TSM

Texas Journal of Microscopy

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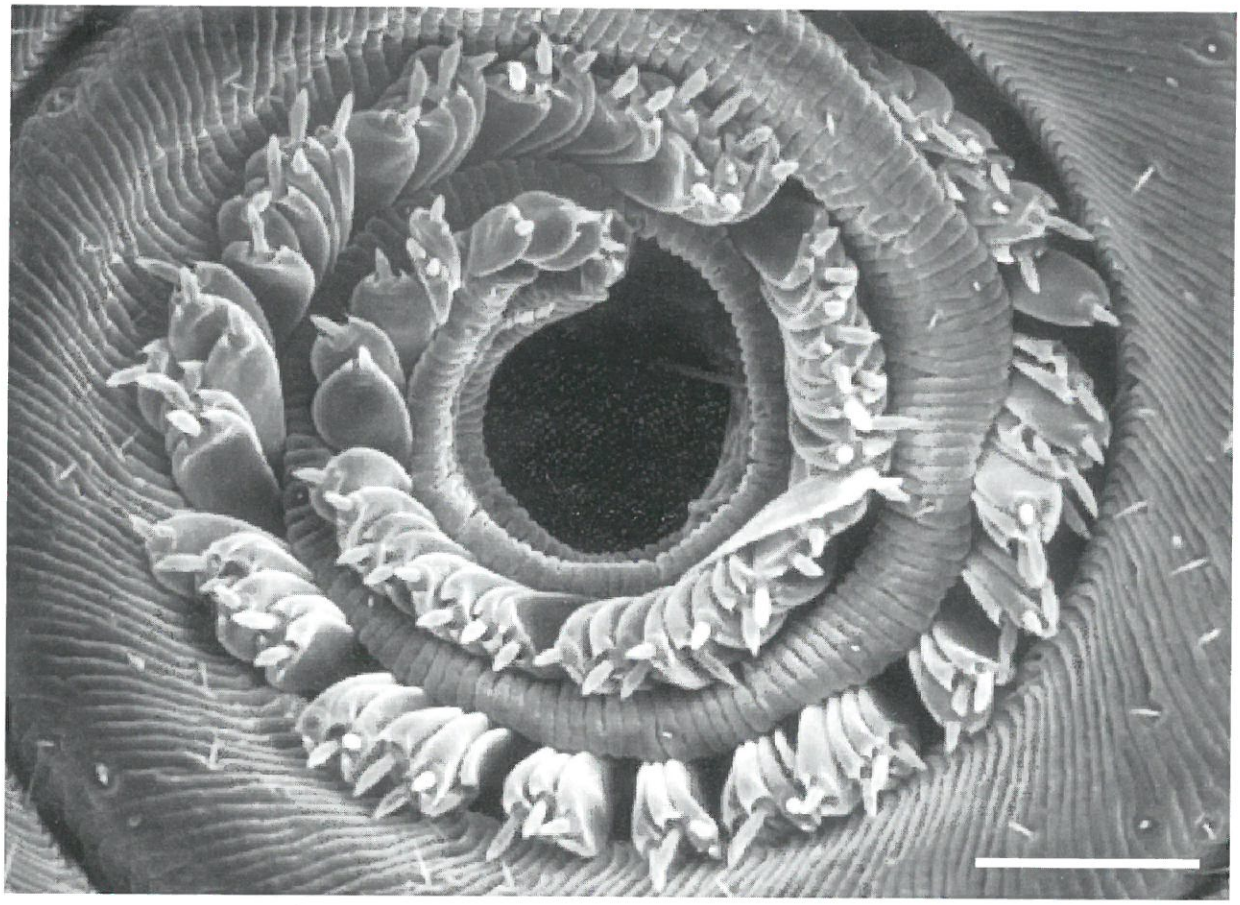

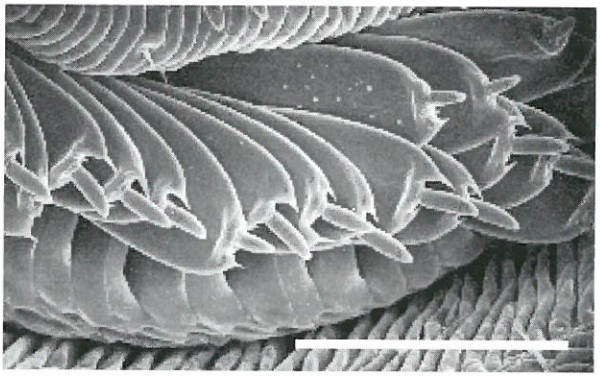

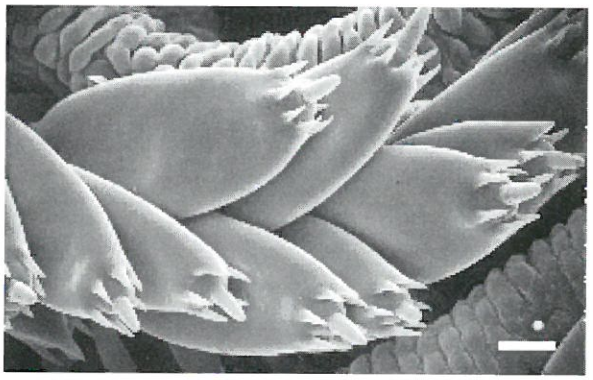

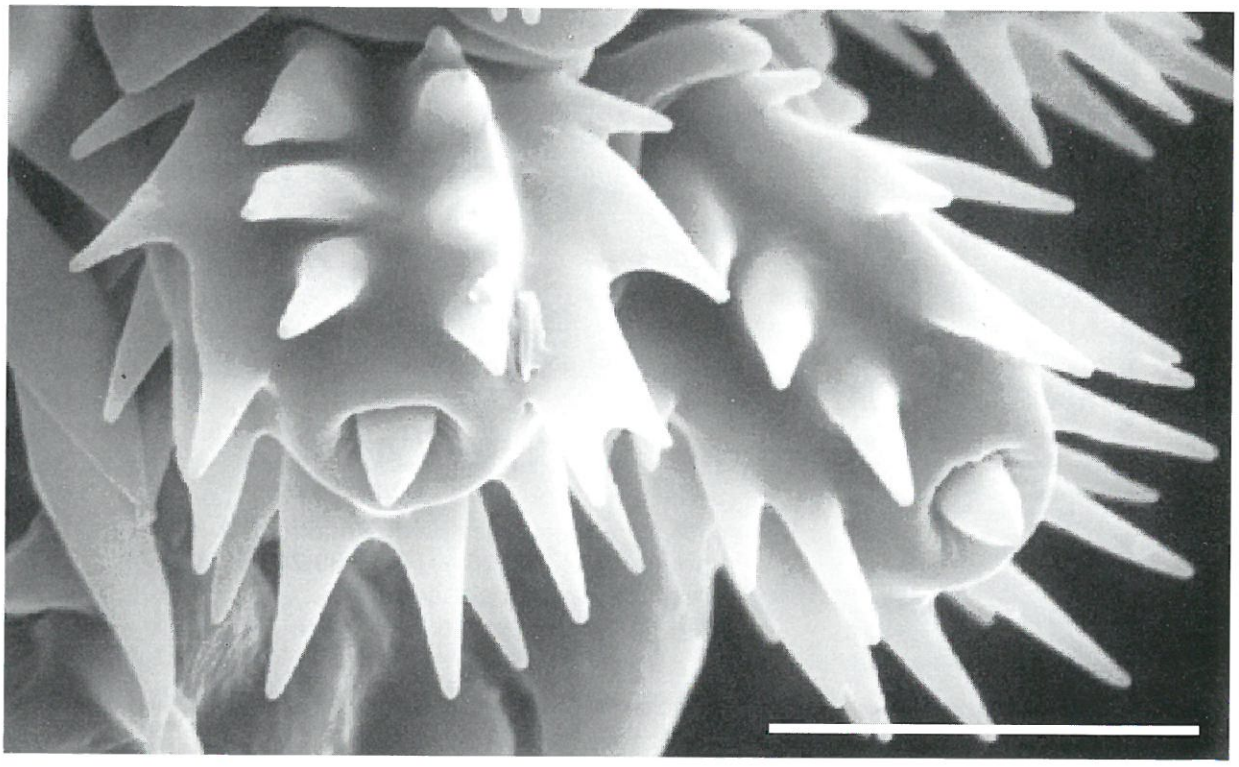

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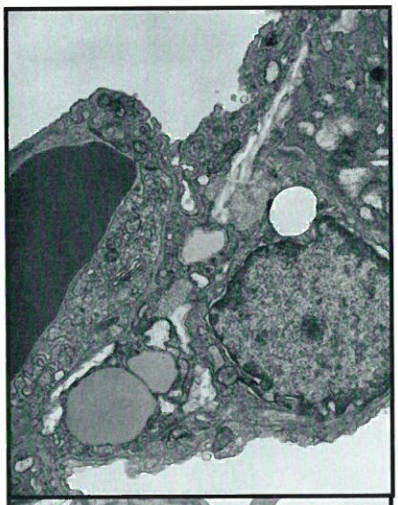

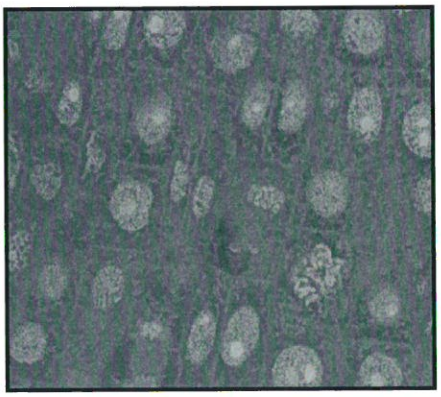

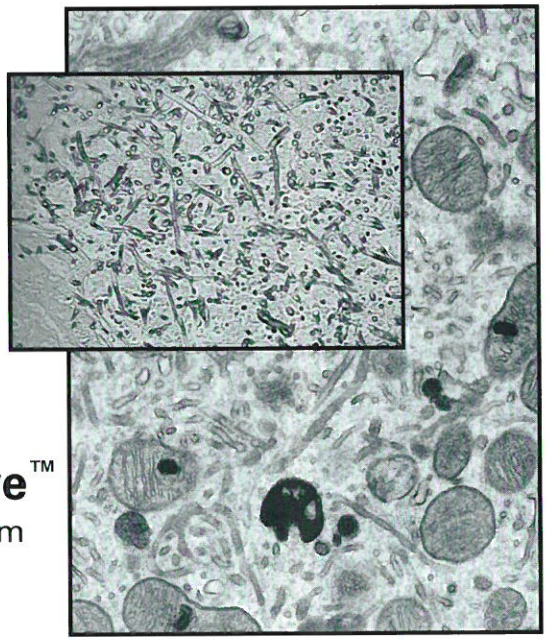

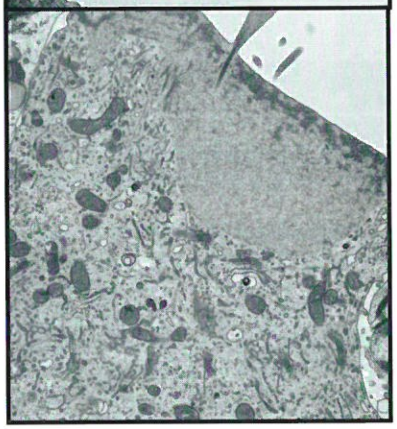

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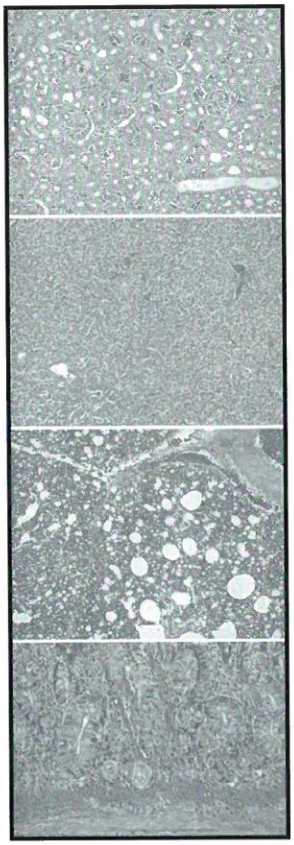

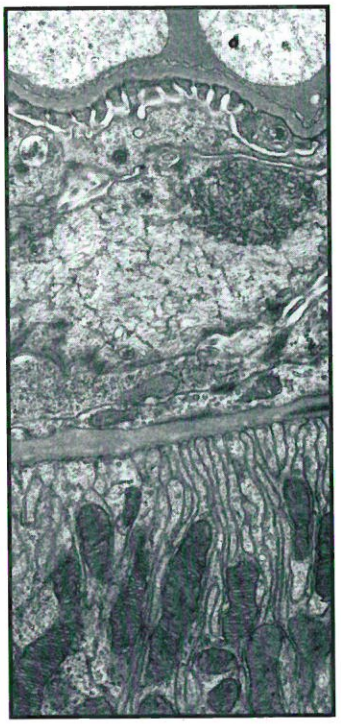

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TEXAS JOURNAL OF MICROSCOPY
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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

The four images represent specialized sensory structures on the butterfly proboscis. Top picture represents the coiled-up distal portion of a nymphalid butterfly proboscis showing densely packed sensilla styloconica. These chemo-mechanoreceptors - "taste buds / tactile sensors" are characteristically found in most nymphalids on both sides of the tongue tip near the center of the coil. This micrograph was obtained from a hybrid specimen, *Limenitis arthemis* x *L. lorquini* - White Admiral x Lorquin's Admiral (Nymphalidae: Limenitidinae). Bar = 100 µm. The right picture on the second row represents sensilla styloconica at the proboscis tip of *Limenitis arthemis* or White Admiral butterfly (Nymphalidae: Limenitidinae). Sensory peg is flanked by two apical shoulder spines at the terminal end of the stylus. Bar = 100 µm. The picture to the right on the second row shows sensilla styloconica at the proboscis tip of *Nymphalis antiopa* or Mourning Cloak butterfly (Nymphalidae: Nymphalinae). Sensory peg appears to be protected by a crown of apical shoulder spines at the terminal end of the stylus. Bar = 10 µm. Bottom picture represents sensilla styloconica of *Gyrocheilus patrobas* or Red-bordered Satyr (Nymphalidae: Satyrinae). These sensilla are ornamented with serrated ridges adorned with many spines. Bar = 10 µm. All specimens were coated with gold or gold-palladium alloy in a Polaron Instruments E 5100 SEM coating unit and visualized with a JEOL JSM-T 300 scanning electron microscope at the UNT Electron Microscope Center. Images were obtained using Polaroid Type 55 P/N 4x5 sheet film, converted to digital images with the Microtek E6 ScanMaker and edited using Adobe PhotoShop 5.5 software. Daniel Petr, Department of Biology, Southwestern Adventist University, Keene, Texas 76059.

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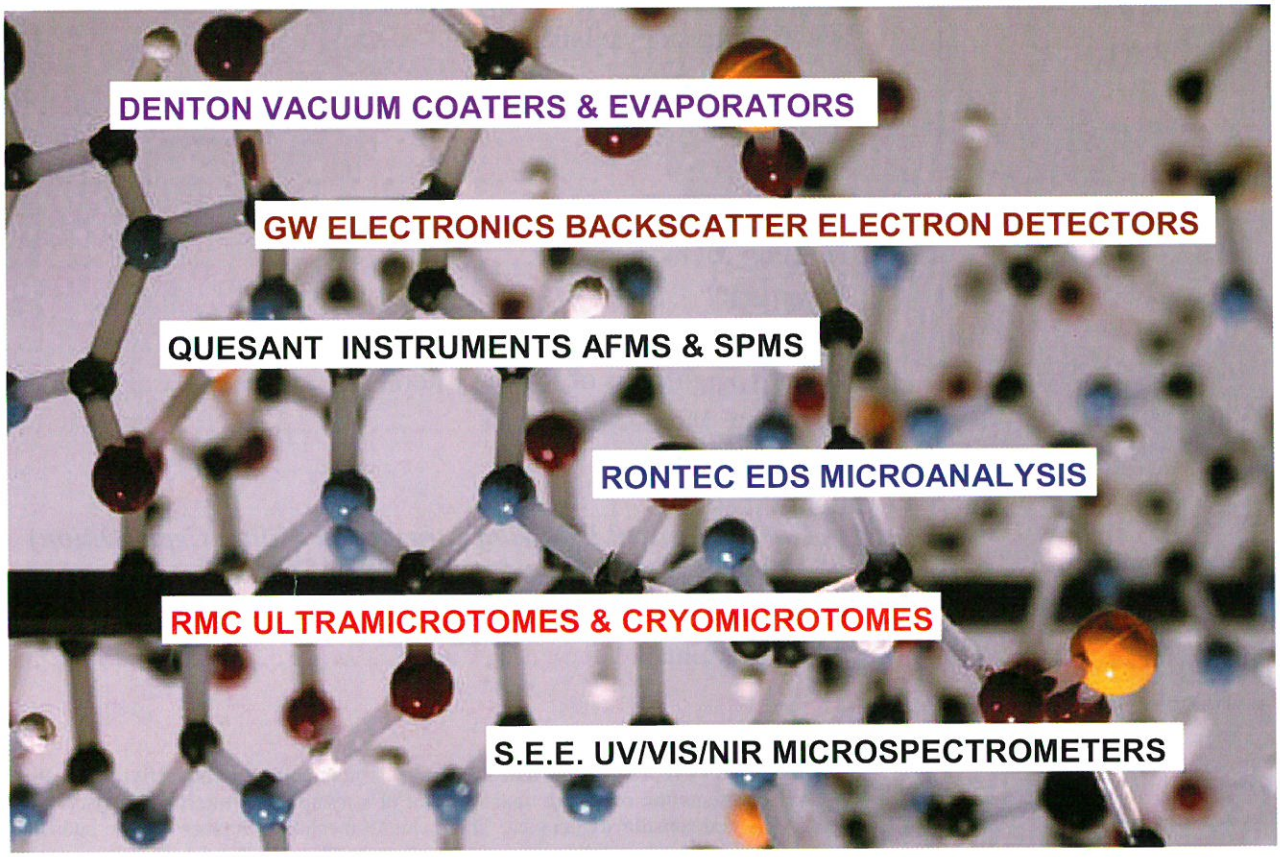

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President's Message

It has been almost a year since we last gathered to exchange ideas, present our research and learn from each other. It is good to see that many of the presentations scheduled for El Paso will be presented at the Ft. Worth meeting. I am especially looking forward to Dr. Chianelli's talk on the analysis and duplication of Mayan blue pigments. It is also comforting to see former regular and student members serve the Society in a different capacity. James Long will be making his first appearance representing Ted Pella. Following a cancellation of the scheduled workshop, former student members from Dr. Arnott's lab Mike Davis and Mike Johnson, now employed by Nikon, stepped up and put together a workshop with little lead time. Mike Crowley of Oxford Instruments will be joining the Executive Council as our new Corporate Representative replacing Cathy Ryan from Micro Star Technologies. Cathy thanks for a job well done.

The Texas Society for Microscopy has the advantage of a strong and committed core group of members but the overall membership rolls have decreased in recent years. The challenge facing the Society in the coming years is how to become more relevant to the professional development of our members and to the needs of the general community. We have always provided an excellent stage for the young researcher to gain experience with platform presentations, which is not often available at national meetings. We need to provide this same encouragement to those wishing to hone their workshop presentation skills. We can pull our vast knowledge from individual labs to provide a concentrated outreach presence. By bringing together individual parts of the Society to tackle a common goal a rejuvenated Society is possible.

There are several opportunities available to members of TSM that need to be discussed in the near future. If you are planning on attending the annual Microscopy & Microanalysis meeting in Quebec in August be sure and let Pam Neill know as she will be appointing our representative to Local Affiliated Societies meeting. It is also time to nominate candidates for the post of MSA Director of Local Affiliated Societies. Ev Osten the current LAS director and Local Arrangements Committee chair was scheduled to attend our Ft. Worth meeting to discuss the need for volunteers to serve on the LAC for Microscopy and Microanalysis 2003 in San Antonio but will be unable to attend. If you are interested in serving on this committee contact Ev Osten at efosten@mmm.com or contact me for additional details.

I would like to take this opportunity to thank all of the members that have provided valuable assistance and worked tirelessly during the past year. Without their help my job would have been impossible. It has been a privilege to serve TSM as President

Sincerely,

David C. Garrett
TSM President, 2001-2002

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:

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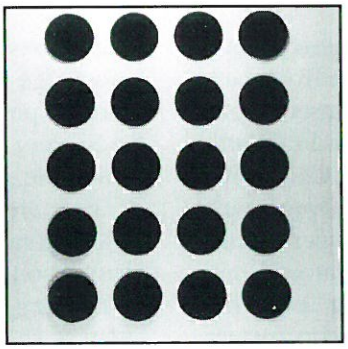

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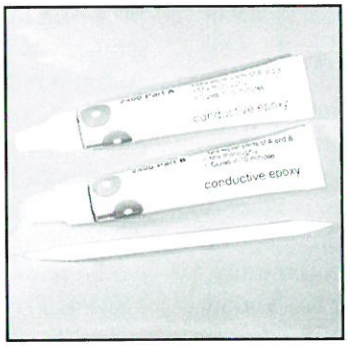

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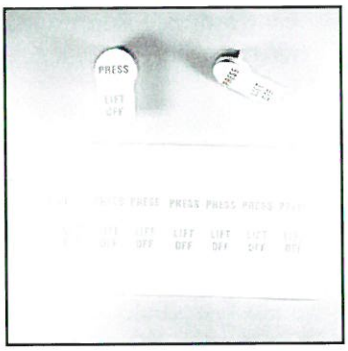

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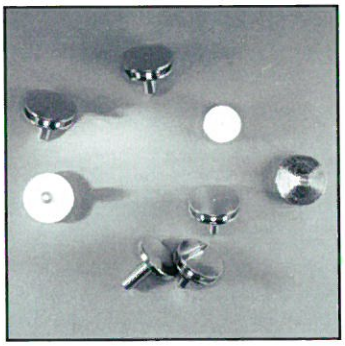

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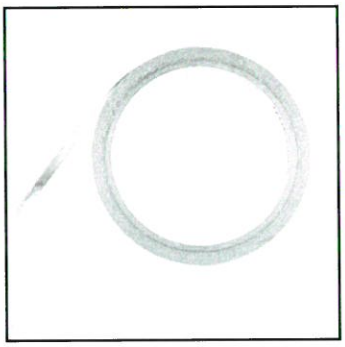

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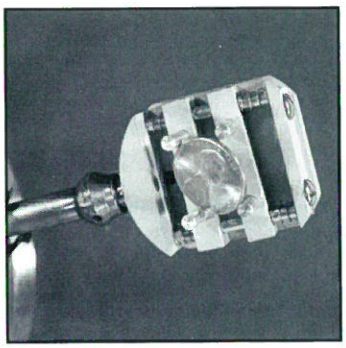

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ASSETS AS OF MARCH 31st, 2001:

Checking Account No. 005772227833 (Bank of America)	$2,738.42
Certificate of deposit No. 1882289323	$4,079.37
TOTAL	**$6817.79**

Income:

Dues	$995.00
Spring Meeting Registration (Houston)	$1295.00
Journal Advertisement Revenue (Vol. 32:1)	$1152.50
Journal Advertisement Revenue (Vol 32:2)	$500.00
Donations	$100.00
Checking Account Interest	$1.52
Total Income	**$4044.02**

Expense:

Journal Printing:	
32:1	$2218.08
Student Travel	$401.00
Secretary's Account	$75.00
Spring Meeting 2001 Expenses	$2683.75
Hospitality Expense	$56.66
Guest Speaker's Hotel Room Charge	$184.86
Past President's Plaque	$67.66
Postage	$47.65
Postal Permit Renewal	$125.00
Mailing & Office Expense	$107.68
Bond	$144.59
Checking Account Service Charge	$103.13
Total Expense	**$6215.06**

ASSETS AS OF DECEMBER 31st, 2001

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Total Income $1415.23

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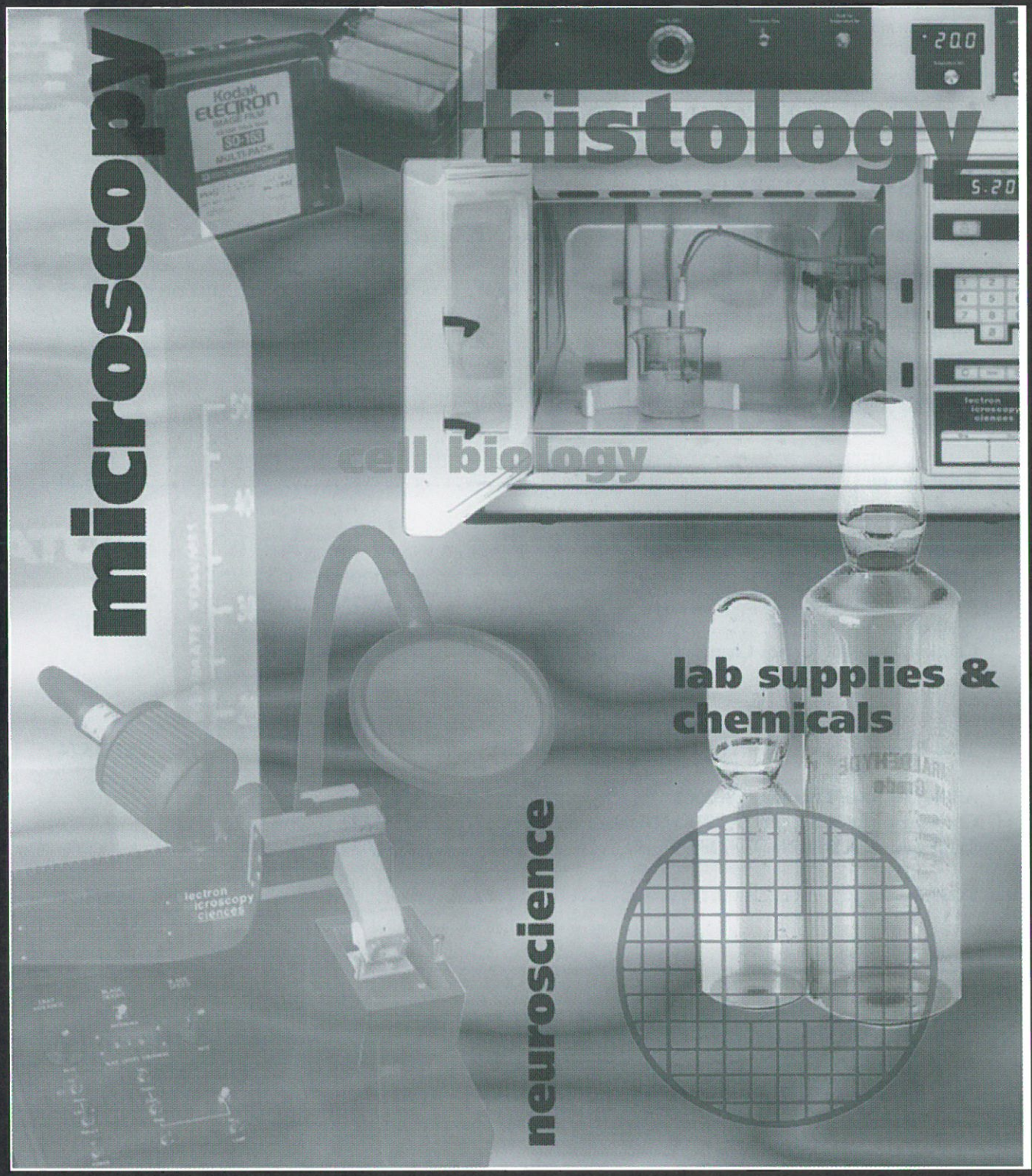

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Abstracts

BIOLOGICAL SCIENCES
FALL 2001 — SPRING 2002

LIGHT AND ELECTRON MICROSCOPY AS TOOLS FOR STUDYING CELL GROWTH AND MORPHOGENESIS IN FUNGI. R.W. ROBERSON, Dept of Plant Biology, Arizona State University, Tempe, AZ 85287

Fungi progress through significant morphological stages during development. This study will illustrate selected morphological milestones (e.g., cell (hyphal) growth, spore formation) utilizing live cell imaging (e.g., video enhance contrast, laser confocal) and electron microscope (e.g., cryopreparation, 3-D analysis) methods to determine and/or better understand the organization and function of the tubulin cytoskeleton. Of particular interest is the behavior of a unique fungal organelle, the Spitzenkorper, during cell morphogenesis. The role of the Spitzenkörper in cell growth and morphogenesis has long been enigmatic. As a microtubule-organizing center in some fungi, the Spitzenkorper is directly involved in regulating the nucleation and distribution of cytoplasmic microtubules and thus, specific cytoplasmic functions. These observations, along with analysis of cytoskeletal deficient mutants, will form the basis for discussion of the roles of tubulins in fungal cell growth and morphogenesis.

COMPARATIVE MORPHOLOGY OF SENSILLA STYLOCONICA ON THE PROBOSCIS OF NORTH AMERICAN NYMPHALIDAE AND OTHER SELECTED TAXA (LEPIDOPTERA): SYSTEMATIC AND ECOLOGICAL CONSIDERATIONS. D. PETR AND K. W. STEWART, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Sensilla styloconica on the proboscis of 107 species of North American and tropical butterflies were comparatively studied using the scanning electron microscope. Focus was on 76 species of North American Nymphalidae representing 45 genera and 11 subfamilies. Nomenclature for generalized and specific types of nymphalid sensilla is proposed. Written descriptions and micrographs are presented for each species studied. Morphological features were generally consistent for all or most species within genera and sometimes within subfamilies, with specified exceptions.

Statistical analysis revealed significant differences for six of eight variables tested between two distinct feeding guilds of North American Nymphalidae. Average number, density, extent of proboscis coverage with sensilla, their total length, and shoulder spine length were all significantly greater in the non-nectar feeding guild than in nectar feeders, and may indicate adaptation for greater efficiency in feeding on flat surfaces. The greater frequency of apical shoulder spines in non-nectar feeders may represent adaptation for protection of sensory pegs from mechanical abrasion during feeding, or for anchoring the flexible proboscis tip to the surface. Sensilla styloconica in nymphalid butterflies appear to function as extensions that provide greater sensory reach during feeding. The role of these sensilla in liquid uptake, pollen feeding, and host plant selection is discussed.

The presence of sensilla styloconica in all subfamilies of Nymphalidae, except Danainae, largely supports Ehrlich's (1958) higher classification concept for the family. The presence of only sensilla basiconica in the Danainae, and other characteristics are presented as further evidence that they should be reconsidered for full family status.

BIOMINERALIZATION IN DIOECIOUS MULBERRY, *Morus* sp. *(Moraceae).* DIEDRE L. SHEPARD, DAVID C. GARRETT, HOWARD J. ARNOTT, NATHANIEL MILLS, AND CAMELIA G.-A. MAIER. Department of Biology, Texas Woman's University, Denton, Texas 76204 (DLS, NM, CGAM), Department of Biological Sciences, University of North Texas, Denton, Texas 76203 (DCG), Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019 (HJA).

Continued interest in sexual dimorphism of dioecious plants as part of ongoing phytoestrogen research lead us to compare developmental morphology, quantitative tissue localization, and chemical composition of calcium depositions in male *vs.* female plants of two mulberry species, *Morus alba* and *Morus rubra (Moraceae)*. Previous work on this project included observation of crystal distribution, analysis of prismatic crystals using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX), and histochemical detection of calcium carbonate depositions in leaf primordia in winter buds. In this research, we continued studying the morphology, distribution, and chemical structure of calcium depositions in mature leaves and summer buds. For both species, cystoliths appeared to reside in the upper epidermis and upper mesophyll in the midsection of both male and female leaves. Cystoliths were not evident in buds developed during summer months, suggesting that they are formed later in the leaf development. At least three types of calcium depositions were found, including prismatic crystals, druses, and lithocysts. Calcium depositions were isolated from mature leaves by breaking plant cells in ethanol and their morphology will be analyzed under polarized light and SEM. EDX along with histochemical analysis will be used to determine the chemical composition of calcium depositions. Comparison of soluble and insoluble oxalate in relationship to sex and species will be accomplished using a Sigma Urinalysis Diagnostic Kit. This study will allow evaluation of sexual dimorphism and allocation of resources in the dioecious species of mulberry.

BACTERIOLYTIC ACTIVITY FROM A CONTINUOUS FLOW CULTURE CHEMOSTAT CONTAINED WITHIN CONCENTRATED 0.22μm FILTERED CHEMOSTAT CONTENTS. R. E. DROLESKEY, K. M. BISCHOFF, R. B. HARVEY and D. J. NISBET, USDA, ARS, Southern Plains Agricultural Research Center, College Station, TX 77845

Fluid from a characterized continuous flow culture chemostat containing 29 microorganisms was subjected to a low speed centrifugation, 4000 x g, followed by sequential filtration through 0.45 and 0.22μm membranes. The sterilized chemostat fluid was then centrifuged for 3h at 150,000 x g, washed in buffer and re-centrifuged. Pellets were either re-suspended in buffer or fixed for ultrastructural evaluation by the addition of a glutaraldehyde fixative. Examination of glutaraldehyde preserved pellets revealed numerous single membrane-bounded vesicles of between 50-100nm in diameter. Vesicles varied with regard to contents and membrane electron density. Re-suspended vesicles were utilized in a bacterial killing assay in which isolates of *Salmonella typhimurium* and *Escherichia coli* were incubated with microvesicle suspensions. Bacteria incubated with microvesicle suspensions exhibited a 2-log drop in colony forming units per ml (CFU/ml) within two hours of inoculation while no reduction in CFU/ml was observed in control preparations. These results suggest that the isolated microvesicles are bacteriolytic towards some enteropathogenic bacteria. Bacteriolytic vesicles present in the culture probably result from extrusions of outer membrane from Gram negative bacteria present in the culture.

THALLUS GROWTH AND BRANCHING OF THE LICHEN *RAMALINA STENOSPORA*. TIFFANY B. FOWLER and ANN E. RUSHING, Department of Biology, Texas Lutheran University, Seguin, TX 78155 and Department of Biology, Baylor University, Waco, TX 76798.

The fruiticose lichen *Ramalina stenospora* is characterized by an erect, highly branched thallus. Growth of the thallus is by apical extension of spherical meristem initials, or growing regions, comprised mainly of fungal hyphae. Branching of the thallus is by a combination of dichotomous divisions of these apical growing regions and by the lateral formation of new spherical initials. The first visible sign of branching at the apex of the thallus is a slight enlargement of the spherical meristem followed by the formation of a centrally located indentation or crevice that divides the meristem into two nearly equal units. Each new meristem gives rise by apical extension to a narrow thallus branch. Lateral branching results from the outward protrusion and subsequent extension of a portion of the thallus margin. After this initial extension, the meristems of lateral branches become spherical and are characterized by dichotomous divisions similar

to those of main thallus branches. Based on these observations, *R. stenospora* displays evolutionarily advanced features such as the small, spherical meristem and the extensive branching. *Ramalina stenospora* is similar in overall branching pattern to the closely related species *R. willei* although *R. stenospora* has more extensive lateral branching. In comparison, *R. celastri*, with it's broadly ovoid meristem and broad thallus that rarely branches is considered to be a more primitive species.

MICROSCOPY OBSERVATIONS ON CARBOHYDRATE AND PROTEIN IN PEPPER FRUIT. R. GOMEZ, R. I. RUIZ and A. KUANG, Dept. of Biology, The University of Texas – Pan American, Edinburg, TX 78539.

Capsicum annuum is a very important and valuable crop. Different varieties are grown for vegetables, spices, and condiments. *C. annuum* is a great source of vitamins, especially vitamins A and C. Its fruits also contain carbohydrate and protein. This study will illustrate microscopic observations on carbohydrate and protein present in young and ripened pepper fruits. Pericarps of pepper fruits at different developmental stages are fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer. Fixed pericarps are embedded in L. R. White resin and sectioned with a Leica microtome. Sections are stained with Periodic acid – Schiff's reagent for carbohydrate and Aniline Blue Black for protein. The results will provide additional information on nutrients in pepper fruit. Supported by NASA grant NAG2-1375 and Student Financial Aid Office.

STORAGE RESERVES IN GREEN PEPPER (*CAPSICUM ANNUUM*) SEEDS. R. I. RUIZ AND A. KUANG, Dept. of Biology, The University of Texas – Pan American, Edinburg, TX 78539

Storage reserves in seeds play an important role during early development in plants. The deposition of major reserves such as protein, starch, and lipids during seed development is a critical process that determines seed quality. The present study investigates reserves deposited in green pepper (*Capsicum annuum*) seeds at late developmental stages. Seeds are fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1M phosphate buffer. Fixed seeds, after ethanol dehydration, are infiltrated and embedded in L. R. White resin. Seeds then are sectioned and stained for carbohydrate, protein, and lipids. Selected seeds are post-fixed in 1% buffered osmium tetroxide and processed for electron microscopy observation. Preliminary study shows that seeds mainly contain starch at an earlier developmental stage and protein, in the form of protein bodies, at maturity. A detailed study in the deposition of reserves during seed development is in progress. The results will provide unique information on seed nutrients since available literature merely highlights the nutrients of pepper fruits (pericarp). Supported by NASA grant NAG2-1375 and The University of Texas-Pan American Graduate Office.

CALCIUM OXALATE CRYSTAL DISTRIBUTION AND DEVELOPMENT IN THE SECONDARY PHLOEM OF SALIX NIGRA. PHILLIP WELLS AND HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The black willow (Salix nigra Marsh.) is common along waterways in north Texas. It grows very rapidly and produces a substantial tree in a relatively short time. The specimens we are studying are growing in a limestone-based soil. Hence calcium is relatively abundant in the soil solution and this leads to large deposits of calcium oxalate crystals in the plant. The leaves, secondary phloem and cork are the sites of crystal deposits. In the leaves twin prismatic crystals of calcium oxalate are abundant both in the mesophyll and associated with veins. In the secondary phloem crystal containing cells are laid down in discreet tangential rows separated radially by non-crystal containing cell layers. In the tangential plane the crystal cells form "boxes" consisting of five to six cells which are separated from each other by phloem ray cells. In each crystal cell a twin prismatic "kinked" crystal is found. Because these crystals are highly birefringent we expect them to be calcium oxalate monohydrate, however we are checking that by x-ray diffraction studies. The calcium oxalate crystals within their crystal cell walls are stable for rather long times and we have found them in wood attached by fungi and in other decay processes they are still present. The development of alternating layers of calcium oxalate crystal cells in the secondary phloem appears to be a rather complicated developmental sequence as yet not well understood. In most living plant cells the concentration of calcium ions must be kept to a low level (usually 10-7 molar) and it is believed that the formation of calcium oxalate crystals represents a mechanism for marinating the low concentration of calcium in the cell sap. This detoxification is maintained as long as the tree is living by the continued formation of new calcium oxalate crystal. In terms of the calcium concentration in the soil solution it is important to note that the phloem crystals maintain their integrity for a substantial time, especially in dead branches and decaying stems. In terms of individual plants the loss of the leaves and the outer bark represent ways of removing the calcium stored as calcium oxalate from the tree.

CALCIUM OXALATE CRYSTAL DISTRIBUTION IN THE LEAVES AND STEMS OF SCHINUS MOLLE SEEDLINGS. HOWARD J. ARNOTT AND PHILLIP WELLS, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The California Pepper Tree (Schinus molle L.) is common cultivar in California and Arizona although it is native to Peru. It grows rapidly and produces a robust tree in a relatively short time; in many cases individual trees develop into long-lived specimens which live to be over one hundred years of age. These trees produce the pink peppercorn of commerce. The specimens currently under study are two year old plants growing in the UTA greenhouse. These young plants developed from seeds obtained from the Carter Seed Co. Vista, California. The seeds germinated after being soaked in very hot water to break dormancy. We have examined the leaves and stems using light, polarization and scanning electron microscopy. In the pinna of these pinnately compound leaves the majority of calcium oxalate deposits occur in association with veins. A series of crystal idioblasts are associated with veins and each cell produces a single druse. The druse crystals are actually multiple-interpenetrant crystals of calcium oxalate monohydrate and can be isolated using techniques previously described. Each druse appears to occupy about two thirds of the volume of the living cells that contain them. The druse cells are packed together in about four to five files directly over the vein. In addition to the crystal idioblasts that are associated with the veins there are "nests" of druse containing idioblasts found in a random distribution pattern in the inter-vein portion of the leaf. The stems of S. molle have exceptionally large pith surrounded by primary and secondary xylem with large vessels, but calcium oxalate deposits are not found in any of these tissues. Two year old stems still have the cortex and epidermis in tact. The cortex contains several latex canals, which have a structure similar to those reported in the flowers and seeds. A few druses can be found in the secondary phloem along with a ring of highly birefringent fibers.

CHEMICAL SIGNALING IN THE HACKBERRY BUTTERFLY (ASTEROCAMPA CELTIS). MARTHA I. GRACEY AND HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019.

Chemical signaling in insects provides a large and diverse field for study. There is already a substantial body of research on this subject and it continues to be a focus in many laboratories. Agriculturists study insect pheromones in order to boost crop production, increase crop yield and profits from their crops. They even pit insects against one another by making the chemicals produced by the insects into insect repellants and natural herbicides. Direction, location, and over-all safety can be determined by the insect through the use of pheromones. Sensory organs for olfaction and thermal regulation located on the antennae and wings of butterflies provide the insect with critical data about the surrounding area. Their detection repertoire includes searching for suitable foods, mates and nesting plants; they can even detect repellants. It seems obvious that the structure of antennae will play a great part in these abilities. In order to learn about their structure, the antennae of the Hackberry butterfly were air dried and examined under the scanning electron microscope. Many sensory hairs, scales and pores are present on the surface of the antennae. The scales and pores provide an avenue through which the pheromones can affect the associated nerve bundles; as the scales and pores cover the antenna, the detection process can give the insect input similar to a global positioning system. Using this information the insect has an opportunity to fly toward or away from a region depending on the chemical "inhaled." A careful examination of the location of scales and pores on the antenna will give us information, which can support or reject the positioning hypothesis.

CALCIUM OXALATE CRYSTAL DISTRIBUTION IN BRISTLECONE PINE (PINUS LONGAEVA) LITTER FROM MOUNT. WASHINGTON, NV. LORI EARLEY, HOWARD J. ARNOTT AND CATHERINE ARNOTT-THORNTON, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Mount Washington is a peak in the South Snake Range of Eastern Nevada. The mountain is formed mostly by a "large block of limestone" that has been uplifted and thus is different from the quartzite substrate found

on Wheeler Peak. Both mountains support substantial stands of bristlecone pines (Pinus longaeva D.K.Bailey). Part of Mount Washington is a part of Great Basin National Park and two of us (HJA and CA-T) received permission to collect litter samples from the park land. Six collections of litter were made between 3,080 and 3,390 m. The summit of Mount Washington is at 3,553m and is above timberline. We have begun to sample the litter from these collections using light and electron microscopy combined with efforts to culture the fungi found in the litter. For this presentation we will focus on one of the six collections, namely: Arnott 597, collected at 3,390m with GPS readings of longitude 114°18' 51"W and latitude 38°54' 49"N. The collection site consisted of a large isolated bristlecone pine tree with abundant litter under its low-lying branches. This tree, although not affected by fire, was within 200m of an area recently burned. Pieces of the leaf litter were examined under the scanning electron microscope and many bi-pyramidal and plate-like calcium oxalate crystals were observed in direct association with fungal hyphae. The plate-like crystals formed on top of one another so as to give these particular crystals a very intricate, rose-like appearance. In order to determine which fungi produced these crystals, small pieces of leaf litter were placed directly on agar plates to encourage fungal hyphae growth and spore production. Once the different types of fungi were identified, the actual culture that was grown on the agar plate was critical point dried and observed under the scanning electron microscope. By looking at isolated and identified fungi, we hope to determine which fungus is producing calcium oxalate crystals or if different species of fungi produce different types and/or shapes of calcium oxalate crystals.

A STUDY OF CASTS FROM EPOXY EMBEDDED EGGSHELL OF WHITE LEGHORN CHICKENS. SANDRA L. WESTMORELAND. The 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.

VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN ATHEROSCLEROTIC PLAQUE AND ARTERIALIZED VEIN GRAFT. GLENN C. HUNTER, *ANN S. BURKE, XIANG YING XUE, SCOTT D. LICK, *ROBERT A. COX, HAL K. HAWKINS. UTMB, Depts. of Vascular Surgery and Pathology, Galveston, TX 77555, *Shriners Hospital for Children, Dept. of Electron Microscopy, Galveston, TX 77550

Vascular endothelial growth factor (VEGF) is implicated in the pathogenesis of atherosclerosis and restenosis: in addition it has a potential therapeutic role in promoting angiogenesis in skeletal and cardiac muscle. To define the role of VEGF in plaque evolution, we analyzed 20 atherosclerotic plaques and 20 saphenous vein grafts.

All specimens were evaluated with immunohistochemistry and in situ hybridization to localized VEGF and its receptors (FLT-1 and FLK-1). Cell specific localization was determined using antibodies against macrophages (CD68), smooth muscle cells (a-actin), endothelial cells (Factor VIII related-antigen) and cell proliferation (PCNA). The presence of VEGF m-RNA was confirmed using in situ hybridization. RT PCR was used to identify the different isoforms of VEGF.

VEGF expression was detected in microvascular endothelial cells (ECs), macrophages and smooth muscle cells (SMCs) in complex plaque. Normal veins showed diffuse staining for VEGF, where as, in stenotic veins, staining was limited to adventitial microvessels and SMCs and ECs in areas of endothelial thickening. Both receptors were identified in macrophages, ECs and SMCs in complex lesions and vein grafts. The FLT-1 receptor was more widely distributed than the FLK-1 (KDR) receptor in atherosclerotic tissue and in vein grafts. RT PCR demonstrated increased expression of all isoforms (121, 165, 189) in diseased arterial specimens compared to arterialized and normal vein.

All 3 VEGF isoforms are present in atherosclerotic plaque and normal and arterialized vein grafts but their expression is variable. The predominance of FLT-1 vs. FLK-1 receptors may account for the absence of proliferative activity in arterialized and stenotic vein grafts and atherosclerotic lesions.

MATERIALS SCIENCES

FALL 2001

THERMAL PRECIPITATOR FOR THE COLLECTION OF SUBMICRON ATMOSPHERIC PARTICULATE MATTER (PM) FOR TRANSMISSION ELECTRON MICROSCOPY EXAMINATION. JOHN J. BANG, E.A. TRILLO, L.E. MURR. Environmental Science and Engineering, Metallurgical and Material Engineering. The University of Texas at El Paso.

A device called a thermal precipitator (TP) was designed and utilized in order to determine its potential applicability for the collection of representative particulate matter in the air, especially those whose aerodynamic diameter less than 1 micron (PM1.0), with subsequent transmission electron microscopy (TEM) analysis. In this study, we were particularly interested in the TP's capability of collecting individual PM that are believed to exert harmful effects on the human respiratory system. After a calibration process, field tests were performed under different weather conditions, locations, and time frames. TEM, selected area electron diffraction (SAED), and electron energy dispersive X-Ray spectrometry (EDS) analyses were performed on individual samples and chemical species were analyzed. During this investigation, individual air PM with different sizes ranging from 10 microns to 10 nanometers for TEM analysis were collected. However, the detection limit may go down to the level of a few nanometers. Two interesting observations were made: 1) a large fraction of collected particulates were complex aggregates of very small particles and 2) a large fraction of the collected particulates were crystalline or polycrystalline. Specifically, two particle compositions, iron silicon boride (Fe5SiB2) and lithium silicon nitride oxide (Li9SiN3O2), from the air samples were identified during this preliminary study. Thus, this study has demonstrated, by utilization of the TP and TEM, the potential for collecting individual air particulate matter in the nanometer scale range, in addition to identifying the components via combined analyses of TEM, SAED, and EDS. This research was supported by Mr. & Mrs. MacIntosh Murchison Endowment and funds provided to UTEP by the Texas Tobacco Industry Settlement.

CHARACTERIZATION OF IMPACT CRATERING OF SOFT 1100 ALUMINUM THROUGH OPTICAL AND TRANSMISSION ELECTRON MICROSCOPY. V.S. HERNANDEZ, O.L. VALERIO, AND L.E. MURR. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968

The projectile/target behavior and crater formation of soft 1100 aluminum (r = 2.7 g/cm3) impacted by stainless steel (SS) (r= 7.8 g/cm3), soda lime glass (slg) (r= 2.2 g/cm3) and tungsten carbide (WC) (r= 15 g/cm3) 3.175 mm diameter sphere projectiles at velocities ranging from 0.5 km/s to 3.99 km/s, has been studied by light optical microscopy, transmission electron microscopy (TEM), and microhardness testing. Dense WC projectiles created anomalously elongated craters at impact velocities above 1 km/s, while slg projectiles showed an ideally hemispherical shape as a consequence of projectile/target density ratio ((rp/rt)1/2) similarities. In addition, crater depth/crater diameter ratio (p/Dc) anomalies were more pronounced at lower impact velocities with higher projectile densities.

The microstructure, observed by metallography and TEM, surrounding the crater walls is characterized by a dynamic recrystallization zone. This recrystallized zone facilitates the material flow necessary for the cratering

process. The width of this zone increases with the impact velocity and corresponding shock pressures. The microstructure was correlated to residual microhardness mappings. Research supported in part by NASA-MURED Grant NAG- 9-1171 and NASA Grant NAG-9-1100 from NASA Johnson Space Center, Houston TX.

THE EFFECT OF SOLUTIONIZING TIME ON THE AGING BEHAVIOR OF 7075 ALUMINUM ALLOY AND ITS COMPOSITE CONTAINING 10% VOLUME FRACTION OF ALUMINA PARTICLES. E. V. ESQUIVEL and S. K. VARMA, Metallurgical and Materials Engineering Department, The University of Texas at El Paso, El Paso, TX 79968-0520

Metal matrix composites are considered advanced engineering materials that are finding use in an increasing array of applications. Higher strength-to-weight ratios, increased wear resistance, and improved strength make the composite material more attractive over the conventional unreinforced metal (or alloy). Aluminum and aluminum based alloys are the most commonly used matrix material due to its inherent high strength-to-weight ratio. An additional advantage, strength-wise, of some aluminum alloys is the age hardening response.

The 7075 aluminum alloy is among the age-hardenable series that has been used as the matrix in this study with a 10% volume fraction of alumina particles as the reinforcement. It has been observed that an increase in solutionizing time for the 7075 aluminum alloy causes a decrease in the time required to achieve peak hardness (TPH), but this behavior is not observed in the 7075 composite. Hardness plots will be presented to observe the TPH dependence, or lack of, with solutionizing time. Optical and transmission electron micrographs will be shown to observe microstructural changes that might contribute to the deviant behavior of the composite from the un-reinforced alloy. This project has been sponsored by the NASA-MURED project NAG9-1171.

MATERIAL AND NUMERICAL ANALYSIS OF SPHERICAL Al 2024 PROJECTILES IMPACTING LIMESTONE TARGETS. R. M. FENOMANANA, S. LAIR, L. E. MURR, S. QUIÑOÑES. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, TX 79968

Three spherical 2024Al projectiles having each an original diameter of 19.5mm were impacted into limestone targets at velocities ranging from 0.80 to 1.30 km/s. Conventional microscopic analysis on these samples show void formations at the region diametrically opposed to the impact surface. Transmission electron microscopy (TEM) was performed on the deformed edge and far away from the impacting surface at the highest velocity. The deformed edge region had dislocation cells in very small grains and dense dislocation structures in the non-deformed region. Microhardness tests were also performed to map the residual yield stress of the samples. These experimental results were compared with those resulting from the AUTODYN simulations.

The purpose of this research is to initiate these experimental results so that the ultimate shapes and yield stress maps of spherical Al 2024 projectiles having velocities out of the experimental velocity range can be predicted. This research is supported by the Institute of International Education (IIE), the Fulbright Scholarship, and a NASA research grant (NAG9-1171).

TOOL WEAR DURING FRICTION STIR WELDING OF AL METAL MATRIX COMPOSITES. D.J. SHINDO, R.A. PRADO, K.F. SOTO, A.R. RIVERA, L.E. MURR. Metallurgical & Materials Engineering Dept., The University of Texas at El Paso, El Paso, TX 79968

Friction stir welding (FSW) is a method that has been practiced since 1991 and produces a better quality weld than other conventional methods (i.e. fusion welding). FSW can be used for a variety of applications in automotive, marine, construction and other industries. The technique involves joining two pieces of metal, and the weld is created in the solid state with no melting. The current research focuses on tool wear during the welding of a Al 6061 T6 + 20% Al2O3 particles, and a system of Al A339 + 20% SiC. Both materials are metal matrix composites (MMCs).

The tool steel being used in this project is an oil hardening, AISI O-1 tool steel. The rate of tool wear was determined using optical macrographs. The rotational speed of the tool pin was 500, 1000, 1500, 2000 and 2500 rpm for the Al 6061 MMC, with a traverse speed of 60 mm/min.

Optical microscopy was used to examine the microstructure of the weld zone confirming the solid phase nature of the technique; the microstructure shows a characteristic dynamic recrystallization phenomena. The rotating tool makes contact with the material creating frictional heat and forging a bond between the two plates of Al alloy. Transmission electron microscopy was also performed on the Al 6061 T6 + 20% Al2O3 system, revealing the internal microstructure. This research was funded by General Services Administration Grant PF-90-018.

ANALYSIS OF STAINLESS STEEL AND IRON LOW VELOCITY IMPACT CRATERS. ANDRES. A. BUJANDA, NATALIE. E. MARTINEZ, ELIZABETH. A. TRILLO, LAWRENCE.E. MURR, Department of Metallurgical and Materials Engineering, The University of Texas, El Paso, TX 79968

Low Earth Orbit (LEO) is becoming increasingly crowded due to greater and greater numbers of satellites and probes and there is an inherently greater risk of existing debris striking and damaging these valuable and necessary instruments. Most research has been conducted on hyper-velocity (5 km/s or greater) impacts with focus on the relationship between the density of the projectile and that of the target material. However, it has been discovered that low velocity impacts produce a curious "hump" in the P/Dc (depth to diameter) measurements on Cu and Al targets. This research analyzes the effects of low velocity impacts on systems of equal target and projectile density in stainless steel and Fe materials.

Impacted stainless steel and Fe targets were analyzed using optical, scanning electron and transmission electron microscopy. The geometry of each crater was measured to give a depth to diameter ratio (P/Dc). These ratios indicate hemispherical crater geometry. Optical microscopy reveals a larger area of deformation in the Fe samples than in the stainless steel. Dynamically recrystallized regions were observed at the bottoms of the craters followed by a heavily deformed region in both target metals. Deformation twins were observed in the Fe while stainless steel formed microbands. This research was funded by NASA MURED Grant NAG-9-1171 and NASA Grant-NAG-9-1100.

MICROSTRUCTURAL EVALUATION OF TITANIUM-TANTALUM ALLOYS FOR BIOMEDICAL CONSIDERATION. G. GONZALEZ, C.R. ORTIZ, R. VILLA, A.S. TAPIA, E. TRILLO, S.W. STAFFORD, L.E. MURR, Metallurgical and Materials Engineering Department, University of Texas at El Paso

Titanium-tantalum alloys are currently being considered for biomedical applications. These alloys exhibit high strength and excellent corrosion resistance when heat-treated. Ti40Ta and Ti50Ta are being studied as an alternative to Ti6Al4V, a popular biomedical material that has been shown to release toxic metal ions. Aging treatments on Ti40Ta were performed at 400°C for 20, 30, 40, 50, and 100 hours in an attempt to find an ideal heat treatment. Unlike the Ti50Ta, the Ti40Ta samples did not show a very large increase in hardness. In addition, the corrosion tests performed on these materials revealed very good corrosion resistance in comparison with the Ti6Al4V standard. Overall the microstructural analysis conducted through optical and transmission electron microscopy demonstrates a variation of martensitic characteristics. This research is funded by the General Services Administration Grant GSA - #PF-90-018.

HARDENING EFFECTS DUE TO SURFACE ALTERATIONS ON THE SKIRT PORTION OF A CARIBBEAN STEEL DRUM. NATALIE. E. MARTINEZ, ANDRES A. BUJANDA, NORMA PADILLA, ELIZABETH. A. TRILLO, LAWRENCE. E. MURR, Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX, 79968.

Previous experiments on the steel drum have uncovered many different aspects of the drum's manufacturing process that result in its characteristic sound. However, these experiments have concentrated on the head of the drum, the portion where most fabrication processes occur. This research has examined the effects of peening deformation on the skirt portion of the steel drum in order to reveal possible advantages of this procedure to the drum's overall tonal quality. The drum skirt of a standard, undeformed 29-note lead pan was peened utilizing a hexagonal close-packed pattern. Vickers and dynamic hardness measurements were taken before and after peening and results show a general increase in skirt hardness. Acoustical analysis performed on the drum before and after peening using Digi-Design software programs, showed harmonic changes to all note spectra. After peening, the drum was Nitrogen heat-treated at 800°C for one hour. Dynamic hardness readings were taken of note regions and color spectrums of hardness regions were created. These hardness spectra show a general hardness gradient towards the center of the note, a trend that is opposite to an undeformed, untreated drum. This research was supported by the U.T.E.P. Pandemonium Steel drum Gift Fund.

EDUCATION
SPRING 2002

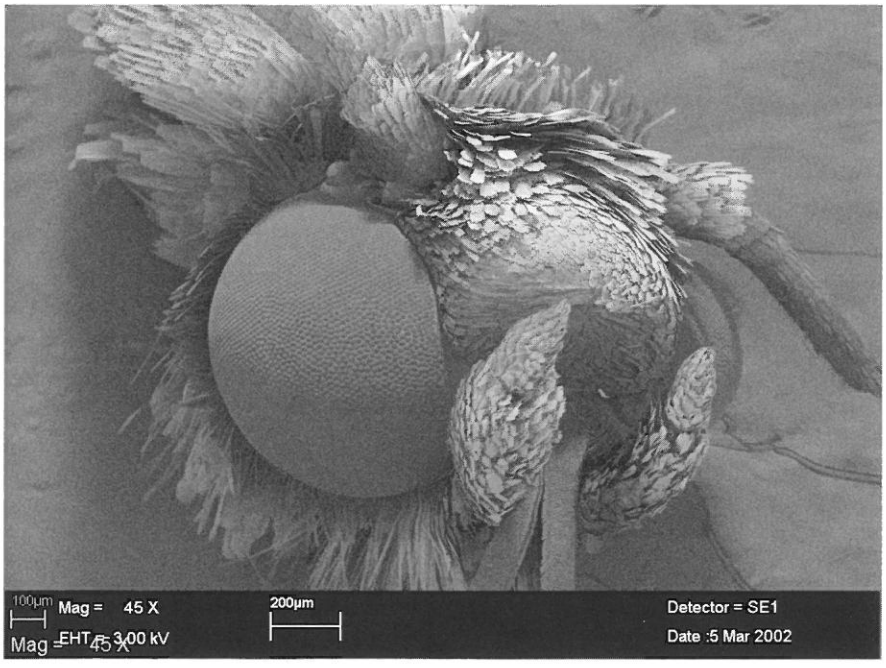
EXAMPLES OF A COMBINATION OF DIGITAL PHOTOMICROGRAPHY AND POWERPOINT PRESENTATIONS USED AS SUPPLEMENTS IN THE TEACHING OF GENERAL BOTANY AND PLANT ANATOMY. NATALIE E. HUBBARD AND HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Original micrographs of plant specimens were made for use as supplements in Botany laboratories with different levels of difficulty. The micrographs were made from selected specimens imaged using an Olympus Vanox microscope equipped with a Spot Digital Imaging System. Light microscopy was supplemented by Scanning electron Microscope images. All images were cropped, adjusted for brightness and contrast in PhotoShop and incorporated into PowerPoint presentations designed to enhance the materials available to students. As an example, for this presentation, we chose a laboratory that represents the broad area, namely the structure of stems and leaves as it would be presented in a General Botany Laboratory (course). The micrographs used for this exercise were made from permanent (often commercial) slides equivalent to those used by the students in their required study of the course materials. Definitions of basic terminology used in this laboratory lesson were added to the PowerPoint presentation. The second course we selected as an example is an advanced course in Plant Anatomy. In the laboratory described the topic discussed is the shoot apex. Because of the academic level of this course the presentation was made more specific and substantially more detail was given in comparison to that designed for the more general laboratory. Micrographs of shoot apices, taken from a research collection and from living material, were made in the same manner, however, different levels of magnification and more elaborate labeling was used in this presentation. Likewise the text accompanying this presentation is more robust. Both of these PowerPoint shows were designed for UTA students to view from the UTA Biology website via the internet as supplement their laboratory course work.

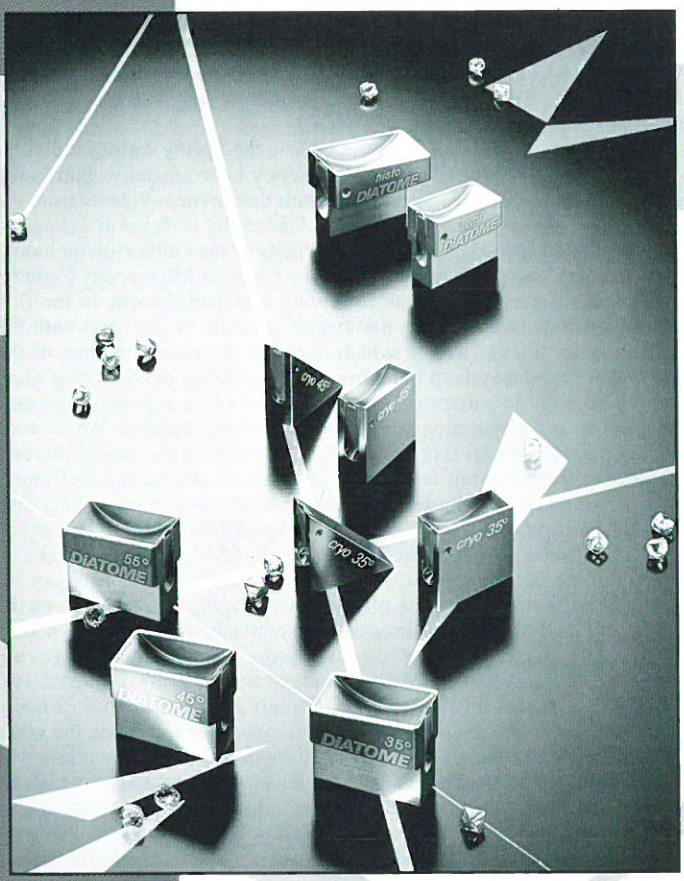
AN ELECTRON MICROSCOPIC LABORATORY CONTRIBUTION TO "YOUNG PEOPLE'S UNIVERSITY." HOWARD J. ARNOTT, MARTHA GRACEY AND LORI EARLEY, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Over the last 20 years on two weekends in the Spring various individuals from The Center for Electron Microscopy have annually contributed to "Young People's University," a program that invites students from the third through the sixth grade to visit The University of Texas at Arlington. Students attend special classes from all areas of the University including both Arts and Sciences. Over the years the Electron Microscopy Center's contribution has evolved to the following four-part format. In the first part all students (we limit the number of students to 12) meet with the instructors for a short lecture which explains the general format of the course and introduces them to microscopy in general and Scanning Electron Microscopy in particular. This session involves a power point presentation as well as questions and answers from the students. We are constantly amazed at the level of questions, which these grade school students can ask. A group portrait is taken at this time. In the second and fourth parts of the course the students are divided into two groups which take turns in the exercises. The third important part is a well-needed "coke" break. After separation, one group meets in the EM Lab and is introduced directly to the Scanning Microscope. Our demonstration consists of inserting a specimen, starting the microscope, imaging the specimen on the screen and capturing digital images on the attached computer. Students are allowed a chance to "drive" the microscope and additional images are captured. Magnification, brightness, contrast and focus are discussed with regard to the subject (often an insect). Usually a second and occasionally a third specimen are examined in the microscope. Meanwhile the other group is visiting the photo lab where they are introduced to the printing of electron micrographs. They learn about developers and fixation, paper's contrast and exposure times. They make and develop shadowgraph of objects and/or their hand. Students keep the prints they make and we provide them with copies of scanning micrographs. A labeled class portrait is given to them to mark their attendance.

OUR STUDENTS



The micrograph represents a head view of the beet armyworm, *Spodoptera exigua*, a destructive insect attacking a broad range of hosts including vegetables, weeds and agronomic crops, particularly cotton. If left unmanaged, beet armyworm populations could cause severe outbreaks and cost agricultural growers millions of dollars. Photographed with LEO VP435 by **Peter Carreon**, graduate student at **The University of Texas-Pan American**, Edinburg, Texas.



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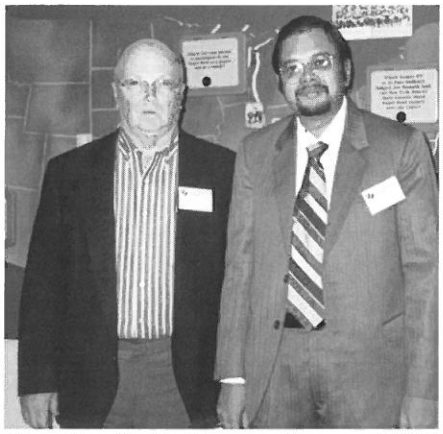
Meeting Memories

Banquet dinner at the 1997 TSM spring meeting in Fort Worth. David C. Garrett, TSM President (left), Jo Long with FEI Company, former Corporate Member Representative (middle) and Susan Robbins, Baylor College of Medicine (right). (Pictures by Nabarun Ghosh)



Drs. Louis H. Bragg, former TSM President and journal editor and Nabaruri Ghosh, Treasurer at the 1999 TSM spring meeting in Waco.

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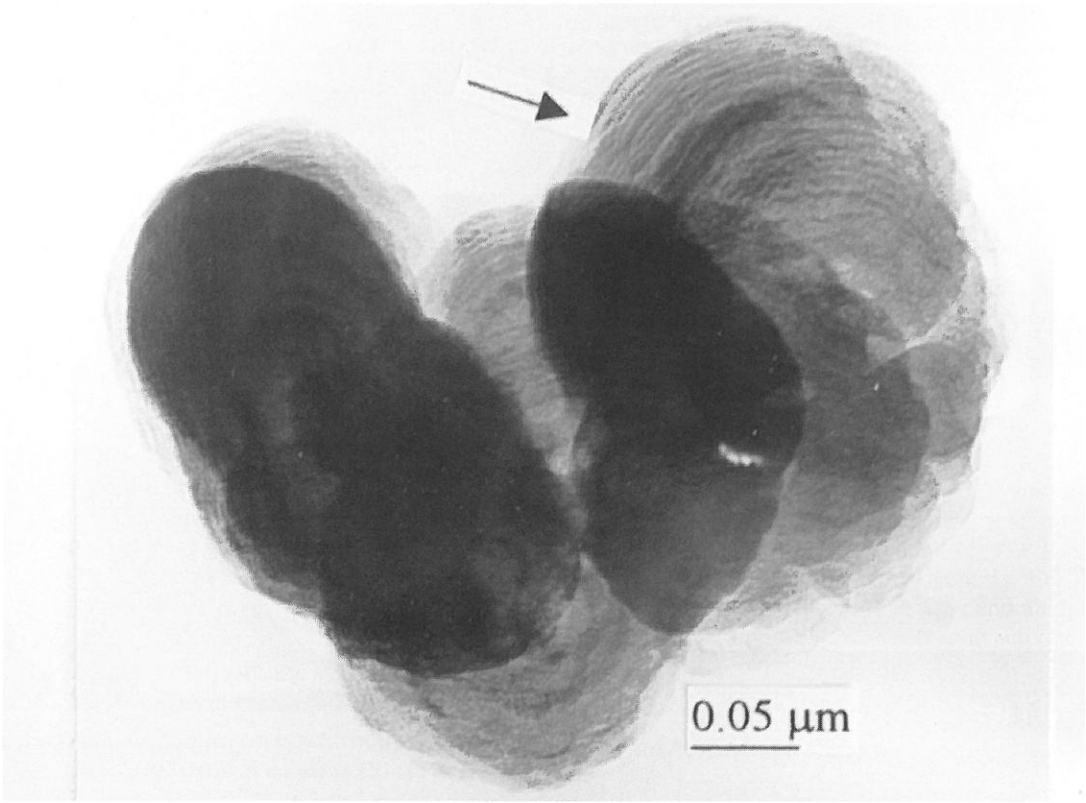

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THE CYTOLOGICAL EFFECTS OF ATRAZINE ON *ALLIUM CEPA* (ONION)

SAMANTHA USNICK[1], NABARUN GHOSH[1], and DON W. SMITH[2]

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For over 25 years, atrazine has been used as a selective broadleaf herbicide in many capacities, from preplant to pre-emergence to post-emergence, depending on the crop and application. Currently, 96% of all atrazine used is for commercial applications in fields for the control of broadleaf and grassy weeds in crops such as sorghum, corn, sugarcane, pineapple and for the control of undesirable weeds in rangeland. Atrazine is also a preferred treatment in reforestation projects in coniferous forests and Christmas tree plantations as well (HSDB). It has been estimated that eighty million pounds of atrazine are applied to soil crops annually in the U.S. Farmers prefer to use atrazine in place of other similar herbicides due to its versatility and effectiveness at a relatively economical cost. With the use of atrazine at such high levels in the U.S., environmental concerns are on the rise. Rainfall or irrigation shortly after atrazine application leads to runoff that can easily and quickly enters lakes, streams, and drinking water supplies. In Texas alone, atrazine has been detected in lakes and rivers at levels high enough to be considered a potential health threat to the public (TNRCC, 2001). Many panhandle wells have also detected atrazine in samples taken. In the early 1990s, the Texas Department of Agriculture sampled wells in eleven Texas counties and found "seventy five cases of pesticide-contaminated groundwater" (TNRCC, 2001). The pesticides detected in the study included atrazine, but at this time were not at levels high enough to be in violation of state pesticide regulations. The concern for the public is, of course, what will happen to concentrations over the long term with the popularity of atrazine increasing, and what impact atrazine levels will have on public health.

We investigated the effect of atrazine on the standard plant test system (Bhanja et. al '98), *Allium cepa* (onion). In a field situation, *A. cepa* would not be a target plant for the action of the chemical herbicide atrazine, since it affects largely broadleaf weeds. We established a control with the *Allium* bulbs grown on hydroponics culture. The control set was compared with the atrazine treated sets in terms of Mitotic Indices and cytological behavior with fixed intervals. The root tips were excised from the control set of bulbs and pre-treated them with saturated solution of para-Dichlorobenzene (p-DB) for 3 hours. After pre-treatment the root tips were washed with distilled water and fixed with 1:3 Aceto-ethanol for overnight. The fixed root tips were stained with 2% Aceto-Orcein solution and squashed in 45% acetic acid (Sharma and Sharma, 1980). A well-scattered somatic metaphase plate was selected for karyotypic analysis. 2-3 metaphase plates (Fig.1-3) were used to determine the karyotype of *A. cepa*. The karyotypic analysis revealed standard 2n=16 chromosomes.

The experimental treatment with atrazine followed the procedure of root tip squash preparation. *Allium* root tips were grown on hydroponics culture until growth was evident. At the phase of active growth they were placed in a solution of 1% atrazine. We excised the root tips from both the control and treated sets of bulbs After 24 hours; the root tips were fixed with 1:3 Aceto-ethanol and stained in an Aceto-orcein solution. We used the very tip meristematic portion of the tip (0.5mm) for the squash preparation. The Mitotic Index was determined by counting the total number of cells and the total number of dividing cells per microscopic field. We observed the cells at oil immersion lenses to analyze the chromosomal abnormalities from the squash preparations from the treated root tips. We captured digital images using a BX-40 Olympus microscope attached to the DVC Camera. We took the photographs of the specific types of chromosomal anomalies recorded at the particular dilution and interval.

There was a noticeable trend of decrease of Mitotic Index and increase in Abnormality Index in the treated sets. We observed many chromosomal anomalies including laggard chromosomes (Fig. 4, 10), diplochromatids (Fig. 9), fragmentation of chromosomes (Fig. 11), sticky metaphase, anaphase bridges (Fig. 5-7), polyploidy, and the formation of micronuclei (Fig. 8). The laggard chromosomes and sticky bridge formation were noticed at the 24 hour-interval after treatment with 1% Atrazine whereas fragmentations, diplochromatids and micronuclei formation were frequently recorded after 48 hours with the treatment of the same dosage. In this investigation we observed significant cytological effect of atrazine on the *Allium* test system.

References:

Bhanja, E., Mishra, J.R., Rath, S.P. (1998) Genotoxic effects of two organophosphorus insecticides, quinalphos and malathion on root meristem of *Allium cepa* L. *Advances in Plant Sciences*. Vol.11 (1): 279-284.

HSDB: *Hazardous Substances Data Bank*: Full Record. http://toxnet.nlm.nih.gov/cgi-bin/sis/search

Sharma, A. K. and Sharma A. (1980) *Chromosome Technique:* Theory and Practice. 3rd Ed. Butterworths, London, pp. 68-470.

TNRCC (2001). Texas Natural Resources and Conservation Coalition
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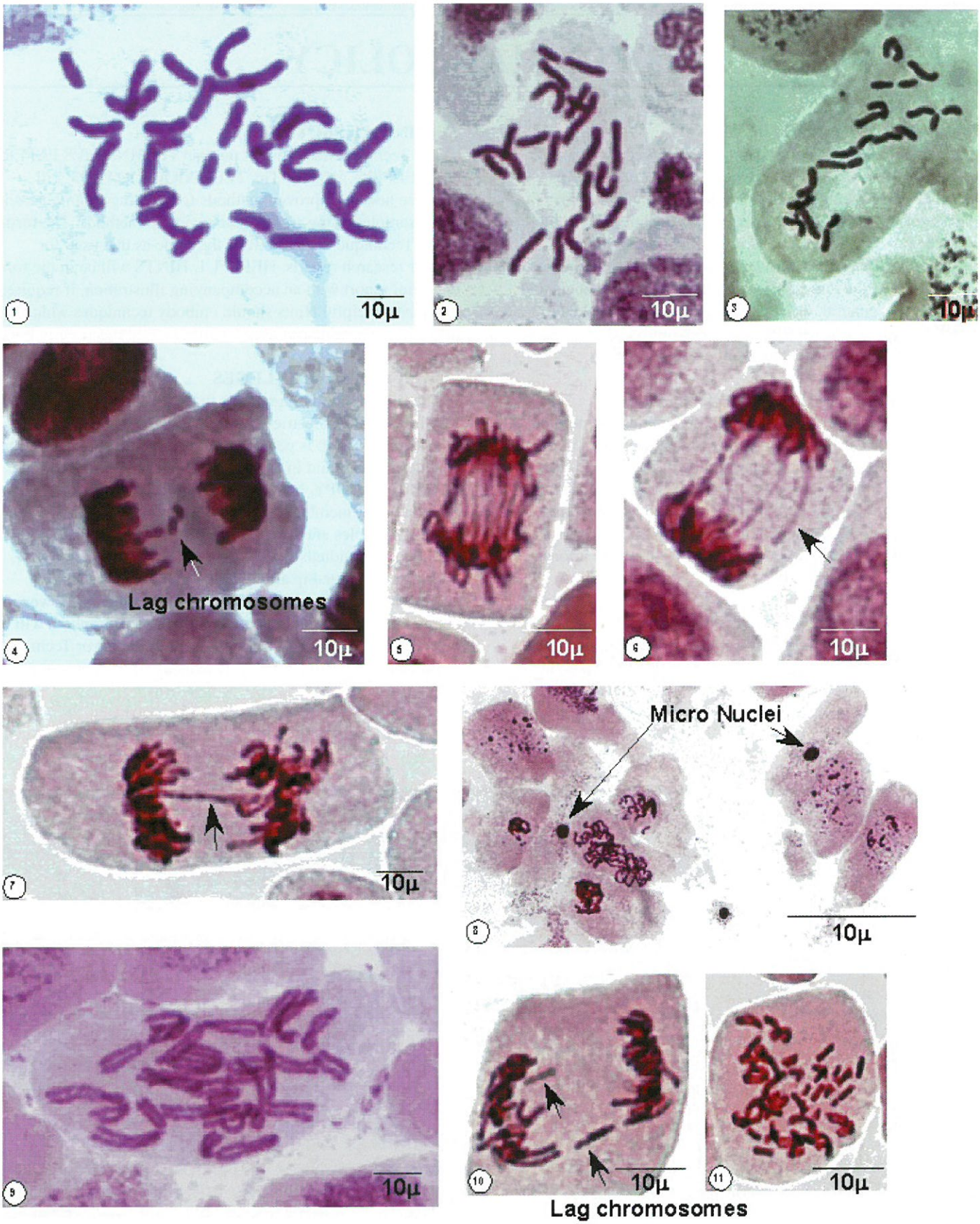
http://www.tnrcc.state.tx.us/index.html



Figures 1-3 showing 2n=16 chromosomes from mitotic metaphase plates from *Allium cepa* root tips. Figs. 4 & 10 showing Laggard Chromosomes and Figs. 5,6 & 7 showing Sticky Bridges from 24h treatment with 1% Atrazine. Fig. 8 showing Micronuclei, Fig. 9 Diplochromatids and Fig. 11 Fragmentations with 48h treatment with 1% Atrazine.

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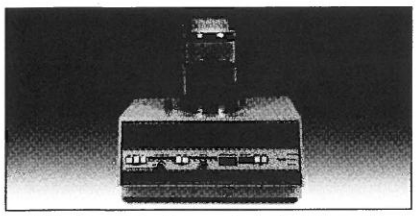

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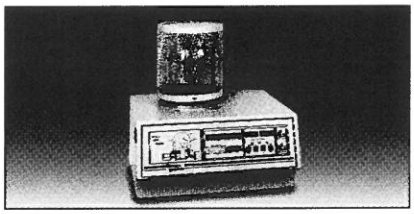

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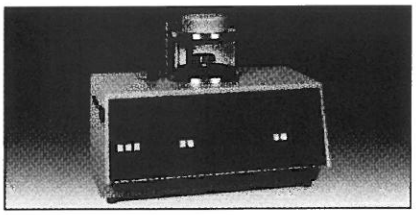

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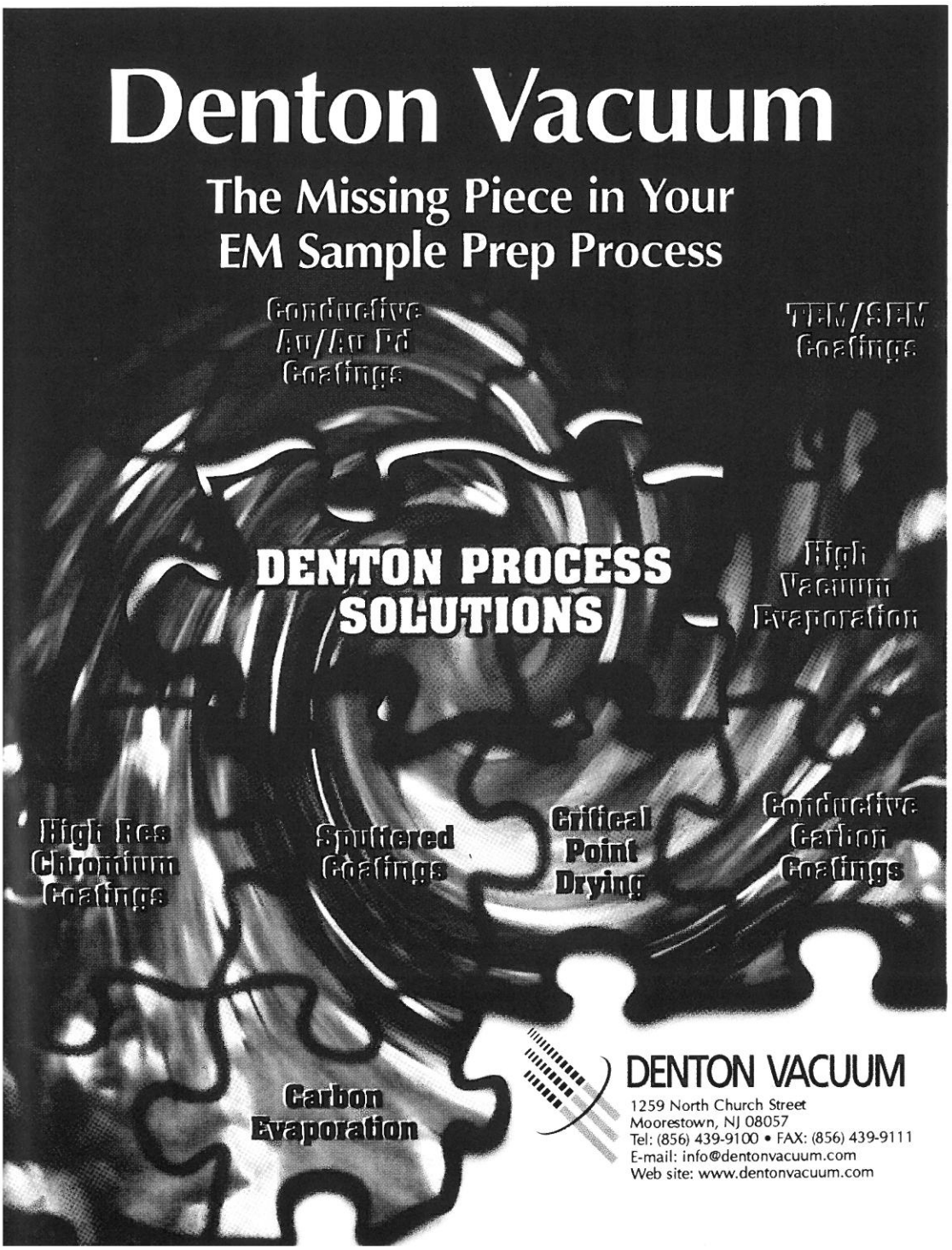

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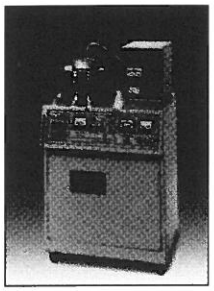

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