

## “Objects Worthy of Notice”

# Microscopical Anatomy of Selected Plants Collected by The Lewis & Clark Expedition

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### Introduction

Plants that are used for taxonomic research are generally preserved by drying them out while they are being pressed flat for easy storage. This pressed plant material, is placed in a herbarium, in phylogenetic order, based on the species, genus, family and order designations.

Herbaria are essential for the study and verification of plant classification, the study of geographic distributions, and the standardizing of taxonomic nomenclature. Lately, herbarium materials have been utilized in biochemical and genetic studies. The key to these studies is the state of preservation of the plant material. The preservation of the reproductive and vegetative structures (hairs, stomata, cuticle, etc.), internal anatomy (vascular tissue size and arrangement), biochemistry and genetic material is essential to make accurate observations, to collect representative data and answer pertinent research questions. To investigate the potential of herbarium specimens to be used in non-taxonomic studies, we examined herbarium material collected by the Lewis & Clark Expedition (1803-1806) and evaluated their state of preservation through microscopic examination of the external and internal features.

Plant material from ten specimens was selected from the plants collected by the Lewis and Clark Expedition and housed at The Academy of Natural Sciences in Philadelphia.\* For the sake of space, only six of these samples will be described. Preliminary microscopic examination of the specimen surfaces revealed exquisite preservation of trichomes, stomata, and cuticle in most samples. Detailed examination of the surfaces and the internal anatomy of the plants, using digital image analysis (gradient pseudocoloring), have revealed fungal hyphae on, and in, all of the samples examined.

### Materials and Methods

**Light Microscopy:** Samples were photographed on a Wild M420 Makroskop prior to embedding or carbon coating. Samples were embedded in Spurr's resin (Spurr, 1969), sectioned at 10-15  $\mu\text{m}$  with glass knives, mounted on glass microscope slides and stained with toluidine blue-O. Slides were viewed in a Leica DMRX research microscope. Images were captured on film (macro photography) with a Nikon DXM-1200 camera.

**Electron Microscopy:** 16 mm diameter disks of carbon conductive double-sided adhesive material were applied to the center of carbon stubs (26 mm X 8 mm). Sample fragments were arranged on the adhesive material. Occasionally, curved or irregular fragments were mounted on minimal amounts of carbon paste to insure good contact with the adhesive surface and facilitate conductivity. The mounted samples were then placed in an Edwards E306A vacuum coating unit and given a carbon coating approx. 30 nm thick. Samples were viewed on a JEOL JXA-840A Scanning Electron Microscope with a Thermo-NORAN TN-5502 energy dispersive analytical attachment and, NORAN Vantage spectrum processing software. With a few exceptions (see specs. on photos), samples were photographed between 8-15 kV at a working distances ranging from 7-12 mm.

**Colorization of SEM Images:** Gradient pseudocoloring is a method for bringing out edges and their orientation in the image. The gradient image is created in Hue-Saturation-Intensity (HSI) space. The original image is used to modulate the intensity, and local variance is used to calculate saturation, or the range of color to grayscale. The hue of the image is calculated from a derived edge, such as a Sobel, variance, Roberts, or other edge detecting filter. A gradient phase is calculated from the edge image, indicating the direction of changes in intensity. This phase is then converted to color hues, so that edges sloping in one direction will have a color distinct from edges sloping in other directions. The effect is similar to illuminating a three dimensional object with a ring of colored lamps, so that edges oriented towards different lamps will reflect the different colors. The three images are combined in HIS space, and converted to RGB to produce the gradient pseudocolor image.

### Silver Buffalo-Berry

(*Shepherdia argentea* [Pursh] Nutt.)

#### Oleaster Family (Eleagnaceae)

This specimen was collected on September 4<sup>th</sup>, 1804 at the mouth of the Niobrara River, south of the current border of Nebraska and South Dakota (Fig. 1). The collection site is near the current southern range of the plant (Fig. 2) inferring that it was most likely collected shortly after being seen.

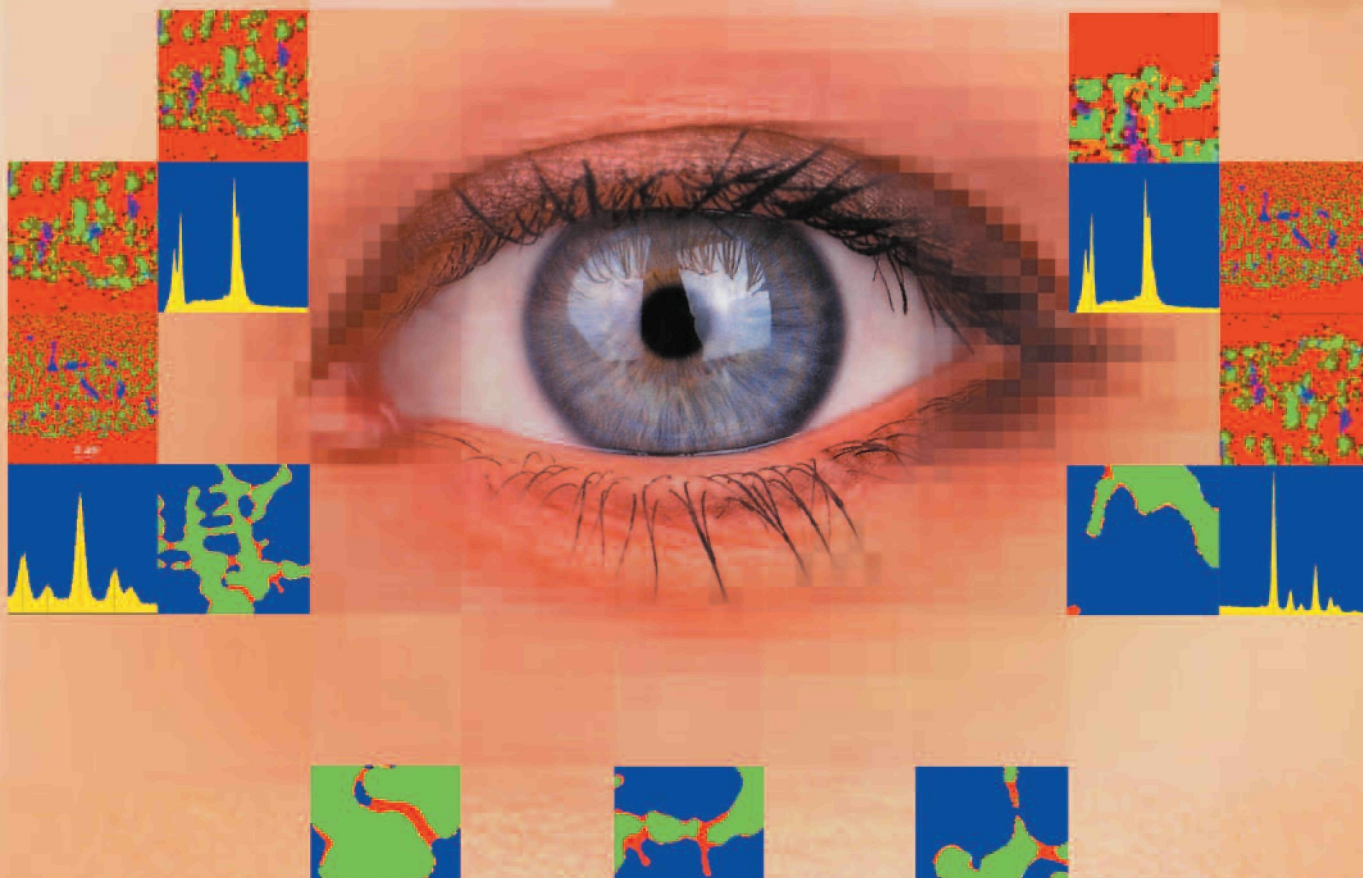


Fig. 1. Collection site for silver buffalo-berry.

Fig. 2. Current range map for silver buffalo-berry.



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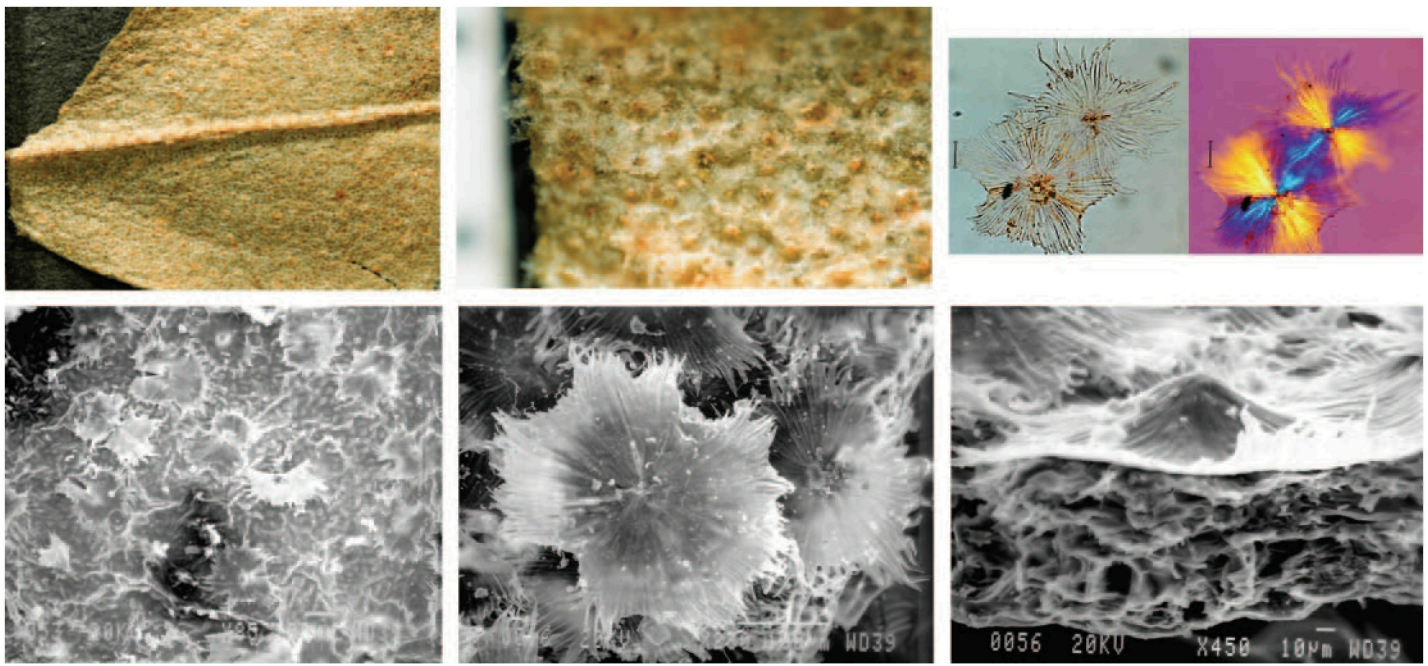


Fig. 3. Macrophotography of silver buffalo-berry, showing midvein. Upper left.

Fig. 4. Macrophotography of silver buffalo-berry, showing numerous peltate trichomes. Upper center.

Fig. 5. Peltate trichomes in normal light (l) and polarized light (r). Upper right.

Fig. 6. SEMs of Peltate trichome. Lower left and center.

Fig. 7. SEM of Peltate trichome, showing its close association to the leaf surface. Lower right.

Microscopy shows a well preserved leaf (Fig. 3) covered in peltate (shield-like) trichomes (Figs. 4 – 7). They are tightly packed and closely appressed to the surface of the leaf (Fig. 7). The trichomes are an adaptation to a xeric (dry) environment, where they impede the loss of water by creating a boundary layer of humid air at the leaf surface.

### Interior Wild Rice

(*Zizania palustris* L. var. *interior* [Fassett] Dore)

#### Grass Family (Poaceae)

This specimen was collected on September 8<sup>th</sup>, 1804 along the Missouri River, near the current border of Nebraska and South Dakota (Fig. 8).

Microscopy shows another well preserved leaf (Fig. 9), with intact cuticle, knobby protuberances called papillae and bow-tie shaped silica cells (Fig. 10). Also apparent are numerous fungal hyphae on the surface of the leaf (Figs. 10 & 11). Fungal hyphae extend into the stomata (Fig. 11).



Fig. 8. Collection site for wild rice.

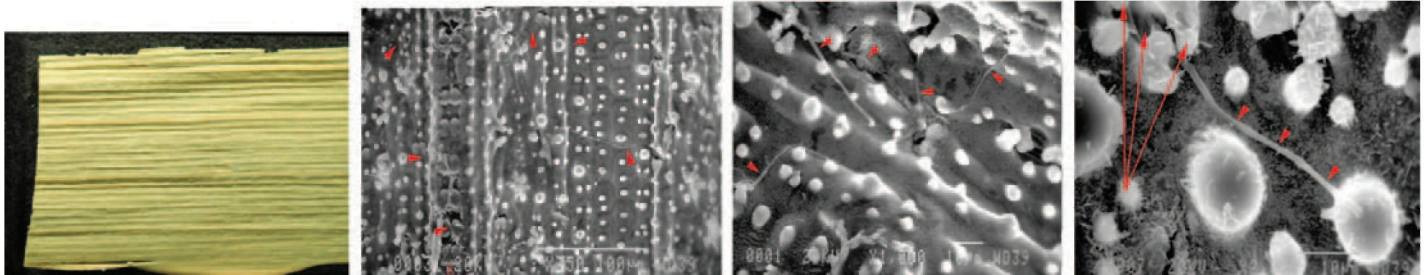


Fig. 9. Macrophotograph of wild rice leaf. Upper left.

Fig. 10. SEM of wild rice, showing fungal hyphae (arrowheads) and silica cells (S). Upper right.

Fig. 11. SEM's of wild rice, showing fungal hyphae (arrowheads) at a stomatal complex (arrows).

### Indian Tobacco

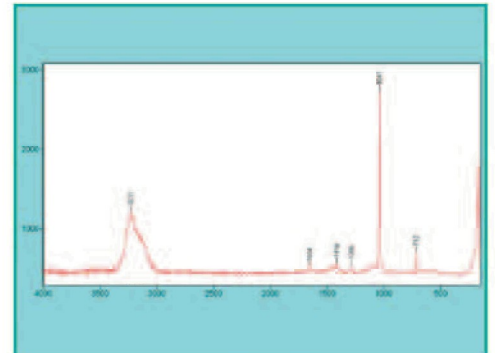
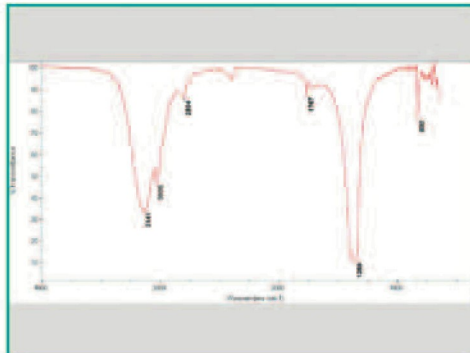
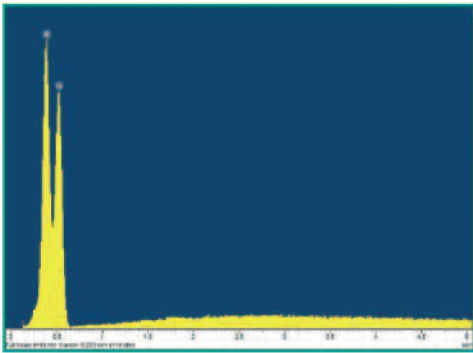
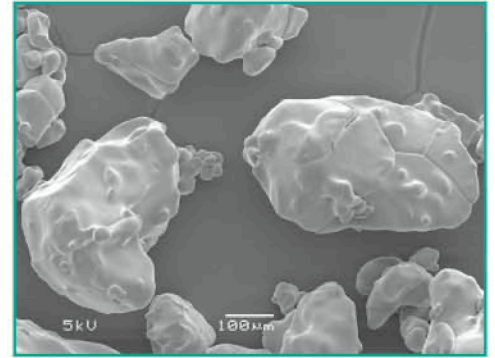
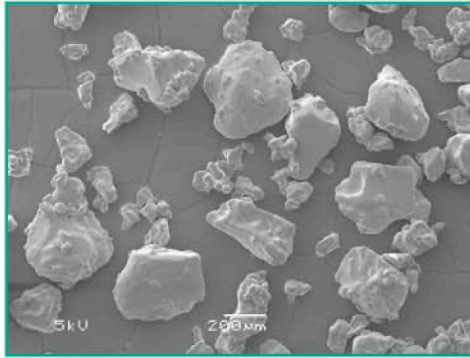
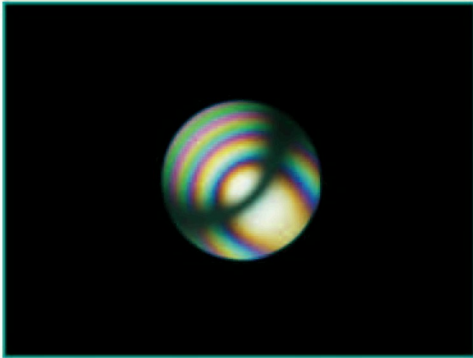
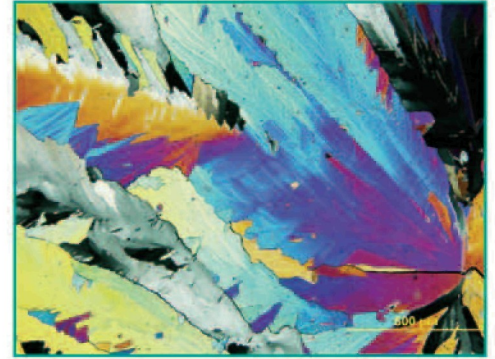
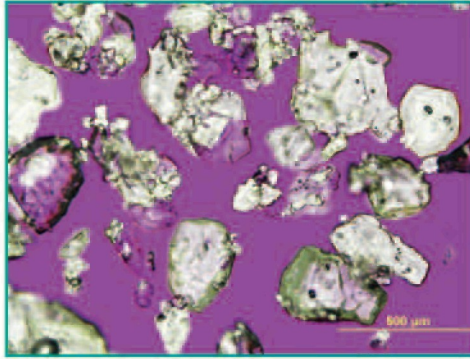
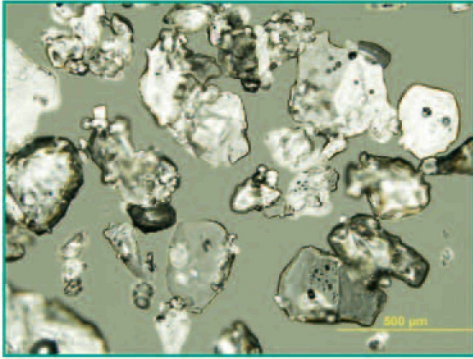
(*Nicotiana quadrivalvis* Pursh)

#### Potato Family (Solanaceae)

This specimen was collected on October 12<sup>th</sup>, 1804 at the Arikara Camp near the mouth of the Grande River (Fig. 12.). The herbarium sheet shows that the plants were dried while being hung, and not pressed in a book (see the sheet at: [http://clade.acnatsci.org/mccourt/Lewis%20and%20Clark/flattened%20jpgs%2012\\_10/146.jpg](http://clade.acnatsci.org/mccourt/Lewis%20and%20Clark/flattened%20jpgs%2012_10/146.jpg)).

The samples of tobacco were not well preserved, with few discernable trichomes and stomata (Figs. 13 – 15). The leaf surface appeared altered and contained considerable extraneous material (Figs. 15 – 17).

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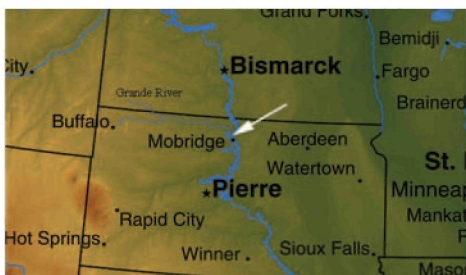


Fig. 12. Collection site for Indian tobacco.

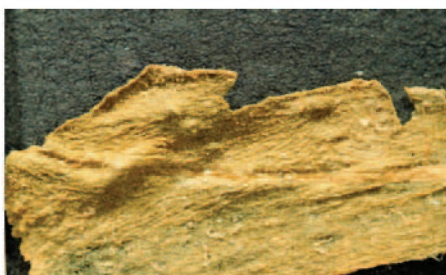


Fig. 13. Macrophotograph of Indian tobacco leaf.



Fig. 14. Macrophotograph of Indian tobacco leaf, showing several trichomes.

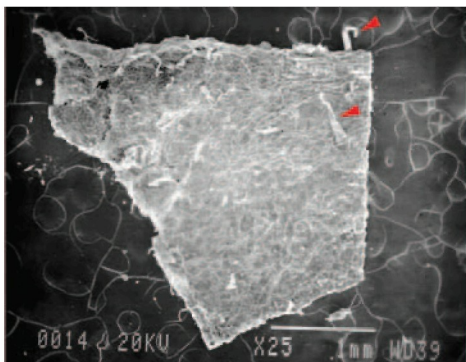


Fig. 15. SEM of Indian tobacco leaf showing remnants of trichomes (left, arrowheads) and cluttered leaf surface (center).

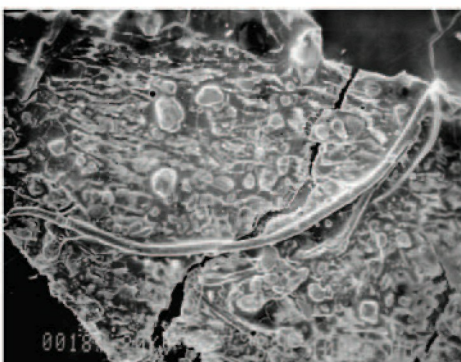


Fig. 16. SEM of Indian tobacco, showing fungal hyphae (right, arrowheads).

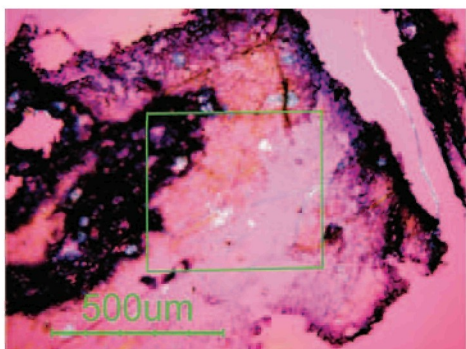
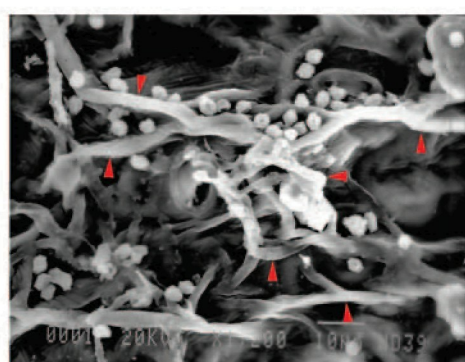
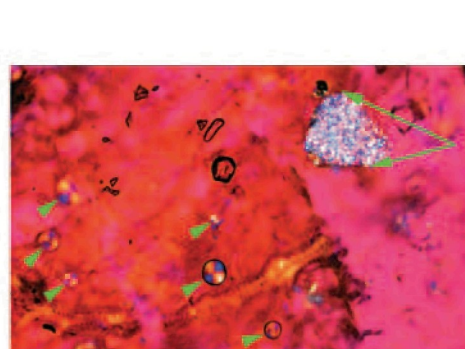
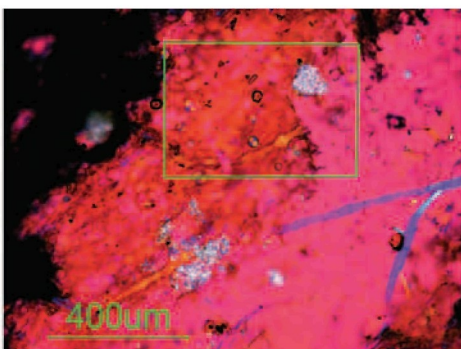


Fig. 17. Polarized light micrographs (with  $\lambda$  plate), showing extraneous, granular (arrows) and spherical (arrowheads) birefringent materials.



## Oregon Boxleaf

*Paxistima myrsinites* (Pursh) Raf.

**Bittersweet Family (Celastraceae)**

This specimen was collected on November 16<sup>th</sup>, 1805 at Cape Disappointment (Fig. 18).

Microscopy shows leaves that are well preserved (Fig. 19-21). The stomatal apertures are overarched by rodlets of cuticle and the leaf surface is covered with fungal hyphae (Fig. 22 - 23).



Fig. 18. Collection site for Oregon boxleaf.



Fig. 19. Macrophotograph of Oregon boxleaf leaf.

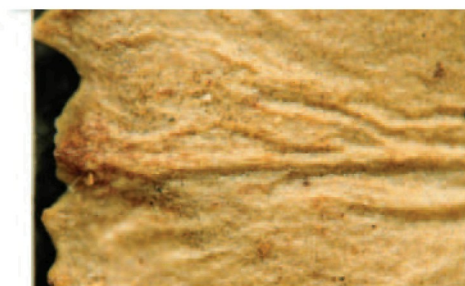
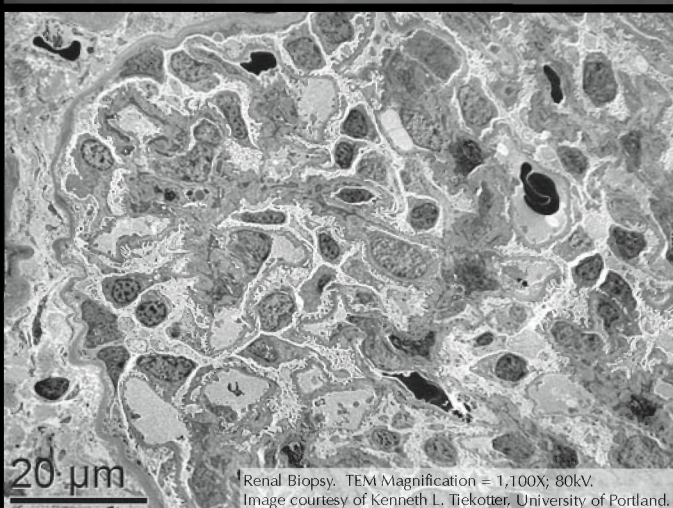
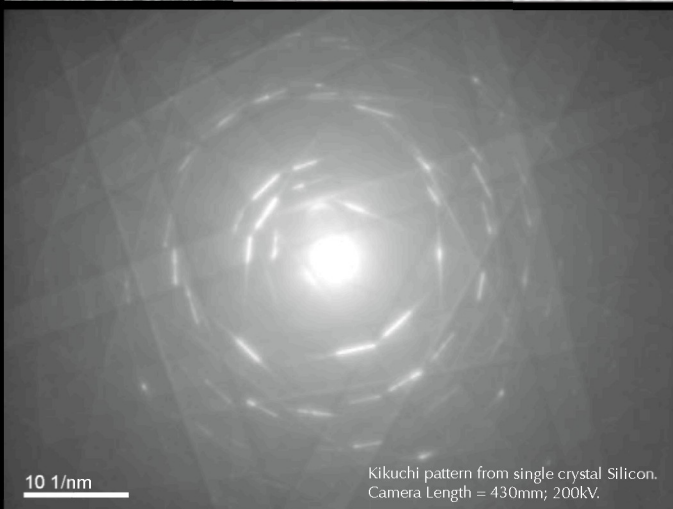
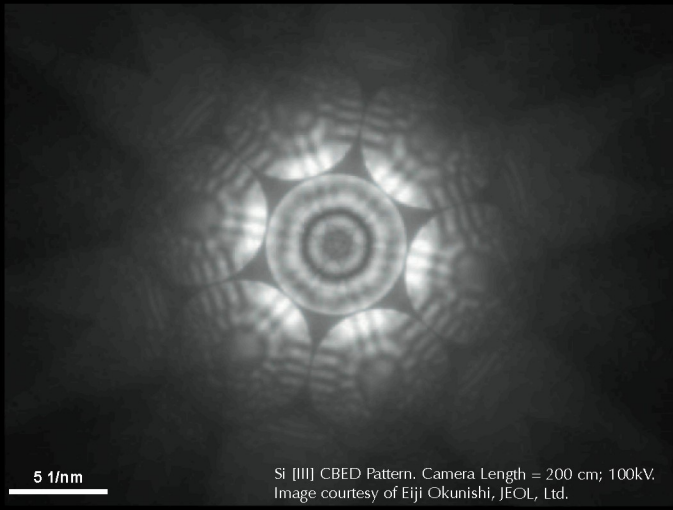


Fig. 20. Macrophotograph of Oregon boxleaf leaf.



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Fig. 21. SEM's of Oregon boxleaf leaf, showing stomata (white flecks). Left.

Fig. 22. SEM's of Oregon boxleaf, showing fungal hyphae (arrowheads). Normal SEM (center) and gradient pseudocoloring (right).




Fig. 23. SEM's of Oregon boxleaf stomatal complex, showing cuticular rods (arrows) overarching the guard cells and fungal hyphae (arrowheads). Normal SEM (left) and gradient pseudocoloring (center).

Fig. 24. Collection site for American Dune Grass.

### American Dune Grass

*Leymus arenarius* (L.) Hochst.

#### Grass Family (Poaceae)

This specimen was collected on December, 1805 at Fort Clatsop, Oregon (Fig. 24, above).

Microscopy shows yet another well preserved leaf (Figs. 25 – 28), with an intact cuticle and tough, pointed trichomes (Figs. 27 & 28). Apparent are numerous fungal hyphae on the surface (Figs. 27 & 28) and in the cavity formed by the rolled leaf (Fig. 29). The septate hyphae (Fig. 29) (hyphae with cross walls) indicate it is a basidiomycete (club fungus). There are also numerous spherical structures, likely conidiospores on the inner surface (Figs. 27 & 28).




Fig. 25. Macrophotograph of American Dune Grass (left).

Fig. 26. SEM of American Dune Grass, showing vascular bundles (arrows) and abaxial surface (AB) Second image..

Fig 27. SEM's of American Dune Grass, showing numerous, pointed trichomes and fungal hyphae (arrowheads) on the inner (adaxial) surface. 3rd & 4th




Fig. 28. SEM's of American Dune Grass, showing pointed trichomes, a single fungal hypha (arrowhead) on the adaxial surface and thin cuticle covering the surface. Normal SEM (left) and gradient pseudocoloring (second).

Fig. 29. Light microscopy showing septate fungal hyphae (arrowheads) from an airspace formed by the curled leaf of American Dune Grass (3rd). Image on the right is a digital compilation of 9 focal planes using the Extended Depth of Focus feature in ImagePro Plus© software.



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## Big-leaf Maple

*Acer macrophyllum* Pursh

### Maple Family (Aceraceae)

This specimen was collected on April 10<sup>th</sup>, 1806 along the Columbia River near The Great Rapids (Fig. 30). The collection site is near the current eastern range of the plant (Fig. 31), showing that it was most likely collected shortly after being seen.

Microscopy reveals a well preserved leaf with large hairs (Figs. 32 – 34) on the veins of the lower (abaxial) surface, cuticle (Fig. 35) and stomata (Figs. 36 & 37). The leaf shows macroscopic signs of being infected with Tar Spot fungus (Fig. 32). The leaf surface is covered with fungal hyphae (Fig. 36). Hyphae can be seen inserted into the stomatal pore (Fig. 37).



Fig. 30. Collection site for big-leaf maple.

Fig. 31. Current range map for big-leaf maple.

Fig. 32. Macrophotograph of big-leaf maple, showing trichomes (arrowheads) and tar spot fungus (arrows).

Fig. 33. SEM's of big-leaf maple.



Fig. 34. SEM's of big-leaf maple, showing trichomes (arrowheads) and leaf veins (V). Adaxial surface (left) and abaxial surface (center)

Fig. 35. Adaxial surface of big-leaf maple, showing intact cuticular ridges. This image is the same as Fig. 2E in Teece, et al (2002). Right.



Fig. 36. SEM's of big-leaf maple, showing fungal hyphae (arrowheads). Original image (left) and digitally enhanced image (2nd). Normal SEM (left) and gradient pseudocoloring (2nd). The normal SEM image is the identical one used in Figure 2B in Teece, et al (2002).

Fig. 37. SEM's of big-leaf maple, showing fungal hyphae (arrowheads) at two different stomatal apertures (outlined in red). 3rd & 4th.

### Discussion

It is clear from the above images that the plants collected by Meriwether Lewis, while structurally sound, have been contaminated by fungi on the surface and internally. This finding is in disagreement with previously published research (Teece, et al, 2002). ■

### Acknowledgements

A gracious acknowledgement to Dr. Richard M. McCourt, Department of Botany, The Academy of Natural Sciences of Philadelphia\* for providing samples for this study. Range maps of woody species were scanned from Little, Jr. (1976), digitally cleaned up and colorized.

\*The Academy of Natural Sciences, an international museum of natural history operating since 1812, undertakes research and public education that focuses on the environment and its diverse species. The mission of the Academy is to create the basis for a healthy and sustainable planet through exploration, research and education. The Academy of Natural Sciences' Lewis & Clark Herbarium is a collection of 226 sheets of dried, pressed plants collected by Meriwether Lewis during the Lewis & Clark Expedition of 1804-06. These plants are the most well documented and historically important collection of American plants in existence. Recognizing their value, the federal government declared the herbarium an "American Treasure" through its Save America's Treasures program.

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... Continued from page 28.

diatom a helpful test sample for accessing the quality of the microscope images [6]. Figure 2 shows fine structure of three fragments of *Pleurosigma angulatum* taken from different sources.

A variety of living cells have been observed and photographed with this technique, including cancer cells (Figures 3, 4), pollen (Figure 5), and bacteria (Figure 6). Spirochete (Figure 6), a microscopic bacterial organism, has a worm-like, spiral-shaped form. It wiggles vigorously when viewed under a microscope. The photograph shows a fine structure of bacterium, represented by 12 small additional "coils" between major periods of a spiral body.

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## Acknowledgements:

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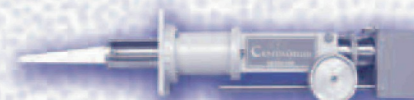
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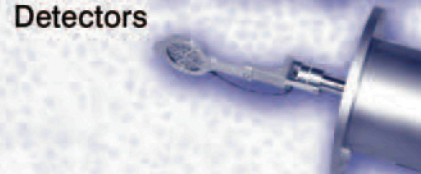
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