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A Sample Transfer Procedure For Scanning Electron Microscopy[1]

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Abstract

Scanning electron microscopy (SEM) preparation procedures can limit and restrict sampling and transportation of plant tissue for SEM analysis. At nurseries throughout the United States leaf samples of *Acer rubrum* L. 'October Glory,' were placed in glutaraldehyde and transported by parcel post at ambient temperatures in mailing sacks. Similar leaf samples were fixed in glutaraldehyde, shipped by small package air express in ice 4 °C (40 °F) within stainless steel thermos bottles. Upon receipt, both groups of samples were processed in the laboratory using standard SEM procedures and examined with an SEM. Laboratory controls were performed simultaneously at Delaware, OH under standard SEM procedures. Samples shipped by air express in ice appeared similar to specimens from the laboratory controls when evaluated with the SEM. Downy surface wax was not altered. Leaf surfaces of specimens that were shipped by parcel post at ambient temperatures were altered, lacking downy wax and possessing lesion-like areas. Rapid transfers of fixed specimens in ice reduces fixation and temperature-induced artifacts when shipped by express air freight.

Index words: Epicuticular leafwax, *Acer rubrum,* specimen preparation.

Introduction

Although scanning electron microscopy (SEM) is a useful tool, collection and subsequent handling of specimens can restrict SEM applications. Since the primary use of SEM is to examine surface structures, the main aim in specimen preparation is to insure that the lifelike, original topography of the specimen is well-preserved. If plant tissues are not handled properly, delicate surface features can be distorted, creating artifacts (1,6). While some botanical species (2) do not require or cannot tolerate fixation, dehydration, and critical-point-drying (CPD), most botanical tissue must be immediately preserved in fixative after sample collection. Damage can occur if plant tissue is in contact with fixative longer than 18 hr (5) at ambient temperatures. Therefore, time and temperature can be limiting factors in the design of long distance sample collection procedures. The purpose of the current study was to evaluate possible methods of long distance transport of botanical samples for SEM analysis.

Materials and Methods

Leaves of *Acer rubrum* L. 'October Glory' were collected at Calloway Gardens, GA; Urbana, IL; Princeton, NY; and Gresham, OR during growing seasons from 1976 through 1980. Specimens were fixed in 3% glutaraldehyde, in 0.1 M phosphate buffer at pH 7.2 in screw top vials. Five specimen vials were placed in mailing envelopes and shipped parcel post (U.S. Postal Service) at ambient temperatures. The same day 5 additional vials were sealed in a stainless steel thermos bottle in ice at approximately 4 °C (40 °F) and shipped via small package air express. Small package express service is available from all common air carriers who guarantee "next flight out" priority to desired destinations. Federal aviation laws and airline workers union rules precluded routine use of dry ice (solid CO_2) as an inexpensive coolant system.

Upon receipt, all samples were washed 3 times in buffer, post-fixed in 1% osmium tetroxide (OsO_4), rinsed in buffer, dehydrated in 30, 70, 80, 90, 95, 100% ethanol and critical-point-dried (CPD) in an Autosamdri (Tousimis Corp., Rockville, MD). Specimens were mounted on stubs and sputter-coated with 400-500 Angstroms of Au and examined on a Hitachi S-500 SEM (Hitachi Corp., Mountain View, CA), at 20kV with a 40 °tilt. The control treatment consisted of 'October Glory' leaf tissue sampled at Delaware, OH and prepared according to a strict regime of 18 hr fixation followed by post-fixation in osmium tetroxide, ethanol dehydration and CPD as described above.

The leaf surfaces of the control treatments and the fixed, iced samples that were shipped by small package air freight and then prepared, appeared identical when examined with SEM. In all replications, cuticles were covered with downy epicuticular wax and appeared uniformly tufted and convex (Figs. 1,3) as previously described (3).

Specimens in fixative that were shipped by parcel post at ambient temperatures appeared to be altered when observed with SEM. Epicuticular wax lacked the downy appearance (Fig. 2), as shown by black arrows. Lesion-like areas were visualized as void of fluffy wax crystals (white arrows, Fig. 2). At higher magnification, epicuticular wax appeared to be an amorphic covering over the epidermal cells (Fig. 4). The long distance handling procedure described in the current study has been successfully used in transferring *Populus deltoides*

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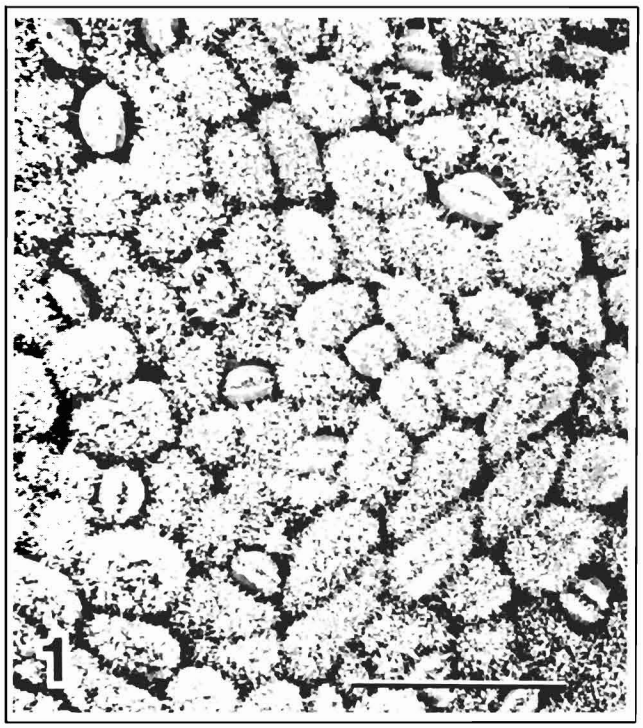

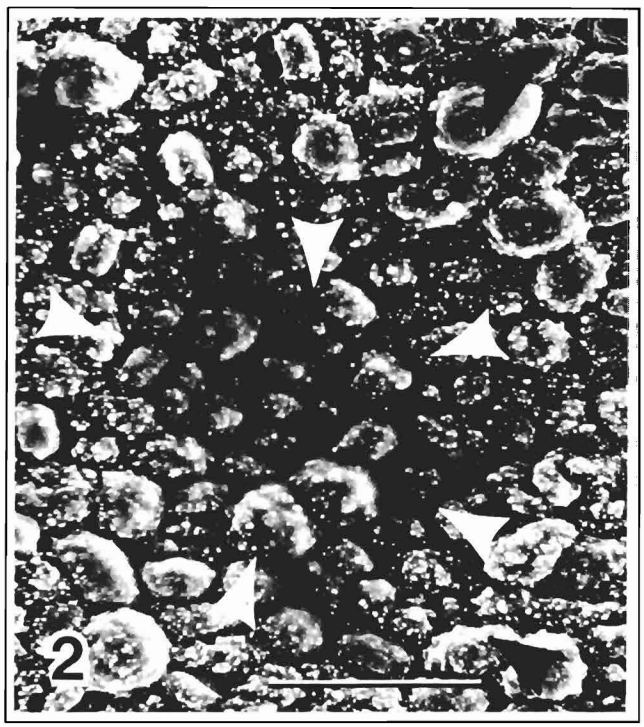

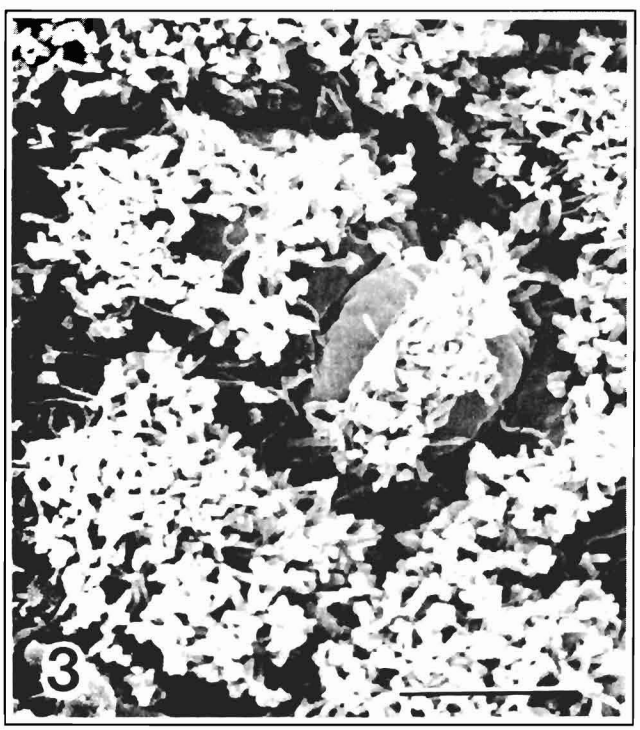

Fig. 1-4 Scanning electron micrograph of *Acer rubrum* abaxial leaf surfaces. Figures 1 and 3, an iced, fixed and prepared specimen that was shipped by small package air freight. Note downy epicuticular wax. Specimens that were prepared entirely in the laboratory (control) according to standard SEM procedures appeared identical to fixed, iced and prepared specimens that were shipped by air freight. Fig. 2, specimen that was shipped in fixative at ambient temperatures appeared to lack downy epicuticular wax (black arrows). Lesion-like areas (white arrows) lacked epicuticular wax crystals. Fig. 4, epicuticular wax appeared to be an amorphic covering over epidermal cells. Fig. 1 and 2, Bars = 50 μm; Fig. 3 and 4, Bars = 5μm.

specimens (micrographs not included in this paper) damaged by gaseous air pollutants to our laboratory for evaluation (4).

Rapid transfer of fixed specimens to reduce fixation and temperature-induced artifacts can best be achieved by small package express air freight in iced thermos bottles. With knowledge of airline schedules, sampling can be scheduled in close proximity to flight departures. Within hours after sampling, remaining SEM preparatory procedures can be completed. Utilization of "Plant

Fingerprinting'' with SEM can be more practical, accurate and accessible utilizing the method of sample transfer described in the study.

Significance to the Nursery Industry

The scanning electron microscope (SEM) is becoming an important tool in cultivar identification or ''plant fingerprinting.'' Often nurserymen or scientists obtain and transport plant samples hundreds of miles to analytical SEM laboratories. For maximum success, specimens of most plant species should be placed in fixative (i.e. glutaraldehyde) within an iced thermos bottle. More than 18 hours of immersion in fixatives can alter fine surface detail of plant structures when viewed with SEM. Since several days were required for delivery of specimens placed in mailing envelopes and shipped by standard United States Postal Service parcel post, it was determined to be an inadequate method of transfer. On the other hand, small package air freight, which guarantees overnight delivery, optimized sample transfer to the SEM laboratory and minimized surface artifacts to red maple leaves.

Further research is being conducted on several nursery genera to determine required transfer and preparation procedures. Utilization of ''plant fingerprinting'' with SEM can be more practical, accurate and accessible with the method described in this study.

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