Differentiation of Various Pollen Grains Using Scanning Electron Microscopy

Jonathan D. Hulse and Kristen A. Lennon

Department of Biology, Frostburg State University, Frostburg, MD 21532

INTRODUCTION

- Each and every plant is unique in that they all have pollen grains with different surface textures that can be used to indentify that specific species, much how every human is differentiated by their finger print.
- Pollen houses the male reproductive cells that germinate to produce a pollen tube and transfer their genetic information when they come in contact with the female reproductive structure, the pistil.
- The Scanning Electron Microscope allows for great detail to be observed that far out compares the capabilities of a typical light microscope.
- Comparison of pollen collected from various species of plant by the use of a Scanning Electron Microscope was the ultimate goal of this study.
- Pollen grains were collected from the greenhouse in Compton Science Center at Frostburg State University from Calypso, Geranium, Passion Flower, and Marigold.
- Special interest was focused on surface morphology of pollen grains.
- Care was taken to collect pollen fresh off each plant and scan immediately after harvesting.
- Another interest was to eliminate the use of harmful chemicals typically used to process organisms for Electron Microscopy.

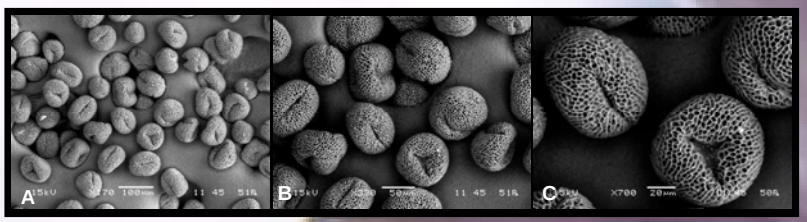

Figure 1: Unfixed, Uncoated Calypso pollen taken at 15kV (Magnification from left to right: 1.A 170X, 1.B 350X, 1.C 700X)

METHODS

Pre Sampling

- Four 10 mm diameter aluminum SEM stubs were cleaned by bathing twice in acetone (the only actual waste product), then double-sided, conductive adhesive tabs were applied and stubs were stored in container prior to use to prevent contamination.

Sample collection

- Samples of pollen from Calypso, Geranium spp., Passion Flower (Passiflora spp.), and Marigold (Tagetes spp.) were collected from the greenhouse in Compton Science Center, Frostburg State University.
- Pollen was transferred to stub by removing the stub from the container, exposing the adhesive surface and holding it underneath the flower while gently tapping the flower to release the pollen, or by gently pressing the stub against the stamens to collect the pollen by direct contact.

Microscopic Observation

- No chemical fixation was used for this experiment to eliminate the production of hazardous waste and prevent exposure to heavy metals.
- Samples were observed directly after collection under low vacuum on a JEOL JSM-6060LV scanning electron microscope at 15kV.
- Selected pictures were taken in order to maximize usefulness of surface morphological features and orientation of the pollen to show dimensional detail. Pictures were captured digitally using the JEOL software and imported to Adobe Photoshop CS4 where they were optimized for brightness and contrast.

RESULTS

- Calypso (Fig. 1): Notable surface features: Spherical in shape with obvious three corner inward fold on side position, and numerous poriferate surface depressions surrounded by reticulation.
- Marigold: (Fig. 2) Notable surface features: Spherical shape with many actinate projections spaced regularly, with three way inward fold on end position.
- Passion Flower: (Fig. 3) Notable surface features: Twice as long as is wide, three single inward folds on side, covered in numerous poriferate surface depressions surrounded by reticulation.
- Geranium: (Fig. 4) Notable surface features: rounded cubical shape with four single inward fold, and numerous poriferate surface depressions surrounded by reticulation.
- Each pollen has characteristics that are unique to itself, although some similarities are noticed between grains.

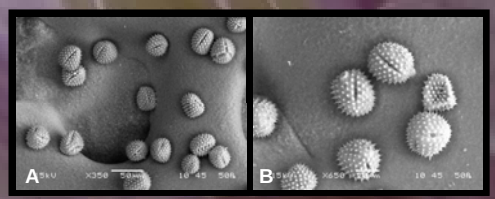

Figure 2: Unfixed, Uncoated Marigold pollen taken at 15kV (Magnification from left to right: 2.A 350X, 2.B 650X)

Abstract

The focus of this experiment was to analyze the surface morphology of various species' pollen grains by the use of Low Vacuum Scanning Electron Microscopy. A goal of this experiment was to minimize the production of hazardous waste and to view the samples without the use of heavy metals typically used for Electron Microscopic Techniques.

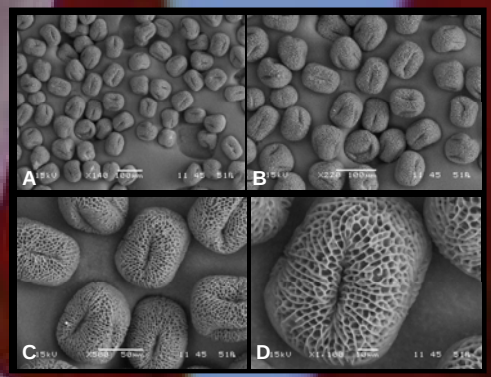

Figure 4: Unfixed, Uncoated Geranium pollen taken at 15kV (Magnification from top left to bottom right: 4.A 140X, 4.B 220X, 4.C 500X, 4.D 1100X)

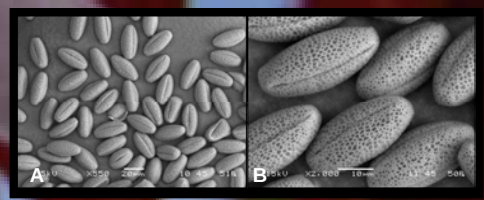

Figure 3: Unfixed, Uncoated Passion flower taken at 15kV (Magnification from left to right: 3.A 550X, 3.B 2000X)

DISCUSSION

- Examination of surface structures with the use of Low Vacuum Scanning Electron Microscopy is a effective way to minimize waste and obtain top quality micrographs that allow the examination of the surface structure of pollen.
- This study provided insight into the complexity and variation between pollen grains of different species. Surface morphology can suggest modes of pollination, as well as a means of classification.
- The analysis of pollen has many practical applications in determining biodiversity in forest systems, using in forensic botany studies, or identification of air born pollen allergens .
- Further research can be completed to show the similarities and differences between like taxa and to show a possible correlation between evolutionary lineages due to pollens surface morphology.

ACKNOWLEDGEMENTS

Give special thanks to Tim Pegg and David Valenta for much needed assistance and patience in the laboratory! Some days just aren't ultramicrotome days! Background picture of a Blackberry Lilly/Virginia Creeper taken by Jonathan Hulse.