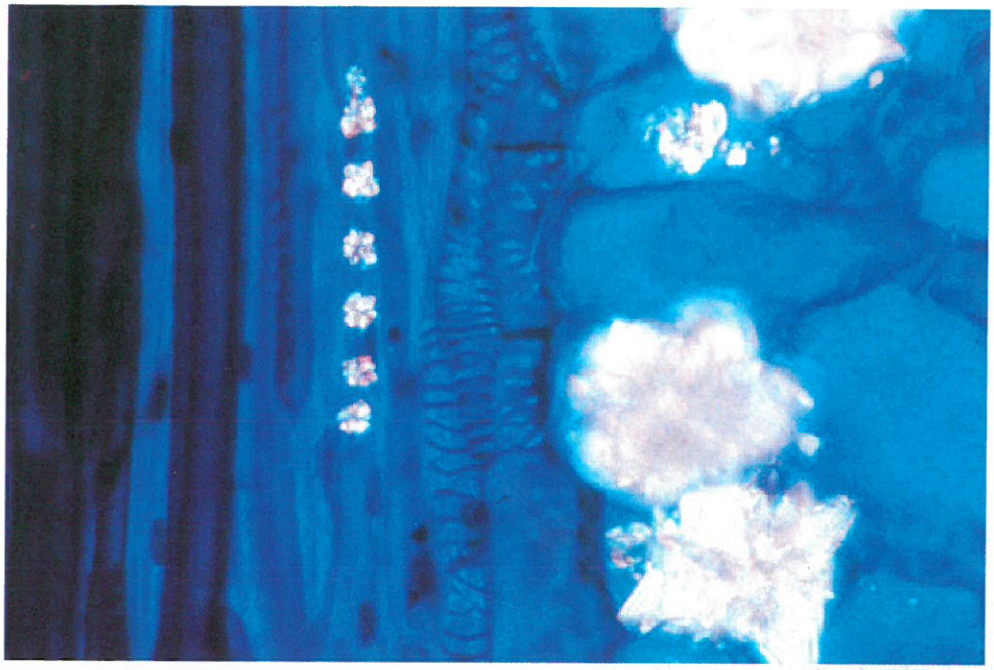

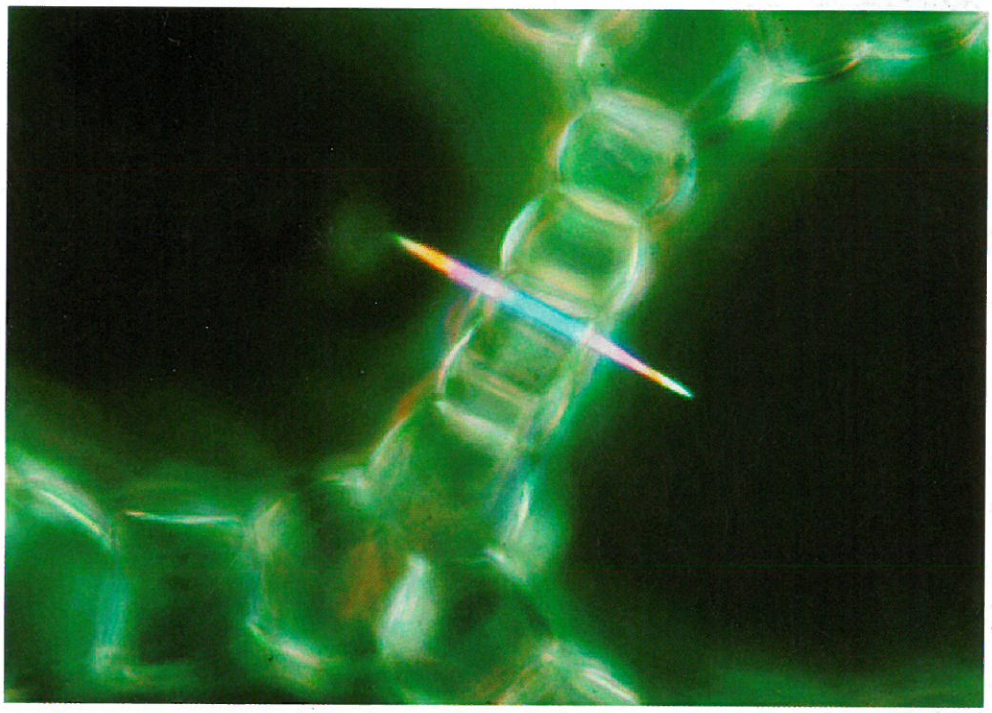

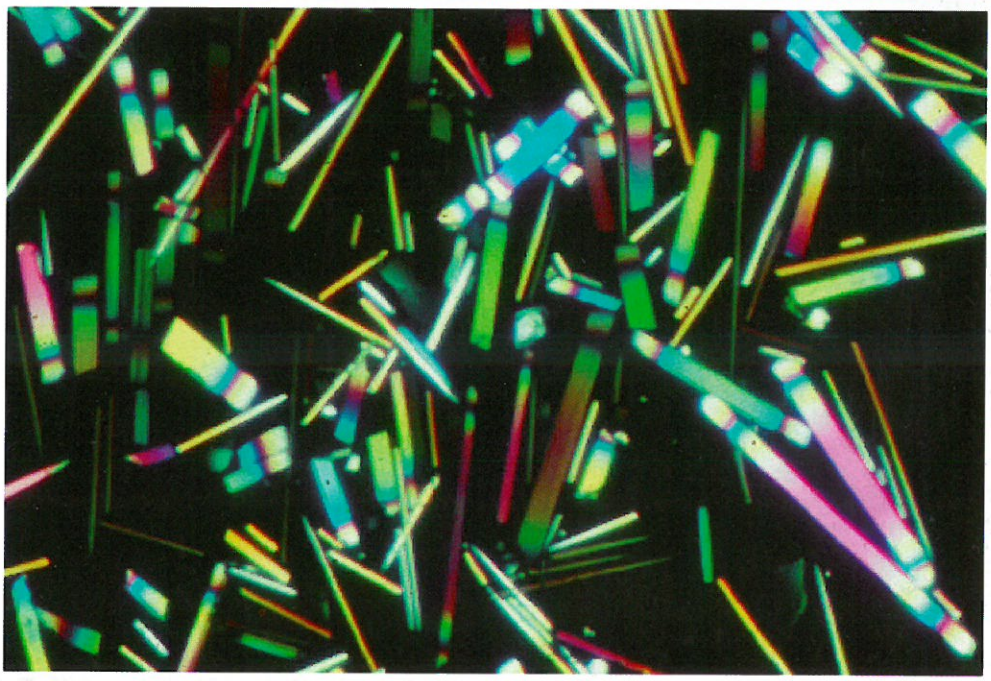

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TEXAS JOURNAL OF MICROSCOPY
VOLUME 33, NUMBER 2, 2002
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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

The three light microscopy images on the cover are illustrations of biomineralization in plants. Top picture represents a section through a Ginkgo (Ginkgo biloba) leaf showing a file of small druses as well as a few large druses in the vicinity of vascular tissue (140X). Middle picture is a light micrograph of a fresh section through water hyacinth (Eichhornia crasipes) leaf (320X). A raphide is visible under crossed polarizers sticking out in the air spaces of the aerenchyma. Bottom picture illustrates isolated raphides from water hyacinth leaf under crossed polarizers (375X). For more details on biomineralization in plants, see 'Educational Tips' on page 51. Howard J. Arnott, Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

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President's Message

The 2002-2003 year will be a great time for TSM with meetings in Austin (Fall, 2002) and Denton (Spring, 2003). Bring your families to Austin, our state capital. Jo Taylor, the Program Chairman, has gotten good rates at the Embassy Suites and worked with Graham Byrd (ASI, Inc.) on great workshops to start things off on Thursday, October 24.

The Spring 2003 meeting in Denton will be a very good opportunity to visit with our old friends from the Oklahoma Microscopy Society (OMS). We are arranging a joint meeting with OMS at Texas Women's University in Denton. Start making preparations to attend and present papers and/or posters. Encourage your students and technicians to participate as well and stay tuned to TSM's website, www.texasmicroscopy.com, for more details and updates. Yes, TSM has its very own domain name now and we have Becky Holdford, the webmaster, to thank for her hard work and dedication to TSM. If you have comments or would like to help with the website, please contact Becky.

I would like to thank everyone who participated in the emergency election for TSM officers for 2002-2003. We are now back on track and I look forward to working with the newly elected officers. They are Anne Rushing, President Elect, Bob Droleskey, Treasurer Elect, and Susan Robbins, Program Chairman Elect. The Executive Council appointed Sam Ho from Baylor University Medical Center, as the new Student Representative.

I would like to close this message by physically asking each TSM member to consider participation in TSM by attending and presenting papers at the meetings, submitting manuscripts and illustrations to the journal, and holding office. The Journal is peer-reviewed and has national circulation so, please inundate Camelia Maier, the editor, with your manuscripts and support our Society. If you are interested in helping out at the meetings or if you have students that could do this, please contact the Program Chairman and volunteer. This is a great way to meet fellow TSM members. We are also open to suggestions for new topics for speakers and workshops for our meetings. Your officers strive to meet the scientific needs of the Society by addressing current issues in science, so we need your input. After all, this is your society and it will not survive without you. See you in Austin!

Pamela Neill
TSM President 2002-2003

Call For Papers

Manuscripts are needed for the next edition of the Texas Journal of Microscopy. Please send your work as short communications, full articles or review articles in biological sciences, material sciences or education to:

Camelia G.-A. Maier
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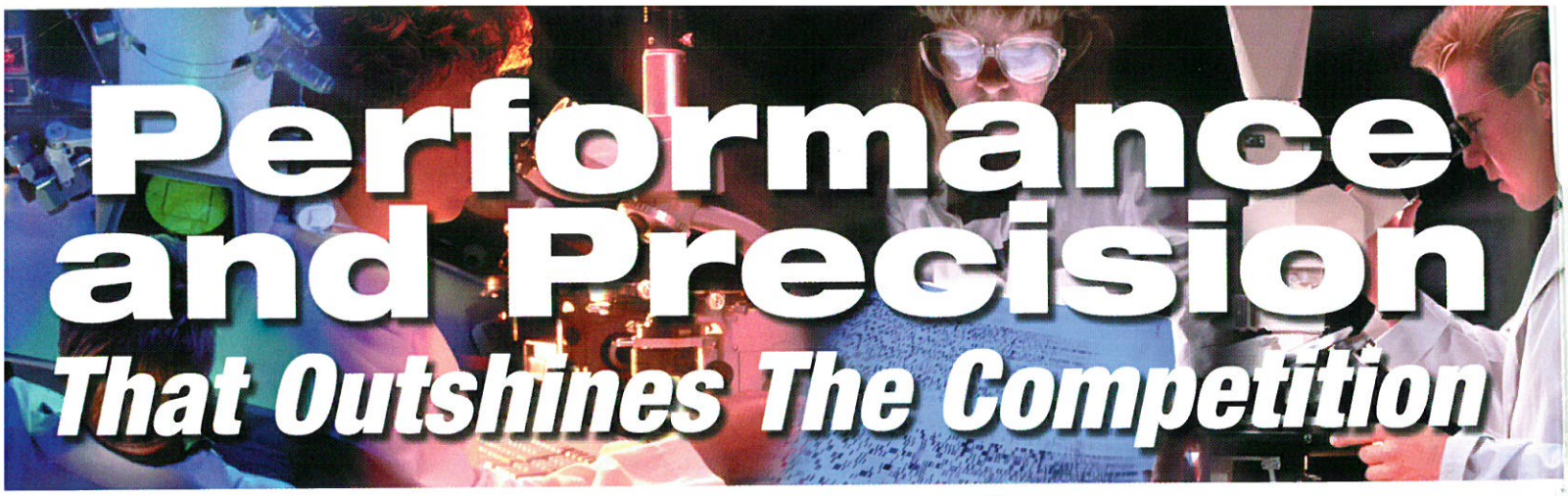
cmaier@twu.edu

Manuscript deadline is January 15, 2003



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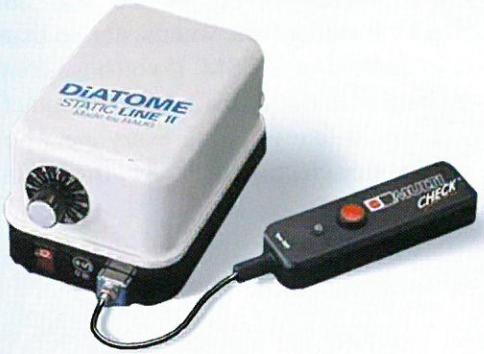

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Treasurer's Report

TREASURER'S 2002 YEAR REPORT
For Period beginning March 1st and ending August 31st, 2002

ASSETS AS OF MARCH 1st, 2002:

Checking Account No. 005772227833 (Bank of America)	$448.87
Certificate of deposit No. 1882289323	$4,079.37
TOTAL	**$4528.24**

Income:

Dues	$1450.00
Spring Meeting Registration:	$1100.00
Journal Advertisement Revenue 33:1	$1850.00
Color plate for journal article	$450.00
Donations	$415.00
Checking Account Interest	$1.01
CD Interest	$244.77
Total Income	**$5510.78**

Expense:

Journal Printing:	
33:1	$1290.43
Color plate for journal article	$450.00
Spring Meeting 2002 Hotel Expenses	$2361.49
Guest Speaker's Travel	$504.50
Secretary's Account	$500.00
Postage	$16.25
Checking Account Service Charge	$72.00
Total Expense	**$5194.67**

ASSETS AS OF AUGUST 31st, 2002

Checking Account No. 005772227833 (Bank of America)	$764.98
Certificate of deposit No. 1882289323	$4,079.37
TOTAL	**$4844.35**

Abstracts

BIOLOGICAL SCIENCES
FALL 2002

A RELIMINARY SEM STUDY OF LEAF STRUCTURE IN SPECIES OF *SALVIA*. MARTHA I. GRACEY AND HOWARD J. ARNOTT, The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019

Salvia species, also known as sages or salvias, are a diverse group of plants in the Lamiaceae that grow abundantly throughout the world in diverse habitats. These plants are used for many purposes: in the United States, they are used mainly as kitchen herbs, as bedding plants in gardens and flowerbeds and as minor crops. *Salvia divinorum* is a species found in the more temperate regions of Mexico. This species produces a type of hallucinogen, a small organic molecule called Divinorin A, and it is used by aficionados in several countries (sagewisdom.com). It is currently legal to possess *S. divinorum* in the United States. The leaf structure of *Salvia* species is diverse; simple observation shows that some species are smooth while others are obviously covered with trichomes; some of this variation may be due to habitat differences of the species in nature. This paper will compare the leaves of the hallucinogenic *S. divinorum* with those of other non-hallucinogenic species of *Salvia*. The characteristics of the leaves will be examined using the SEM and LM. The leaves of *S. divinorum* that were studied included specimens resuscitated from dried "drug samples" and others from living plants. Associated with the veins in *S. divinorum* we found elongate trichomes composed of 5 or 6 cells; each cell has numerous small pegs on its surface. This type of trichome is also occasionally found in the intervenal areas. Short, bulbous "glandular" trichomes are found commonly in the intervenal areas along with many stomata.

APOPTOSIS IN OVINE LUNG AFTER SMOKE INHALATION AND BURN INJURY. Ann S. Burke, Robert A. Cox, Robert E Barrow, Daniel L. Traber and Hal K. Hawkins. Shriners Burns Hospital, Galveston, TX 77550 and University of Texas Medical Branch, Galveston, TX 77555

Apoptosis, programmed cell death, is characterized by changes in the cell membrane, condensation of cytoplasm and DNA cleavage. In this study, we used caspase 3 active antibody and a TUNEL assay kit to assess apoptosis in sheep lung after combined smoke inhalation and burn injury. Lung tissue was collected using a standard protocol. We used 5 normal sheep, 8 at 24 hours, 6 at 48 hours and 5 at 72 hours after smoke inhalation and burn injury. Tissues were collected at autopsy, fixed in formalin and processed into paraffin. Four micron sections were cut for the TUNEL assay and immunohistochemistry using rabbit anti-human/mouse caspase 3 active antibody. Sections of normal lung had intermittent caspase 3 positive cells in the epithelial lining of airways and macrophages. After 24 hours, injured lung tissue had focal areas of strong caspase 3 positive staining in airways, surrounding blood vessels, macrophages and parenchyma. After 48 hours, most bronchioles also showed positive staining for caspase 3. The TUNEL assay showed a progressive increase in nuclear staining with time at 24, 48 and 72 hours post injury. Cells committed to entering the apoptotic pathway, shown by caspase 3 staining and those already dead, shown by the TUNEL assay increase with time after combined smoke inhalation and burn injury. Bronchi showed significantly increased apoptosis after 24 hours. After 48 hours, bronchioles were also adversely affected. Immunostaining for activated caspase 3 correlates with TUNEL staining in showing an increase in the number of cells dying via apoptosis after smoke and burn injury. Additional studies are in progress to study the caspase cascade in the apoptotic pathway. Interruption of an early caspase could contribute to recovery from pulmonary inhalation injury that may have therapeutic value.

OBSERVATION OF *SALMONELLA TYPHIMURIUM* FIMBRIAE BY NEGATIVE STAIN. R. E. DROLESKEY[1], A. D. HUMPHRIES[2], M. RAFFATELLU[2], A. J. BÄUMLER[2], R. B. HARVEY[1] and D. J. NISBET[1], [1]USDA, ARS, Southern Plains Agricultural Research Center, College Station, TX 77845 and [2]Dept. of Medical Microbiology and Immunology, College of Medicine, Texas A&M University System Health Science Center, College Station, TX 77843.

Washed and unwashed overnight cultures of *Salmonella typhimurium* were examined for the expression of fimbriae using negative stain. In the course of the evaluation, it was noted that the distribution of bacteria on formvar coated grids was dependent on the negative stain utilized for visualization. With phosphotungstic acid, bacteria tended to aggregate along the grid bars on both carbon coated and uncoated formvar grids. Aggregation of bacteria hindered the observation of individual bacteria for the determination of fimbrial expression. Alteration of stain concentration and pH did not effectively enhance the distribution of bacteria on the grid. Several surface tension modifiers – poly-L lysine, bovine serum albumin and bacitracin – as well as glow discharge, were able to slightly improve bacterial distribution. However, in the case of protein modifiers the appearance of protein molecules in the background interfered with the observation of expressed fimbriae. Staining of bacteria with either 1 or 0.5% aqueous ammonium molybdate produced grids with evenly spread bacteria with a relatively clean background. Inclusion of protein surface tension modifiers did not enhance the distribution of bacteria but did contribute unnecessary background to the images. Staining bacteria with aqueous uranyl acetate produced grids with bacteria more evenly distributed than with phosphotungstic acid. However, grids stained with uranyl acetate had lower numbers of attached bacteria than when stained with ammonium molybdate. In the case of *S. typhimurium*, ammonium molybdate proved to be a superior stain for the visualization of fimbriae. Its superiority was due to its ability to produce grids with evenly stained and distributed bacteria without the need for surface tension modifiers.

***IN VITRO* PROPAGATION OF *SESBANIA GRANDIFLORA*, A TROPICAL LEGUME.** GREG T. LEWELLEN[1], BRIT PATTEN[1] and NABARUN GHOSH[1]. [1]Department of Life, Earth and Environmental Sciences, West Texas A& M University, Canyon, TX 79016

Research in the area of biotechnology and genetics could aid in the preservation and restoration of endangered or threatened species. Techniques including gene banking, cytogenetic analysis and tissue culturing maybe used to enhance reforestation and conservation efforts. One particular species, *Sesbania grandiflora*, has significant potential to stimulate the agricultural development of many arid countries, as well as enhancing the agricultural economy of the southwest United States. Reforestation projects in Taiwan and Indonesia have replanted *S. grandiflora* in hopes of preventing soil erosion and replenish soil damaged by slash-and-burn agricultural techniques. *S. grandiflora* seeds were surface sterilized using 2% Tween20 and 2% sodium hypochlorite. These seeds were then germinated *in vitro* in MS media containing 8% (v/v) coconut milk. We used cotelydons as the explant source. Explants were inoculated into MS media containing coconut milk (8% v/v), IAA (0.25 mg/L) and BAP (2.0 mg/L). Shoot regeneration occurred directly from the explants as well as from callus. The microshoots were transferred to MS media containing 5% (v/v) Coconut Milk, 2.0 mg/L NAA, 1.0 mg/L BAP, 80.0 mg/L casein hydrolysate and 4% (w/v) T. C. agar.

We observed organogenesis in several calli batches and recorded the stages by capturing digital images with an Olympus SZ-CTV Stereoscope attached to a DVC camera. This organogenesis occurred spontaneously without further modification of hormonal

concentrations. Regeneration of micro-shoots occurred from callus of 1 to 1 months of age. Rhizogenesis occurred 60 days after initial shoot transfer. Somatic metaphase plate from the squash preparation of the root tips revealed 2n =24 chromosomes.

ASSESSMENT OF POLLEN CONCENTRATION IN THE ATMOSPHERE OF TEXAS PANHANDLE THROUGH THE USE OF A BURKARD VOLUMETRIC SPORE TRAP. BRIT PATTEN[1], GREG LEWELLEN[1], NABARUN GHOSH[1], C. SAADEH[2] and MICHAEL GAYLOR[2]. [1]Department of Life, Earth and Environmental Sciences, West Texas A& M University, Canyon, TX 79016, [2]Amarillo Center for Clinical Research/Allergy A.R.T.S. 1901 Medi Park, St. 40, Amarillo, TX 79016

The purpose of our analysis of pollen data is to assess and enumerate the impact of airborne pollen and mold spores on the breathing and causes of allergic rhinitis in individuals that are carried on the atmospheric oscillations of the exterior environment. Our objective is to survey the type and concentration of pollen and spores on a daily basis and correlate these concentrations with both the weather on a particular day and the incidence of allergic reactions. Aeroallergens are often the cause of serious allergic and asthmatic reactions, affecting millions of people each year.

The analysis of air was performed through the collection of pollen and spores using a Burkard Volumetric Spore Trap. We mounted the trap on the flat roof of the Agriculture and Natural Sciences building of West Texas A&M University in Canyon, Texas. This area has adequate exposure to the prevailing winds of West Texas, and is above the trees of the surrounding community.

Collection and transfer of the pollen sampling tape takes place at the same time, 9:00 a.m. CDT, on a daily basis. Tapes are analyzed with a minimum of five latitudinal traverses, and daily concentration is assessed.

The most significant allergens present during these summer months were *Alternaria* ascospores, and pollen from *Cladosporium*, *Dreschlera* (*Poaceae*), ragweed (*Ambrosia*) and pine (*Pinus*). Temperature was found to have an inverse relationship with mold spores. The number of reported cases of allergic rhinitis increased proportionally to the increases in overall allergen counts. Most significant was the increase in number of patients corresponding with increases in mold and *A. artemisiifolia* counts.

CALCIUM DEPOSITION AND IDIOBLAST DYNAMICS DURING LEAF DEVELOPMENT IN DIOECIOUS MULBERRY SPECIES. ZHE ZHOU, DORI GREENWALD, DAVID C. GARRETT, DIEDRE L. SHEPARD, HOWARD J. ARNOTT, and CAMELIA G.-A. MAIER, Department of Biology, Texas Woman's University, Denton, TX 76204 (DLS, ZZ, and CGAM), Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019 (HJA), and Department of Materials Science, University of North Texas, Denton, TX 76203 (DCG)

Biomineralization is widespread among microorganisms, plants and animals. The diversity of calcium deposition shapes and sizes, their tissue distribution and prevalence have led to a number of hypotheses regarding their functions in plants. However, sexual variations in calcium depositions and their significance have not been addressed. Our interest in sexual dimorphism of dioecious plants prompted us to investigate calcium deposition and idioblast dynamics in developing leaves of dioecious mulberry. Leaf primordia from winter and summer buds as well as leaves in different stages of development from male and female individuals of *Morus alba* and *M. rubra* (*Moraceae*) showed at least three distinct forms: prismatic crystals, druses and lithocysts. Oxalate levels were determined by using an oxalate diagnostic kit. Both male and female showed the same pattern of prismatic crystals in leaf primordia illustrating developmental differences. Although no significant differences between *M. rubra* male and female were observed in the distribution of prismatic crystals in summer buds, significant higher number of crystals were found in female leaf primordia of winter buds than in the male counterparts. This significant higher level of calcium oxalate in female winter buds may suggest differential resource allocation between sexes. Mature leaves in both sexes of the species under study showed at least two different types of lithocysts, classical calcium carbonate ones, and 'reticulate' lithocysts composed of silica, not previously reported in mulberry. More studies are needed to elucidate the formation and significance of silica lithocysts. Although no significant differences were found in total oxalate between sexes, females of both species showed significant higher levels of insoluble oxalate compared to males suggesting differences in resource allocation and/or in defense against herbivory between sexes.

LUNG DEVELOPMENT OF CHICKS UNDER NORMOXIC AND HYPOXIC CONDITIONS. KNIERIEMAN L.[1], DANG L. [1], NGUYEN D[1]., BURGGREN W. [2], and MUIRHEAD D. [3], [1]North Garland Maths, Science and Technology High School, Garland, Texas, [2]Biology Department, University of North Texas, Denton, Texas, [3]Cellular Pathology department, Texas Scottish Rite Hospital for Children, Dallas, Texas

The ultrastructural morphology of type II pneumocytes in lungs of fetal *Gallus domesticus* (domestic chickens) were studied after being incubated in normoxic (21% oxygen) and hypoxic (15% oxygen) conditions. The fetal chicks used in this study were of the same gestational age. They were subdivided into three groups: group-1: normoxic (control), group-2: day 6 to 12 under hypoxic and returned to normoxic, group-3: hypoxic conditions.

Animals were sacrificed at day 21; lungs were removed, fixed and processed for electron microscopy. Group-1 revealed ultrastructural appearance characteristic of normal fetal lungs. However, group 2 and group3 revealed features consistent with underdeveloped lungs, including ultrastructural changes. An increase in the numbers of type II pneumocytes in groups 2 and 3 was noted. Lamellar bodies were in various stages of development with loss of lamellar whorls. The changes seen in group 3 were more severe. An interesting finding was that of congested capillaries filled with red blood cells. This explained the differences in the mass of the lungs observed in the groups 2 and 3 compared to the control group 1.

The above observations indicate that the fetal development of, type 11 pneumocytes and their lamellar bodies are effected by concentration of oxygen in the immediate environment. Changes seen in this fetal chick model may represent the changes seen in human infants with respiratory distress syndrome (RDS). These findings may provide a basis for the development of an animal model for the study of this disease.

ULTRASTRUCTURAL EFFECT OF COPPER ON THE BRAIN AND LIVER OF *CARRASIUS AURATUS*. ASHIMI L[1], RAMOLIA S.[1] AND MUIRHEAD D. [2], [1]North Garland Math's, Science and Technology High School, Garland Texas, [2]Cellular Pathology Department, Texas Scottish Rite Hospital for Children, Dallas, Texas.

Pollution of waterways, lakes and oceans with excess copper is a major environmental problem. It is known that excess copper is toxic to aquatic life and can result in death. The purpose of this study was to document the effects of excess copper on the ultrastructure of the central nervous system and liver of *Carrasius auratus,* the common goldfish.

This study was designed in two stages. Stage one involved the characterization of normal ultrastructural features of the central nervous system and liver of healthy goldfish. Stage two involved testing various levels of copper required to induce abnormal ultrastructural changes. It is hypothesized that copper at excess levels will cause ultrastructural changes in the brain tissue and the hepatocytes and Kupffer cells of the liver.

Tissues were harvested and fixed in 2% glutaraldehyde using filtered tank water as a buffer, processed according to standard electron microscopy processing and embedded in Spurr's resin. Sections of 1 (m were stained with toluidine blue and examined with light microscopy. From the same sections representative areas were selected, ultrasectioned (60 nm) and stained with heavy metals, and then examined in a LEO 906E electron microscope.

Representative electron micrographs of the brain and liver of *Carrasius auratus* demonstrated normal ultrastructure. The features of the normal ultrastructure will be used as the baseline, upon which comparisons will be made when examining tissue samples from goldfish exposed to copper in stage 2.

ULTRASTRUCTURAL CHANGES SEEN IN *NICOTIANA TABACUM* INFECTED WITH TOBACCO MOSAIC VIRUS. K GONZALES K[1], MALHI J[2], BORLAND J[3], MUIRHEAD D.E.,[4][1]Garland High School, Garland, Texas, [2]North Garland Math's, Science and Technology High School, Garland, Texas, [3] Austin Academy for Excellence, Garland, Texas, [3] Cellular Pathology Department, Texas Scottish Rite Hospital for Children, Dallas, Texas

Tobacco Mosaic Virus (TMV) is found worldwide and was first described by Wendell Stanley in 1935. It was the first large macromolecule aggregate shown to be capable of self-assembly from its component parts. This virus is a large rod in which a cylinder of protein is arranged around a helical RNA core. The assembly process is unexpectedly complex and includes the formation of double rings of protein, which serve as intermediates, adding to the growing virus coat.

Two plant types, *Nicotiana tabacum* glurk and *Nicotiana tabacum* turk, were used to study the infection of TMV. The extent of viral infection, its effects on cellular ultrastructure, and the effects of fertilizer on the spread of the virus were compared. TMV caused local lesions in glurk and systemic infections in turk. Plants were divided into three groups: group-1 systemically, group-2 locally and group-3 were fertilized, and inoculated. All plants were examined by TEM using leaf dip negative staining (LDNS) and ultra-sections of biopsied samples of lesions from infected leaves. LDNS viral particles were counted in the sap and rated on a scale of scanty, mild, moderate, and abundant. All specimens were examined and photographed on a LEO 906E electron microscope

TMV produced three times as many lesions on locally infected plants compared with systemically infected. The lesions were isolated to the inoculated leaf in the first group and widespread in the second. Plants with the greater number of lesions contained less viral particles in the sap. Possibly, because in plants with a large number of lesions, viral particles had already entered the cells and were not present in the sap. The fertilized plants were larger in size and had more surface area for infection explaining the larger number of TMV particles. Examination of thin sections of infected plant cells revealed aggregates of viral particles surrounding the nucleus and chloroplasts. The TMV particles appear to destroy the nuclear envelope and the chloroplast double membrane.

THE EFFECTS OF DISTRACTION ON THE SARCOMERIC LENGTH OF SKELETAL MUSCLE. [1]BRANDON, J.[1] KIM C., [2]MAKAROV, M.[2], SAMCHUKOV M.[2], MUIRHEAD D.[2],[1] North Garland Electron Microscope Department, North Garland Math, Science, and Technology Magnet High School, Garland, Texas, [2] Department of Research, Texas Scottish Rite Hospital for Children, Dallas Texas

Distraction is the process of femoral or tibial bone lengthening to correct for limb length discrepancies due to congenital defects. An osteotomy is used to cut the bone, an external Ilizarov frame is applied and the bone ends are gradually moved apart at an optimal rate over time to result in a total lengthening of up to 30% of the entire bone. Osteogenesis, the process of regeneration of bone tissue, occurs as the bone is distracted, filling the gap between the bone ends. Surrounding muscle must adapt to the distraction forces. Actin and myosin are the major contractile proteins in muscle and form structures known as sarcomeres. The purpose of this study was to examine the effects of distraction on the length of the sarcomeres. It is hypothesized that sarcomeres may increase in length to adapt to the distraction or more sarcomeres may be generated to acquire length in response to distraction. Two methods were used to measure sarcomere length: a neon laser technique and electron microscopy (EM) with Soft Imaging System software (SIS). Measurements from both methods were generated and statistically analyzed. Due to the contractile nature of muscle fibres, collection and processing of the tissue required optimization in order to maintain the ultrastructure of the sarcomere. The two collection techniques used revealed an artifactual discrepancy in the sarcomere length. The first method (removal of muscle from the distracted site, consequent contraction and direct fixation in formalin) resulted in the average sarcomere length being 1.7 μm. This was below expected values based on the neon laser calculation of 3.18-3.2 μm. The second collection technique involved use of custom muscle forcepts to maintain the length of muscle during fixation. This gave a normal sarcomeric pattern. Using the latter method of collection, tissue was examined with both neon laser technique and EM with SIS. Data were generated for normal muscle and distracted muscle. Average sarcomeric length in non-distracted muscle was 3.2 μm. In comparison, the average sarcomere length in a distracted muscle was 2.8 μm. In conclusion, there was a 14% decrease in length of sarcomere in distracted tissue suggesting that new sarcomeres are being generated rather than elongated to adapt to the distraction.

ULTRASTRUCTURAL THREE-DIMENSIONAL RECONSTRUCTION OF MITOCHONDRIA FROM PIG LIVER. Joslin, C[1]., Buttle, K., Muirhead, D.E.[2], Mannella C.[3], [1]North Garland High Electron Microscopy Unit, Garland, Texas, [2]Cellular Pathology Department, Texas Scottish Rite Hospital for Children, Dallas, Texas, [3]Wadsworth Center, Resource for visualization of Biological Complexity, Albany, New York

Mitochondria are known as the powerhouse for the cell, and are thus responsible for the production of the cell energy. Cristae (foldings of the inner membrane) within the mitochondria are the sites of the Kreb's cycle and oxidative phosphorylation, which are essential pathways for energy production. The purpose of this study was to construct a three-dimensional image of the mitochondrion in order to establish whether mitochondria are multiple ultrastructural organelles or a single organelle undulating throughout the cytoplasm of the cell. Pig liver was prepared using standard methodology for tomography fixation and EM processing. An example series of 150 serial ultrasections were cut at 300 μm. A second set of liver tissue was prepared using 2% glutaraldehyde in cacodylate buffer, followed by a refined processing schedule. A second example series of 250 serial sections were generated from this tissue. Serial section reconstruction and automated electron tomography with and without double-tilt reconstruction was used to generate three-dimensional imaging of the mitochondria. Following these reconstructions, the computer program Slicer Dicer was used to color-enhance the images. Serial section reconstruction and automated electron tomography (tissue processed with standard fixation) yielded an inadequate level of detail. Serial section reconstruction and automated electron tomography with double-tilt reconstruction (tissue from the improved fixation and processing schedule) resulted in a higher level of detail and thus an improved three-dimensional image of the mitochondria. The video imaging of the three-dimensional reconstruction suggests that mitochondria consist of a double membrane structure of which the inner membrane appears to form cristae. However, further sectioning would be required to capture the entire mitochondrial organelle. A portion of this work was performed and supported by the electron tomography research at Resources for Visualization of Biological Complexity, National Center for Research Resources, NIH Grant RR01219.

NODULE FORMATION AND ULTRASTRUCTURAL CHANGES OF THE ROOT CELLS OF ALFALFA PLANTS INFILTRATED BY *SINORHIZOBIUM MELILOTI* BACTERIA. Chen, R.[1], Dingrando, L. [1], Gonzalez J.[2], and Muirhead, D.E[3,1] North Garland Electron Microscope Department, North Garland Math, Science, and Technology Magnet, Garland, Texas, [2]Faculty of Science, University of Texas at Dallas, Richardson, Texas, [3]Cellular Pathology Department, Texas Scottish Rite Hospital for Children, Dallas, Texas 75219

Nodule development in plants is thought to occur as a result of the symbiotic interaction of bacteria with plants during nitrogen fixation. The nitrogen fixation process begins in the soil, where plants are emitting flavonoids. The flavonoids stimulate bacteria to produce chemicals, which causes the plant's root hairs to curl around the bacteria. Bacteria enter the plant's root cells but it is not known whether the bacteria enter by phagocytosis or tunneling. Plants then emit nod factors, which induce nodule development. The purpose of this investigation was to characterize the events leading to nodule formation and related ultrastructural changes in the roots of alfalfa plants (*Medicago sativa*) inoculated with *Sinorhizobium melilotis*.

Root samples were fixed in 2% Gluteraldehyde in caccodylate buffer (pH 7), at 4°C for two hours followed by preparation for standard electron microscopy. Alfalfa plants inoculated with the *Sinorhizobium meliloti* grew nodules. Changes were observed in the ultrastructure of the roots and root cells of inoculated plants. Root hairs were originally straight, but curled at the locations of bacterial infiltration. Nodule growth increased and became more abundant as time progressed. In the infiltrated root cells, bacteria were located at the perimeter of

the cells when infiltration began and continued to spread throughout as the nodules matured. The ultrastructural organelles in the infiltrated root cells were displaced. In the cytoplasm, each infiltrating bacterium was membrane-bound, giving the appearance of a vacuole. Some vacuoles contained more than one bacterial cell. From this study it was found that an alfalfa plant exhibits nodular formation due to bacterial infiltration and consequently shows ultrastructural changes within the cells of the nodules.

THE SEQUENCE OF DEVELOPMENT OF AND THE EFFECTS OF VARIED CONDITIONS ON PROPAGULE FORMATION IN *BRYOPHYLLUM DAIGREMONTIANUM*. LARISSA C. PARSLEY and ANN E. RUSHING, Department of Biology, William Carey College, Hattiesburg, MS 39401 and Department of Biology, Baylor University, Waco, TX 76798.

Bryophyllum diagremontianum (Hamet & Perrier) Berger is a succulent plant native to tropical and temperate environments. Its regenerative abilities allow the plant to produce small plantlets (propagules) on the margins of leaves for the purpose of vegetative reproduction. These propagules develop shoots, leaves, and roots, allowing them to grow independently from their parent plants. This study focuses on the morphological sequence of development for these propagules while on their parent plants (control plants) and for propagules under certain experimental conditions. In the control plants we found the general order of organ initiation to be a swollen leaf notch which produced one pair of leaves, followed by a second pair perpendicular to the first, root primordia, and a third pair of leaves perpendicular to the second. This sequence did not change in the experimental leaves. Propagules formed in leaves both attached to and detached from their parent plants. However, detached leaves still attached to a section of stem developed leaf notches but no propagules.

A CYTOLOGICAL AND HISTOLOGICAL EXAMINATION OF POST OAK (*QUERCUS STELLATA*) ROOT. Blake C. Boling and Don W. Smith, Department of Biological Sciences, University of North Texas, P.O. Box 305220 Denton, Texas 76203-5220

Ecologically and economically, *Quercus stellata* (post oak) is an important member of the North American family of deciduous hardwoods, yet its numbers are rapidly dwindling. The post oak fruit, the acorn, is an indispensable hard mast, along with the pecans, chestnuts, beech nuts, and pine seeds. The chestnut blight fungus has decimated native American chestnut and native chinquapin from our eastern forests. As a result, these food resources are no longer available to foraging animals in many parts of the country. Remaining hard mast producers such as the post oak need to be protected. Post oak wood is no longer in demand. However, economically the tree is still very important. A single majestic post oak can add tens of thousands of dollars to a property's value. Empirical data suggests that human encroachment upon the root system of post oak is one of the leading causes of post oak death. We wish to start our investigation of post oak death by examining the histology of its root tips. Furthermore, we have augmented classic squash techniques in order to calculate the mitotic index of post oak root tips. We will present an overview of post oak root histology and discuss our improved squash technique.

***IN VITRO* CALLUS AND ROOT FORMATION FROM DIVERSE EXPLANTS OF COWPEA [*VIGNA UNGUICULATA* (L.) WALP].** GEORGE OMWENGA AND DON W. SMITH, Department of Biological Sciences, University of North Texas, P.O. Box 305220, Denton, Texas 76203-5220

With slow progress being made in the development of varieties resistant to insects and diseases of cowpea, a nutritionally important crop in most African countries, genetic engineering combined with tissue culture methods offers hope to improve resistance to attack. Genes for transfer have been identified but we still lack a reproducible tissue culture regeneration system. Our aim was to find conditions that stimulate regeneration from tissue culture in selected cowpea genotypes by working out combinations and amounts of phytohormones. We evaluated (i) auxins (IAA, NAA, 2,4-D, Dicamba, Picloram, 2,4,5-T, and IBA), (ii) cytokinins (BAP, Kinetin and Zeatin) and (iii) substituted urea derivatives (Thidiazuron and 4-CCPU), in initiation media such as MS, B-5 and BM. Explants were cultured in media plus phytohormone combinations either (i) continuously in the same media, (ii) a shock treatment before being transferred to media without hormone or (iii) stepwise decreased or increased phytohormone levels. We induced callus, roots or roots and callus from leaf, hypocotyl, and cotyledon sections. Root organogenesis occurred with MS and NAA (1.0-2.0mg/l) and kinetin (0.02-0.1mg/l), IBA (0.1-0.2mg/l) and kinetin (0.1-0.2mg/l). When roots did not form callus texture was mucoid to hard compact. Subculturing resulted either in continuous proliferation of the callus or death. So far we have been unable to prescribe phytohormone combinations that results in the development of shoots from callus or explants. Successful regeneration of cowpea plants from tissue culture would greatly enhance the application of genetic engineering in cowpea and improve the quality and quantity of production.

A STUDY OF CASTS FROM EPOXY EMBEDDED EGGSHELL OF WHITE LEGHORN CHICKENS. Sandra L. Westmoreland, Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019

High-pressure epoxy casts of eggshell samples from eggs of White Leghorn chickens were prepared to study the pore system, through which gas exchange occurs during embryogenesis. In addition to the details of pore structure that were revealed, an exceptional three-dimensional view of the interior of the mammillary cone, made possible by plastic replicas, has provided a clearer picture of this important eggshell region where nucleation for biomineralization occurs. Unfertilized eggs of Hy-line W98 White Leghorn chickens, obtained from the Poultry Science Department of Texas A & M University, were emptied and the shell was treated with Clorox bleach to remove the organic shell cuticle and shell membranes. Small shell fragments were placed in holders, covered in CIDA 506 resin epoxy with Polycon hardener, and placed in a pressure vessel in a closed vacuum at 1,200 psi until set. Epoxy-embedded shell blocks were cut in half to expose radial shell surfaces and were then placed in concentrated hydrochloric acid to dissolve the eggshell. Shell casts viewed on the JOEL 35C scanning electron microscope were observed to contain a replica of the upper shell surface, the mammillary cone region, and the pores. The pores in the plastic casts were seen as solid columns of plastic that were continuous connecting the upper and lower shell surface replicas. The replica of the mammillary cone surface, when viewed from the side, contained a wave-like pattern, which indicated the impression of the spaces of the adjacent mammillary cone junctions. When the mammillary cone casts were viewed from directly above, replicas of individual mammillary cones could be seen. The basin-like structures contained replicas of shell membranes, which were seen in a woven, crisscrossing pattern. The shell membrane fibers were of varying sizes in diameter. Some fibers were individual, while others were in bundles. The cast of a single spherical body could be seen in many of the mammillary cone replicas. These bodies are proposed to be the mammillary cores, sites of shell nucleation and of calcium translocation during embryogenesis.

AN SEM STUDY OF FROST RINGS IN A BRISTLECONE PINE (*PINUS LONGAEVA*) FROM SHEEP MOUNTAIN, CALIFORNIA

HOWARD ARNOTT AND CHRISTINE HALLMAN

**Department of Biology and Center for Electron Microscopy,
The University of Texas at Arlington, 76019 and
The Laboratory for Tree-Ring Research, University of Arizona, Tucson, Arizona, 85721s**

In the woody stems of many plant species annual rings occasionally exhibit growth abnormalities termed "frost rings." Recent frost ring studies have been concerned with "frost ring chronologies" in an attempt to relate frost rings with major physical events (volcanic eruptions) that affect climate (LaMarche and Hirschboeck, 1984). Investigation of the anatomy of frost rings for the sake of understanding plant structure and development has been rare. However, studies in the early part of the 20th century utilized standard plant anatomy techniques: sections cut with a microtome, staining with dyes, careful light microscopic observation and the making of photomicrographs. These investigations were made in an attempt to understand both the timing and the cellular changes associated with the formation of frost rings. Rhoads (1923) Bailey (1925), Glock (1951) are examples of anatomical studies which illuminated the frost ring structure and development. However, to this time the SEM has not been widely applied to the study of frost rings. In the year 2000, one of us (CH) produced a core from a tree on Sheep Mountain, White Mts., Inyo National Forest, California. This core (from LTRR 2002-268) presented an opportunity to study both normal and frost rings using the SEM. Prior to making specimens for examination in the SEM, the core was carefully inspected and frost rings as seen in the dissecting microscope were noted. Subsequently, the cores were scanned using a HP ScanJet 4200 at 1200dpi. The scans were made to insure that after the core was dissected for SEM preparations, the position and structure of all rings could be monitored by using the scans. For use in the SEM, the core segments were sectioned by hand, attached to aluminum stubs, sputter coated, and then studied in a JEOL 35C SEM with a VitalScan digitizing unit; all SEM images are digital. The core had annual rings beginning in 1925, the innermost frost ring occurred in 1928. Subsequent frost rings were found in 1941(Fig. 5), 1952 (Fig. 4), 1964, 1965 (Fig. 3), and 1978. When all rings from 1925 to 2000 were examined in the SEM, only six frost rings were found. The remaining 69 annual rings exhibited normal structure similar to the 1977 ring (Fig. 1). In normal annual rings the rows of tracheids extend from the late wood into the following spring wood in a straight line, each row having been initiated by successive tangential divisions in a single fusiform cambial cell. In normal rings rays also follow straight through from one growth ring to the next, as they are derived by successive divisions of a single ray initial cell. Over a limited space, one to four growth rings, the same number of tracheid-rows pass from one ring to the next (Fig. 1). Observation of the frost rings shows a quite different pattern of differentiation. The number of tracheid-rows may be different, the rows are usually "tilted" to one side, sometimes by more than 20 degrees from radial, and often very large cells with especially thick cell walls are found in frost rings (Figs. 2-5). When the frost ring is "mild" it is possible to follow the tracheid-rows through the frost area and into the next annual ring where they are displaced in which ever way the tracheid-rows are tilted. The way in which this frost rings occur has often been attributed to the freezing of water in one or more cell compartments of the vascular cambial area. Whatever the specific cause of frost rings, centrifugal growth always returns to normal as new tracheids and rays are produced by the "reestablished" vascular cambium.

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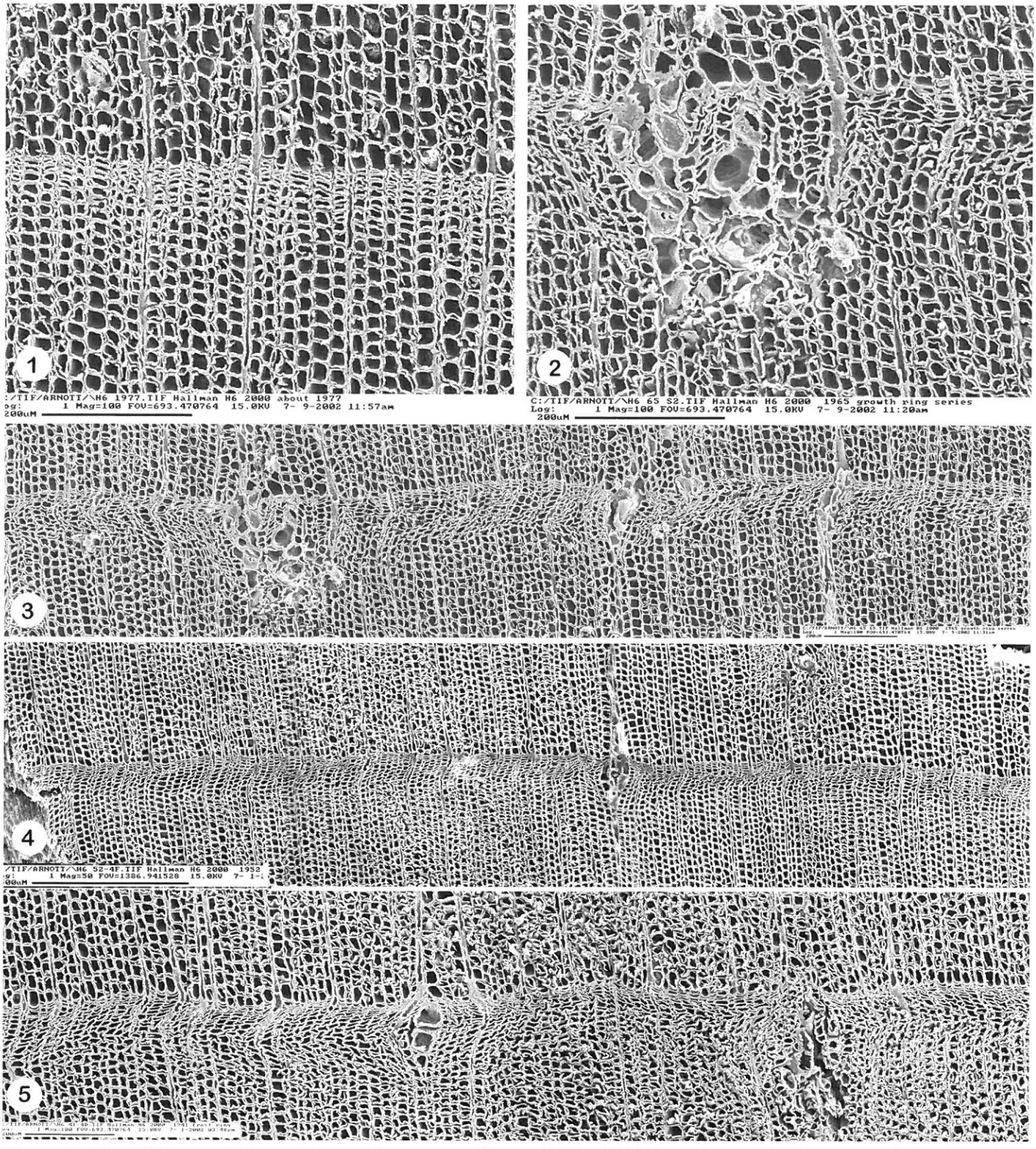


Figure 1. The 1977 annual ring with normal structure. **Figure 2.** The 1965 frost ring exhibiting many of the aberrations found in frost rings: crushed and enlarged cells, thick cell walls, diverted tracheid files and enlarged rays. **Figure 3.** Montage showing many aberrations in the 1965 frost ring. **Figure 4.** A montage of the 1952 frost ring showing limited aberrations characteristic of a "mild frost ring." Note that the tracheid files are all "pushed" toward the right at an angle of about 20 degrees but that they can be followed through the ring interface. **Figure 5.** A montage of the 1941 frost ring, which exhibits an opposing divergence on the right verses that seen on the left. Note the expanded ray, with three large thick-walled cells, near the middle of the figure.

MATERIALS SCIENCES
FALL 2002

INTERNAL MORPHOLOGY AND SURFACE AREA OF NATURAL MANGANESE OXIDES. JOE B. DIXON, G. NORMAN WHITE, ZHIPING LUO, J.S. JACOB, and J. G. KIM. Soil and Crop Sciences Department, Texas A&M University, College Station, TX 2474.

Manganese oxides are known to be important oxidizing agents because they contribute to the oxidation of Cr(III) to Cr(VI) and to the polymerization of organic compounds in soils. The characterization of these minerals is difficult because of their scarcity and their poorly defined morphological properties. Indeed the detailed morphological properties of the most common of Mn oxides in soils, birnessite have not been described. The combination of high magnification transmission electron microscopy and digitized images offers some insight in how to better investigate these Mn oxides. Mn oxide crystals tend to be small and less perfect compared to layer silicates of the same size. Inconsistencies of tunnel sizes in todorokite have been known for many years. The internal fabric of todorokite, birnessite, and lithiophorite particles in model natural samples is surprisingly porous as indicated by high surface area and internal openness shown by high magnification transmission electron micrographs. Manganese oxides observed thus far lack the crystal faces (e.g. ~5 nm in length) evident in submicron crystals of goethite. Although the data set for Mn oxides crystals is relatively small it seems clear from direct electron microscopic evidence and indirectly from x-ray diffraction and surface area data that Mn oxide crystals in soils owe part of their reactivity to their large exposed surface area that is both internal and external. Twelve specimens representing todorokite, birnessite, and lithiophorite from soils indicate a range of crystallinity indicated by electron diffraction spot versus ring patterns. Internal heterogeneity of the particles indicated by varied density in electron micrographs (except for one lithiophorite specimen) indicates folds, contortions and turbostratic stacking of layers contribute to internal porosity of these oxides. Birnessite is the least crystalline of the three oxides investigated.

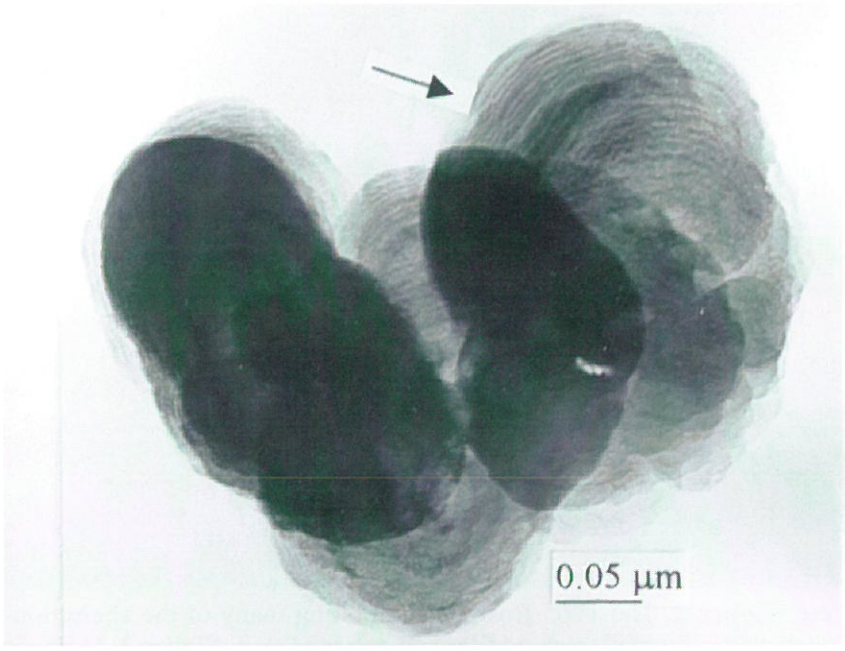
EDUCATION
FALL 2002

AN INTERDISCIPLINARY INQUIRY LABORATORY ON CALCIUM BIOMINERALIZATION IN PLANTS. CAMELIA G.-A. MAIER and HOWARD J. ARNOTT, Department of Biology, Texas Woman's University, Denton, TX 76204, and Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019

This educational paper was presented as a poster for the Fall 2002 meeting. Abstract and more details on page 51.

Answer to "What Is It?"

from Texas Journal of Microscopy 33:1



This TEM by John J. Bang, Environmental Science and Engineering (ESE) Ph.D. program in the Department of Metallurgical and Materials Engineering, University of Texas at El Paso, represents graphitic material or graphite with a very unique and well-defined layered morphology (arrow). The specimen in the picture was collected one foot above the ground level and five feet away from a heavy traffic intersection in El Paso, Texas, during one morning rush hour in 2001. The raw sample collected on a 3-mm carbon/formvar coated Ni grid was examined by TEM within an hour afterwards.

Car brake shoes and pads use large amount of graphitic material. In addition to its impacts on human health in chronic exposure scenario, graphitic material has a potential to serve as a major vehicle for other catalytic reactions in the air due to its high surface area to volume ratio per unit mass. Particle number count or mass of collected material has been used as a feature to correlate any group of particle/material with its impact on human health. Nowadays, the surface area to volume ratio feature is believed to be a better predictor for the level of detrimental impact on human health than particle number count or mass.

Carbonaceous/graphitic material by itself is inert in terms of chemical reaction. However, exposure to high concentrations of this material even for a period shorter than one year has been known to cause respiratory illness, both obstructive (chronic bronchitis, emphysema) and restrictive types as well in people with various occupations. Chronic exposure to the material, even at lower concentrations, raises public health concern especially because human activities generating carbonaceous material have been increased lately (nanotechnologies using graphitic material, for example). Further follow-up studies are needed for source identification and characterization of any chemical reactions facilitated on the graphitic material surfaces.

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Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSM member and do not necessarily reflect the opinions of the Editor or the Society. The content of the letters should be concerned with the philosophical or operational aspects of the TSM, the Journal and its contents, academic or national policies as they apply to TSM and/or its members and microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

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Micrographs submitted for cover photos should be marked as such. The choice of photographs will be made by the Editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used for cover photos and text fillers are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

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The Technical Section will publish TECHNIQUES PAPERS, and HELPFUL HINTS. The TECHNIQUE PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with methods. The format of the Technique Papers will be the same as that used for regular research reports. HELPFUL HINTS will be in the form of a brief report with an accompanying illustration, if required for clarity. Helpful Hints should embody techniques which will improve or expedite processes and/or procedures used in EM.

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Letters to the Editor

MSA Technologists' Forum

The Technologists' Forum is a special interest group that provides a channel for personal growth and development of technologists within MSA. The Forum organizes a special topic presentation, a symposium, a roundtable discussion and an exhibit booth at the annual Microscopy and Microanalysis meeting. The sessions are presented so as to maximize the technical information useful to today's lab worker. A semiannual newsletter, published electronically by the Forum, increases contact among its members and expands their participation in and contributions to MSA. The Forum also sponsors the Professional Technical Staff Awards, which is a competitive program to encourage participation of technologists at the annual meeting (see below for details). Any MSA member is welcome to belong to the Technologists' Forum for free and contribute to its activities. For more information, please contact Technologists' Forum Chair Jeanette Killius at 330/325-6311, by e-mail at jkillius@neoucom.edu, or visit the Tech Forum web pages through the MSA web site at www.msa.microscopy.com.

MSA Professional Technical Staff Awards

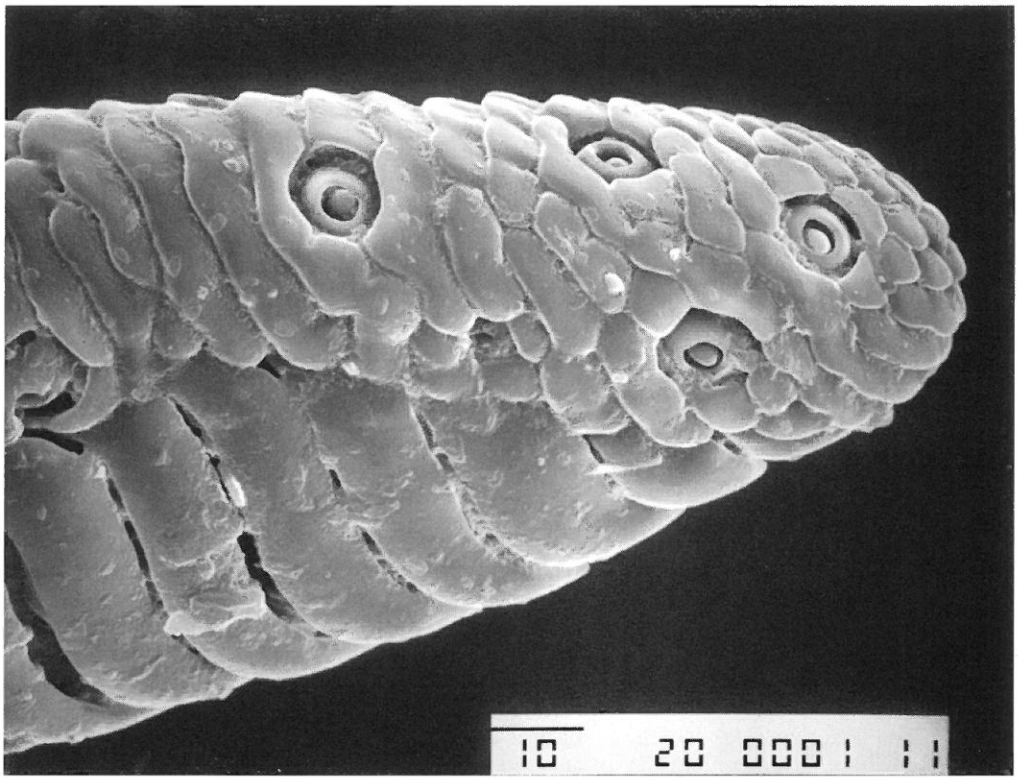
The Professional Technical Staff Awards (PTSA) were created to stimulate attendance at the Annual Meeting of MSA for professional technical staff who ordinarily might not participate in a national meeting, and to encourage supervisors to support their staff in professional activities. There will be up to four awards given, based on the quality of a first-authored paper submitted for presentation at the meeting. The awards consist of free full registration for the meeting, a copy of the Proceedings and the Sunday evening social event. MSA will reimburse awardees up to $600 for travel, lodging and other expenses. Applicants must be full paid-up members of MSA at the time of application. Abstracts will be judged by the MSA Technologists' Forum. Successful applicants must present their papers personally at Microscopy and Microanalysis 2002 in order to receive the award. They are expected to attend and participate in the entire meeting. Former winners will not be eligible for another award. Complete information about the application process, including application deadlines, will be available in the Registration Bulletin and Call for Papers for Microscopy and Microanalysis 2003, which will be sent to MSA members in November 2002. For further information, contact the Chair of the Technologists' Forum: Ms. Jeanette Killius, at 330/325-6311 or by e-mail at jkillius@neoucom.edu.

Jeanette Killius
Technologists' Forum Chair

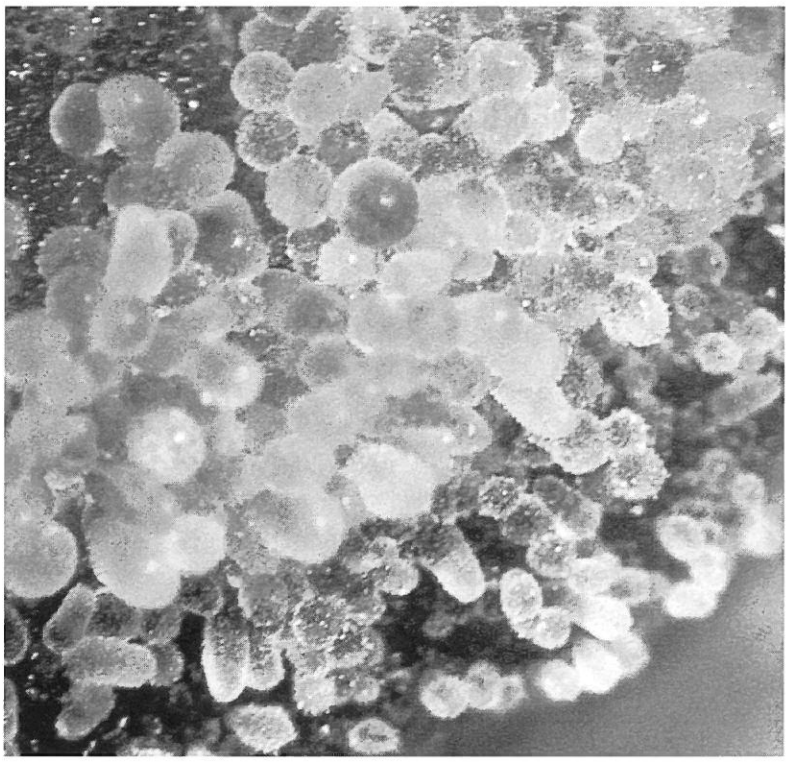
ADVERTISER'S INDEX

What Is It?

Answers In Next Edition

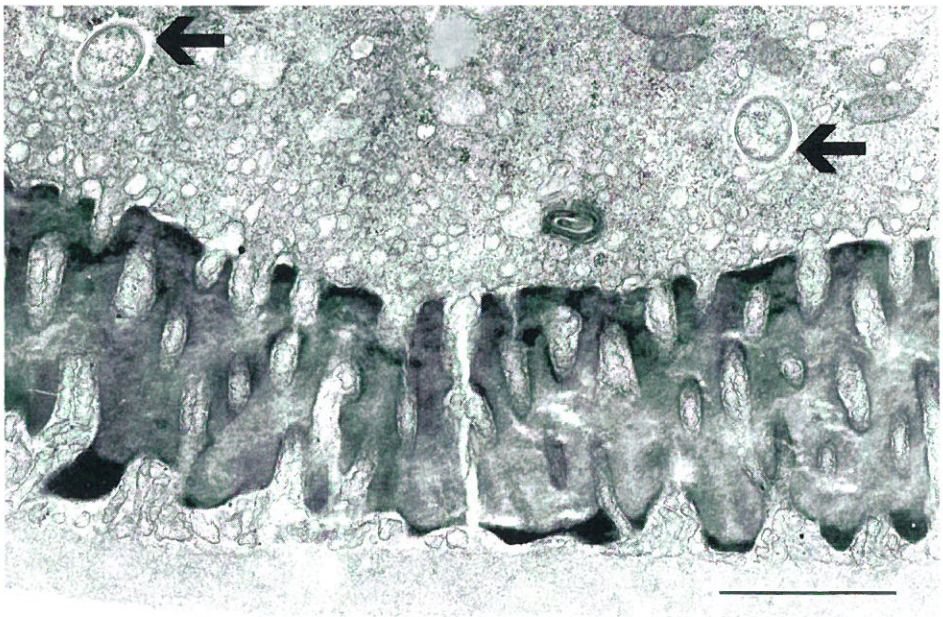


SEM by Daniel Petr, Department of Biology, Southwestern Adventist University, Keene, Texas 76059.

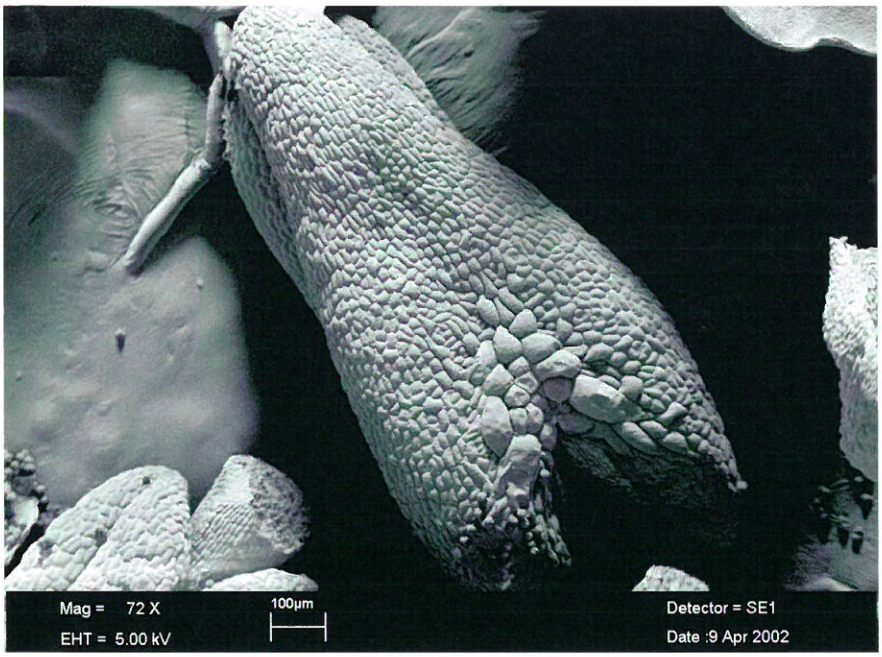


Light micrograph by Camelia G.-A. Maier, Department of Biology, Texas Woman's University, Denton, Texas 76204-5799

OUR STUDENTS

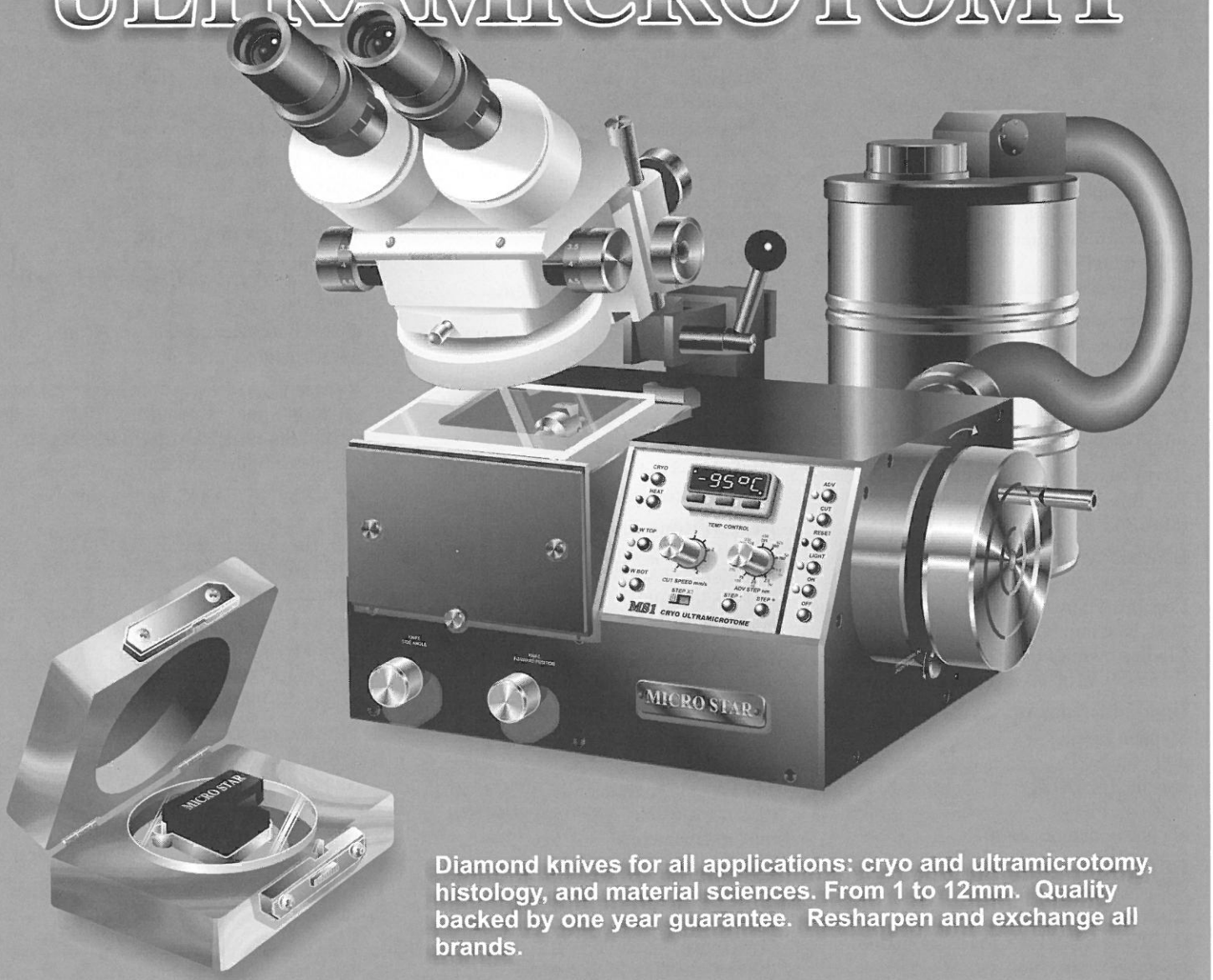


TEM by **Ted J. Whitworth**, at the **University of Texas Medical Branch in Galveston**, of a 70-nm section through an immature tick (*Aponomma hyrdrosauri*) egg. The electron dense material beneath the egg surface is the vitellin envelope. Arrows are pointing to two *Rickettsia honei* bacteria, a spotted fever group rickettsia. The tick specimen was collected from an Australian blue-tongued lizard on Flinders Island, Australia. The presence of rickettsia inside the immature tick egg suggests the possibility that *R. honei* can be transovarially transmitted, from mother tick to its progeny. Furthermore, no other reptilian tick has ever been shown to be a host of a rickettsial species. Ticks were dissected in a drop of fixative, and tissues such as salivary glands, midguts, malpighian tubules, and ovaries were then fixed for two days in Ito's fixative. The picture was taken with a Phillips 201 electron microscope at 60 kV and the scale bar represents 1μm. Ted's mentor is **Dr. David H. Walker**, Professor and Chairman, Department of Pathology at the above university.



This SEM represents a young anther from a bud of turnip plant, *Brassica rapa* (*Cruciferae*). The anther is a pollen-containing organ made up of two bags with pollen grains. The anther together with its connecting stalk or filament makes up the stamen, which is the male reproductive organ in flowering plants. All the stamens in a flower are disposed as a whorl called the androecium. *Brassica rapa* plants have six stamens which are tetradynamous, that is, four of them have long filaments, while the other two have short filaments fixing their anthers onto the floral receptacle. The second anther in the micrograph exposes a short filament and some pollen grains. Photographed with LEO VP 435 SEM by **Onur Dundar**, graduate student at the **University of Texas-Pan American,** Edinburg, Texas. Onur works with **Dr. Anxiu Kuang** in the Department of Biology at the above university.

MICRO STAR
ULTRAMICROTOMY



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Educational Tips

AN INTERDISCIPLINARY INQUIRY LABORATORY ON CALCIUM BIOMINERALIZATION IN PLANTS

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Abstract

Few plant biology laboratory manuals mention the presence of calcium crystals and none of them offer structured laboratory activities to study calcium deposits in detail. We have developed a three-hour interdisciplinary laboratory during which students learn to develop a strategy for studying the localization, morphology, and elemental composition of calcium deposits in plants. They also learn basic microscopic techniques, digital photography for recording experimental results, and the integration of plant anatomy and physiology with chemistry and physics. The activities proposed require use of and coordination between a plant biology laboratory and an electron microscopy facility. The rapid and efficient method of isolation described uses common laboratory equipment. Few alternative methodologies, such as: light and polarization microscopy, SEM, Energy Disperse X-ray Analysis (EDX), histochemical detection *in situ* and chemical staining of isolated calcium deposits are suggested in determining the structure and the chemical compositions of the deposits. Students are asked to take pictures and write a detailed laboratory report. These activities encourage students to adopt interdisciplinary and team approaches, useful in their future activities in the plant biology and other laboratories.

Introduction

Biomineralization is widespread among microorganisms, plants and animals. Common plant minerals are calcium oxalate, calcium carbonate and silicon dioxide. Calcium oxalate is the most abundant and widespread mineral deposit found in over 240 gymnosperm and angiosperm families. Calcium carbonate is common in a limited number of flowering plants, while silicon dioxide is common in grasses and a limited number of other plant families (Arnott and Pautard, 1970). Calcium oxalate and calcium carbonate deposits form within the vacuoles of specialized cells called crystal idioblasts, which thus play an important role in the calcium metabolism of plants. The diversity of mineral deposit shapes and sizes, their tissue distribution and prevalence have led to a number of hypotheses regarding their functions in plants, such as: calcium sink, calcium detoxification, plant defense, light gathering and reflection, tissue support, etc. (Franceschi and Horner, 1980; Franceschi, 1989; Bradbury and Nixon, 1998; Webb, 1999; Nakata and McConn, 2000).

Although biomineralization in plants and fungi have been known and studied for so long, little is known about the formation of crystals and other deposits. Therefore, the need for improving our understanding for such an important biological process should be reemphasized. Despite the prevalence and biological significance of calcium biomineralization in plants, only a few plant biology laboratory manuals mention the presence of calcium crystals and none of them offer structured laboratory activities to study calcium deposits in detail. We have developed a three-hour interdisciplinary laboratory during which students isolate, study the morphology and determine the chemical composition of calcium deposits in leaves.

Students are asked to develop their own strategy for studying biomineralization in their choice of plants. They also learn digital photography for recording experimental results, and come in contact with the integration of plant anatomy and physiology with chemistry and physics. Basic chemistry, physics, and microscopy concepts, as well as the calcium cycle in plants, oxalate metabolism, and the ecophysiological significance of biomineralization in plants can be discussed with students during the suggested experiments. These activities designed to around a study of calcium biomineralization in plants, and to encourage students to adopt interdisciplinary and team approaches, useful in their future activities in the plant biology and other laboratories. By employing plant species in which biomineralization has not previously been (carefully) studied, students have the opportunity to discover new information. Furthermore they may continue their study as undergraduate research participants, and later they may even publish their findings, enhancing our knowledge of biomineralization in plants.

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Plant Material and Equipment

The following list of plants known to contain mineral deposits is proposed for this laboratory. Leaves are used in general for the proposed plant species, but other organs can be employed, such as germinated seeds for bean, for example. In addition, students should be encouraged to study their own choice of experimental plants and plant organs.

Bean, *Phaseolus vulgaris*
Pecan, *Carya illinoiensis*
Begonia, *Begonia* sp.
Persimmon, *Diospyros* sp.
Ginkgo, *Ginkgo biloba*
Redbud, *Cercis canadensis*
Grape, *Vitis mustangensis* or *V. vinifera*
Rose, *Rosa* sp.
Mulberry, *Morus* sp.
Water Hyacinth, *Eichhornia crasipes*
Okra, *Hibiscus esculentus*

For the isolation of the mineral deposits, the following equipment and chemicals are necessary: blenders, glass beakers, cheese cloth, ethanol, watch glasses or micro spot plates (Fisher Scientific, catalog no. 21-379), stereoscopes, Pasteur pipettes with bulbs, and glass vials with caps. SEM analysis requires stubs, preferably carbon ones if EDAX is attempted. For light microscopy of fresh plant material, the basic microscopy materials and equipment is necessary: microscopes, razor blades, slides and cover slips, water, pipettes. Microscopes equipped with crossed polarizers and digital cameras are necessary for the students to be able to perform polarizing light microscopy and take pictures for their reports. The following staining techniques can be used with both fresh plant material and isolated mineral deposits for histochemical analyses: silver nitrate (Sugimura *et al.*, 1999), periodic acid – Schiff's (PAS) (Sigma Procedure No. 395, catalog no. 395-B), potassium iodine, toluidine blue, etc.

Laboratory Setting and Activities

Working teams are composed of 2-4 students. Although the same plant species can be used for all teams, students are encouraged to bring their choice of plant species to compare results. A typical schedule of the laboratory activities is as follows:

1) Students start by examining the fresh plant material under the light microscope for the presence, tissue location, and morphology of the mineral deposits (15 minutes).
2) They will then continue their study by following the protocol for the isolation of mineral deposits (30-45 min). The rapid and efficient method described for this step is described in detail in Fig. 1B.
3) After isolating calcium deposits, students may choose activities appropriate to their laboratory circumstances (see Suggested Activities diagram in Fig. 1A) in order to determine the structure and chemical composition of the deposits. They can use a few alternative methodologies, such as: light and polarization microscopy of leaf mounts and/or isolated deposits, SEM, (EDX) and backscattered electron imaging of isolated deposits. The later methods require use of and coordination between a plant biology laboratory and an electron microscopy facility. Histochemical detection *in situ* and chemical staining of isolated calcium deposits can be attempted as well.
4) Students are asked to take light microscopy and SEM pictures and write a detailed laboratory report.

In general, most groups will carry out polarization microscopy, while only a few may perform SEM, and EDAX of isolated deposits in approximately 2-h time period. Histochemical detection *in situ* takes more than one lab period, therefore it can either be performed over two lab periods, be suggested for a final course project, or as a continuation of the lab as undergraduate research by some students. Histochemical detection of calcium carbonate deposits in green leaves with silver nitrate should be performed only after the leaves are cleared (Ilarslan *et al.*, 1997). Leaf primordia in closed buds do not need clearing since they do not contain large amounts of chlorophyll.

An example of light microscopy of fresh sections of water hyacinth leaf is shown on the cover (middle picture). A raphide is visible under crossed polarizers in the aerenchyma. Also on the cover are a section through Ginkgo leaf (top picture) showing a file of small druses as well as larger ones, and isolated raphides from water hyacinth (bottom picture) under crossed polarizers. Examples of isolated deposits under SEM are provided in Fig. 2. A calcium map showing the disposition of calcium in isolated raphides from grapevine leaves and an EDAX spectrum are shown in Fig. 3.

This laboratory should be of much interest and utility to instructors ranging from high-school to college levels, who are looking for a way to interest students in plant anatomy and cell biology. Participation in this laboratory should be of special interest to many instructors, since most have not had a personal opportunity to isolate and study plant mineral deposits.

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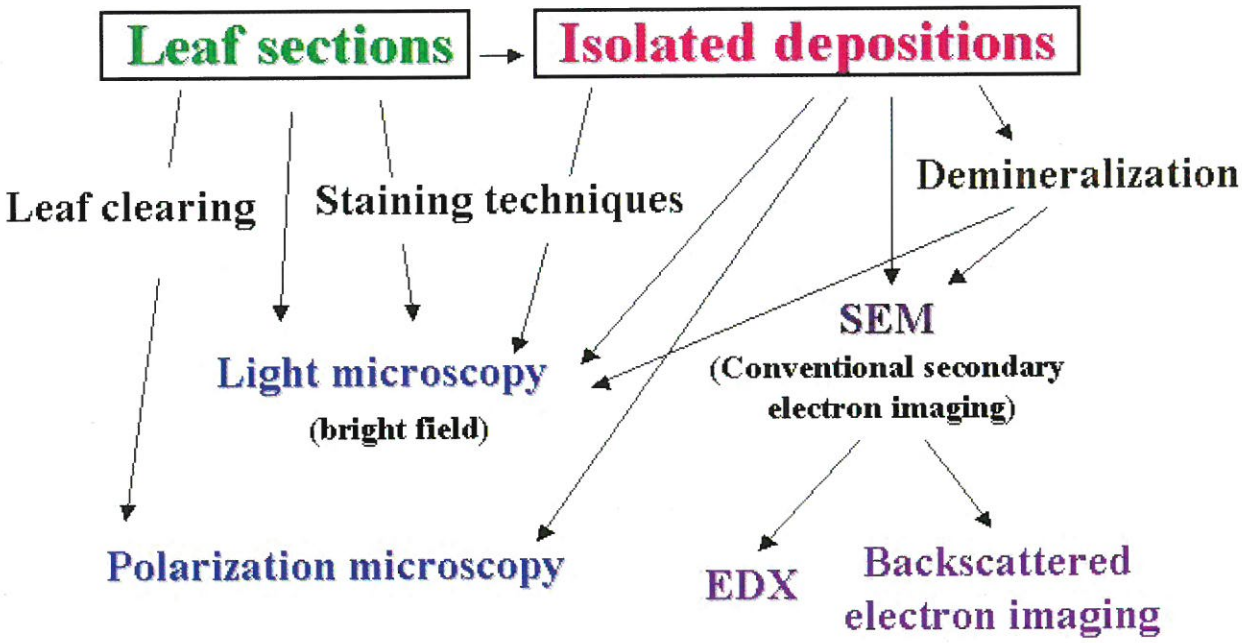
Figure 1 – Suggested activities for a 3-h laboratory and other studies on plant biomineralization (A) and detailed protocol for the isolation of plant mineral depositions (B).

A

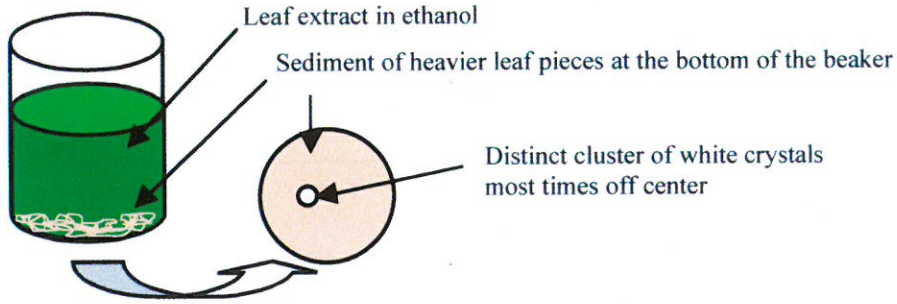
Suggested Activities



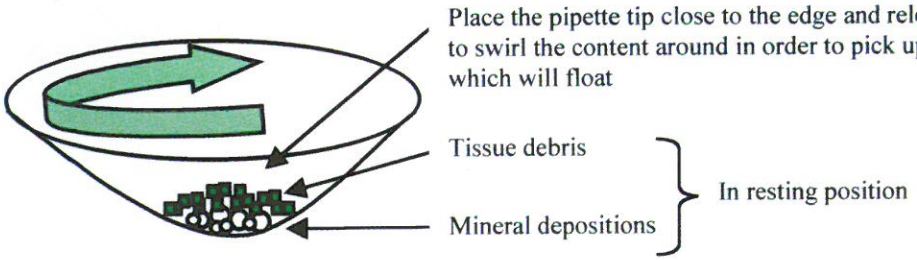
B

Protocol for Isolation of Mineral Depositions from Plant Tissues

For the purpose of this lab, we adopted the method described in Webb *et al.*, 1995 for the isolation of mineral depositions in leaf tissue. In short, after being washed and blotted dry, 20-40 leaves are extracted in 250-300 ml absolute ethanol by grinding them in a blender for approximately 5 minutes. The extract are strained through 8 layers of cheesecloth in a glass beaker and the cluster of isolated mineral depositions is located in the sediment at the bottom of the beaker by gently swirling the content of the beaker.



"Cleaning" of isolated mineral depositions can be done in a watch glass, but better off in a micro spot plate under a stereoscope by repeatedly washing a small sample of mineral depositions with ethanol. The washing procedure by sucking-releasing the ethanol with a Pasteur pipette separates the mineral depositions from tissue and cell debris.



The cleaned crystals can then be taken into a glass vial with cap for storage and/or further cleaning and analyses.

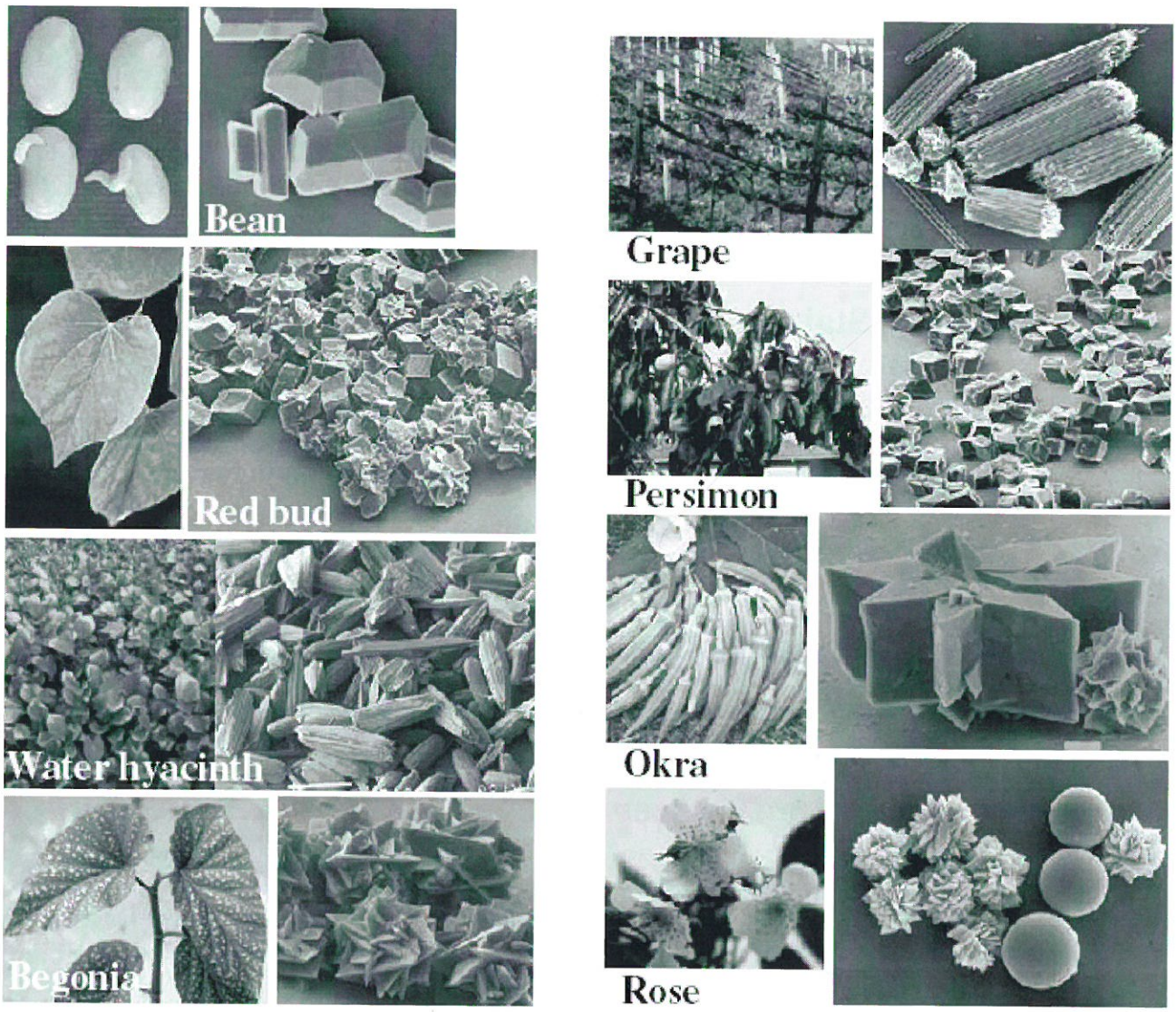


Figure 2 - Examples of isolated crystals and depositions under SEM. Grape and water hyacinth contain raphides, crystals in bundles of several hundred per cell, thought to function in defense against herbivory (Bradbury and Nixon, 1998). Bean, persimmon, and redbud contain mostly prismatic crystals of calcium oxalate. Okra, rose, redbud, and begonia contain druses of calcium oxalate. Rose also exhibits spherical depositions.

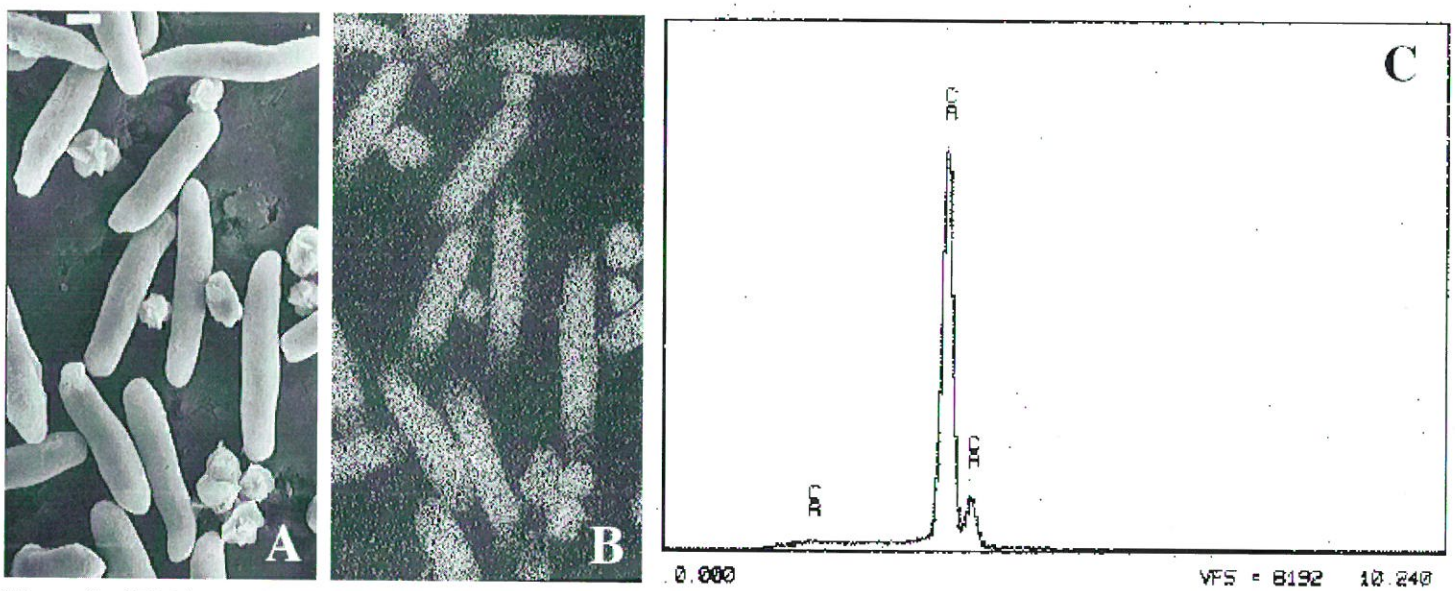


Figure 3 - SEM image (conventional secondary electron imaging) of isolated raphides in grape (*Vitis mustangensis*) (A) and elemental dot map for calcium (B). Calcium can also be detected by using EDAX (C).

Meeting Memories

April 2002 TSM meeting in Fort Worth. Jo Taylor (bottom row), Bob Droleskey (upper row, left), Alice Stacey, Pam Neill, Becky Holdford, Ann Rushing, and Robbie Roberson, invited speaker from Arizona State University, in downtown Fort Worth.

Spring 2001 meeting in Houston. Howard J. Arnott (in the middle) with Jean, his wife and Scott Russell, President of the Botanical Society of America, invited speaker at the time.

Banquet picture at TSM meeting in Forth Worth, spring 2002. Ann Rushing and Sam Ho, Baylor University, to the right and Rumpa and Monica Ghosh, wife and daughter of Nabarun Ghosh, TSM Treasurer, to the left.

Executive Meeting at April 2002 TSM meeting in Fort Worth. From left – Camelia Maier, Journal Editor; Don Smith, Past President; and Mike Crowley, Corporate Member Representative.

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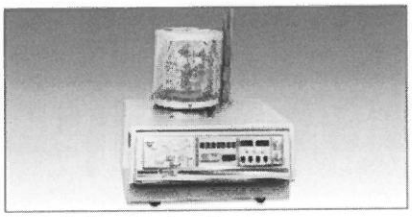

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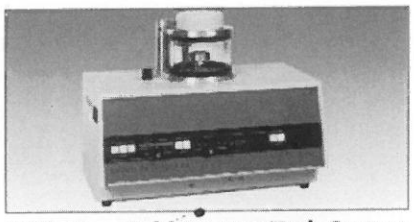

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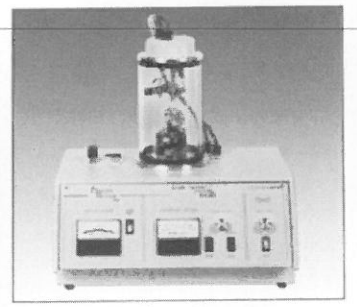

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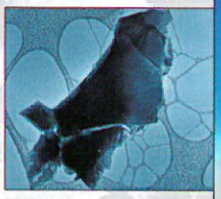

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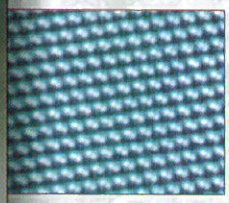

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