leaf epidermis. A drop of acetone on the film will further soften it so much that all but the smallest air bubbles are removed, and an impression of the leaf is formed. After ten to twenty minutes the film dries and releases itself from the specimen. It may then be trimmed, if necessary, and mounted and coated for viewing by SEM in the normal manner.

As the material actually examined is a negative replica, it must be noted that papillae, micropapillae and ridges appear on the pictures as hollows of various shapes, and stomatal cavities appear as humps. Wax deposits appear 'right way round', but are seen

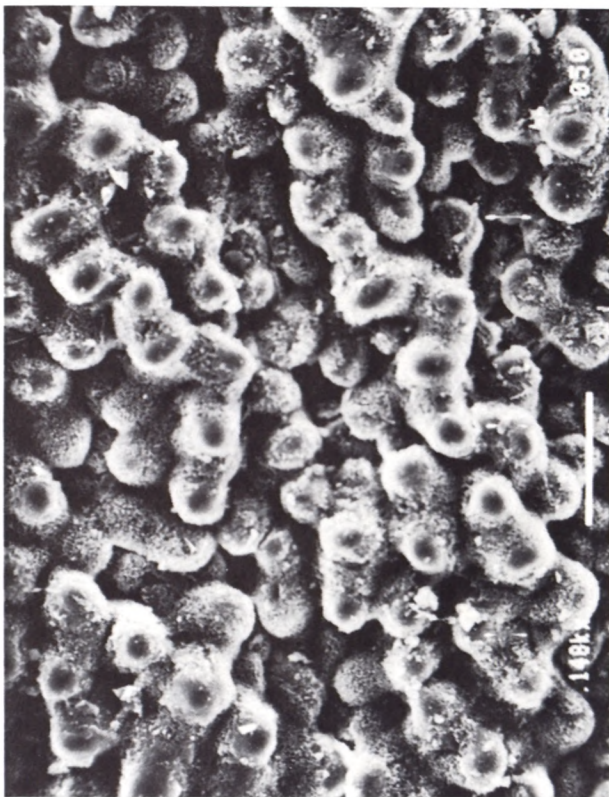


FIG. 13. — Live material of leaf of *Aloe namibensis*, Hardy 6330 in PNBG 28193. The scale bar is approximately  $135 \mu\text{m}$  long.

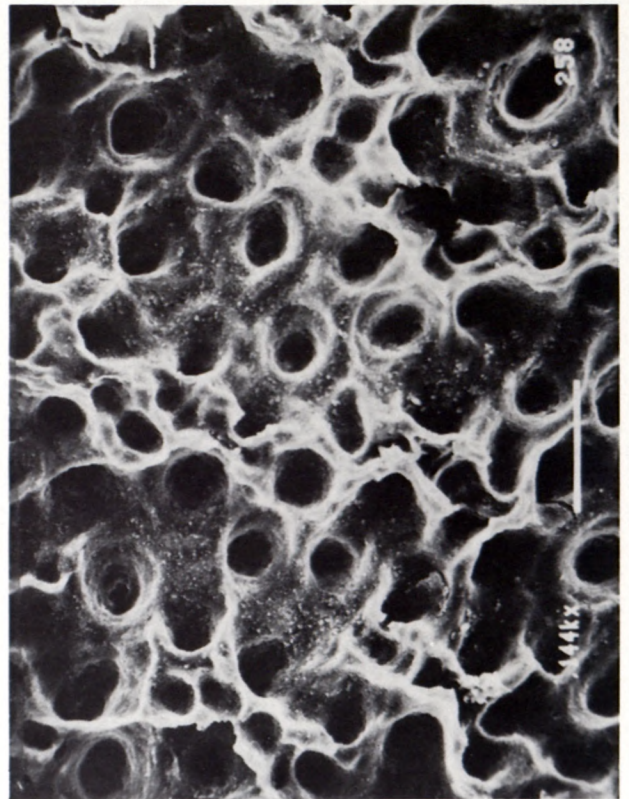


FIG. 14. — Acetate replica of a specimen of *Aloe namibensis*, Giess 10459 in PRE. The scale bar is approximately  $140 \mu\text{m}$  long.

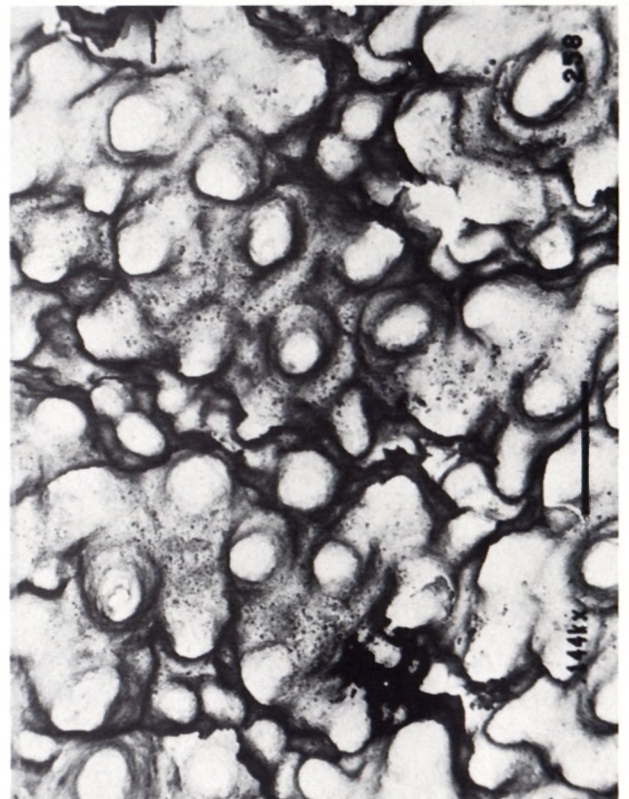


FIG. 15. — Negative of Fig. 14. The scale bar is approximately  $140 \mu\text{m}$  long.

from below rather than from above. The practical effect of this may be seen by comparing the picture of live material of *Aloe namibensis* in Fig. 13 with the replica in Fig. 14.

An interesting optical illusion may be noted when using these replicas (see Fig. 15). This is the negative of Fig. 14. In negatives of SEM pictures of replicas made as described above, hairs, papillae, micropapillae, ridges and hollows are seen in an approximation to their correct perspective; this is seen when Fig. 15 is compared with Fig. 13.

An example of the use of characters made available by this method, that would otherwise have been unavailable to the investigators, is given by Glen & Hardy (in press).

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