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Localization of UV Absorbing Compounds in Nuttall Oak (*Quercus nuttallii*) Leaves Using Naturstoffreagenz-A (NA) and the Leica DMI6000 B Inverted Robotic Microscope

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Protection from the stratospheric ozone layer is essential to survival of plants, humans, animals, and other life forms of organisms on this planet. Depletion of stratospheric ozone, mainly due to man-made pollutants, such as chlorofluorocarbons and other halogenated ozone depleting substances, has resulted in the increased solar ultraviolet-B (UV-B, 280-315nm) radiation at high and mid-latitudes in both the Southern and Northern hemispheres [1, 2]. Trees growing under these conditions must have evolved strategies for coping with such detrimental stress in order to survive. The increased concentrations in leaf UV-B absorbing compounds, mainly flavonoids and related phenolics, may help protect and filter plants against the enhanced UV-B levels during the growing season. Research has shown that flavonoids and related phenolics play an important role in plant defense against UV-B radiation [3].

Nuttall oak (*Quercus nuttallii*) is native to Southern USA as one of the best oak tree species for adaptability to a wide range of environmental stresses. The aim of this study is to introduce the scientific community to some useful microscopic techniques using the Leica DMI6000B Inverted Microscope to locate UV absorbing compounds in tree leaves, using Nuttall oak as an example. Over the years, light optical microscopes have refined their technology, offering noninvasive alternatives with high resolution power images that can be used to locate fluorescence compounds instead of the expensive confocal microscopes [4, 5].

Leaves was collected from the sun portions, at the terminal 10-50 cm of a branch, of five individual Nuttall oak trees in spring (April) 2009 on Southern University and A&M College campus in Baton Rouge, LA, USA. The fresh leaves were sectioned into approximately 0.5mm x 0.5 mm squares pieces using a sharp razor and embedded on a specimen disc using OTC-compound (Jung Tissue Freezing Medium, Leica Microsystems, Nussloch, Germany) and placed in the chamber of a cryostat (Leica CM-1850 UV Microtome, Nussloch, Germany) for sectioning ranging from 30-40 µm thickness. Rapid freezing at various low temperatures ranging from -20°C to -22°C was utilized depending on leaf moisture content. One drop of 0.2% Naturstoffreagenz-A (NA) (from Sigma Aldrich, St. Louis, MO) [0.2%, w/v, 0.2 g amino ethyl diphenyl boric acid in 100 ml distilled water stock solution] was added to a slide that was covered with a glass cover slip. Images were taken by monochrome and color cameras using the green fluorescence protein (GFP) cube (Leica DMI6000 B Inverted Research Automated Robotic Fluorescent Imaging Microscope, Cambridge, United Kingdom).

Naturstoffreagenz-A (NA) is a well-known reagent used in paper and thin layer chromatography to visualize flavonoid compounds where secondary fluorescence is induced. The reagent can also be applied in histochemistry for detection of flavonoid compounds. In this study, the NA-stained Nuttall oak leaves showed that the wall-bound UV absorbing compounds were rendered very bright and visible under both monochrome and color cameras (Figs. 1-2). The UV absorbing compounds were present primarily in upper and lower leaf epidermal layers of leaf cross-sections (Fig. 1a, Fig. 2a) and main vein petioles (Fig.

1b, Fig. 2b), as well as in vascular bundles and sparsely in the palisade tissues in leaves. The strategic locations of UV absorbing compounds could play an important role in screening out harmful solar UV-B radiation and enable Nuttall oak leaves to be tolerant to UV radiation. [6]

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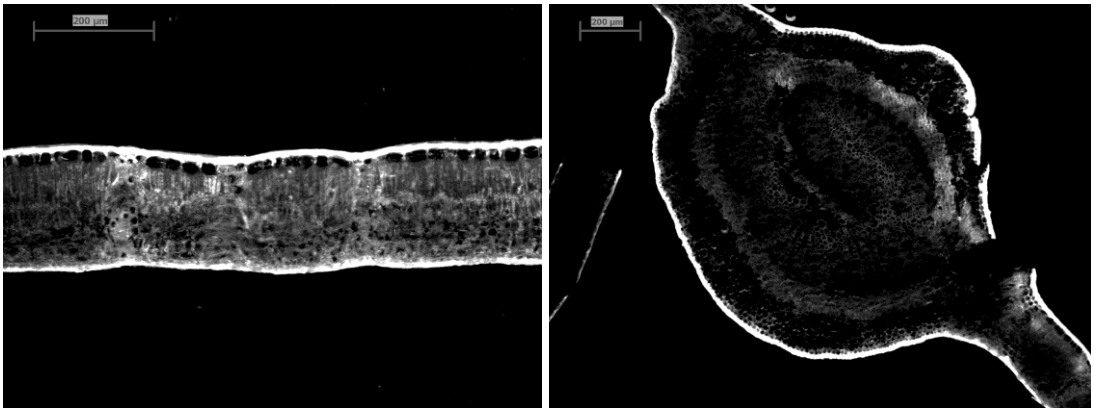
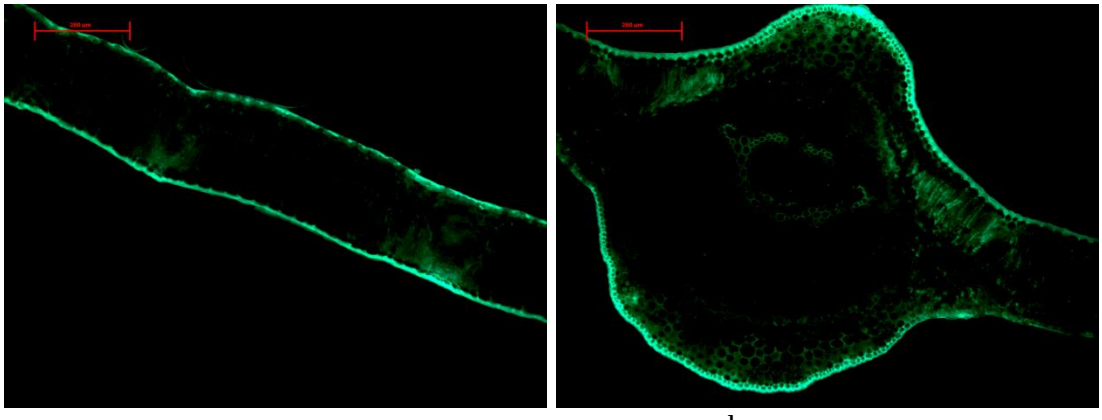


a b

Figure 1. Visualization of Leaf UV absorbing compounds in Nuttall oak leaf cross-section (a) and petiole (b) using GFP-NA-stain taken with monochrome camera.



a b

Figure 2. Visualization of Leaf UV absorbing compounds in Nuttall oak leaf cross-section (a) and petiole (b) using GFP-NA-stain taken with color camera.

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