Journal of the Minnesota Academy of Science

Volume 63 | Number 3 Article 4

1999

Winchell Posters

IN VITRO TESTING FOR ANTIBACTERIAL PROPERTIES OF SOME COMMON MEDITERRANEAN PLANTS

D. B. Andretta, S. Akbulut, D. Timonen, and Al R. Karim, University of Minnesota - Duluth, 211 Life Science Building, 10 University Dr., Duluth, MN 55812

The antibacterial effects of some plants have been known for years, and the medicinal uses of plants has been the subject of much debate. Five plants, and their extracts, namely oleander *(Nerium oleander),* chamomile *(Anthemis nobilis),* mint *(Mentha spicata),* sage *(Salvia officinalis),* and thyme *(Thymus vulgaris)* were tested for their inhibitory effects on the growth of 8 species of bacteria. The bacteria used in the experiment include: *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Micrococcus luteus, Pseudomonas aerugrinosa, Proteus vulgaris,* and *Staphylococcus aureus.* Four of the plants tested are regarded to be medicinal, and are widely known for their medicinal folk preparations. Oleander *(N. oleander),* however, is poisonous. It contains poisonous glycosides that are similar to cardiac glycosides. The most effective method in inhibiting the growth of bacteria was mixing ground plants directly into agar and then autoclaving. Water extracts, excluding sage (S. *officinalis)* and thyme *(T. vulgaris),* were ineffective in preventing bacterial growth. Thyme (T. *vulgaris)* and sage (S. *officinalis),* which inhibited the growth of all bacteria at a level of 5% (w/v), were the most inhibitory of the 5 plants studied. Oleander *(N. oleander)* was found to be the least effective plant in inhibiting the growth of bacteria. M. *luteus, P. vulgaris,* and *B. cereus* were found to be the most sensitive bacteria in each growth medium containing ground plants . *E. aerogenes* was the most resistant bacteria at all concentrations of ground plants. At a level of 5% (w/v) of ground plants/agar only sage *(S. officinalis)* and thyme *(T. vulgaris* inhibited the growth of E. *aerogenes.* The combined use of sage (S. *officinalis)* and thyme *(T. vulgaris)* water extracts exhibited a synergistic antibacterial effect.

DO TALL GRASS PRAIRIE GRASSES GENERATE GENE DIVERSITY THROUGH POLYPLOIDY? *CLONING AND SEQUENCING THE GENE FOR PPDK FROM BIG BLUESTEM*

Gary Bailey, Biotechnology Program, Moorhead State University, MN, 56563

The tallgrass prairie represents a rich source of floristic biodiversity. A large fraction of the flora of this ecosystem are grasses that use the C4 photosynthesis. Most of these "C4"grasses are polyploid (6N, 8N). In order to explore the theory that high ploidy number is related to adaptive value in a prairie ecosystem, we are cloning the gene for Pyruvate Orthophosphate Dikinase (PPDK), a key C_4 photosynthesis gene from the C_4 grass *Andropogon gerardii* (Big Bluestem). This is being accomplished by screening a Big Bluestem cDNA library using a maize PPDK gene probe. Positive clones have been selected and will be sequenced. This will be the first C4 photosynthesis gene cloned from a native tallgrass prairie species. We will then use the cloned PPDK gene to probe a Big Bluestem genomic Southern blot to observe PPDK gene diversity in the Big Bluestem genome. This will test the "polyploidy and adaptation to harsh environment" theory.

IMPACTS OF EUROAMERICAN SETTLEMENT ON PHOSPHORUS ACCUMULATION RATES IN LAKE VOLNEY, MINNESOTA

Eric J. Bergman and Charles E. Umbanhowar Jr., St. Olaf College, Department of Biology, 1520 St. Olaf Ave., Northfield, MN 55057

We quantified historic phosphorous accumulation rates in sediments from Lake Volney, Minnesota, a 113.2 ha, 22 m deep, hypereutrophic lake located in Le Sueur Co. Five cores were collected in 1995, and cores were sectioned into 8 cm intervals. Sediment composition was analyzed using loss-on-ignition, and concentrations (mg/gm) of organic, apatite, and non-apatite inorganic (NAI) phosphorous were determined based on serial acid digestion and analyzed by the absorbic acid method. Sediment accumulation rates were estimated based on evaluation of sediment magnetics, pollen, and Pb-210 dating. Our results suggest significant increases in organic and NAI-P fluxes in recent years, consistent with other studies of Lake Volney that point to large amounts of phosphorous (1382 kg/yr) coming from agriculture-dominated watersheds surrounding the lake.

GENETIC ENGINEERING PHOTOSYNTHETIC COLD TOLERANCE IN MAIZE BY SITE DIRECTED MUTAGENESIS OF THE GENE ENCODING PYRUVATE-ORTHOPHOSPHATE DIKINASE.

II. MULTIPLE MUTATIONS AND THEIR EFFECT ON COLD STABILITY OF PYRUVATE-PI-DIKINASE.

Derrin Birch and Jeff Clauson, Department of Biology, Moorhead State University, Moorhead, MN, 56563

Compared to other cereal grains, maize is a remarkably productive crop species because it has a highly advanced form of photosynthesis called C4-photosynthesis. A key enzyme of C4 photosynthesis is pyruvate, orthophosphate dikinase (PPDK). Unlike other enzymes in the pathway, this enzyme is particularly susceptible to cool temperatures and becomes inactive at about 12 °C. However, it was recently discovered that another C4 plant, *Flaveria brownii,* has a PPDK that is insensitive to cold. Comparisons of the maize and *F. brownii* PPDK genes suggests a difference of only four amino acids in the C-terminal portion of the enzyme is responsible for conferring tolerance to cold. We have used site- directed mutagenesis to change these four specific amino acids in maize. Results on how combinations of two, three, and four amino acid changes effect cold stability of the maize enzyme will be presented.

AN INVESTIGATION OF THE INFLAMMATORY RESPONSE THE RELATIONSHIP BETWEEN IRON, NITRIC OXIDE AND MACROPHAGE KILLING ACTIVITIES.

Adrienne Boire, Macalester College 1600 Grand Avenue, Saint Paul, MN 55105

Macrophages are key players in the inflanunatory response. There is considerable evidence pointing to the role of iron in macrophage activity. When stimulated with interferon gamma (IFN-λ) Macrophages actively take up serum iron and deposit it into ferritin removing iron from the site of infection. Tissue damage and hemolysis cause inflammatory sites to have high local iron concentrations. Additionally, NO synthesis through the inducible nitric oxide synthase (iNOS) pathway is linked to iron metabolism. NO is synthesized by macrophages to kill bacteria, tumor cells and parasites after phagocytosis. Increased iron levels lead to increased ferritin and NO production.

This relationship between iron and macrophage killing activities was investigated. My working hypothesis was that increased extracellular iron will increase killing activities

(phagocytosis and oxidative burst) in stimulated macrophages. RAW264.7 macrophage cells were cultured in DMEM containing 10% fetal calf serum. After stimulation with IFN-λ, these cells were treated with haptoglobin and desferrioxamine B and were then rinsed with Krebs-Ringer bicarbonate buffer (KRB) to remove extracellular iron. Iron (ferrous citrate in KRB) was added in concentrations of 0μM, 1μM and 8μM. Phagocytic activity was assayed via latex bead ingestion and NO production was assessed through the use of the Greiss reagent.

Ultrastructural Localization of NGF Producing Cells in Laminae I & II of the Rat Spinal Cord

Jason Bomberger & Ben Goltz, Neuroscience Program, Department of Biology, Macalester College, 1600 Grand Ave., St. Paul, MN 55105

Peripherally administered nerve growth factor (NGF) induces hyperalgesia and local inflammation. Retrograde transport brings NGF to the neuronal cell body in the dorsal root ganglion (DRG) where it changes gene expression and increases neuropeptide concentrations in the dorsal horn of the spinal cord. TrkA receptors bind NGF and have been localized in laminae I and II of the dorsal horn of the spinal cord. Our laboratory has shown that the number of trkA receptors increases during carrageenan induced inflammation. In addition, intrathecal administration of NGF blocks this hyperalgesia without having an analgesic effect in normal animals. The presence of trkA receptors in the dorsal spinal cord and the fact that intrathecal NGF has an antihyperalgesic effect suggests that endogenous NGF is involved in nociception. This study attempts to identify the specific cells that produce NGF acting in the spinal cord. Electron microscopy is being used to examine the localization of NGF-like immunogold labeling. If NGF is made in the spinal cord rather than being transported from the periphery, we hypothesize that NGF immunoreactivity will be found associated with the Golgi apparatus. Double labeling with glial fibrillary acidic protein (GFAP) is also used to distinguish if NGF production is associated primarily with glial cells. In order to optimize immunogold labeling tissue has been blocked in both epoxy and Lowicryl embedding media.

Exploring the functional properties of a maize regulatory protein kinase by directed mutagenesis of its target protein, pyrovate,orthophosphate dikinase

Monty Botschner, Department of Biology, Moorhead State University, Moorhead, MN, 56563

Pyruvate,orthophosphate dikinase, PPDK, is a central enzyme of the C4 photosynthetic pathway found in maize. As a photosynthetic enzyme, it is inactivated at night and activated during the day. A specific protein kinase called PPDK regulatory protein (RP) is responsible for this light/dark regulation via reversible phosphorylation of PPDK. Little is known about the functional properties of RP because of its extreme instability once removed from the leaf for study. For example, despite numerous attempts, it has never been purlfied to homogeneity. We are using directed mutagenesis of it's target residues in PPDK to more fully elucidate how RP functions to inactivate the PPDK at night and activate it during the day. Results will be presented on the mechanism of PPDK inactivation/activation and RP substrate requirements for inactivation/activation.

Production of Systemin Binding Protein in Response to Wounding of Tomato Plants

Gina M. Brandel, Minnesota State University, Mankato, Department of Chemistry and Geology, PO Box 8400, MSU 40, Mankato, MN 56002-8400

Systemin, the first peptide hormone to be isolated in plants, has been shown to be a primary signal of protease inhibitor induction in response to wounding. A 50-kDa systemin binding protein (SBP-50) has previously been identified to bind systemin *in vitro;* however, the precise function, mechanism and regulation of the binding protein remain unknown. To determine, in part, the role of SBP-50 in the wound response, relative SBP-50 concentrations were measured in wounded versus non-wounded tomato leaves. A polymer-two phase system of Dextran T500 and PEG 3350 was used to isolate the components of the tomato leaf plasma membranes. A biotinylated systemin derivative was incubated with the plasma membrane fraction to specifically bind SBP-50, and the two proteins were crosslinked. The membrane components were then separated by gel electrophoresis, and Western blotting was employed to detect the biotin label, and therefore any protein bound to systemin.

Optimization of Ethanol Production from Corn in Wet Mill Fermentation

Alex Campbell and John Giannini, Ph.D., St. Olaf College, Northfield, MN 55057

While humans have been fermenting alcohol for thousands of years, the production of ethanol as a renewable fuel source is a relatively new science. To make the process as efficient and economical as possible requires large scale, multi-step fermentation performed by high-tech industrial processing plants. Although the basic principles of fermentation are well understood, the many steps in the process could theoretically be manipulated to improve the amount of ethanol produced. Working in conjunction with Al-Corn, an ethanol producing CO-OP in Southern Minnesota, we attempted to optimize the fermentation process and increase their overall ethanol production. There were three objectives to this study. The first was to accurately duplicate on a small scale the fermentation process occurring at the CO-OP. Second, the effect of fermentation time on overall ethanol production was studied. Finally, the effect of adding different nitrogen sources when the fermentation was at maximum ethanol concentration was studied. It was found that it is possible to accurately reproduce the fermentation process on a small scale, and that the amount of ethanol produced levels off after 48 hours of fermentation to an average concentration of 13.12 percent. The effect of different nitrogen sources on ethanol production is still being studied, but preliminary results indicate that adding nitrogen (ammonium sulfate) late in the fermentation process may further increase ethanol levels.

The Enthalpy of Hydration of Polyacrylamide Granules

Ed Cox, Gordon McIntosh, Jim Olson, University of Minnesota-Morris, 600 E 4th Street, Morris, MN 56267

The purpose of this research project was to determine the enthalpy of hydration of polyacrylamide granules commercially sold as Terawet. In the project, a microcalorimeter, microvoltmeter, analog-to-digital converter, and Data Logger software were used to determine the heat evolved when dry Terawet was wetted with an excess of water. Using the Peltier effect, the heat was converted to a voltage that was logged as a function of time, and the amount of heat released upon hydrating was determined. The temperature dependence in the enthalpy of hydration was investigated by running the

experiment at a lower temperature. Results indicate that more heat is released per unit mass when the Terawet is hydrated at lower temperatures.

LOCALIZATION OF MELATONIN IN PLANTS: QUANTIFICATION IN PLANT CYTOSOL, MITOCHONDRIA, AND CHLOROPLASTS
Claire de la Cova, Macalester College, 1600 Grand Ave., St. Paul, MN 55105

Plants have been shown to produce significant amounts of melatonin, but its role in plants is not known. Melatonin levels are highest in leaf tissue of plants, and have been found to fluctuate on a daily cycle. Interestingly, melatonin levels peak during the middle of the day, also the time of greatest photosynthetic activity. Because melatonin has been shown to be an effective scavenger of free radicals which can be produced during photosynthesis and metabolism, it has been suggested that it may act as a protective antioxidant in plant cells. In order to investigate this possible role, I hypothesized that melatonin may be associated with the photosynthetic organelle of plants, the chloroplast. In my experimental study, I determined the melatonin levels of the total cell, cytosol, mitochondria, and chloroplasts of plant cells.

Pea plants were used to isolate plant cellular fractions. A total of 64 pea plants of the same age were harvested at two times: half were collected at midday, and half harvested at midnight. Immediately after harvest, four cellular fractions were separated in each plant group: a total cell fraction, a cytosol fraction, a fraction containing intact mitochondria, and a fraction with intact chloroplasts. Melatonin was extracted from each aqueous fraction using chloroform. High pressure liquid chromatography was used to identify and measure the amount of melatonin in the cellular fractions. The levels of melatonin present in the total plant cell, the cytosol, the mitochondria, and the chloroplasts, were compared for both day and night groups.

FLUORESCENCE MEASUREMENTS OF DNA-QUINONE COMPLEXES
Mamatha Derarapalli, Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

Carbazoquinones are known to interact with DNA by DNA intercalation. Since the discovery that quinones cause anti-cancer activity, a great deal of research has been directed toward developing a novel set of quinone analogs. In a collaborative effort, we obtained newly synthesized carbazoquine analogs to quantify their affinity for DNA. We demonstrate the effects of the synthetic carbazoquinone derivatives intercalating in the DNA by fluorescence spectroscopy. The base-pair specificity and the nucleic acid binding constants distinctly vary for each of the cabazoquinone analogs due to the specific interaction of different substituents with the grooves of the DNA complex. The DNA-carbazoquinone complexes contribute to the existing information concerning the mode of biological action of quinones.

PROPAGATION OF *ARCTOSTAPHYLOS UVA-URSI* FOR NORTHERN MINNESOTA'S TETTAGOUCHE STATE PARK LANDSCAPE RESTORATION PROJECT
Elizabeth Diederichs, St. Olaf College, 1500 St. Olaf Ave., Northfield, MN 55057-1001

We have tested methods of propagating *Arctostaphylos uva-ursi* (Bearberry) plants from Minnesota's Tettagouche State Park on the north shore of Lake Superior. Bearberry plants have been damaged by human traffic in sensitive areas of the park. State park personnel are working to restore trampled areas by seeking a method of propagating local Bearberry plants. Our objective is to develop a propagation technique that can be implemented by personnel at Tettagouche Park. We collected Bearberry cuttings in October 1998 that included both root and foliage and stored them in moist peat until January 1999. At that time we constructed four variations in soil conditions including two treatments of sand and two treatments of soil where half of the cut material was treated with root growth hormone. Eighty of the cuttings were placed on a greenhouse bench for daily watering and the second eighty cuttings were placed under a mist table providing water at ten-minute intervals. Our preliminary results show that propagation in sand with no root growth hormone and daily watering gives the highest percentage (70%) of new growth. All treatments, both bench and mist table, yield new growth, with percentages ranging from 10% to 70% success. Our preliminary conclusion for this experiment is that State Park personnel can successfully propagate Bearberry plants in sand with no growth hormone using methods we have developed.

RAPD-PCR ANALYSIS OF FOUR FRESHWATER MUSSEL SPECIES: A SEARCH FOR POPULATION SPECIFIC GENETIC MARKERS
Lucas M. Dunklee, Macalester College, 1600 Grand Ave., St. Paul, MN 55105

Freshwater mussel (Bivalvia: Unionoidae) species of North America are declining sharply in species richness and abundance. Conservation efforts are currently hampered by insufficient knowledge of the reproductive patterns of unionids. After spawning, mussels are dispersed in a larval form (glochidium) which is microscopic in size and parasitic on fish gills during maturation and dispersal. The small size of the glochidia makes visual species identification difficult or impossible, and invites development of genetic identification techniques. Considerable work has already been done to construct a "species key" based on DNA polymorphisms in the ITS-1 region. It would be of value to unionid conservationists and population ecologists alike if a complementary technique were developed to identify the geographic population of origin of glochidia using molecular techniques. While the ITS-1 is an ideal region for identifying the species of a particular individual, it has not demonstrated polymorphism below the species level. The current study investigated the possibility of locating an alternative region of the genome which could be used as a "population key," which would enable unambiguous determination of the population of origin of an unknown individual. RAPD-PCR (Randomly Amplified Polymorphic DNA) techniques were used to screen the genome of multiple populations of four species *(Truncilla truncata, Quadrula pustulosa, Elliptio dilatata, Fusconaia flava)* for suitably variable regions. RAPD-PCR profiling provided not only possible sources for new "key regions," but information about the genetic diversity between and within these four species.

IS SIBLICIDE A POSSIBILITY IN BLACK-CROWNED NIGHT HERONS?
Emily Emond and Matthew Medeiros, Hamline University, 1536 Hewitt Ave. St. Paul, MN, 55104

Older siblings kill younger siblings (siblicide) in some egrets and herons but not others. The prey size hypothesis predicts that siblicide should occur in species where food intake can be monopolized by one chick (usually the senior). Whether siblicide occurs in black-crowned night herons *(Nycticorax nycticorax)* has not been explored. We compared the aggression and feeding behaviors of seniors and juniors in two nests of black-crowned night herons in a heron colony near Ashby, MN. We observed some fights and two cases of a senior chick swallowing the head of a junior chick, immobilizing it until it seemed near death. Siblicide often happens when the junior chicks starve following attacks by

seniors that make juniors too intimidated to eat. We found that the senior chicks tended to get more food than the junior chicks, but our sample size was too small to draw firm conclusions. Our results suggest the possibility of siblicide occurring in this species. The results are surprising because the bolus sizes were large, so fighting should be rare according to the prey-size hypothesis. Our findings make black- crowned night herons a good candidate for further studies of nestling aggression.

REGULATION OF THE *SUPEROXIDE DISMUTASE* GENE BY BINDING OF THE FERRIC UPTAKE REGULATORY PROTEIN

Ashley Evans, University of St. Thomas, 2115 Summit Avenue, St. Paul, MN 55105

The intracellular changes made by cells due to changing external environments is an area of increased study. One proposed mechanism responsible for this phenomenon is gene regulation by the binding of specific proteins. The research performed examined the Ferric Uptake Regulatory (FUR) protein and its regulation of the *superoxide dismutase (sodA)* gene in an attempt to more completely understand gene regulation. The *sodA* gene functions in the breakdown the dangerous free radical superoxide. The *sodA* gene was isolated, purlfied, and amplified using Polymerase Chain Reaction. The FUR protein was also isolated and purlfied using standard techniques and its molecular mass determined using SDS-PAGE. Finally, the *sodA* gene was labeled for chemi-luminescent detection in order for gel shift assays to be carried out to determine binding constants of the FUR protein to the *sodA* gene. Further experiments will be performed to examine the mechanism by which FUR binds *sodA*, as well as to explore whether the FUR protein serves an activating or repressing role in the cell.

CALCITE TWINNING CONSTRAINTS ON THE STRESS-STRAIN FIELDS ALONG THE MID-ATLANTIC RIDGE, ICELAND

David W. Farris, Department of Geology, Macalester College, 1600 Grand Ave., St. Paul, MN 55105 USA

Three dimensional in-situ stress-strain fields associated with the mid-Atlantic rift in Iceland were analyzed using the Groshong strain-gauge method to analyze calcite twins found in amygdoidal moberg basalts (>0.7 Ma). European plate samples (positive expected values (PEV)) exhibited an average E1 magnitude of -2.58 ±1.28% and the North American plate (PEV) showed an E1 magnitude of -5.17 ±3.15%. NEV (negative expected values) E1 magnitudes for the European and North American plate are -2.61 ±1.41% and -9.85 ±1.2% respectively. Both the European and North American plates exhibit two distinct stress-strain fields. On each side of the rift, the first strain (PEV) was ridge sub-normal (indicative of ridge-push) and the secondary strain (NEV) was ridge sub-parallel. The Jamison and Spang method was used to calculate differential stress in each sample. PEV values of 47 ±34 mPa for the European Plate and 46 ±14 mPa for the North American plate were found. NEV differential stress was calculated to be 80 ±64 mPa for the European Plate and 49 ±6 mPa for the North American plate. The shortening strain magnitudes and differential stresses are thought to represent regional tectonic conditions (i.e., ridge-push), and not local geologic phenomena such as the hotspot or glacial loading.

ENHANCING THE EFFICIENCY OF DNA TRASFECTION INTO NEONATAL CARDIAC MYOCYTES IN CULTURE

Grimes, A.M., L.V. Nustad, C.E. Broadwell, S.M. Olmschenk, C.C. Eichelberger, W.W. Wegh, and M.A. Wallert, Department of Biology, Moorhead State University, Moorhead, Minnesota 56563.

Transfection of an antisense oligodeoxynucleotide, specific to the initial coding sequence of the Na^+-H^+ antiporter (NHE-1), into cultured neonatal cardiac myocytes has been shown to decrease antiporter activity. The rate of recovery following an acid load was decreased from 0.080 pH units/min in control cells to 0.033 pH units/min in antisense treated cells. This is a decrease in activity of 41 percent. It was not clear why a further reduction was not obtained, but one concern was the transfection effficiency. The process of optimizing the transfection procedure was undertaken in an effort to obtain a further reduction in antiporter activity. The major challenge with any transfection method comes in the fact that obtaining optimal transfection is an empirical process. Liposomal transfection reagents which incorporate synthetic cationic lipids with neutral lipids are currently the most successful methods of transferring nucleic acids into cells. Five different lipid transfecting agents (Tfx-10, Tfx-20, Tfx-50, Fugene-6 and Lipofectin) have been tested to determine which gives the best transfection. The pSV-β-galactosidase gene was used as the reporter gene in these experiments. Cells which have been successfully transfected with pSV-β-galactosidase will create a blue reaction product when treated with X-gal. Variations in the amount of DNA used, the charge ratio of transfecting agent to DNA, and incubation times have been tested. Transfection efficiency was determined by counting stained and unstained cells. In preliminary experiments Fugene-6 and Lipofectin gave the best results.

EFFECTS OF SPINALLY ADMINISTERED BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) ON THERMAL NOCICEPTION IN MICE WITH CARRAGEENAN-INDUCED PERIPHERAL TISSUE INFLAMMATION

Anica Bowe, Tawni Epperson, Rachel Groth, Department of Biology, Macalester College, St. Paul, MN 55105

Nerve growth factor (NGF) has been implicated as playing a key role in inflammation-induced hyperalgesia. Further, it was previously demonstrated in another laboratory that peripheral inflammation induced an NGF-mediated increased expression of brain-derived neurotrophic factor (BDNF) mRNA in the dorsal root ganglion (DRG). As such, the present study served to further elucidate the role of BDNF in nociception in the spinal cord. The effects of intrathecal administration of BDNF (25 ng/μl) on thermal nociception were assessed in a hotplate assay. Changes in hotplate latency from baseline were taken at 30 minutes, 4, 24, and 48 hours following treatment. Preliminary results suggest that BDNF may have a hyperalgesic effect in mice with carrageenan-induced peripheral inflammation, although this effect was not significant at the concentration of BDNF used in the present study. In normal (non-inflamed) mice we observed an analgesic trend. We will further explore these possible effects by increasing the number of subjects and the concentration of BDNF used. Additionally, in order to determine if the BDNF receptor trk B is localized and modulated in areas of the spinal cord that are involved in nociceptive processes, trkB receptor distribution will be examined using immunohistochemistry.

EFFECTS OF FRESHWATER MUSSELS ON NUTRIENT DYNAMICS IN A SMALL STREAM

Faith Hareldson, Macalester College, 1600 Grand Ave., St. Paul, MN 55105

Freshwater mussels have been shown to have significant effects on nutrient cycling in lakes and rivers, due to their high filtration rates and potentially high population densities. However, little attention has been given to the influence of mussels on nutrient processing in smaller streams. In the present study, total suspended solids and chlorophyll a were measured in water samples taken from a high mussel density section and a low mussel density section along the Sunrise River in Minnesota. Within each section, samples were obtained both at the sediment-water interface and the water's surface at sites upstream, above, and downstream of mussel beds. The data will be analyzed to discern whether the high-density mussel bed is having a significant influence on the concentration of suspended matter and chlorophyll a throughout the water column. The low mussel density section will be used as a control in the analysis. Relationships between mussel distribution and physical characteristics (such as flow and depth) of the stream at the high- density site will be examined as well. This information will provide insights into freshwater mussels' role in streams, and how changes in mussel densities, often consequences of human activities, may affect nutrient cycling in these systems.

ENZYMES AS ENVIRONMENTAL INDICATORS: DETERMINATION OF AMBIENT LEAD LEVELS VIA δ-AMINOLEVULINIC ACID ALA-DEHYDRATASE ACTIVITY

D. Christopher Harmes and Kyla Hayford, Macalester College, 1600 Grand Ave., Saint Paul, MN

It is known that the metabolic processes of organisms are affected by toxicants in the organisms' immediate surroundings. It should then be possible to determine biologically available toxicant levels in the environment by evaluating the biochemical system of the affected organisms. Working under this hypothesis, we developed a sensitive assay for the enzyme δ-aminolevulinic acid (ALA)-dehydratase, an enzyme incredibly sensitive to the presence of lead. ALA-dehydratase is involved in heme synthesis (step 2), converting δ-aminolevulic acid into porphobilinogen (PBG). ALA-dehydratase exists at high levels in the liver of an organism, a major site of porphyrin synthesis. The presence of dead would likely inhibit the hepatic enzyme activity of an indicator species. To asses the level of inhibition and thus the organism's level of exposure to lead, the liver of an indicator species can be removed and assayed with a known quantity of ALA. Measurement of ALA-dehydratase activity by directly measuring PBG levels is in itself difficult. However, converting PBG into Uroporphyrin I forms a fluorescent product that is quantifiable with a great deal of sensitivity. This conversion is done with the addition of Uroporphyrinogen I Synthase, which forms a tetrapyrrole from four PBG's. The tetrapyrrole is then non- enzymatically converted into Uroporphyrin I. The number of moles of Uroporphyrin produced is proportional to the activity of ALA-dehydratase. Studies with the ubiquitous fathead minnow *(Pimephales promelas)* have been conducted in order to form a standard that would enable the quantification of biologically available lead levels from ALA-dehydratase activity.

NEUROTRANSMITTER SYSTEMS IN PLANTS? *THE SEARCH FOR A PLANT ACETYLCHOLINE RECEPTOR GENE*

Grant Harrington, Craig Nerby, Biotechnology Program, Moorhead State University, MN, 56563

The physiological role for acetylcholine, along with the acetylcholinereceptor (AChR) and acetylcholine esterase, in neurotransmission of animals is well known. Until now, there has been little evidence of similar systems existing in plants. We have screened a tobacco cDNA expression library for a plant AChR gene in an effort to show the existence of this system in plants. This was done using AChR monoclonal antibodies raised against the δ-subunit of AChR to find positive clones. These clones will be sequenced and the sequences will be compared to known animal AChR sequences using a gene and protein database. The goal of this project is to provide the first evidence of the existence of AChR in plants and its obligatory physiological system.

IN VITRO EFFECTIVENESS OF AR-100 AGAINST FIVE DIFFERENT BACTERIAL SPECIES

Michael J. Hoffman, M.A. Rashid And M.R. Karim, University of Minnesota - Duluth, 211 Life Science Building, 10 University Drive, Duluth, MN 55812

The development of resistance to antibiotics in many microorganisms has provoked a search for new substances that inhibit bacterial growth. One substance that is proving to be effective against microbial growth is referred to as AR-100. This substance was used to attempt to inhibit the growth of bacteria including *Escherichia coli, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus* and *Staphylococcus epidermidis.* The effectiveness of AR-100 was determined by using the Kirby-Bauer procedure. Bacteria were fawned onto plates of Mueller-Hinton Agar. Three filter disks were impregnated with 4ug, 6ug and 8ug of AR-100 and placed on the agar containing the respective bacteria. Three controls were used in conjunction with the AR-100 disks. The controls were Penicillin (10ug/disk), Tetracycline (30ug/disk), and Streptomycin (10ug/disk). The plates were incubated at 37 degrees Celsius for 12 hours. Findings showed AR-100 was effective against both *Staphylococcus epidermidis* and *Staphylococcus aureus* as well as *Bacillus subtilis.* Disks containing 4ug had an average zone of inhibition of 18mm for *Staphylococcus epidermidis* and 16mm for *Staphylococcus aureus.* The same disks showed a zone of inhibition average of 13mm for *Bacillus subtilis.* These low AR-100 concentrations proved ineffective against *Escherichia cold* and *Proteus vulgaris.* When compared with the control antibiotics, AR-100 proved to be most effective against the *Staphylococcus* species. Results to this point have been promising, however more work is in progress to determine the effectiveness of AR-100 in different concentrations and against other microorganisms.

GLUCOSE-INDUCED ENHANCEMENT OF SPATIAL MEMORY IN HUMANS

Adam Johnson, Minnesota State University, Mankato Department of Psychology - MSU 35, P.O. Box 8400 Mankato, MN 56002-8400

Glucose has repeatedly been shown to enhance learning and memory processes for a variety of tasks in both humans and non-human animals. However, the memory modulatory effect of glucose on spatial memory tasks has not received much attention. The present study examined the effect of glucose on human spatial memory. Thirty-two young adults were randomly assigned to one of three treatment conditions (100 mg/kg or 50 g glucose or saccharine). Participants consumed a lemon-flavored beverage containing the appropriate sweetener and were subsequently presented with 16 pictures arranged on a 4 x 4 grid. After studying the location of these pictures for 20 seconds the pictures were arranged in a pseudo-random order and handed to the participant who was instructed to place them in the correct location on the grid. Participants received a total of three consecutive learning trials of this nature and one recall trial 24-hrs later. Results indicate

that only participants receiving 100 mg/kg of glucose performed significantly better than the saccharine control group. These findings are consistent with results from both human and non-human studies examining the effects of glucose on other forms of memory, and extend the effects of glucose to a spatial memory task in humans.

AN INVESTIGATION OF THE EFFECT OF THE LECTIN *BANDEIRAEA SIMPLICIFOLIA*-I ON PORCINE AORTIC ENDOTHELIAL CELLS *IN VITRO*

Jason S. Johnson, Hamline University, 1536 Hewitt Ave., St. Paul, MN 55104

Xenotransplantation, the transplant of organs from one animal species to another, has recently become the focus of researchers around the world. Its success would end the transplantation crisis arising from a severe lack of compatible human donor organs. However, in order for xenotransplantation to succeed, researchers must prevent the various modes of rejection of xenografts by the human immune system. The focus of my research project was to investigate a means of alleviating a form of organ rejection known as hyperacute rejection. Hyperacute rejection is mediated by preformed natural antibodies, which are found in the organ recipient's bloodstream, that recognize antigens present on the cells of xenografts. It was found that treatment of porcine aortic endothelial cells in an *in vitro* model of a porcine-to-human heart transplant with *Bandeiraea simplificolia*-I, a plant lectin, resulted in the alleviation of hyperacute rejection as initiated following incubation with human serum. The results of my research reveal certain aspects concerning how BS-I acts on the cells to prevent hyperacute rejection.

pKa DETERMINATION OF A LONG CHAIN ALKANOIC ACID IN A NORMAL PHASE MICROEMULSION

Karin Jolivette and D. Scott Nelson, Department of Chemistry, St. Olaf College, Northfield, MN

n-Alkanoic acids and n-alcohols are amphipathic molecules with a hydrocarbon tail terminated in a carboxylic acid and alcohol headgroup respectively. The pKas of alkanoic acids are known for the shorter chains monomeric acids in water. The focus of this project is to measure the pKa of very long chain alkanoic acids that can form a two dimensional assembly. When the alkanoic acid headgroups are close together, hydrogen bonding is expected between the -OH of one headgroup and the C=0 of an adjacent headgroup which is expected to increase the pKa of the acid. The surface charge of an acid assembly will be negative which will attract protons and lower the surface pH. To test the contribution of these two phenomena, two normal phase microemulsions consisting of 25 nm spheres of hexadecane (330 μM coated with 250 μM palmitic acid or with palmitic acid and hexadecanol 125 μM each, were prepared in 150 mM NaCl. A pH indicator dye was added to the solution and the absorbance spectrum was measured and compared to a standard curve to determine the pH. The pH was used to solve a simultaneous equilibrium in which the only unknown was the pKa of the alkanoic acid. Using Oregon Green 500 indicator dye, the pKa of palmitic acid has been calculated to be 6.4 ± 0.1 for the first system. This is significantly higher than that of the free acid in solution (~4.8). The cause of this rise in apparent pKa is being explored using the second system.

ANHYSTERIC MAGNETIC SUSCEPTIBILITY STUDIES OF FLOW DIRECTIONS IN RIDGE PARALLEL DIKE SWARMS: ICELAND

Bryan C. Kennedy, Macalester College, Geology Department, 1600 Grand Avenue, Saint Paul, MN 55105

A series of oriented mafic dikes were collected from the North American (west) and European (east) portions of Iceland which are separated by the active Mid-Atlantic Ridge. Magmatism along this tectonic feature produces ridge-parallel mafic dike swarms. As this is an active tectonic system, and these dikes are young and unmetamorphosed, the primary igneous flow fabrics could be studied by Anisotropy of Magnetic Susceptibility (AMS) techniques as a proxy of igneous flow during emplacement. Seventeen samples were collected (7 N. American; 6 European) and 153 cores were analyzed by AMS at the IRM at the Univ. of Minnesota. Average magnetic susceptibilities are 19.59%, and our samples preserve a clear vertical Kmax orientation in every sample, indicating vertical emplacement. This strong signature is interpreted to support the relationship between a shallow magma source with overlying thin crust and vertical dike intrusion. This is the opposite of previous AMS dike studies in continental crust (thick crust, horizontal dike intrusion). (Ernst, 1990) Kmax plotted sub-horizontally, perpendicular to the dike margin in two of the dikes measured. These anomalous results represent a possible non-laminar flow during dike emplacement.

SYNERGISTIC ANTI-VIRAL EFFECT OF BETULIN AND ACYCLOVIR AGAINST HERPES SIMPLEX VIRUSES

Keyel, P.A., M. Amjad, M.R. Karim, Department of Biology, University of Minnesota, Duluth, MN 55812.

Betulin, a pentacyclic triterpenoid compound isolated from the bark of the Minnesota White Birch, was found to have anti-viral activity. Betulin and some derivatives exhibit significant anti-viral activity against Herpes Simplex Virus type 1 and 2 (HSV-1 and HSV-2) both *in vitro* and *in vivo*. Acyclovir and its derivatives are currently the drugs of choice for treating HSV infections. In this study the synergistic anti-viral effects of Betulin and Acyclovir were observed against HSV-1 and HSV-2. African green monkey kidney (Vero) cells were grown to monolayer in 12-well tissue culture plates in Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. The cells were infected with HSV-1 and HSV-2 at a multiplicity of infection (MOI) of one. The infected cells were treated with varying concentrations of Betulin and Acyclovir and plates were incubated for 18-36 hours at 37 °C in the presence of 5% CO_2. Virus-induced cytopathic effects (CPE) were observed at 18, 24 and 36 hours after infection. A significant antiviral effect against HSV-2 was observed resulting in the 95% reduction of virus-induced CPE, when Betulin and Acyclovir were used at a dose of 125 ηg/mL each as compared to treatment with Betulin and Acyclovir alone at the same dose. Similarly, Betulin and Acyclovir at a combined dose of 125 ηg/mL was found to be more effective against HSV-1. This study indicated that combination therapy using Betulin and Acyclovir against HSV infection is possible.

G-TOO: THE SOCIAL WORLD OF THE SECONDHAND INDUSTRY

Scott Kjar, Hamline University, 1536 Hewitt Avenue, St. Paul, MN 55104

The purpose of this study on the secondhand goods industry was to explore a topic that hasn't had a lot of academic exposure. More specifically, this research examined the setting of G-Too, a division of Goodwill Industries because of its uniqueness compared to other previously owned shopping venues such as garage sales, flea markets, and thrift stores. This study analyzed shopper typologies, the idea of community, gift giving, desire for uniqueness, and fear of contagion. Participant observation and interviews were utilized to collect the data. The results yielded both corresponding and conflicting results with the prior research.

The Effect of Betulin, Allobetulin, and Derivatives against Three Different Genera of Bacteria

Benjamin R. Koch and M.R. Karim, Department of Biology, 211 Life Sciences, University of Minnesota-Duluth, Duluth, MN 55812

Betulin, a pentacyclic triterpenoid derived from the outer bark of white "paper" birch, has been shown to have antiviral properties against the herpes simplex viruses, namely HSV-I and HSV-II. The purpose of this study was to find the *in vitro* effectiveness of betulin, allobetulin, and seventy-five derivatives against three genera of bacteria commonly associated with humans. Bacteria used included *Escherichia cold (E. colt), Staphylococcus aureus,* and *Bacillus subtilis.* The sensitivity of the bacteria to the chemical was assessed using the standard Kirby-Bauer method. Eighteen-hour old bacteria, cultured in nutrient broth, was spread over the surface of a Mueller-Hinton agar plate. Five 400ng dried discs of betulin derivatives were applied to the surface of the agar. Plates were incubated at 37°C for 14 hours. The zone of inhibition, if any, for each chemical was assessed, and compared with the control. Plates were done in triplicate to ensure reproducible results. Betulin 3,28-dimaleate was shown to inhibit the growth of *Staphylococcus aureus.* Two other derivatives, betulin 28-phthlate and allobetulin ethanolamine, also have potential as antibacterial agents. Currently these chemicals are being tested at a higher concentration to assess their effectiveness as chemotherapeutic agents. Further studies are in progress to test the effectiveness of more betulin and allobetulin derivatives. This research is the first step in using derivatives like betulin 3,28-dimaleate for treating bacterial infections in human beings.

Influences of Gender and Power on Mixed-sex Dyads

Lori Korte, Kelly Cornell, Brenda Degner, Nikki Moore, and Jason Mrozek, St. Cloud State University

Previous research has shown that a violation of conversational norms, such as an interruption, results in a speaker being viewed differently depending on his or her gender (LaFrance, 1992). This study looked at how a speaker was viewed after interrupting or being interrupted depending on gender and a specific status role that was assigned to them. Students from four psychology classes rated speakers in four mixed-sex dyed tapes. Results showed that there was no difference in how speakers of different genders were rated when in the same power role. There was a significant difference between how the interrupter and interruptee were rated.

Genetic Fingerprinting in Mussel Conservation

Jesse Kroese, Department of Biology, Macalester College, 1600 Grand Ave., St. Paul, MN 55105.

Freshwater mussels have an unusual life cycle that includes a period of parasitic encystment on fish hosts during their larval stage. Understanding these parasite-host interactions is integral to mussel conservation. Mussels in their larval form, however, are difficult to distinguish from one another. Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) techniques provide an alternative means of distinguishing among these larval forms. The goal of this project is to create an identification key of the genetic fingerprints of the St. Croix River mussels. To do so, DNA is extracted from mussel specimens, amplified via PCR, and cut by restriction enzymes. A diagnostic suite of restriction fragments is found for each mussel species to form the identification key. To date, 21 of the St. Croix River mussel species have been genetically fingerprinted, including the federally endangered *Quadrula fragosa.* Preliminary data suggest that all 38 St. Croix River mussels can be distinguished with this method and that genetic fingerprinting may serve as a valuable larval identification tool for mussel conservation.

Development of an Invivo Fluorescent Phospholipase D Assay

D. Loban. A. Krider and J. Provost, Department of Chemistry, Moorhead State University, Minnesota, 56563

Phospholipase D (PLD) is a ubiquitous enzyme that hydrolyzes the head group from phosphatidylcholine (PC). PLD plays an important role in the signal transduction of many different hormones and growth factors. Activation of PLD can lead to the generation of several bioactive second messenger lipids. There are a variety of mechanisms by which the activity of PLD is controlled, however the exact role and regulation of this enzyme is not well understood and is under intense investigation. The current method for determining PLD enzymatic activity in intact cells involves the incorporation of radio-labeled fatty acids in cell cultures. While this is a reliable method of PLD enzymatic analysis, the use of radioactivity in the assay is expensive and potentially dangerous. In order to avoid the use of radioactive lipids, an invivo method using fluorescent labeled PC incorporation of fluorescent lipid and its potential use will be reported.

The Role of Polysialated Neural Cell Adhesion Molecules in Inflammation-Induced Hyperalgesia

Jessica Maddox and Dana Moody, Macalester College, 1600 Grand Ave., St. Paul, MN 55105

The role of polysialated neural cell adhesion molecules (PSA-NCAMs) in a carrageenan-induced inflammation model of chronic pain in mice is the current focus of research. Previous investigators have shown that PSA-NCAM is necessary for long term potentiation (LTP; a molecular model of memory and learning) in the hippocampus and spatial learning and memory. Endoneuraminidase is an enzyme that specifically cleaves sialic acid from NCAM, leading to decreased LTP, learning, and memory. It is theorized that the increased excitability of the C-fibers in the dorsal laminae of the spinal cord due to chronic pain may be similar to the molecular mechanism underlying LTP. Mice received either intrathecal saline or neuraminidase and either intraplantar saline or carrageenan. Their pain thresholds were then evaluated over a course of 48 hours using a hotplate behavioral assay. It was determined that neuraminidase did not significantly inhibit hyperalgesia in the carrageenan-treated mice. However, neuraminidase produced hyperalgesia in the intraplantar-saline mice. Immunohistochemical studies will be used to examine the levels of total NCAM and PSA-NCAM in the brains and spinal cords of these animals. It is possible that neuraminidase interferes with memory consolidation in these animals, or is, itself, a pain-inducing agent.

Verifying the Presence of Archaeal Nucleic Acids in Picoplankton from the North American Great Lakes by Amplifying the DNA of Archaebacteria using the Polymerase Chain Reaction

Angela Malley and Randall Hicks, University of Minnesota - Duluth, 211 Life Science Building, 10 University Drive, Duluth, MN 55812

In recent experiments, samples taken from the North American Great Lakes, indicated that 1-2% of the total nucleic acid present from picoplankton were archaeal acids. These results were quite unexpected and perplexing, due to the fact that *Archaea* are usually found in extreme environments; those distinguished by anoxic, hypersaline, and severely hot conditions. This discovery is surprising since these conditions are not found in waters of the North American 1 treat Lakes, where the samples were taken. The purpose of this study was

to verify the rRNA hybridization results, which suggested that archaeal nucleic acids were present in the picoplankton. If archaeal nucleic acids were genuinely present in these samples, the archaeal DNA should have amplified. In order to confirm the results of the rRNA hybridizations, samples previously collected were used for DNA amplification. These samples were collected from various North American Great Lakes. With previously extracted picoplankton nucleic acid, DNA was provided and was amplified by the Polymerase Chain Reaction using DNA primers specific to archaebacteria. Both positive and negative controls were amplified to confirm the presence of archaeal nucleic acids. The amplification products were then run on an electrophoresis gel to verify that DNA amplification occurred. Various samples amplified, while other samples did not. This indicates that archaeal nucleic acid may be present in some samples, but not others.

Regulation of Cardiac Myocyte NHE-1 Activity by Potential Agonist Stimulation and Antisence Expression

I. Manke, C Broadwell, L Nustad, A Grimes, M. Baumgartner, M.A. Wallert and J. Provost, Departments of Biology and Chemistry, Moorhead State University, Moorhead, MN 56563

Maintenance of intracellular pH is the physiological function of several ion transporters one of which is the Na+/H+ exchanger (NHE). NHE is a multifamily protein expressed in nearly all cells. NHE-1 is the only exchanger expressed in ventricular myocytes, and is believed to be the chief transporter responsible for intracellular pH maintenance. The regulation of neonatal cardiac myocyte pH is investigated in response to agonist stimulation and down regulation of NHE-1. The regulation of the exchanger is important to understand the means by which hormonal changes, cellular stress and disease states such as diabetes and ischimia affect intracellular H+ concentration. We show that several agonists which act through receptors coupled to the G-proteins increased the activity of NHE-1. Addition of the same agonists resulted in differential translocation and activation of RhoA, a potential upstream effector of NHE. The relative concentration of RhoA in membrane is only slightly increased over the control cells with phenylepherine treatment, while RhoA is substantially increased in membranse were treated with endothelin and angiotensin. Additionally, we have used antisense oligo-nucleotides to down regulate NHE-1 in myocytes. These cells displayed a reduced ability to recover from intracellular acid load as compared to control cells. Alterations in regulation of the exchanger has been implicated in hypertension especially in vascular smooth muscle cells. These data support the role of NHE-1 as the dominant regulator of pHi in cardiac myocytes and that its regulation is potentially under the control of different signaling pathways.

An *In Vitro* System for Assessing the Effects of Chemical Agents on Transgene Expression in *Drosophila melanogaster*

Emory H. Matts and Presley Martin, Hamline University, 1536 Hewitt Ave., St. Paul, MN 55104

The pattern and level of alcohol dehydrogenase *(Adh)* expression is frequently altered in transformed lines in which the *Adh* gene is inserted at a new location in the genome of *Drosophila melanogaster.* Chromatin structure is thought to play an important rôle in causing this abnormal expression. For example, chemical agents known to cause chromatin decondensation (DMSO and sodium butyrate) lead to activation of the *Adh* transgenes in these lines. The goal of this study was to develop an *in vitro* system for testing the ability of chemical agents to alter transgene expression. Fat bodies from third instar larvae of *Drosophila melanogaster* were cultured in 96 well plates in Schneider's *Drosophila* medium. The medium was supplemented with DMSO concentrations ranging from 100 mM to 400 mM and incubated for 24 hours. Both histochemical staining and spectophotometric determination showed that *Adh* expression was increased by DMSO. This system will be used to determine whether Trichostatin A, which alters chromatin structure by specifically inhibiting the enzyme histone deacetylase, increases *Adh* expression levels.

Growth of Legumes Interseeded in Vegetatively Mature Maize

Neil Mattson[1], A. Olness[2], D. Lopez[1], [1]University of Minnesota, Morris, MN 56267, [2]USDA ARS, Morris, MN 56267

Legumes are a source of nitrogen as a result of symbiosis with various species of *Rhizobia.* A two-year field study. was conducted to determine the amount of legume biomass produced from planting legumes during the flowering stage of maize *(Zea mays* L.). Hairy vetch *(Villa vilosa* L.), alfalfa *(Medicago sativa* L.), white clover *(Trifolium repens* L.), medic *(Medicago lupulina* L.) or lupin *(Lupinus albus* L.) was grown in a randomized block design with four replications at 4 sites. Legume vegetation was clipped at ground level in mid-October. Samples were dried at 60° C in a forced draft oven and weighed. Nitrogen content of the legume was determined using a LECO model 2000 HCN carbon analyzer. Regression analyses show that hairy vetch is rather insensitive to competition by maize. Alfalfa showed moderate sensitivity to competition by maize and medic clover was highly sensitive to competition from maize. White clover and lupin germinated but died in the early vegetative stages, perhaps due to lack of water or light or both. Therefore, choice of legume depends on the expected maize canopy cover which affects the response of the legume. Average yields of interseeded legume ranged from about 460 kg per hectare for alfalfa and medic to about 640 kg per hectare for vetch. Average N content of the legume biomass was about 4%.

Movement Patterns of a Larval Caddisfly over Tile and Sand Substrates in Artificial Flumes

Jean M. Miesbauer, Deric Deuschle, and Dr. Roger Haro, University of Wisconsin - La Crosse, 1725 State St., La Crosse, WI 54601

Macroinvertebrates face many challenges crawling in a running water environment, including water currents and substrate stability. We constructed behavioral experiments to assess the effects of substrate stability on the mobility of a larval caddisfly, *Glossosoma intermedium* (Trichoptera: Glossosomatidae). We placed small groups (8-10) of starved caddisfly larvae in artificial flumes with bottoms of unglazed ceramic tiles (stable substrates) and cleaned stream sand (unstable substrates). We assessed movement behavior for both second and fourth instar larvae independently. We videotaped caddisfly movements and interactions over a 24-hour period with a time-lapse recorder and low-light surveillance cameras.

Preliminary results suggest there are differences in behavior between the two instars. Both instars spent most of their time moving near or along tile edges, however fourth instar larvae spent more time on top of the exposed tile and in the sand. While in the sand, second instar larvae commonly emigrated out of camera view in a straight line while the fourth instar larvae circled in wide-angled segments in a fashion similar to search behavior. Behaviorally, the fourth instar larvae contacted each other frequently while direct contact among the second instar larvae was rare. So far, our results suggest that *Glossosoma* locomotion and behavior is influenced by substrate condition and larval stage.

SCANNING ELECTRON MICROSCOPY OF MUSSEL LARVAE
Benjamin D. Miller, Macalester College, 1600 Grand Avenue, St. Paul, MN 55105

The conservation of mussels requires an understanding of their dispersal by fish hosts; thus, successful species identification during their larval state is crucial. Limited data exists for the determination of a species from its larvae. While general identification of subfamilies, for example Ambleminae verses Anodontinae, may be possible using standard light microscopy, determination of the species level is unreliable by these means. Scanning electron microscopy (SEM) may offer reliable classification of glochidia to respective species by observation of small but quantifiable differences in mussel valve structures and sizes. Using SEM, I studied glochidia from eight species of mussels from the St. Croix River, including the federally endangered Q. *fragosa*. Analysis of hinge and valve dimensions suggests that an accurate differentiation between species can be made. In addition to this quantitative analysis, qualitative examinations (pictures from the SEM) prove to be useful in identification as well. Although positive field identification of glochidia is not yet possible; the methods used in this study offer a reliable means for identification in lab. This data forms a substantial framework from which further studies concerning fish host identification is possible.

SEGREGATION PHENOMENA IN BINARY MIXTURES OF GRANULAR MATERIALS IN A HORIZONTAL ROTATING DRUM
Kevin A. Parendo, University of Minnesota - Morris, Morris, MN 56267

Materials composed of macroscopic grains, such as sand and fine powders, exhibit a wide variety of behaviors that cross the boundaries between conventional solids, liquids, and gases. One striking phenomenon is the tendency of mixtures of granular material to segregate by size when agitated. For example, when uniform mixtures of two sizes of glass beads are placed in a horizontal rotating drum, the beads will sometimes segregate by size into bands spanning the length of the drum. This relatively slow "axial" segregation is coupled to a very rapid "radial" segregation in which one component of the mixture will preferentially reside on the surface whereas the other component will tend to stay submerged. It is challenging to understand the full dynamics behind the observed axial segregation solely from observations of the avalanching surface of the beads in the drum mixer.

We are investigating the effect on axial segregation caused by varying the overall concentration of the two components in mixtures that segregate. There appear to be reproducible concentration thresholds beyond which axial segregation is not observable, though it may still occur beneath the surface. We have also sieved mixtures axially to document the degree to which surface bands actually correspond to overall axial concentration fluctuations. Preliminary results will be presented.

DO PHYOTPHAGES RESPOND TO THE PRESENCE OF GALLS ON GRAPE LEAVES?
Jennifer Parish, Hamline University, Department of Biology

The purpose of this research was to measure the effects of interspecific competition between insects that depend on the river bank grape. Being that early-spring gall making insects reduce the area of leaf tissue that is available to other insects throughout the rest of the growing season, I hypothesized that those grape leaves with galls would have less predation by phytophageous (leaf eating) insects. The grape plants used in this study grow along Pierce Butler Route in the Hamline/ Midway area of St. Paul. Digital images of the leaves were captured daily over the course of a three week period. The relative leaf area that had been consumed by phytophages was determined by using NIH image to compare the final relative area of tissue eaten to the initial area of tissue that had been eaten. Correlation tests and the Mann-Whitley U-test show that there is no correlation between the number of galls present on a leaves and the area of leaf tissue consumed. There is a trend that shows that the leaves with galls had less predation than those leaves without galls, although this is not a statistical difference.

DOES THE CYCLIN DEPENDENT KINASE INHIBITOR $p57^{kip2}$ PROTECT FIBROBLASTS FROM APOPTOSIS?
Vadim Pisarenko, University of Minnesota, Departments of Genetics and Cell Biology and Surgery, Box 120 FUMC, Minneapolis, MN 55455.

The cyclin dependent kinase inhibitor (CKI) $p57^{kip2}$ has multiple roles in mammalian development; transgenic null mice lacking $p57^{kip2}$ have multiple defects, including inappropriate cell proliferation and higher than normal levels of apoptosis. During the late phase of wound healing, a portion of the wound fibroblasts have elevated levels of $p57^{kip2}$. I examined the role $p57^{kip2}$ plays in fibroblast apoptosis. Cells were induced to produce $p57^{kip2}$-green fluorescent protein (GFP) fusion proteins or antisense $p57^{kip2}$ and GFP, or GFP alone. Protein expression was induced by transient transfection of Rat1R12 fetal fibroblasts with mammalian expression vectors. The cells were then treated with agents which stimulate apoptosis, i.e., camptothecin, etoposide and wortmannin. Cells expressing $p57^{kip2}$ were protected against apoptosis in that fewer of the apoptotic cells were seen to be expressing GFP. On the other hand, introducing deleted versions of $p57^{kip2}$ and $p57^{kip2}$ level-lowering mRNAs, did not protect cells from apoptosis. Furthermore, we have shown that inducing $p57^{kip1}$ production (another related CKI) in cells produced similar results as $p57^{kip2}$. These results are consistent with the concept that $p57^{kip2}$ protects fibroblasts from apoptosis during the late phase of wound healing and conserves a portion of the wound fibroblast population.

FIBROBLAST EXPRESSION OF $p57^{kip2}$ DETECTED BY IMMUNOCYTOCHEMISTRY AFTER TRANSIENT TRANSFECTION.
Samuel A. Roiko, University of Minnesota, Departments of Genetics and Cell Biology and Surgery, Box 120 FUMC, 420 Delaware St. SE, Minneapolis, MN 55455

The cyclin dependent kinase inhibitor $p57^{kip2}$ binds to cyclin/cyclin-dependent kinase complexes during the G1 phase of the cell cycle and inhibits cell proliferation. In wounds, $p57^{kip2}$ is expressed in a subset of fibroblasts during the late phase of healing. The goal of this study was to characterize $p57^{kip2}$ in a fetal fibroblast cell line (Rat1R12) using immunocytochemical staining procedures, paving the way for examination of $p57^{kip2}$ using flow cytometry. In addition, I wanted to inhibit $p57^{kip2}$ expression using antisense expression constructs. Rat1R12 fibroblasts were transfected with mammalian expression vectors encoding $p57^{kip2}$ fused to the green fluorescent protein (GFP), antisense $p57^{kip2}$ RNA/ GFP constructs, or GFP alone. The transfected cells were stained with affinity purlfied or unpurified rabbit antisera to $p57^{kip2}$. The bound antibodies were then detected with biotinylated donkey-α-rabbit Fab2, and streptavidin conjugated to Cy-3. Both antibody preparations demonstrated specific staining of cell nuclei, as expected. However, the affinity purified antibody preparation was surprisingly unstable. Expression of $p57^{kip2}$/GFP fusions greatly enhanced immunostaining. Attempts to antagonize the expression of $p57^{kip2}$ by transient transfection of antisense mRNA vectors proved unsuccessful. Further study will involve attempts to

antagonize the expression of $p57^{kip2}$ by transient transfection of specific antisense oligonucleotides.

COMPUTER CCD ANALYSIS OF MUTATIONS IN MAIZE AND WILD RICE MICROSPOROGENESIS
Baumgardt A. Jennifer, Sedgwick R. Michael (Qin Qin Liu). University Minnesota-Duluth Department of Biology, Duluth, MN 55812

CCD computer imaging analysis is an effective technique for identifying subcellular defects caused by mutations in maize and wild rice microsporogenesis. With the use of a DAPI fluorescent probe, each stage of microsporogenesis can be observed and related to anther development. For example in maize, the defects of chromosome and cell wall formation in transposon induced mutants was identified by such a technique. In addition, the first documentation of cytological defects in mutant wild rice microspores has been produced by this methodology.

THE DETERMINATION OF A THRESHOLD LEVEL OF EXOGENOUS METHYL JASMONATE EFFECTING AN INCREASE IN PEROXIDASE ACTIVITY IN PROTOPLASTS OF *AVENA SATIVA*
Dwight R. Stoll, Minnesota State University, Mankato, Department of Biology, PO. Box 8400, Mankato, MN 56001

Methyl jasmonate plays a dual role in plant systems by participating in both developmental and defense mechanisms. Recent studies have shown that methyl jasmonate can specifically alter gene expression, while wounding and specific elicitors can cause a buildup of methyl jasmonate itself. Peroxidase enzymes are ubiquitous to all plant systems and have long been associated with plant stress and defense responses. The objective of this research was to establish a threshold concentration of methyl jasmonate effecting a maximum increase in peroxidase activity less than twenty-four hours after treatment. While previous experiments have explored the activity of methyl jasmonate on peroxidase activity using whole leaf tissue, we developed a system for testing peroxidase activity in oat protoplasts after isolation and subsequent treatment and incubation in a suitable culture media.

NEED ABSTRACT TITLE
Steve Svoboda, Hamline University

Noscapine ($C_{22}H_{23}NO_7$) is a primary alkaloid component of opium. Its pharmaceutical effects are similar to that of codeine, yet it lacks any addictive, depressive or analgesic side effects. Noscapine is water-soluble and can be administered orally, which has allowed for its use as an anti-tussive agent in over-the-counter cough syrups around the world since the 1950s (excluding the United States.

Recent work conducted by Ye et al (1998) suggests that noscapine may also be used as an anti-mitotic agent in the division of human breast and bladder cells implanted in nude mice. Noscapine was found to halt the construction of microtubules used in the formation of the mitotic spindle necessary for mitotic cell division and ultimately induce a programmed cell death, or apoptosis. Ye et al found that an 80% regression of tumor growth occurred over a 3-week treatment with noscapine, with 60% of the test subjects treated with noscapine showing a complete loss of tumor formation. Ye et al conclude from their study that noscapine's chemotherapeutical potential in human cancer development merits thorough evaluation.

The purpose of this study is to determine the effectiveness of noscapine as an anti-mitotic agent on a simpler *in vivo* eukaryotic system. These results are compared to the effectiveness of anti-mitotic behavior exhibited by taxol (a standard chemotherapy agent which displays a similar anti-mitotic behavior, along with harsh side effects) on the same system to determine a relatively feasibility of substituting noscapine for taxol's chemotherapeutical applications.

EFFECT OF TOPPING ON THE MANAGEMENT OF MAIZE
[1]Dan Swenson, A. Olness[2], D. Lopez[1], [1]University of Minnesota, Morris, MN 56267, [2]USDA-ARS, Morris, MN 56267

Grain moisture at harvest is often too great in northern regions of the cornbelt to permit storage without drying. Topping of maize (*Zea mays* L.), that is, removal of foliage above the ear late in the growing season, results in faster grain dry down in the field. However, it may cause a loss of yield. Topping was conducted at 4 sites in 1997 and at 3 sites in 1998 to determine effects of this practice on grain yield. Corn was topped in the R-4 growth-stage in 1997 and in the R-5 growth-stage in 1998. Grain yields were converted to relative yields by dividing sample yields at a site by the largest yield obtained at that site; this eliminates the effect of climatic variation between years and sites. The effect of topping varied with growth-stage and site. Topping caused yield losses $\leq$ 35% in 1997 and produced variable effects in 1998. Yield gains with topping were probably due to an interaction with the late summer drought stress in 1998. Topping resulted in reduction of grain moisture at harvest by about 1 to 6.7%. Price dockage of about $0.049 per bushel for moisture content > 15.5 % means that topping increased the value of the harvested crop of $0.05 to $0.30 per bushel. This decrease in drying cost can, under certain conditions, offset grain yield loss and costs of the topping operation.

A COMPARISON OF ENVIRONMENTAL INFLUENCES ON TERRESTRIAL AND BENTHIC HABITATS WITHIN A WATERSHED, EXAMINED THROUGH PAST GROWTH RATES OF OAK TREES, *QUERCUS SPP.*, AND FRESHWATER MUSSELS, FAMILY UNIONIDAE.
Phoebe B.S. Vanselow, Advisor: Daniel J. Hornbach, Department of Biology, Macalester College, St. Paul, Minnesota 55105

As the threat of global climate change becomes more of a reality, it is important to try to predict how ecological systems will cope with increased and novel environmental stresses. The effects these factors have on growth of organisms is one way of measuring potential ecosystem response. These effects can be predicted using knowledge of growth rates during past years of extreme temperature and precipitation levels. Previous studies have shown the ability to recognize the influence of temperature and water stress on tree growth through the interpretation of tree cores. Studies have also analyzed the ability of bivalve shell records to describe past climates. This study examined the relationships between the terrestrial and benthic habitats within the St. Croix River watershed. It determined the influence of past environmental disturbances on the growth rates of organisms in both habitats and how these rates varied between the two communities. This was accomplished using tree cores, thin- sectioned mussel shells, and meteorologic and hydrologic data. Cores were gathered from two species of oak trees found growing on slopes, making them especially sensitive to water stress. Mussel thin-sections were made after collecting two species of mussels from the family Unionidae from the St. Croix River and one of its tributaries, the Sunrise River. Overall past growth patterns for both the mussel and tree populations were determined. Time-series analysis will be used to analyze the relationship between growth rates of oaks and mussels and meteorologic and hydrologic factors. The sensitivity of these communities to the potential effects of climate change can then be assessed.

ISOLATION, SEQUENCING AND CHARACTERIZATION OF AN OOCYST WALL PROTEIN GENE FROM *CRYPTOSPORIDIUM PARVUM*

Vladimir Vigdorovich, Department of Veterinary Pathobiology, University of Minnesota, 295 AnSci/VetMed, 1988 Fitch Avenue, St. Paul, MN 55108

Cryptosporidium parvum is a protozoan parasite, which causes severe intestinal disease in all mammals. The importance of this pathogen in human medicine is highlighted by the chronic infections it causes among the AIDS patients. *C. parvum* persists in the environment outside the host in a structure called the oocyst. The oocyst wall is responsible for exceptional stability of the oocyst, allowing it to withstand harsh chemical treatments, such as the methods used for disinfection of drinking water. We have obtained the complete coding sequence for a novel gene (Cp102), whose sequence displays limited homology to a previously described oocyst wall protein gene (COWP). We have determined that the pattern of Cp102's expression during parasite development is consistent with that known for oocyst formation. However, this pattern is distinct from that for COWP. Further, we have shown both genes map to the same chromosome. Work is currently under way to produce a recombinant protein from the parts of the coding sequence, for future use in generation of antibody reagents. These reagents will be used to characterize protein localization within the oocyst wall structure.

GENETIC ENGINEERING PHOTOSYNTHETIC COLD TOLERANCE IN MAIZE BY SITE DIRECTED MUTAGENESIS OF THE GENE ENCODING PYROVATE, ORTHOPHOSPHATE DIKINASE.

I. SINGLE MUTATIONS AND THEIR EFFECT ON COLD STABILITY OF PYROVATE-PI-DIKINASE.

Adam Vossen and Erin Watkin, Department of Biology, Moorhead State University, Moorhead, MN, 56563

Compared to other cereal grains, maize is a remarkably productive crop species because it has a highly advanced form of photosynthesis called C4-photosynthesis. A key enzyme of C4 photosynthesis is pyruvate,orthophosphate dikinase (PPDK). Unlike other enzymes in the pathway, this enzyme is particularly susceptible to cool temperatures and becomes inactive at about 12 °C. However, it was recently discovered that another C4 plant, *Flaveria brownii,* has a PPDK that is insensitive to cold. Comparisons of the maize and *F. brownii* PPDK genes suggests a difference of only four amino acids in the C-terminal portion of the enzyme is responsible for conferring tolerance to cold. We have used site-directed mutagenesis to change these four specific amino acids in maize. Results on how various single amino acid changes effects cold stability of the maize enzyme will be presented.

THEORETICAL AND EXPERIMENTAL INVESTIGATION OF ANNEXIN V BINDING TO NEGATIVELY CHARGED PHOSPHOLIPID VESICLES

Justin D. Wheeler and N. London, Department of Biology, St. Olaf College, Northfield, MN 55057. Advisor: Dr. Anne Walter

Annexin V is a 36-kDa peripheral protein which binds in a calcium-dependent manner to phosphatidylserine (PS) containing vesicles. To understand protein-membrane interactions we investigated annexin V binding using a molecular graphics program, mathematical predictions of electrostatic forces, and fluorescence spectroscopy. Since the $[Ca^{2+}]$ at the membrane surface will (a) vary with surface charge and (b) alter annexin binding, we calculated surface $[Ca^{2+}]$ as a function of solution [NaCl]. Mathematical modeling predicted that a decrease in the sodium chloride concentration in solution would result in an increase in calcium surface concentration and a corresponding increase in annexin surface concentration. By fluorescence spectroscopy assay we are able to measure annexin V binding by a red shift and intensity increase in emission due to energy transfer from annexin's tryptophan residue to dansyl-PE in the vesicle bilayer. This assay confirmed an increase in binding with reduced NaCl concentration, but not to the magnitude predicted by mathematical calculations. In order to confirm energy transfer results, we have begun to examine annexin binding using fluorescence anisotropy. Preliminary experiments indicate an increase in anisotropy values upon binding, consistent with parallel changes in tryptophan-dansyl energy transfer. By varying the ionic strength of our solution we hope to maximize annexin's affinity, allowing us to construct a supported planar bilayer of phospholipid bound annexin on mica, which may then be imaged using atomic force microscopy to test by direct observation our theoretical and fluorescence results.

THE SUPPRESSIVE EFFECTS OF *STREPTOMYCES* ON *RHIZOBIUM MELILOTI*

Amy M. Willert, Hamline University, 1536 Hewitt Ave. St. Paul, MN 55104

The objective of this study is to determine the effects of *Streptomyces,* used in plant disease control, on *Rhizobium meliloti,* the symbiotic bacteria responsible for nitrogen fixation. Fifteen suppressive strains of *Streptomyces* were tested for their ability to suppress the growth of *Rhizobium meliloti* using *in vitro* and *in vivo* assays. For the *in vitro* assay, a double-layer agar method was used. *Streptomyces* strains displaying the strongest suppressive effects were assayed *in vivo by* co-inoculation onto germinating alfalfa seeds. Correlation of *in vitro* and *in vivo* results will be discussed.