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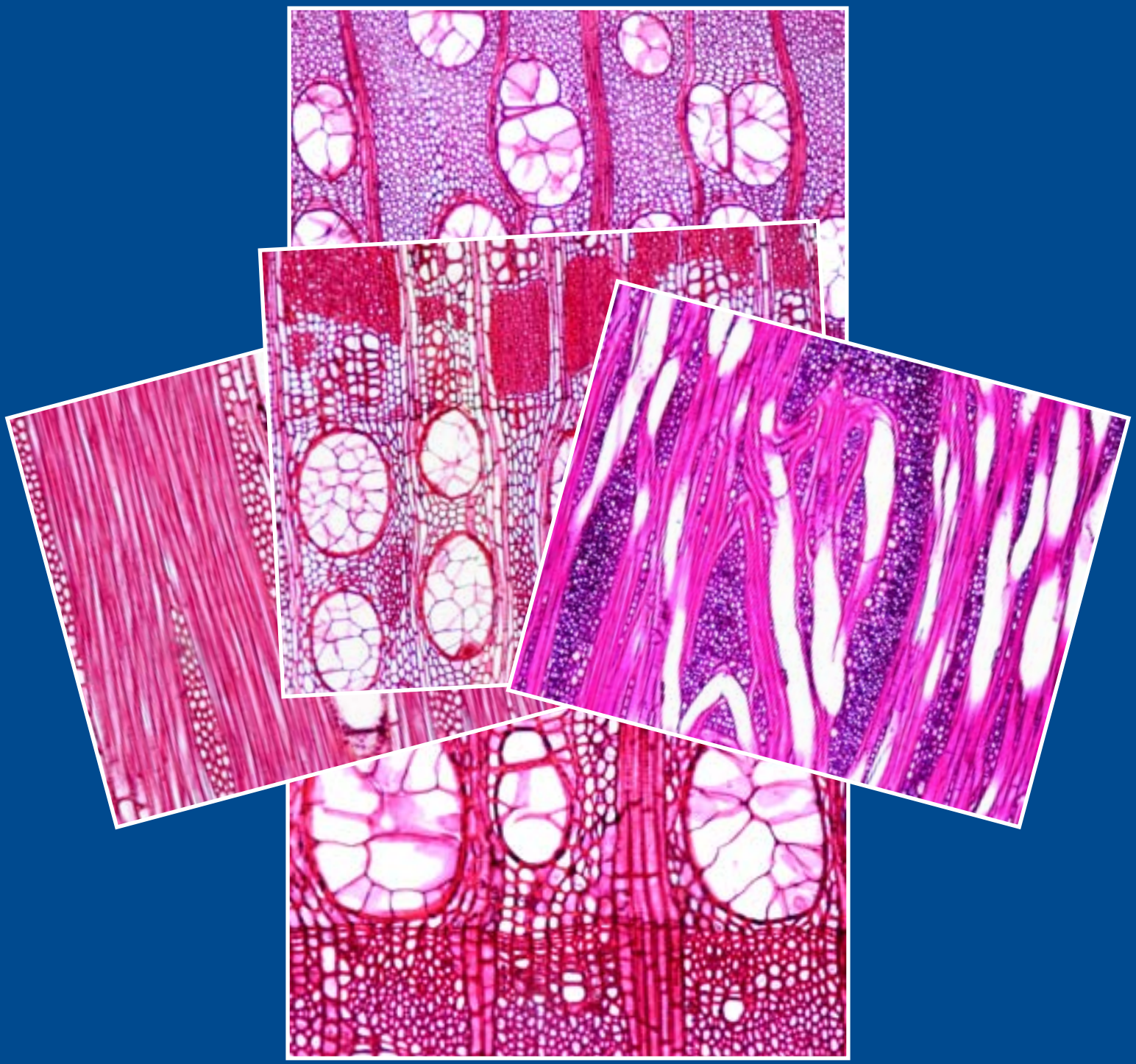

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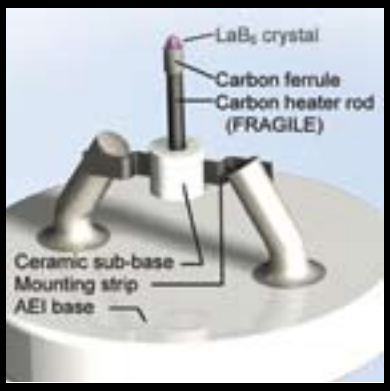

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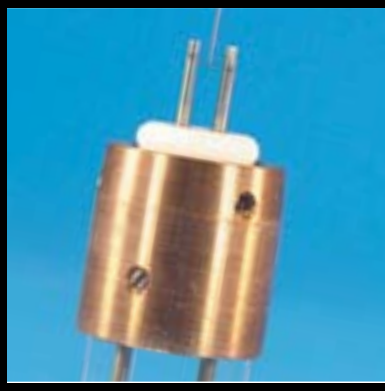

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Contents

TEXAS JOURNAL OF MICROSCOPY
VOLUME 41, NUMBER 1, 2010
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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ADVERTISER INDEX

ON THE COVER

Light micrographs of wood sections from slides of three tree species, beginning on the left, and moving clockwise: *Morus rubra, Maclura pomifera, Morus rubra, Fagus ferruginea,* and *Morus rubra*. The histological slides dated back to 1911 were prepared by William E. Blades and micrographs were taken by Howard J. Arnott.

President's Message

It has been an exciting year for the Executive Council. It is with much enthusiasm that we forge ahead making advancements in our approach to the TSM strategy. Since the meeting in April of 2009, your Executive Council has been hard at work planning the 2010 Meeting.

TSM's mission statement was our guidance in our planning - "The purpose of this Society is to further the use, understanding and knowledge of all aspects of microscopy and their applications as they apply to life sciences, materials sciences and industry."

For the 2010 meeting, the Executive Council has placed a focus on furthering the understanding and knowledge of microscopy in our youth. It was decided at the 2009 Executive Council meeting to create a new position on our council titled Youth Ambassador. The goal was to pull in our youth and focus on the education of our future. To accomplish this goal, TSM has partnered with Marine Reef International, Hitachi and Raytheon to create a workshop involving CSI type hands-on activities utilizing a Table Top SEM and Raytheon's "Math Move U" initiative. At the workshop, we will have Lynn Mortensen, Raytheon, NCS Engineering Regional Director as our guest speaker. In addition, the students will attend the vendor reception where area universities will be present to provide degree information.

At the 2010 meeting, we will have two guest speakers. Lee Hughes is an assistant professor in the Department of Biological Sciences at the University of North Texas. Lee will speak about "Freshman Phage Hunters: Integrating a Research-based Experience into Freshman Biology for Majors". Our second speaker will be Dr. Puligandla Viswanadham who recently retired Principal Scientist from Nokia Research Center in Irving, Texas. His talk will be "Failure Analysis Strategies in Electronics Industry - Past, Present and Future".

As always, I would like to extend a Thank You to our Corporate Sponsors. Your dedication to our society contributes to our success. It is through you that we gain our insight into new technologies and their applications. You have been the backbone to the society and for that we are very grateful.

I would like to take a moment to say THANK YOU to the Executive Council. Without their limitless efforts and energy, we would not have been able to position ourselves for a successful meeting or make progress in growing our society as we have done over the past year. Thank you to David Garrett, our Program Chair. He has done an excellent job working with the Hilton Garden Inn and creating a memorable program. Thank you to Robert Champaign, our Youth Ambassador for his efforts on putting together the Student Microscopy workshop. Thank you to Jo Taylor our President Elect. Jo has been a very valuable resource with her knowledge of the Society and enthusiasm to contribute to change. Thank you Nabarun Ghosh our Past President. Your guidance and experience has been valuable. Thank you to Mike Pendleton, our Secretary, for your efforts in updating our member list, taking care of the Secretarial duties, organizing the elections with Nabarun and always volunteering to take on more work when needed. Thank you to Alice Stacey our Treasurer for maintaining the budget. Thank you to Camelia Maier our Journal Editor. As always, she has shared her talent with us and put together a very high quality journal. Thank you to Becky Holdford, our Web Master, for maintaining our website. Thank you to Jennie Wojtaszek, our Student Representative. I am very appreciative of her efforts made towards reaching out to our student population and volunteering to take on more when needed. Thank you to Linda Dailey our Corporate Representative and Kevin Cronyn. Your willingness to create the CSI microscopy workshop has had an impact on the Society.

A special Thank You to Marine Reef International, Hitachi and Raytheon for their contributions to the student workshop activities. I know this workshop will impact the students and open the door of possibility for future events. Also, thank you to our vendors that have supplied giveaways for the students attending the workshop.

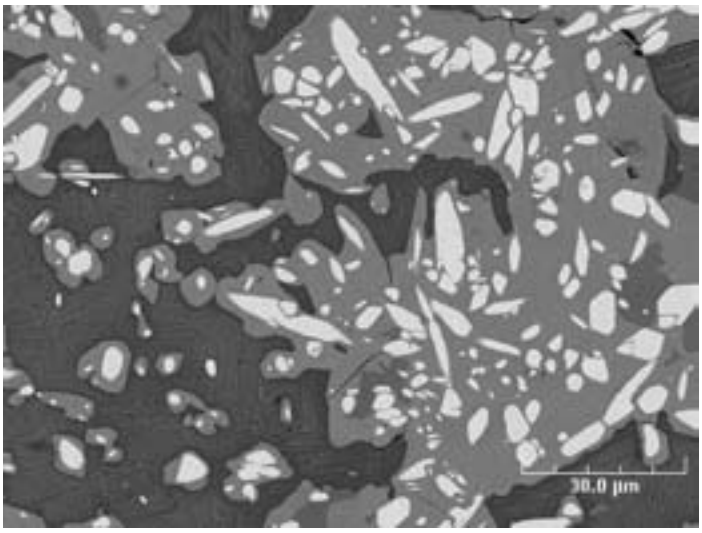
It has been a great honor to serve you and the Society in the capacity of the TSM President. I am very excited about the future of TSM and look forward to the years to come.

Jodi Roepsch
2009-2010 TSM President

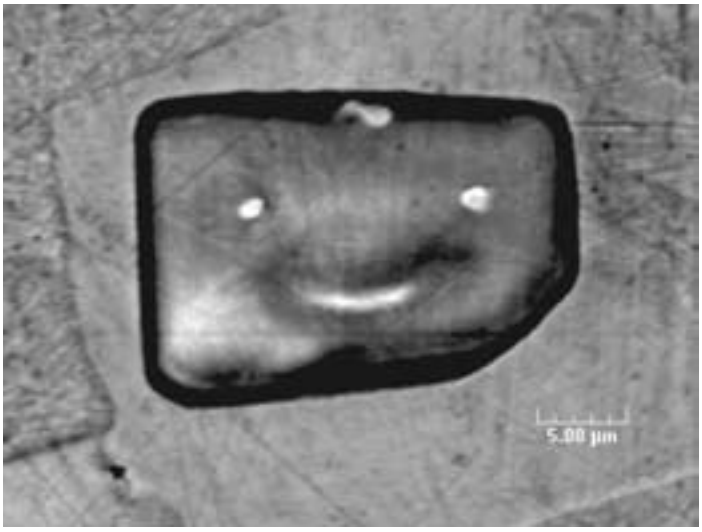
Answer To "What Is It?"

FROM JOURNAL 40:1.

Micrographs submitted by **Paul Mews**, staff member,
Nuclear Engineering Department,
Texas A&M University, College Station, Texas 77843



Backscatter scanning electron micrograph of 76% Zr - 13% Re - 11% Fe metal alloy (by weight). The light white islands are a rhenium rich intermetallic phase. The two medium phases (medium gray scale) are Fe-Zr-Re intermetallic compounds of lesser rhenium concentration, and the darkest phase (black gray scale) is virtually pure alpha-zirconium metal. It was taken at 25 Kv, 15mm working distance with a backscatter detector on a JEOL 6400 SEM at the Microscopy & Imaging Center, TAMU, College Station, Texas.



Ytterbium crystal (the dark central area) surrounded by titanium (the lighter area). The bright areas that define the "eyes" and the "smile" and the "curly hair" are simply charging effects. Taken at 20 kV, 15mm working distance, 3300x magnification on a JEOL 6400 SEM in backscatter mode at the Microscopy & Imaging Center, TAMU, College Station, Texas.

Abstracts

BIOLOGICAL SCIENCES
SPRING 2010

LEAF SEXUAL DIMORPHISM IN DIOECIOUS MULBERRY SPECIES. CARMEN AYALA and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76201

Mulberry plants are dioecious, having the sexes separated in individual male and female trees. Previous studies in our laboratory have shown that sexual dimorphism of mulberry trees affect the development of silkworm caterpillars and their silk structure. The objective of this study is to characterize the sexual dimorphism traits of leaves, which are feed for silkworms, in order to explain the caterpillars' preference for male leaves. The morphology and anatomy of leaves, especially the presence, types, and elemental analysis (EDS) of trichomes, were studied with light and scanning electron microscopes on herbarium specimens. Fresh mulberry male and female leaves will be compared in spring. Leaf blades are broadly ovate, sometimes irregularly lobed, 10-18(-36) × 8-12(-15.5) cm, with base rounded to nearly cordate, sometimes oblique, margins serrate or crenate, apex abruptly acuminate. Leaf surfaces abaxially are sparsely to densely pubescent, and adaxially with short, hooked trichomes. Mulberry leaves possess multicellular glandular trichomes of varying sizes and shapes and unicellular non-glandular trichomes with a bulbous base and elongated tapering tip. All leaf trichomes contained silicon (Si) as shown by EDS. The trichomes on the female red mulberry (*Morus rubra* L.) leaves contained an average of 68.72% Si, along with 18.76 % K, 7.45% Ca, 4.52% Cl, 1.9 % P, and 1.9% Al. The white female mulberry (*Morus alba* L.) trichomes contained an average of 70.05% Si, 17.71% K, 6.74% Ca, 4.39% Cl, 2.13% Al, 2.05% P, and 0.15% Cu. Silicon makes the hair sturdier and sharp and may serve as a means of protection against caterpillar herbivores.

COMPARING *RHYNCHOSPORA INDIANOLENSIS* (SMALL) AND *RHYNCHOSPORA SCUTELLATA* (GRISEB.) ACHENE MORPHOLOGY USING SCANNING ELECTRON MICROSCOPY. STEPHANIE CAMPOS (Eastfield College) and DALE KRUSE (Texas A&M University)

A current taxonomical debate exists over the species *Rhynchospora scutellata* of the sedge *(Cyperaceae)* family. Past studies suggest a variety of ways to taxonomically group *R. scutellata*, with some researchers treating as many as four species as conspecific. Achene morphology has been used in several past studies to differentiate between two species. This preliminary study utilized scanning electron microscopy to examine sedge achenes of two of the controversial species, *R. indianolensis* and *R. scutellata,* in order to determine whether they are actually the same species. Several dimensions of achenes were measured and compared. The measurement data shows that the two species demonstrate distinct traits of separate species. However, due to only slight variation in measurements an expanded study with a greater number of specimens is necessary to better understand the taxonomy of *R. indianolensis* and *R. scutellata*.

LEAF STOMATA AS BIOINDICATORS OF GLOBAL WARMING. NATALIE CERVANTES and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76201

Global warming is a current environmental issue that is under scrutiny by scientists, politicians, environmentalists, and general population. Recent studies have indicated that stomatal densities change in response to changing atmospheric CO_2 levels, a greenhouse gas. The goal of this project is to study the effect of global warming on the leaf morpho-anatomical characteristics of two native plants, Eyebane (*Chamaesyce nutans*, *Euphorbiaceae*) and Texas bluebonnet (*Lupinus texensis*, *Fabaceae*). Specimens in the TWU herbarium from the 1920s will be compared to current field specimens from same locations. The number and size of stomata and other epidermal cells were determined from scanning electron micrographs of herbarium specimens. Stomata indexes will be compared between old herbarium specimens and current field specimens. It is expected that 2010 field specimens of both plant species have lower stomata densities compared to 1920 herbarium specimens indicating an increase of CO_2 concentration in north-central Texas over the last 90 years. The number of epidermal cells and stomata of Eyebane were determined on leaf herbarium specimens at 600X on a surface of 0.435 um^2. The length and width of twenty-five epidermal cells and twenty-five stomata complexes per leaf were measured and recorded for Eyebane herbarium specimens. This in-progress study will provide data on morpho-anatomical changes in two Texas native plants due to global warming.

LOSS OF GERM CELLS DUE TO ANDROGEN WITHDRAWAL IN ADULT RAT TESTES. DIBYENDU DUTTA, IN PARK, HIWOT GUILILAT, SAMUEL SANG, NATHANIEL MILLS, Dept. of Biology, Texas Woman's University, Denton, TX 76204

Spermatogenesis is maintained by testosterone (T), the androgen produced from mature Leydig cells. Ethylene dimethane sulfonate (EDS) is an alkylating agent that selectively kills adult Leydig cells, thus creating an environment devoided of testosterone inside testes. In the absence of testosterone germ cell loss is a well-documented phenomenon. However the molecular mechanism(s) for this germ cell loss is (are) not well understood. Adult male rats were injected

with EDS (75 mg/Kg body weight) to kill the Leydig cells and tissues were collected 5 and 7 days post-EDS treatment. To confirm that the results were due to the depletion of T only, a T replacement group after EDS treatment was also studied. For comparison purposes various controls such as (1) untreated controls, (2) vehicle control, and (3) T only groups were used. Serum T level in EDS treated rats was below the level of detection by radioimmunoassay. However, luteinizing hormone receptor (LHR) mRNA as quantified by RT-PCR and real time qPCR revealed that an average of 90% Leydig cells were depleted after EDS treatment in both EDS and EDS+T treatment groups. Histological evaluation confirmed the loss of Leydig cells from the interstitium in EDS treated rat testes. Germ cell loss was due to both apoptosis and premature release from testes to epididymis. The number of germ cells undergoing apoptosis was quantified by **T**erminal deoxynucleotidyl transferase d**U**TP **N**ick **E**nd **L**abeling (TUNEL) assay. Histological evaluation of EDS-treated rats' epididymis confirmed that round spermatids, before differentiating to mature spermatozoa had moved out of testes into epididymis. Such migration of immature germ cells could be attributed to the disruption of blood-testes barrier (BTB) generated by Sertoli cell tight junctions. Further evaluation of BTB mRNA and membrane proteins of round spermatids and Sertoli cells are under investigation. It can be concluded that germ cell loss in testes due to T withdrawal is both due to apoptosis of germ cells and flushing out of immature germ cells into epididymis. Supported by Texas Woman's University 2008-2009 Research Enhancement Program.

LOCALIZATION OF OXIDATIVE AND NITROSATIVE STRESS IN PULMONARY HYPERTENSION IN LUNGS OF BROILERS (*Gallus domesticus*). E. ANN ELLIS[1], JAIME BAUTISTA-ORTEGA[2] and C. A. RUIZ-FERIA[2], [1]Microscopy and Imaging Center and [2]Dept. of Poultry Science, Texas A&M University, College Station, TX 77843.

Pulmonary hypertension occurs in young, rapidly growing broilers and can be exacerbated by hypoxia. The broiler has been bred to convert feed to muscle at a high rate and these birds develop some of the same cardiovascular problems as obese patients. Chronic pulmonary hypertension results in right ventricular heart failure and cardiovascular stress in the lungs. Elevated levels of nitric oxide and superoxide reduce the availability of nitric oxide and contribute to the cardiovascular pathology. Lungs of broilers were fixed by perfusion through the heart, followed by cytochemical localization of NADH oxidase, a biomarker for superoxide, and immunocytochemical localization of nitrotyrosine, a biomarker for peroxynitrite and reduced availability of nitric oxide. NADH oxidase localized in vascular endothelial cells and in parabronchial epithelial cells. Nitrotyrosine localized in the same areas. There was a 1.3 fold increase of NADH oxidase and a 1.6 fold increase in nitrotyrosine in hypoxic birds as compared to normoxic birds. These studies indicate increased oxidative and nitrosative stress which results in reduced availability of nitric oxide in pulmonary hypertension.

IDENTIFICATION AND ANATOMICAL CHARACTERIZATION OF SOME MEDICINAL PLANTS USING DIGITAL MICROSCOPY. NABARUN GHOSH[1], YASEMIN CELIK[1], CHRISTINE PESINA[1], CHRISTIAN D. RIDNER[1] and DON W. SMITH[2], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016. [2]Department of Biological Sciences, University of North Texas, Denton TX 76203.

Medicinal plants represent the local heritage and assets for a country. They are being indexed, characterized and tested for medicinal values. We studied transverse sections of the leaves from *Adhatoda vasica* Nees, stems from *Alstonia scholaris* L. R. Br., *Ephedra sinica* Stapf., and the fine sections of rhizome from *Zingiber officinale* Rosc. We prepared slides from hand cut transverse sections of the stems and leaves after double staining with Safranin (50%) and Light Green (90%) following differential staining technique with alcoholic dehydration. The sections were mounted in Euparal. The characteristic vascular bundle and distribution of tissues helped us identify and standardize anatomical features. The methanolic and ethanolic extracts of tannins and flavonoids obtained from the *Adhatoda vasica* leaves have the potential to treat microbial induced ailments or diseased conditions. It is used to treat cough, colds and asthma. The leaf anatomy shows dorsiventral structures, wavy epidermis with thin cuticle. The epidermis bears scattered 1-3 celled, warty, conical covering trichomes and small sessile quadricellular trichomes. The cylindrical cystoliths, two layers of palisade cells and characteristic wings on two sides can be used for identification. *Alstonia scholaris* bark contains alkaloids, ditamine, echitenine and echitamine and is used as an alternative to quinine. The stem contains characteristic wavy vascular bundles stained dark red with safranin composed of xylems of wide diameters. *Ephedra sinica* has been used to treat respiratory infections. Both the ephedrine and pseudo-ephedrine stimulate the sympathetic nervous system. A solid nodal girdle formed of compact vessels with great density. Ginger, *Zingiber officinale* cures upset stomachs, diarrhea, and nausea. Active components of ginger are volatile oils and pungent phenol compounds, gingerol and shogaol. We found the characteristic non-lignified, spiral or reticulate vessels, long brown pigment cells, fibers, starchy parenchyma and yellow oleoresins. We captured digital images using a BX-40 Olympus microscope attached to a DP-70 digital camera and image pro 6.0 software. The specific anatomical characteristics help to detect the adulterants of the medicinal plants species.

STUDY OF ARTHROPOD ADAPTATIONS USING THE SCANNING ELECTRON MICROSCOPE TO ENHANCE HIGH SCHOOL LEARNING. K.M. GRACE, S.L. WESTMORLAND, Biology Program Texas Woman's University, Denton, TX 76207.

This study focuses on the use of the scanning electron microscope as a learning tool for high school students, using Arthropods as the subject of study. I collected an array of arthropods and then picked species to examine closely under the microscope. The species chosen are common in everyday life and are diverse from each other. Through the electron microscope I explored and compared the different

arthropod species focusing on their adaptations. The adaptations that I focused on were: mouthparts, wings (or lack of), the eyes, and legs. I found that while some arthropods were similar in areas they were different others. Use of the scanning electron microscope in high school gives students a chance to manipulate and explore on their own, which is important in inquiry science.

AN SEM STUDY OF RUST ON SAW GREENBRIER. PAUL M. GRAY and JOSEPHINE TAYLOR, Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

The rust fungus *Puccinia smilacis* was identified from leaves of saw greenbrier (*Smilax bona-nox*) exhibiting chlorotic lesions and marginal necrosis. Symptomatic tissue was prepared for scanning electron microscopy. Sporulating uredia were erumpent on the abaxial leaf surface, with significant damage to the host epidermis and cuticle. Telia were not observed. Uredospores were collected and inoculated onto healthy leaves to monitor spore germination and the infection process. Germ tubes emerged within 24 hours, were typically unbranched, and terminated in appressoria that formed over host stomatal openings. Studies of post-penetration development and the role of the alternate host (*Apocynum* L.) in early infection and disease severity are ongoing.

INVESTIGATION OF MORPHOLOGICAL DIFFERENCES LEADING TO RUST RESISTANCE IN INDIANGRASS. PAUL M. GRAY and JOSEPHINE TAYLOR, Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

The rust fungus *Puccinia virgata* is an obligate parasite of *Sorghastrum nutans* (Indiangrass). Uredospores of *P. virgata* initiate infection via the formation of germ tubes that terminate in penetration structures, appressoria, over host stomatal openings. Previous research has shown that different varieties of Indiangrass have varying levels of resistance to *P. virgata* that, at least in part, may be due to leaf morphological features. Scanning electron microscopy was used to characterize the morphological features of three Indiangrass genotypes with varying degrees of rust resistance. Stomatal distribution, subsidiary cell spacing, and intercostal cell lengths were compared using the abaxial leaf surfaces from replicate plants in a growth chamber study. The susceptible variety Rumsey was characterized as having short intercostal cells that formed prominent papillae and wide spacing between subsidiary cells. Intercostal cells were significantly longer and subsidiary cells were more closely spaced in the resistant variety Osage. The correlation of this more topographically uniform leaf surface with a reduction in the number of uredospore germ tubes that successfully form appressoria over host stomata represents a morphological feature to be selected for in breeding programs to improve rust resistance.

ELEMENTAL ANALYSIS OF PAINT CHIPS: AN OPEN INQUIRY INVESTIGATION BY A VETERAN HIGH SCHOOL CHEMISTRY TEACHER. H.R. HATTORI and S.L. WESTMORELAND, Department of Biology Texas Woman's University, Denton, TX, 76204.

During an open inquiry experience in electron microscopy, paint chips found in public places were collected and analyzed for the presence of heavy metals using the x-ray diffraction unit of a Hitachi-1000 SEM. The goal of this work in progress is to detect potentially harmful heavy metals such as lead and bismuth, as well as health risks for general public and the environment. Paint samples examined included outdoor curb paint, outdoor playground paint, interior wall paint, and paints used in art classes. At the time of submission, no heavy metals had been identified in the samples. All samples tested contained aluminum and silicon. Being faced with years of being in the classroom trying to figure out what works best with students made facing an instrument that only does exactly what you ask it to do through programming an interesting contrast. Textbooks and technical manuals did not adequately prepare the investigator for the microscopy experience. Challenges included examining different objects to select a research topic of an appropriate level for the novice microscopist and dealing with the unexpected questions that arose along the journey. Questions encountered included "Can you see the interface between two layers of different types of paint?"; "When using the x-ray diffraction unit, should the sample be placed on an angle stub instead of a flat stub?" and "Is it possible to destroy the paint sample during the analysis?" Based on this experience I would feel comfortable using an electron microscope with my high school students in a similar manner provided that I had created a detailed reference handout outlining the steps to use the instrument and that adequate access to an electron microscope was available.

ELECTRON MICROSCOPE AS AN INVESTIGATIVE TOOL TO STUDY HUMAN HAIR MORPHOLOGY. MAMTA KUMAR AND DR.WESTMORELAND. Biology Department, Texas Woman's University, Denton, TX 76204

This research paper is my reflection on the inquiry method of learning science using electron microscopy as the investigation tool. This study is an attempt to find the effect of various chemicals components of hair care products on morphology of hair. Hair shaft consists of outer lipid epicuticle and a layer of flattened cuticle cells surrounding the elongated cortical cells. Chemicals in hair products cause progressive degeneration of cuticle cells in the form of longitudinal fissures known as split ends. The hypothesis tested in this experiment is that chemicals such as hydrogen peroxide, lemon juice, and alcohol alter hair morphology. As the control for this study, untreated scalp hair of a 4-year-old girl was used. Hair samples from the same source were treated with chemicals for the same duration of time. The hair micrographs from this study will be compared with published data to test the validity of the hypothesis.

A COMPARISON OF ADAPTATIONS OF TRICHOMES AND STOMATA IN MESOPHYTES AND XEROPHYTES. E.L. LAMBERT, C. MAIER, AND S. L. WESTMORELAND. Department of Biology, Texas Woman's University, Denton, Texas, 76204.

Plants have developed adaptations in response to temperature and availability of water in their environments and are classified as xerophytes, mesophytes, or hydrophytes based on these adaptations. This study focused on the adaptations of xerophyte plants of North Texas compared to those of mesophyte tropical plants. This investigation used scanning electron microscopy to compare the density of stomata and trichomes and their locations on the leaves of study plants. The working hypothesis was that plants native to Texas have fewer stomata and more dense trichomes, and that the location of stomata and trichomes will vary when compared to tropical plants. The preliminary results showed that xerophytes have fewer stomata, more dense trichomes, and strategically located stomata in response to high temperature averages and limited water availability in North Texas.

MINERAL DEPOSITS IN DIOECIOUS MULBERRY LEAVES. ARTHUR MITI and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76201

Calcium oxalate (CaOx), calcium carbonate ($CaCO_3$) and silicon (Si) deposits play important roles in plants. Mulberry, *Morus* sp. (*Moraceae*) contain all the mineral deposit types mentioned above. Previous studies in our laboratory provided data on the presence of Si cystoliths in mulberry leaves that were not reported before. The goal of this project is to determine possible sexual dimorphism in the disposition and abundance of Si cystoliths in order to learn more about the role of Si deposits in mulberry. A correlation between the abundance of $CaCO_3$ and CaOx deposits in dioecious mulberry and herbivory intensity was found in our laboratory by employing the mulberry-silkworm system. Silkworms preferred mulberry male leaves to female leaves, which contain higher concentrations of Ca deposits. Silicon is an abundant soil component and plants absorb it through the roots along with water and other minerals. Silicon deposits are mostly found in monocotyledons, especially grasses and less in dicotyledons, such as mulberry species. Mulberry leaf mineral deposits were extracted in ethanol and studied with a Hitachi TM-1000 SEM equipped with EDS.

STUDIES ON THE PHYTOREMEDIATION PROPERTIES *EVAX VERNA* (*ASTERACEAE*). CHINWE ORABUCHI and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76201

Contamination of the environment by toxic metals poses a threat for the health of humans and ecosystems. Phytoremediation is an innovative 'green' technology that utilizes the natural absorbing properties of plants to remediate soils in agriculture and hazardous waste sites. The goal of this project is to determine the phytoremediation properties of a Texas native plant, Rabbit's tobacco or Cotton rose (*Evax verna*, *Asteraceae*) through elemental analyses (EDS). Also called Spring pygmycudweed, *E. verna* is a small annual plant, usually less than 10 cm tall, gray-pubescent with wooly hairs. Heads are sessile in dense small clusters, inconspicuous and wooly-pubescent. Plants were collected from a prairie outside Denton at the time of flowering in spring of 2009 and 2010. All plant organs studied (roots, leaves and flower) showed accumulation of Al in their tissues. EDS of soil particles around the plant roots also showed high concentration of Al. This study has a practical significance in that *E. verna* could be used in the phytoremediation of heavy-metal contaminated mine and agriculture soils.

IRON CORROSION PRODUCTS IN WOOD RECOVERED FROM A HISTORIC SHIPWRECK CHARACTERIZED BY SCANNING ELECTRON MICROSCOPY. M. W. PENDLETON*, G. FOX**, E. A. ELLIS*, and B. B. PENDLETON***. *Microscopy and Imaging Center, Texas A&M University, College Station, TX. 77843-2257, **Museum of Anthropology, Department of Anthropology, California State University, Chico, Chico, CA. 95929-0400, ***Department of Agricultural Sciences, West Texas A&M University, Canyon, TX. 79016-0001.

The whaling vessel Parker was wrecked in 1842 on the Kure Atoll reef, part of the Northwestern Hawaiian Islands Marine National Monument. Among the scatter of artifacts recovered from the wreck site in 2005 was a copper ship's bell and the wood and iron yoke which supported it. Scanning electron microscopy (SEM) was utilized to examine the wood samples of the Parker bell yoke. Samples were prepared for SEM by fixing with 2.5% (vol./vol.) acrolein in 0.1 M HEPES buffer (pH 7.4), washed in buffer and dehydrated in methanol into hexamethyldisilazane. The dry samples were vapor coated with rhuthenium tetroxide [1] prior to secondary and backscatter SEM imaging using a JEOL JSM 6400 SEM (30 kV accelerating voltage and 15 mm working distance). Crystals were observed on the outer surfaces of the yoke wood samples. During crystal observation, an Energy Dispersive Spectrometry (EDS) detector (Princeton Gamma-Tech (Bruker AXS, Inc., Madison, WI), with a takeoff angle of 31.0 degrees, count rates of 300-500 cps for 100 s and an estimated probe current of 5.0 nA), was used to determine that these crystals contained iron, probably as a corrosion product. The intersecting growth pattern of the iron crystals effectively obscured much of the detail of the yoke wood.

Artifacts described in this abstract are being analyzed at the Heritage Resources Conservation Laboratory, Department of Anthropology, California State University, Chico. This analysis is funded by a federal contract through the National Oceanic and Atmospheric Administration.

[1] A. E. Ellis and M. W. Pendleton. Microscopy Today 15 (2007) 44.

(Continued on Page 14)

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CONSTRUCTION OF NON-PRENYLATABLE MUTANTS TO ASSESS THE ROLE OF RHO GTPASE GERANYLGERANYLATION IN NEURITE OUTGROWTH. J. REDDY, F. SAMUEL, and D.L. HYNDS, Department of Biology, Texas Woman;s University, Denton, Texas 76204-5799

The importance of the RhoA and Rac1 for actin polymerization is well known and documented. A commonly accepted idea is that Rho GTPases must be prenylated to be translocated to the plasma membrane and be made active through GTP loading. We and others in the field have discovered GTP-bound RhoA and Rac1 in the cytosol suggesting this dogma to be untrue. We have tested wild type RhoA and Rac1 on their effects on prenylation in activation and will construct non-prenylatable RhoA and Rac1 mutants to assess the role of prenylation in activation and function of these GTPases using site directed mutagenesis to introduce a single amino acid change, causing the GTPases to be unprenylatable. We will then assess the role of GTPase prenylation in activation and subsequent neurite outgrowth using pull down assays and image analysis. We expect that non-prenylatable Rac1 will decrease neurite outgrowth and non-prenylatable RhoA will increase neurite outgrowth. The results will better define the role of prenylation in Rho GTPase function.

EXTRACELLULAR MATRICES THAT ACTIVATE b1 INTEGRINS FACILITATE RAC1 ACTIVATION AND PROMOTE AXON EXTENSION. K.L. THOMPSON[1], D.L. HYNDS[1], P. MCFETRIDGE[2], [1]Department of Biology, Texas Woman's University, Denton, Texas and [2]School of Chemical Engineering and Materials Science, University of Oklahoma, Norman, Oklahoma.

Axon growth depends on actin rearrangements determined, in part, by adhesion dynamics of growth cones. In non-neuronal cells, extracellular matrix adhesion and integrin activation result in Rac1 activation, which is associated with axon extension. We hypothesized that b1-integrin activation, via adhesion to collagen or laminin, increases neurite outgrowth through membrane localization and activation of Rac1. Time-lapse analyses were used to evaluate extracellular matrix promotion of axon growth in dorsal root ganglion (DRG) neurons and B35 neuroblastoma cells. B35 and DRG growth cones advanced generally in a single direction, but with some stalling and trajectory deviations. We also investigated the distribution of the adhesion protein, paxillin, and observed punctate immunoreactivity throughout growth cone lamellipodia and filopodia. Since membrane localization is thought to activate Rac1, we immunochemically assessed the cellular distribution of activated Rac1. Further elucidation of signaling pathways for axon growth may lead to strategies to promote recovery after neurotraumatic injury.

WAX DEPOSITION AND THRIPS HERBIVORY IN LEEK, *ALLIUM PORRUM (ALLIACEAE).* PALLAVI UPADHYAY AND CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton Texas 76204.

Wax production on the aerial parts of terrestrial plants represents an important evolutionary adaptation to life on land, where water availability is a limiting factor. In addition, being the outermost structural layer of all aerial plant organs, epicuticular waxes (EW) form the first line of defense against insect herbivores. The goal of this work is to study the mechanisms of wax deposition in plants by employing leek, *Allium porrum* (*Alliaceae*) as a model system. EW is synthesized in the epidermal cells and its subsequent deposition on the aerial surfaces may be facilitated by its diffusion or by the action of osmiophilic particles, which may contain lipid transfer proteins. EW, generally, are derivatives of very long chain fatty acid and are embedded on epidermal surfaces of plant in form of crystalline or smooth amorphous film. SEM analysis on the leaf surface has depicted that distribution and the localization of EW is asynchronous. Damage to the leek epidermis was observed during an infestation of the leek plants by thrips (Order Thysanoptera), a piercing-sucking type of plant herbivore. Thrips display a characteristic segmented body with specialized mouth pieces that can puncture and tear the plant epidermal layer to feed on the cell content below. In doing so, they cause deformation and ultimately wilting of the leaves. Under SEM, extensive destruction of the EW layer was seen on the infested epidermis. A pattern of insect infestation was observed which was dependent on the amount of EW present on the surface of epidermis in different morphological areas of the leaf. The highest density of insects was observed on the leaf sheaths, which have just a film of amorphous EW and very few insects were found on the leaf blades, which have a higher load of crystalline EW. The infested leaf tissues also displayed stomatal complexes whose pores were clogged by epicuticular debris. These initial observations suggest an important role for EW as defense against insect herbivores.

A COMPARATIVE ANALYSIS OF DENTITION OF FOUR SNAKES FROM THE BIG THICKET NATIONAL PRESERVE; *ELAPHE OBSOLETA LINDHEIMERI, CROTALUS HORRIDUS ATRICAUDATUS, NERODIA ERYTHROGASTER FLAVIGASTER, AGKISTRODON PISCIVORUS LEUCOSTOMA.* ARACELY VAZQUEZ (Eastfield College), PAUL CRUMP (Houston Zoo) and JEFF HUGHES (Eastfield College)

This research was conducted with the intent to record the different species found at the Big Thicket National Preserve and to compare the dentition of *Elaphe obsoleta lindheimeri* (a non-venomous terrestrial constrictor), *Crotalus horridus atricaudatus* (a venomous terrestrial), *Nerodia erythrogaster flavigaster (*a non-venomous aquatic), and *Agkistrodon piscivorus leucostoma* (a venomous aquatic). The teeth of the terrestrial snakes were expected to have more curvature than those of the aquatic snakes because of the difference in the diets of each species. Comparative analysis of dentition with a scanning electron microscope indicated the amount of posterior deviation of the teeth of the terrestrial snakes versus the teeth of the aquatic snakes was not distinctly different.

ULTRASTRUCTURAL CHANGES IN *PROSOPIS PUBESCENS* DUE TO COPPER TOXICITY. [1]M.N. VIVEROS, [2]J.T. ELLZEY, [3]C. G.-A. MAIER, and [3]C. ORA-BUCHI, [1]Environmental Science and Engineering Program The University of Texas at El Paso, El Paso, TX, 79968; [2]Biological Sciences, The University of Texas at El Paso; El Paso, TX, 79968; and [3]Texas Woman's University, Denton, TX 76204.

Phytoremediation is an emerging technology for cleaning industrial and urban contamination (Peuke and Rennenberg, 2005). To remediate local soils, desert plants have been investigated for their ability to uptake heavy metals. Heavy metals may cause physiological, structural, and/or molecular effects which indicate transport, sequestration or immobilization mechanisms of those metals by the plants. *Prosopis pubescens* (Screwbean mesquite, *Fabaceae*) is a desert plant common in the area and was used in this study. The primary objectives of this project were to a) determine the threshold limits of copper tolerance of Screwbean mesquite and b) visualize the ultrastructural changes induced by copper in plant tissues. Transmission electron microscopy revealed ultrastructural changes within the mesophyll cells and the accumulation of a black precipitate within the leaf cells. Severe cellular damage is clearly seen beginning at 600ppm CuSO4 treatment of seedlings. Preliminary elemental analysis has shown the presence of copper within cotyledons of Screwbean mesquite. Copper concentrations will be determined within the plant using ICP-OES. Understanding detrimental cellular effects of copper exposure in Screwbean mesquite has important implications for predicting the future of desert phytoremediation utilizing this plant.

TEACHERS AS SCIENTISTS: USING SCANNING ELECTRON MICROSCOPY TO ENGAGE PRE-SERVICE TEACHERS IN OPEN SCIENCE INQUIRY. S.L. WESTMORELAND, K. GRACE, H. HATTORI, M. KUMAR, E. LAMBERT, E. WAND, Department of Biology, Texas Woman's University, Denton, Texas 76204-5799.

The Texas State Board for Educator Certification (SBEC) has created standards for beginning educators in entry-level positions. These standards are focused upon the Texas Essential Knowledge and Skills (TEKS), the required statewide public school curriculum, and they reflect current research on the developmental stages and needs of children from Early Childhood through Grade 12. The Science Standards for teachers in grades 4-12 include the following as Standard III: "The science teacher understands the process of scientific inquiry and its role in science instruction." (Texas State Board for Educator Certification, http://www.sbec.state.tx.us) However, an informal poll of K-12 pre-service teachers enrolled in science education courses at Texas Woman's University (TWU) indicates that most students report that they have never experienced science inquiry as a part of their education. The current study was conducted to investigate a science inquiry engagement with pre-service science teachers at TWU. The goal was to determine the impact of an "open" science inquiry experience using scanning electron microscopy (SEM) on the students' knowledge, skills, and attitudes about science inquiry. In open inquiry students state the problem, formulate the hypothesis, and develop their own working plan. Students in this study were enrolled in the Master of Art (MAT) program at TWU, which is a graduate program for students with an undergraduate degree who are pursuing certification to teach in Texas. All students in this study were planning to teach middle school or high school science. Students were taught to use the Hitachi 1000 Tabletop SEM and allowed to self-select a topic for investigation. Journals were kept by each student with a dual purpose. The journals were used in the conventional manner to record scientific data; the journals were also used for reflective writing by reversing the book and writing back to front. By reviewing the journal entries, the students' science inquiry knowledge, skills, and attitudes were tracked. Students ultimately chose a wide variety of topics for investigation, but all reported initial frustration at the lack of direction given by the instructor. All also reported a moment of insight when they located and focused on their research topic. Additional stress was reported by students as they attempted to narrow the scope of the project and design an appropriate research strategy. A preliminary conclusion from this study is that the experience of using SEM for an "open" inquiry experience will reinforce pre-service teachers' understanding of the process of scientific inquiry and make it more likely that they will engage their future students in science inquiry.

MICROSCOPIC STUDIES OF THE MUTUALISTIC RELATIONSHIP BETWEEN THE SUNFLOWER AND THE HONEY BEE. JENNIE WOJTASZEK and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76201

Honey bees (*Apis mellifera: family*) are the main pollinators of sunflowers (*Helianthus sp., Asteraceae*). The mutualistic relationship between sunflower and honey bees is reflected in the interactive adaptations of both organisms. Previously, we reported on the honey bee adaptations for recognizing rewards in the sunflower inflorescences, extracting nectar and collecting pollen. The goal of this study is to identify the morpho-anatomical characteristics of the tubulate and ligulate flowers as well as their pigment biochemistry, which are plant adaptations to attract bees. The two types of flowers in the sunflower inflorescence and their pigments are distributed in a target pattern, readily recognized by honey bees. The sterile ligulate flower at the edge of the sunflower inflorescence have conical cells in the upper epidermis that aid in reflecting light to better form the white circle of the target pattern. Fertile tubulate flowers in the middle of the sunflower head have UV reflective pigments in the epidermal cells on the five fused corolla tips serving as the black middle area of the target pattern. The fused anthers are rich in UV reflective pigments as well and have globular glandular trichomes at the tip of the anthers. These trichomes' secretions may serve a dual purpose in deterring herbivores as well as that attracting the bee pollinators. The bi-lobed stigma has conical adaxial cells and stiff hairs as part of the abaxial epidermis. The scattered pollen grains in the tubulate flowers serve as white pinpoints against the dark background of the tubulate flowers' corolla further attracting the bees to the target pattern of the inflorescence. Methanol (10%)

/formic acid (0.4%) was used to extract the floral pigments by solid phase extraction. Drops of pigment extracts were visualized with the scanning and light microscopes. Under SEM, both ligulate and tubulate pigment extracts revealed globular amorphous structures and branched crystalline structures. The light microscope images of the tubulate extracts reveal a significant number of white branched, densely populated non-polarizing crystal structures. The globules are red under light microscope. The light microscope images of the ligulate extract reveal non-polarizing crystal structures that are smaller in size and less densely populated. There are fewer yellow globules in the ligulate extracts. We assume that the white crystalline structures represent anthocyanins and the globular structures are carotenoids. Further studies will focus on identification of pigments in sunflower through chromatographic techniques.

MICROSCOPIC EVALUATION OF *PRYMNESIUM PARVUM* AND FISH KILL BRIAN YATES, WILLIAM ROGERS and NABARUN GHOSH, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016.

Prymnesium parvum is a unicellular, biflagellated haptophyte whose toxic blooms have caused major fish kills in Texas since 1985. The conditions causing mixotrophs produce toxins are unknown. Aquaria containing water from Baylor Lake, which has suffered from golden algae blooms in the past, was inoculated with atrazine at 0.1, 1, and 10 ug/L and glyphosate at 0.1, 1, 10, and 100 ug/L. Composite samples from each aquarium were observed under a light microscope and the number of each genus was recorded. Tolerance indicator values were calculated for each genus based on the prevalence at the herbicide concentration. Atrazine is a very popular agricultural herbicide widely used in Texas. It targets broadleaf weeds primarily in corn, sorghum, and sugarcane crops. It was detected in many Texas wells and water bodies. From two to four weeks after inoculation with atrazine there was a significantly higher percentage of *P. parvum* cells compared to the control. Microscopic observation, characterization of species density was determined using a BX-40 Olympus microscope attached to a DP-70 Digital Camera and Image Pro plus 6.0 software. Sedgwick-Rafter Cell counter was used to determine the concentration of algae per unit volume. Photoheterotrophs made up the majority of the atrazine and glyphosate-resistant phytoplankton. Atrazine inhibits photosynthesis by stopping electron flow during photo system II, which would eliminate the ability of obligate autotrophs to survive. Mixotrophs and photoheterotrophs survive adverse environmental conditions by depending on organic nutrients and other microbes for survival. Buffer strips between areas of atrazine application and water bodies may reduce herbicide runoff and golden algae blooms. Supported by 2010 REU summer grant and Dr. Ray Matlack.

MATERIAL SCIENCE
SPRING 2010

CHARACTERIZATION BY SCANNING ELECTRON MICROSCOPY OF CARBON STEEL EXPOSED TO CRYOGENIC TEMPERATURES. M. W. PENDLETON*, C. MAZZELLA**, D. NEWTON**, and B. B. PENDLETON*** *Microscopy and Imaging Center, Texas A&M University, College Station, TX. 77843-2257, **Wild Well Control, Inc./Blowout Tools, Inc., 2202 Oil Center Court, Houston, TX. 77073, ***Department of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016-0001.

Wild Well Control, Inc./Blowout Tools, Inc. (Wild Well/BTI) conducts safety training in safe pipe freezing techniques. Pipe freezing is a safe and reliable method of blocking a pipe for maintenance or repair used in many oilfield applications. A heat exchanger, such as a fluid tight metal jacket that surrounds the pipe to be isolated, is filled with liquid nitrogen, which cools the pipe. At the start of the freezing process, water begins to freeze inside the pipe near the heat exchanger attached to the outer pipe surface. As freezing continues, ice in the cooled area of the pipe will grow axially until a plug forms at the center line of the heat exchanger. Further cooling results in a growth of ice along the axial plane away from the centerline in both directions until a stable ice plug with concave ends is formed. During the Wild Well/BTI safety-training course, a demonstration of deliberate pipe failure is performed to illustrate the consequences of improper pipe freezing procedures. Samples of steel from pipe freezing demonstrations were fractured to determine if differences in the steel characteristics were caused by the exposure to different cryogenic temperatures. The fracture surfaces of these steel samples were compared using images produced with both secondary and backscatter detectors a JEOL JSM 6400 Scanning Electron Microscope (operated at 30 kV accelerating voltage, 15 mm working distance).

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A RETROSPECTIVE ON OLD MICROSCOPE SLIDES

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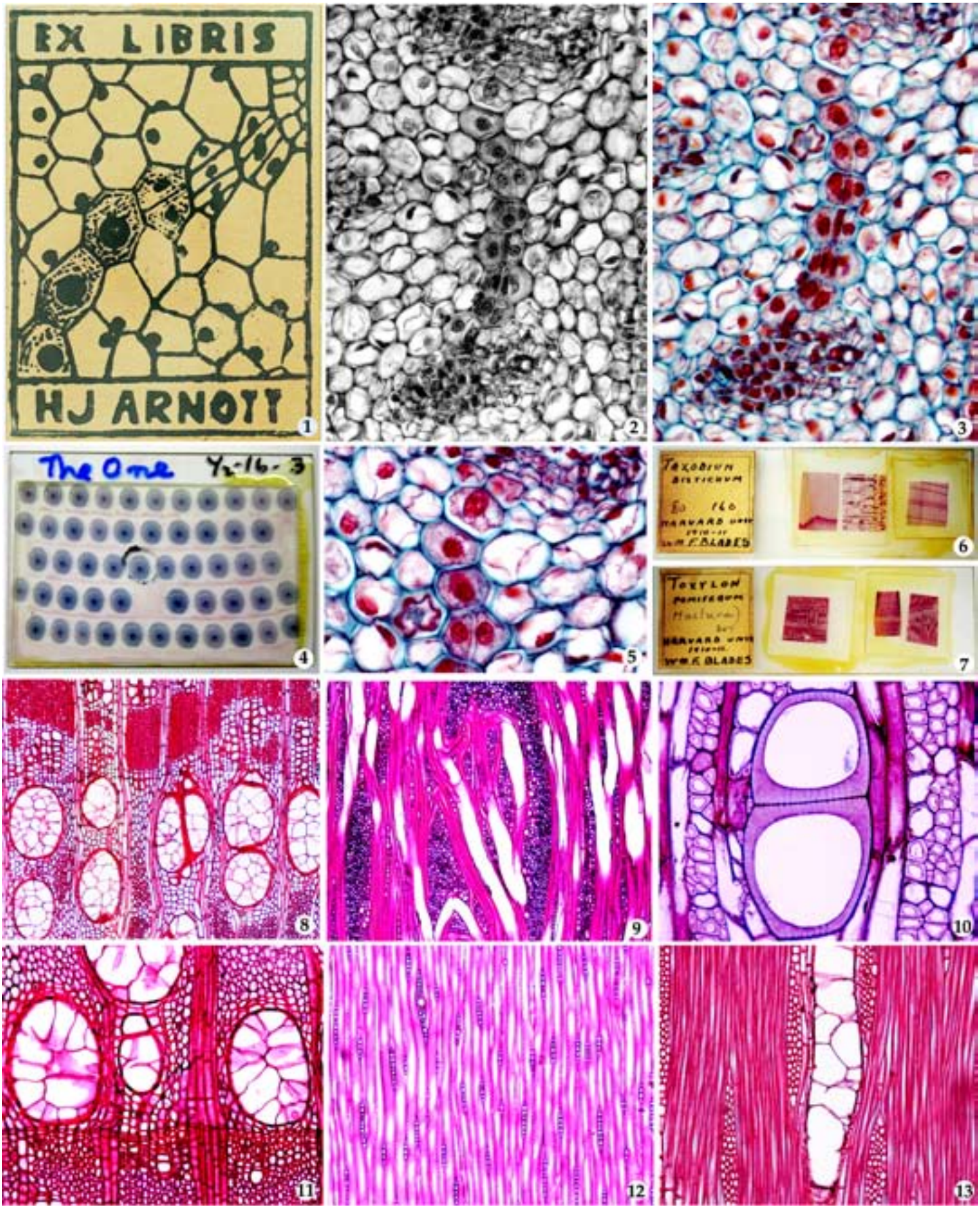
Often microscope slide preparations we examine are old, sometimes very old. As microscopy becomes less central to modern biology and the making of permanent microscope slides retreats into the attic or into oblivion, it is advantageous to examine "the utility and the staying power" of "old microscope slides." What shall or can we do with them? Are "old microscope slides preparations" worth the time and energy that would be required for their conservation? It is a difficult and personal question for me! Perhaps it is worth noting that "old slides" of various types are commonly available on Ebay and therefore have an economic or monetary value.

My latest attention to old microscope slides arose from a "house-cleaning event" in which we found a page of book plates (*Ex Libris* H J Arnott) (Fig 1). The book plates were hand printed from a linoleum block made by the author in the 1970's. The motif for the figure on the plates came from a slide preparation that I made in 1956. The section was photographed by Mr. Victor Durant at UC Berkeley Library' scientific photography division in 1958. A copy of the Durant micrograph is reproduced in Fig 2. It was used in my dissertation and later published. Arnott, Howard J. 1962. The Seed, Germination, and Seedling of *Yucca*. University of California Press. 96 p. Berkeley, CA. Discovery of the book plates sparked my curiosity as to whether I could find the original slide and section made some 54 years ago. The slide was found (Fig 4), and photographed with modern digital imaging techniques (Fig. 3). The slide was still in perfect condition having been kept in a wooden slide box as it was moved from place to place (See Texas J. Micros. 36:1, 2005). In addition to the original observations I found many additional views of commissural (cross) vein development; in fact, my brief study these old slides has led to an apprehension that my initial observations did not provide a 3-D understanding of the process. With some work and my *Yucca* seedling slides (there are several hundred similar to the one shown in **Fig. 4**) it may be possible to better understand the how commissural veins develop in three dimensions. This incident demonstrates to me that old slide preparations are still a rich source of "hidden" data.

In the last few years I have examined several cases in my own slide collection in which careful study provides new insight(s). For example I found that the flowers of *Yucca brevifolia*, *Y. whipplei* and *Y. schidigera* have open carpels, that is, pollen could reach the ovules without passing through any tissue. This has been considered a very primitive floral characteristic and thus generates an interesting conundrum in which primitive flowers are associated with obligate insect (moth) pollination generally considered an advanced character. As in the commissural vein development my old slides are still capable of generating new insights.

Over the years I have studied a series of "old wood slides" that were prepared by William E. Blades of Harvard in 1911. Even though these slides are almost a hundred years old, and even though they received very harsh treatment by some thoughtless zoologists, they are excellent examples of proper botanical microtechnique. The microtomy, staining and overall preparation is of the highest caliber; hence they are a useful today as they were when they were made. Posthumously, I have thanked Bill Blades many times. He came from an era at Harvard in which people like Dr. I. W. Bailey were in their prime. Very few can make slides of his quality. Unlike, even some of the best wood slides available in the trade they never show "tracheid tearing", splitting or other artifacts of sectioning. By a quirk of fate I was present when the slides were being discarded in 1958; they were made superfluous just so the slide boxes could be used by a thoughtless professor. I salvaged them and have used them with care in my teaching ever since. Soon I will pass them to a colleague who will also cherish them and with some luck will give students a chance to see what our predecessors like W. E. Blades could do.

Figure 1. Replica of "EX LIBRIS H J Arnott" book plate. **Fig. 2**. Commissural vein development in *Yucca whipplei* shown in a micrograph Victor Durant made in 1958. 410X. **Fig. 3**. Micrograph showing the identical region made in 2010. 450X. **Fig. 4.** The original slide made in 1956, photographed by V. Durant in 1958 and more recently by the author. 0.8X. **Fig. 5.** Portion of a commissural vein showing the sequential cell changes that lead from a ground meristem to a procambial cells. 600X. **Figs. 6-7.** Slides showing wood sections made by William E. Blades in 1911, please note the details shown on slide labels. 0.8X. **Figs 8-13**. Wood sections of five different species illustrating the quality and to some extent the range, of Blades' preparations. Micrographs of these specimens produce what can only be called "natural art"; art with the colors and textures of the finest art works. **Fig 8.** *Transverse section of the* horse apple *(Maclura pomifera*) wood. Note the large number of tyloses in the vessels. 150X. **Fig 9.** Tangential section of beech **(***Fagus ferruginea*) wood showing large multiseriate rays**.** 150X**. Fig 10.** Transverse section of persimmon (*Diospyrus virginiana)* wood. Note the pits between two vessel members. 600X**. Fig. 11.**



Transverse section of Red mulberry **(***Morus rubra)* wood with tyloses in vessels. 150X**. Fig. 12.** Tangential section of Douglas fir *(Pseudotsuga taxifolia*) wood showing uniform distribution of small rays. 150X. **Fig 13.** Tangential section of Red mulberry (*Morus rubra)* wood showing a large vessel with numerous tyloses. 300X.

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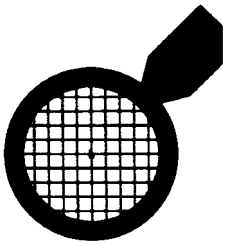

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