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# 13<sup>th</sup> Annual Research Symposium

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Sponsored by  
the Graduate Student Assembly,  
the Graduate School, &  
the College of Engineering

Keynote Address: "*Rescue in Space*"

Fred W. Haise Jr.  
Apollo 13 Astronaut

April 28, 1997  
Commonwealth Ballroom  
Squires Student Center

## PREFACE

*The Research Symposium of Virginia Tech is an annual event devoted to showcasing, documenting, discussing, and reporting accomplished research work conducted at Virginia Tech. It provides a wonderful opportunity for both undergraduate and graduate students campus-wide to present their research in a professional poster format.*

*We congratulate all the participants of the 13th Annual Research Symposium of Virginia Tech for their excellent research work. It is great pleasure to have with us on this occasion Mr. Fred Haise, Astronaut member of Apollo 13, to deliver the keynote speech.*

*We would like to thank all the members of the Research Symposium Committee, Graduate School, Commission on Research, Center for Undergraduate Excellence in Teaching, University Honors Program, College of Engineering, Bell Inc., and the Graduate Student Assembly for their support and encouragement.*

*The research reported in this Symposium's proceeding is a glimpse of the ongoing research work done at Virginia Tech.*



Rajiv Khosla



Alan J. Stesinski

*Co-Chairs: 13th Annual Research Symposium of Virginia Tech-1997.*

**Welcome to the 13<sup>th</sup> Annual Research Symposium of Virginia Tech  
April 28, 1997**

The Graduate Student Assembly is pleased to host this Symposium in order to give undergraduates, graduates, and doctoral candidates an additional opportunity to present their research. This booklet is a compilation of the abstracts they submitted to the Research Symposium Committee.

The Graduate Student Assembly (GSA) is an umbrella organization for the graduate students at Virginia Tech. The Research Symposium is one of the annual activities of the GSA. Other activities include the: Graduate Research Development Project, Travel Fund Award Program, Graduate and Professional School Fair, Virginia Tech Off Campus Housing (VTOCH), and several others. If you are interested to participate in any of the activities, or become a part of the GSA, please contact any of the members of the GSA Board listed below. Thank you.

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## **Undergraduate Category**

## 1 Neural Control: Getting an American Cockroach to Drive a Car

Mr. Steven Bathiche: Senior Undergraduate of Electrical Engineering.

Dr. Jeff Bloomquist: Associate Professor of Entomology.

The connection of the living to the non-living – the interface between humans and machines – has been a recurring theme in the works of futurists such as William Gibson (*Neuromancer*) and Michael Crichton (*The Terminal Man*). The merging of the tools of engineering with the principles of biology may bring these fictions to a reality. The implications of this technology will certainly have a strong impact in society, for better or for worse.

The project's goal was to have a cockroach drive a vehicle via its flight muscle action potentials. We used the American cockroach (*Periplaneta americana*, L.), because of its robustness and large size. The cockroach was tethered to a boom which extends in front of a remote-controlled car chassis.

The control signal path starts from the flight muscles of the cockroach. A thin wire recording electrode is placed in the thoracic flight muscles, and the reference electrode is placed in the abdomen. The recording electrode delivered signals to an amplifier and filter. After the signal is filtered, it is fed into a Motorola 68HC11 EVBU microcontroller. The microcontroller measures the period of the incoming signal and drives the motor of the car forward accordingly via an electronic speed control. The performance of the interface allowed the car to move forward whenever the tethered insect initiated flight behavior. The next immediate developments of the project would include signal processing from the muscles involved in turning to process the left and right intentions of the cockroach.

Future applications include interfacing humans with machines, computers, and each other. This approach has direct consequences for developing interfaces to provide increased mobility and other quality of life applications for the severely handicapped.

## 2 DETERMINATION OF OVULATION TIME IN SHEEP AFTER INJECTION OF P.G. 600

M. A. Cline\*, J. N. Ralston, R. C. Seals, and G. S. Lewis.

Department of Animal and Poultry Sciences

Predicting the time from the end of an estrus synchronization treatment to ovulation is essential for successful timed breeding. Thus, our objective was to determine the time of ovulation in ewes after removal of progestogen implants and injection of P.G. 600 (400 IU pregnant mare's serum gonadotropin and 200 IU human chorionic gonadotropin). Seven days after estrus was observed, ewes received Syncro-Mate-B implants (SMB), and Lutalyse was injected i.m. 6 d later to cause luteolysis. The SMB implants were removed 10 d after insertion, and ewes received i.m. injections of either 2 mL of saline (n = 5) or 5 mL of P.G. 600 (n = 5). Estrus was determined every 12 h after the P.G. 600 or saline. From the time estrus was first observed, ovaries were evaluated with transrectal ultrasonography at 0, 16, 24, 32, and 40 h or until ovulation was detected (i.e., disappearance of the largest follicles). Compared with saline, P.G. 600 reduced the interval from SMB removal to estrus ( $P < .06$ ;  $58 \pm 10$  vs  $41 \pm 12$  h) and to ovulation ( $P < .05$ ;  $100 \pm 9$  vs  $79 \pm 14$  h). However, P.G. 600 did not affect the interval from estrus to ovulation ( $42 \pm 8$  and  $38 \pm 7$  h for saline and P.G.600, respectively). In conclusion, the interval from implant removal to estrus, and hence ovulation, was less in P.G.600-treated than in saline-treated ewes. This information may help improve the success of timed breeding programs for sheep.

### 3 Colonization of soil microarthropods as a potential index for recovery of degraded ecosystems.

Scott Cooney and John R. Heckman  
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Restoration ecology has focused on the development of indices to measure the adequate recovery of a system. By comparing a recovering system with one of similar structure to the site in its predisturbance condition, one can readily draw systemic comparisons with certain structural and functional endpoints. This study looks at the potential for using the soil microarthropod community as an index of recovery. Soil microarthropods, generally fall into two main categories: subsurface soil inhabitants, and surface leaf litter dwellers. By looking at the trends in soil microarthropod distribution and abundance, one can extrapolate these findings and make some assumptions about the nature of the disturbance. This project investigated soil microarthropods as a structural measurement of the relative disturbance of a clearcut and burned forest plot, by comparing it to an adjacent undisturbed plot. Findings indicated significant differences in community structure with taxa richness being greater at the undisturbed site, while raw numbers of individuals were found to be greater in the disturbed site. Possible explanations for this seemingly unexpected result are discussed. The composition of leaf litter dwellers vs. soil dwellers were different in the sites, possibly due to the destruction of habitat for the former in the disturbed site. This is supported by a decrease in litter dwelling mites (Acarina) in the disturbed sites but an increase in soil dwelling springtail (Collembola). Due to these differences, it is concluded that soil microarthropod community structure has merit as a structural measure of ecosystem disturbance and subsequent recovery.

### 4 Transcervical Intrauterine Artificial Insemination in Ewes: Improvement With Exogenous Oxytocin and an Insemination Instrument Designed for Sheep

B.A. Costine\*, M.C. Wulster, and G.S. Lewis  
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Currently, transcervical intrauterine artificial insemination (AI) procedures are neither reliable nor practical for sheep producers. Thus, this experiment was conducted to determine the effects of oxytocin and AI instrument on the time required to perform AI. Ewes (n = 10/group) in synchronized estrus were assigned to randomized treatments that were in a 2 x 2 factorial arrangement; oxytocin and type of AI instrument were main effects. Ewes received either 400 USP units of oxytocin or 20 mL of saline intravenously 20 min before AI with either a standard cattle AI instrument or an instrument that we designed for sheep. Oxytocin was used to cause cervical dilation. The time required to pass the tip of an AI instrument through the vagina and cervix and into the uterus was measured. The following mean times are for each treatment group: 150 s for saline and cattle AI instrument; 135 s for saline and sheep AI instrument; 37 s for oxytocin and cattle AI instrument; and 87 s for oxytocin and sheep AI instrument. Oxytocin reduced ( $P < .05$ ) the time required to pass the tip of an AI instrument into the uterus. The type of AI instrument did not affect the time required to enter the uterus. We conclude that oxytocin-induced cervical dilation and an AI instrument designed for sheep are important improvements for transcervical intrauterine AI and should aid in the development of an AI procedure that can be used successfully on sheep farms.

## 5 Nitric Oxide Modulates Skeletal Muscle Sarcoplasmic Reticulum Calcium Exchange

Lillian M. Diss\*, Christopher W. Ward and Jay H. Williams  
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Nitric Oxide (NO) is a highly reactive, short-lived, biologically active molecule synthesized in numerous body tissues. In fact, NO plays a critical role in modulating physiological processes such as blood pressure, neurotransmission, and immune function. For example, NO donors such as nitroglycerine are often used therapeutically in the treatment of angina. NO can also be synthesized by skeletal muscle and may play a role in the development of force production. If the latter is true, then NO may alter sarcoplasmic reticulum (SR) function, a key determinant of force output. To this end, SR function was assessed by measuring the rates of SR  $\text{Ca}^{2+}$  exchange in the presence of sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetylpenicillamine (SNAP). These compounds spontaneously release NO in aqueous solutions. SR membrane vesicles were isolated from rat *m. gastrocnemius* and treated with 0.01-10 mM SNP and SNAP. Both SNP and SNAP depressed ATP-stimulated  $\text{Ca}^{2+}$  uptake and  $\text{AgNO}_3$ -induced release in a concentration dependent manner. At 1 mM, both SNP and SNAP decreased  $\text{Ca}^{2+}$  uptake by 46.5% and 43.3%, respectively ( $p < 0.05$ ). In addition, 10 mM SNP slowed  $\text{Ca}^{2+}$  uptake by 70.4% and release by 45.5%, while 1 mM SNAP decreased the release rate by 39.4% ( $p < 0.05$ ). Concentrations  $\leq 0.1$  mM were not effective and neither compound, administered alone, triggered  $\text{Ca}^{2+}$  release. These results suggest that NO interferes with the SR  $\text{Ca}^{2+}$  pump and release channel. Such effects could result in depressed force output in whole muscle by altering intracellular  $\text{Ca}^{2+}$  exchange.

## 6 Development of a Transcervical Embryo Recovery Procedure for Sheep

S.F. Flohr\*, M.C. Wulster, and G.S. Lewis.  
Department of Animal and Poultry Sciences.

Despite advances in the development of a transcervical intrauterine artificial insemination procedure for sheep, few studies have been conducted to develop an embryo recovery technique for sheep. This experiment was conducted to determine the feasibility of a transcervical embryo collection method. Ewes were assigned to two treatment groups. During the luteal phase, all ewes were laparotomized, and ten embryo-sized glass beads were injected into each uterine horn. For transcervical recovery, 100  $\mu\text{g}$  of estradiol-17 $\beta$  was injected i.v. on d 5 to increase cervical oxytocin receptors, and 12 h later, 100 USP units of oxytocin was injected i.v. to dilate the cervix. Approximately 25 min after oxytocin, a modified Foley catheter was passed into the uterus for injection and recovery of fluid. For recovery at laparotomy, each uterine horn was occluded posterior to the bead injection site, and fluid was injected into the uterine lumen and flushed through the catheterized oviducts. The number of beads and volume of fluid recovered were recorded. Fluid recovery at laparotomy was greater ( $P < .05$ ) than with the transcervical procedure (74.9 vs 44.8% of the total). Bead recovery at laparotomy was greater ( $P < .001$ ) than with the transcervical procedure (55.7 vs 1.1%). Even though the recovery of fluid and beads with the transcervical procedure was less than at laparotomy, our success indicates that it may be possible to develop an embryo recovery procedure that is appropriate for unanesthetized sheep.



## 7 Interleukin-12 Restores T-cell Interferon- $\gamma$ Production During Tumor Growth

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Department of Biology, Microbiology and Immunology Section

The macrophage (M $\phi$ )-derived cytokine interleukin-12 (IL-12) is vital for a successful antitumor immune response. IL-12 promotes the development of T<sub>H</sub>1-type T-cells, which are important mediators of cell-mediated immunity. These cells typically produce large quantities of interferon- $\gamma$  (IFN- $\gamma$ ), which promotes both T-cell and M $\phi$  antitumor responses. In a tumor-burdened host (TBH), M $\phi$ s are subverted into suppressor cells, producing elevated levels of suppressor cytokines and little IL-12, leading to suppressed T-cell responses. We hypothesize that IL-12 may reverse tumor-induced suppression of T-cell responsiveness through reconstitution of T-cell IFN- $\gamma$  production. To test this hypothesis, splenic CD4<sup>+</sup> or CD8<sup>+</sup> T cells were purified from either normal host or TBH mice and cultured without or with the T-cell mitogen Concanavalin-A and recombinant murine IL-12. Cell-free supernatants were collected and assayed for IFN- $\gamma$  using a specific ELISA. At low doses, IL-12 promoted IFN- $\gamma$  production by normal host and TBH CD8<sup>+</sup> T-cells; more significantly, exogenous IL-12 restored TBH CD4<sup>+</sup> T-cell IFN- $\gamma$  production. Reconstituted IFN- $\gamma$  production could have dramatic therapeutic consequences for a cancer patient. These results suggest that IL-12 may be useful as an anticancer immunotherapeutic agent to restore T-cell IFN- $\gamma$  production, thus promoting antitumor immune responses.

## 8 Generation of Vectors to Engineer Plants for Potential Poultry Vaccines

K. Moffat\*, C. Cramer, and S. Boyle

Departments of Plant Pathology/Physiology and Biomedical Sciences/Pathobiology

Hemorrhagic enteritis virus (HEV) is a DNA virus of the adenovirus family. This virus affects many commercial turkey flocks, causing an acute infectious disease. \$100 million could be lost per year in the United States turkey industry without an adequate vaccine. This live vaccine that is currently available can be immunosuppressive and predisposes turkeys to secondary bacterial infections. We are interested in exploring whether transgenic plants (e.g., corn) can be used to produce and deliver a protective vaccine antigen. As a first step toward this goal, we need to generate plant transformation/expression vectors containing an HEV gene fused to a plant promoter. We plan to use the HEV fiber gene which encodes a component of the viral capsid. The specific objectives of this research are: 1) to fuse the coding region for the HEV fiber gene to a constitutive plant promoter (the 35S promoter from the cauliflower mosaic virus) in the cloning vector pBluescript and 2) to introduce the 35S:fiber construct into the transformation vector pBIB-HYG for *Agrobacterium*-mediated transformation. Strategies for cloning and vector confirmation will be presented.

## 9 The Hypocrisy of American Democracy: Assessing the Effects of International Pressure on the Civil Rights Movement

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Department of Political Science

Opposition to unjust institutional policies has emanated from various sources, whether it be through national organizations, community groups, or individuals. However, these sources have often neglected to explore the potential of international pressure in the struggle to eradicate oppressive United States domestic policies. Our society is so blinded by ethnocentrism that Americans would never imagine international pressure having an effect on the laws within our own borders. A clear example of this ethnocentrism is within areas of research, particularly in the assessment of the forces behind the civil rights struggle. The objective of this study was to use the Amicus Curiae Briefs submitted by the U.S. government in the first civil rights cases following World War II, specifically the landmark decision of *Brown v. Board of Education*, 347 U.S. 483 (1954), to show the effectiveness of international pressure on the U.S. in tearing down racism. Such a position is based on the belief that the *Brown* decision did not derive out of compassion from a white dominated U.S. Supreme Court, but was a result of international pressure both at the United Nations and in international press coverage displaying the hypocrisy of American democracy. It was in the best interest of the U.S. to display to the world that it was moving in the direction of racial egalitarianism; promoting American democracy and fighting international Communism.

## 10 Structure/Function Studies of the *GLP* Repressor in *Escherichia Coli*

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Department of Biochemistry

In *Escherichia coli*, the *glp* operons which encode the glycerol 3-phosphate utilization pathway are negatively controlled by the tetrameric *glpR*-encoded repressor. Genetic approaches were used to define the structure and function of the *glp* repressor. The objective was to locate regions of the repressor important for interactions with the operator and inducer (glycerol-3-phosphate). Specific regions of repressor were altered through mutagenesis of defined segments of the cloned *glpR* gene using two different methods, PCR and a mutator strain. DNA sequence analysis revealed amino acid substitutions responsible for altered repressor function. Substitutions in the N-terminal domain were found that affected operator binding. The inducer binding function was localized to a larger C-terminal region of the repressor.

## 11 Regular Strictly Bi-Transitive Graphs

Karen S. Potanka

Department of Mathematics

While the recent evolution of graph theory can be attributed to its connection to computer science, graph theory has been found to be a key tool in the solution of a wide variety problems in areas such as chemistry, engineering, architecture, and economics. The objective of this research project was to prove or disprove the existence of regular strictly bi-transitive graphs; graphs being a set of vertices and edges, where edges join vertices. Regular strictly bi-transitive graphs must satisfy the following four conditions: 1) *regularity* (the same number of edges meet at each vertex), 2) *bipartite* (the vertex set can be partitioned into two sets S and T so that any edge joins a vertex of S to a vertex of T), 3) *edge-transitive* (given any two edges, there is some symmetry of the graph which maps one edge to the other edge), and 4) *not vertex-transitive* (given any two vertices, there is not necessarily a symmetry which maps one vertex to the other.) The existence of strictly bi-transitive graphs is known, but what happens when we add the condition of regularity? Intuitively, one might think that because all vertices are of the same degree, the symmetry group would act transitively on the vertices. Contrary to this belief, two infinite families of regular strictly bi-transitive graphs were found and several properties of such graphs were noted.

## 12 Determination of Ovulation Time After Injection of Pregnant Mare's Serum Gonadotropin (PMSG)

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Department of Animal and Poultry Sciences

Treatment with PMSG in conjunction with estrus synchronization programs in sheep are designed to increase the number of ewes in heat during a limited time period and better control the time of ovulation for artificial insemination. Thus, our objective was to determine the time of ovulation after PMSG injection. Sheep received one-half of a Syncro-Mate-B progestogen-releasing implant. Six days after implantation, all sheep received Lutalyse to induce luteolysis. The implants were removed after 9 d, and ewes received either 2 mL of saline (n = 5) or 400 IU of PMSG (n = 5). After saline or PMSG injection, ewes were observed twice daily for estrus. After the onset of estrus, the ovaries in each ewe were evaluated with transrectal ultrasonography at 0, 16, 32, 40, and 48 h or until ovulation was observed (i.e., disappearance of the largest follicles). The PMSG, compared with saline, reduced the interval from implant removal to estrus ( $P < .05$ ;  $36 \pm 8$  vs  $57 \pm 10$  h) and to ovulation ( $P < .05$ ;  $76 \pm 15$  vs  $100 \pm 9$  h). However, the interval ( $41 \pm 4$  h) from estrus to ovulation did not differ between treatments. The results of this experiment indicated that even though PMSG did not affect the interval from estrus to ovulation it shortened the interval from implant removal to estrus; thus, it shortened the overall interval from implant removal to ovulation. This information can be used to better determine the time to artificially inseminate sheep.

### 13 Construction of an Expression Plasmid for Introduction of a Fungal Phytase Gene into Soybean

Stacey Smith and Elizabeth Grabau

Depts. of Biology and Plant Pathology, Physiology and Weed Science

Soybean seeds have levels of phosphorus adequate for animal growth. However, the majority of phosphorus is in the form of phytate located in the seed protein bodies. Phytate cannot be digested and utilized by non-ruminant animals. If the gene for fungal phytase, an enzyme that catalyses the removal of six phosphate groups from phytate, were introduced into the soybeans, it should be possible to increase the level of free phosphorus in the seeds. This would eliminate the need for organic phosphate supplements in livestock feed and decrease the amount of environmental phosphorus pollution. The long term goal of my research project is to incorporate the fungal phytase gene (*phyA*) from *Aspergillus niger* into soybeans. An expression plasmid will be constructed containing the *phyA* coding sequence, a seed specific promoter to express the phytase gene during seed development, plant signal and targeting sequences to localize the protein product to the plant vacuole, and a hygromycin resistance gene for selection of transformed material. My work involves insertion of the barley-lectin vacuolar targeting sequence into a *Bgl*II site at the end of the phytase coding region. In addition, the gene for hygromycin resistance will be inserted into the expression plasmid. Colony hybridization will be used to screen transformed colonies. Restriction analysis will be conducted to verify the insertion and orientation of the hygromycin resistance gene.

### 14 Assessing the Accuracy of the Diet Analysis Computer Software Program, Nutritionist III for Zinc Analysis.

A.B. Steenland\* and E. Thomas

Department of Human Nutrition, Foods, & Exercise

The purpose of this study was to assess the accuracy of the computer diet analysis program, Nutritionist III for zinc analysis. Diets containing 3-5mg (zinc3), 7-9mg (zinc7), 14-16mg (zinc 14), and 22-24mg (zinc 22) of zinc were designed based on data from Nutritionist III. According to the computer analysis, zinc3 had 3.96mg of zinc; zinc7 had 8.56mg of zinc; zinc14 had 14.11mg of zinc; and zinc22 had 23.86 mg of zinc. Diets ranged from 1875 kcal to 2331 kcal, with a goal of 55% carbohydrate, 15% protein, and 30% fat. Food items were purchased from a local grocery store and prepared as they would be under free-living conditions. Zinc content for each diet was determined by direct laboratory analysis. Each diet was prepared and analyzed on two separate occasions. The first study yielded results of 6.67mg, 6.81mg, 41.91mg, and 15.26mg of zinc for zinc3, zinc7, zinc14, and zinc22, respectively. The second study yielded results of 5.59mg, 8.45mg, 48.67mg, and 15.07mg of zinc, respectively. All of the laboratory analyses of zinc resulted in different amounts of zinc in foods when compared with the expected values based on the Nutritionist III computer diet analysis. Zinc14 was consistently higher than expected (41.91mg and 48.67mg) and zinc22 was consistently lower than expected (15.26mg and 15.07mg). Based on these findings, the Nutritionist III computer diet analysis program was not a reliable indication of zinc content of diets. Further research needs to be done to determine those food items that contain amounts of zinc different from the Nutritionist III computer diet analysis program.

## 15 Suboptimal Energy Intake is Associated with Inadequate Iron Intake in Young Women

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Department of Human Nutrition, Foods, and Exercise

This research was conducted to examine the relationships between energy intake and iron intake and iron status. Two sample populations were studied to analyze both dietary intake and serum ferritin. The first group (low group, n=9) was characterized by a suboptimal energy intake of <24.5kcal/kd body weight. The second group (high group, n=9) was characterized by a chronic optimal energy intake of >34.0kcal/kg body weight. It was assumed that the high group, having consumed more kcals, would have higher levels of dietary iron, and therefore would also have higher levels of serum ferritin than the low group. The serum ferritin value was determined using an ELISA assay. In both groups the following parameters were assessed: average serum ferritin value from 2 separate blood collections, dietary iron intake, total kcal intake, and kcal/day REE. The average serum ferritin value was not significantly different between the 2 groups (p=0.14). However, none of the women in the low group consumed the RDA for iron while 5 out of 9 women in the high group did. Therefore, a correlation between kcal intake and dietary iron was found in the high group (r=0.50). Furthermore, there was also a correlation between dietary iron and serum ferritin values in the high group (r=0.59). There was a correlation between kcal and dietary iron in the 2 groups (r=0.60, p<0.1). No significant correlations were found between kcal and serum ferritin or REE and serum ferritin in either group. Based on these findings, women who consume suboptimal energy are at risk of inadequate iron intake as well.

## 16 Addition of Soy Protein to Banana Nut Muffins

E. Tompanis\* and R.M. Bakhit

Department of Human Nutrition, Foods and Exercise

Soybeans, one of Virginia's crops, have repeatedly been shown to help lower blood cholesterol levels. This reduction has been attributed to the protein found in the beans. Current research on soy protein has shown this decrease to be substantial for individuals at high risk for developing coronary heart disease. These benefits can be seen when as little as twenty five grams of soy protein is consumed a day. However, few cultures incorporate soy protein into meals, therefore these benefits go unclaimed. Producing food that contains soy protein and that is considered acceptable by diverse populations is a method for implementing soy into the human diet while boosting the demand for soy. The subject for this study is the banana nut muffin. Soy protein, which has a flour like consistency, was added in increments of one gram per muffin to determine the maximum amount which can be added without altering the taste and texture qualities of the muffin. The addition of soy to baked products affects several characteristics of the final product. Soy tends to absorb water, have a small aftertaste, and offers little structure to the final product. To overcome these properties, water, lemon flavor, and gluten were added. Researching and experimenting with these aspects of the functionality of soy protein, a muffin containing seven grams of the protein has been developed and has received good responses from those who have tasted it. Soy protein research involving product development and sensory analysis will continue through the summer.

## 17 Development of a soy muffin in efforts to decrease blood lipid levels and prevent Coronary Heart Disease.

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Department of Human Nutrition, Foods and Exercise

High blood cholesterol and LDL cholesterol are risk factors in the development of Coronary Heart Disease (CHD). In the United States, coronary heart disease remains the leading killer of both men and women. It is known that dietary factors can be used in the prevention and treatment of CHD. For this reason, there is now great demand for foods that contain cholesterol-lowering ingredients such as soy protein. The objective of our study was to develop and evaluate recipes of baked products that contained soy protein isolate (SPI). Two recipes were developed. The first was a citrus muffin that contained 5 grams of SPI per mini-muffin. The second muffin was a blueberry muffin that contained 5 grams of SPI per mini-muffin. Throughout the development process, it was found that the SPI absorbs the moisture in the muffin so applesauce was supplemented for moisture. Evaluators reported during the sampling that large amounts of SPI (greater than 5 grams) left a metallic and gritty after-taste. By adding the natural flavorings lemon zest and pure vanilla extract, the undesirable aftertaste and texture were corrected. Frozen blueberries presented problems with the structure of the muffin. Adding wheat gluten to each recipe solved the structural problem. The volume and compressibility of the samples will be determined by means of a volumeter and penetrometer, respectively. Further research is continuing at this time with the goal of developing high soy protein muffins so that 2 or 3 a day will supply a person at risk of CHD with enough SPI (~ 30-40 grams) to lower their cholesterol levels.

## 18 Identification of Cyst Nematode Responsive Promoters using Transgenic Tobacco

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Department of Plant Pathology, Physiology and Weed Science

Cyst nematodes are estimated to account for 500 - 1000 million dollars of crop damages per year in the U.S. alone. Currently, the most widespread means to control nematodes have been chemicals. Chemical control is not only costly but it also causes severe environmental damage. An alternative to chemical control is designed engineered resistance to root-parasitic nematodes. One strategy for enhancing nematode resistance is to introduce a new gene with a nematode-activated promoter (gene regulatory sequence) and a gene encoding a protein toxic to the nematode. Our goal is to test whether a defense related promoter, tomato *hmg2*, is induced by cyst nematodes. Transgenic tobacco seeds containing an *hmg2* promoter with a GUS ( $\beta$ -glucuronidase) reporter gene construct were used for nematode inoculation. The *hmg2* promoter comes from a gene coding 3-hydroxy-3-methylglutaryl CoA reductase, an enzyme involved in the synthesis of phytoalexin antibiotics. *hmg2* activity, assayed based on GUS activity was determined in tobacco seedlings following inoculation with cyst of *Globodera tabacum*. Our results show *hmg2*:GUS activity localized to the nematode feeding site. Thus, *hmg2* is one of the first cyst nematode responsive promoters identified in plants.

## 19 Taxol Enhances Macrophage-Mediated Cytotoxicity

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Department of Biology

Taxol, a chemical derived from the bark and leaves of the Western yew tree, is used to treat patients with breast, ovarian, and lung cancers. Taxol prevents cell division in cancer cells by stabilizing the microtubules against depolymerization, thus preventing the completion of cell cycling. Taxol also imparts its antitumor efficacy through immunotherapeutic mechanisms. For example, taxol can promote normal host (NH) macrophage (M $\phi$ ) activities in a murine tumor model. However, tumor growth alters M $\phi$  antitumor activities, thus in this study, we investigated the cytotoxic efficacy of taxol-treated tumor-bearing host (TBH) M $\phi$ s against the Meth-KDE tumor cell line. Taxol pretreatment, either with or without interferon- $\gamma$  (IFN- $\gamma$ , a M $\phi$ -priming agent), enhanced both NH and TBH M $\phi$ -mediated suppression of tumor cell proliferation. Pretreatment with both taxol and IFN- $\gamma$  reduced tumor cell proliferation by greater than 50%. Taxol pretreatment increased both NH and TBH M $\phi$ -mediated cytotoxicity against tumor cells in a dose-dependent manner. Most importantly, taxol overcame tumor-induced suppression of M $\phi$ -mediated cytotoxicity. Collectively, these data will enhance our understanding of the immunotherapeutic mechanisms of the anticancer drug taxol and may lead to improved antitumor therapies.

# **Physical Sciences and Engineering Category**



## 20 Acidic Properties of Specific Features on Well-Defined SnO<sub>2</sub> (110) Surfaces

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Department of Chemical Engineering

The relationship between acidic properties and observed chemistry is often investigated on oxide surfaces to give insight into the nature of oxide reactivity. Rutile SnO<sub>2</sub> is a model for defective surfaces because it can be prepared with widely varying surface compositions and two types of oxygen vacancy point defects. In this study, the acidic properties of well-defined SnO<sub>2</sub>(110) surfaces are characterized using NH<sub>3</sub> by investigating its interaction at cation sites of different coordinations using thermal desorption spectroscopy and ultraviolet photoelectron spectroscopy. NH<sub>3</sub> thermal desorption indicates that four-coordinate Sn<sup>2+</sup> cations at bridging oxygen vacancies are more acidic than five-coordinate Sn<sup>4+</sup> cations due to a greater covalent contribution to the bond. The introduction of in-plane oxygen vacancies reduces the Lewis acidity of the associated cations, most likely due to a higher charge density around the in-plane oxygen vacancies. The Lewis acidity of SnO<sub>2</sub>(110) goes through a maximum as the surface becomes more oxygen deficient. This trend matches that observed for the dissociation of methanol on SnO<sub>2</sub>(110).

## 21 Development of An Class Grades Server for the Internet

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Department of Civil Engineering

An intelligent system is being developed to facilitate the posting of students' grades on the internet. This tool allows students to be able to access their grades for a particular course at any time via the world wide web (WWW) browser or via email. It also provides any professor using it, an easy user interface to post the grades on the internet.

A prototype of the system is currently being used in the CE 2214 (Civil Engineering Surveying Class). The current prototype was developed for windows 95 and is running on a 486 DX 50 IBM PC. The IBM PC has been setup temporarily as a server on which an WWW server, a email server and an FTP server are all running. The FTP server allows the course instructor to upload the grades file. The mail server facilitates the checking of grades by students via email and the WWW server facilitates checking grades via a WWW browser.

A complete grade report that is generated for each student includes: a complete summary of currently assigned and graded assignments; tabulation of class average, class high, and class low for each assignment; tabulation of total score for the student compared to the class average; and a separate list of assignments the student has not turned in.

## 22 Microperturbation Effects In Singlemode Optical Fibers: Application to High-Speed Communication and Sensor Instrumentation Systems

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The primary objective of this research was to analyze and quantify the effect of microperturbations in an optical fiber, and to demonstrate novel fiber devices that may be realized through their controlled induction and signal processing. Such periodic distortions or microbends have spatial wavelengths on the order of a few hundred  $\mu\text{m}$  and cause wavelength-selective coupling between the electromagnetic fields propagating in a fiber. Hence by controlling the periodicity of these distortions, any desired transmission spectrum can be obtained. In this work we a) developed a *highly accurate technique* to predict microbend losses in singlemode optical fibers in terms of deformation and optical fiber parameters, nominal errors in less than 2.5% between theory and experiment were shown; the developed algorithm may be used with any fiber, provided the deformation function is known, b) using results from analysis, proposed and demonstrated an *in-line wavelength-tunable fiber optic polarizer* with extinction of  $> 25$  dB and insertion loss  $< 1.3$  dB at an operating wavelength of 1300 nm, c) microbend-loss based *bio-corrosion detection and measurement fiber optic sensor* instrumentation were designed, and d) low-cost techniques to fabricate *long-period refractive-index gratings* in an optical fiber were proposed. The primary applications of these devices are in high-speed fiber optic communication and sensor instrumentation systems which require the use of miniaturized devices for enhanced operation.

## 23 Auditory Icons: Using Representational Sounds to Convey Situational Information in Commercial Motor Vehicles

Steven M. Belz\*, John J. Winters, Gary S. Robinson, and John G. Casali

Industrial Systems Engineering

A study was conducted to define a set of auditory icons to be used in the presentation of information to commercial vehicle operators through a non-speech auditory display. Auditory icons are representational sounds in that they have a specific, stereotypical meaning associated with their sound. While typical non-verbal sounds are defined by their particular acoustic parameters, auditory icons are defined by the object or action that created the sound. Fifty-one icons were evaluated on the basis of perceived meaning, urgency, and association of task with an expert opinion.

## 24 Economics and Design of Recombinant Proteins Factor VIII, Protein C and Human serum Albumin Recovery Process from Milk

S. Elhadj\*

Department of Chemical Engineering

The current recovery of pharmaceuticals from blood plasma is often expensive, insufficient, and presents risks of contamination. It is now feasible to express these proteins in the milk of genetically engineered mammals. The challenge is then to design and scale up a recovery process meeting current regulations, cost minimization goals, and demand for these pharmaceuticals. The objective of this project is to present the results of a model showing the most significant parameters affecting manufacturing costs of Human Serum Albumin (hSA), Protein C, and factor VIII. All the processes presented are based on chromatography columns to achieve separation. Some of the parameters entering the design and influencing costs are superficial velocity, expression levels, production scale, process separation scheme, milk cost, column capacity, and yield of the columns. The sensitivity of the manufacturing cost of the protein to these parameters are illustrated graphically and discussed. It was found that the rate of decrease of the costs becomes negligible as superficial velocity, expression levels, column yields, and scale of production increase while all other parameters remain constant. However the costs decrease observed are significant within a given range of these parameters. Column capacity, even though decreases the costs as it increases, affects cost less as the scale of production is reduced. Milk cost increases protein costs but its impact is reduced as column capacity increases. Finally it was found that a judicious choice of separation scheme, such as Fall Through against Direct Adsorption for hSA production, can decrease the protein cost by over 50%.

## 25 Biodegradable Composites for the Environment from Wood-based Resources

Indrajit Ghosh<sup>1</sup>; Wolfgang Glasser<sup>1</sup>; and R. M. Davis<sup>2</sup>;

<sup>1</sup>Department of Wood Science and Forest Products, and Biobased Materials/Recycling Center, <sup>2</sup>Department of Chemical Engineering,

Polymer composites have been replacing metals as construction materials in the automotive, aircraft, sporting goods, electronics and marine industries. They are characterized by having a high modulus reinforcement held by a thermosetting or thermoplastic matrix material. Biodegradable composites are prepared by impregnation of continuous cellulose fibers (solvent spun 'lyocell' prototypes) with aqueous suspensions of a thermoplastic matrix consisting of a cellulose ester-lignin ester blend by suspension (powder) prepregging. Though comparatively less stiff and less strong than conventional glass, carbon, aramid, or ceramic fibers, highly oriented cellulose fibers offer advantages in terms of elongation (and thus impact resistance), cost, and environmental considerations (i.e. fully biodegradable). The addition of lignin ester to cellulose ester improves modulus and melt flow characteristics along with controlling the glass transition temperature of the matrix material. The prepregs are converted to engineered multi-layered composites by alignment stacking and consolidation under heat and pressure. Moreover, a thermoplastic water-soluble xylan derivative has been found to be a useful dispersant/binder for the composite. The biodegradable composites may find application in the automotive or related high volume consumer industry.

## 26 Electrochemical and Spectroscopic Characterization of Fullerenes

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Department of Chemistry.

Fullerenes have attracted a lot of attention since their discovery. Much of the scientific interest has focused on trying to characterize and understand the physical properties of these materials. Unfortunately, fullerenes are generated in low abundance, making their characterization difficult. Here we describe electrochemical and UV-VIS-NIR spectroscopic methods which allow measurements on solution volumes as small as 50 $\mu$ L. With these micro-techniques, we can begin to study the properties of the less abundant fullerenes.

## 27 Driver Reaction to Unexpected Situations When Using an In-Vehicle Information System that Integrates an In-Vehicle Signing Information System, an In-Vehicle Routing and Navigation System, and an In-Vehicle Safety Advisory and Warning System

Richard J. Hanowski  
Industrial and Systems Engineering

A field experiment was conducted to investigate the benefits and costs of using an In-Vehicle Information System (IVIS) when the driver is confronted with unexpected situations. The IVIS used in the present study included three in-vehicle subsystems that provided signing, navigation, and warning information. Three research questions were investigated, each involving the use of an IVIS and response to unexpected situations: (1) Are there benefits with an IVIS? (2) What impact does IVIS information density have on driver behavior and performance? and (3) What impact does driver age have on system use and driving behavior? As drivers traveled a prescribed route, they were confronted with six unexpected situations, including a car approaching from a hidden entrance, an ambulance approaching from the rear, and a crash scene. The following conclusions and recommendations can be made from this field study: (1) results indicated a clear benefit for drivers responding to external events and vehicle status warnings when using an IVIS, (2) drivers are capable of safely switching attention from an IVIS to the forward roadway, while responding to an external event, (3) the layout of information is an important consideration for IVIS design, (4) older drivers behaved cautiously when using an IVIS and responding to unexpected situations, (5) limitations associated with older driver performance may benefit from an optimally designed IVIS, and (6) drivers should be allowed to select preferred "low urgency" messages and alerting cues, including redundant messages and multiple cues, from a bank of options that cross modalities.

## 28 Performance of Water-Based Epoxy in Circuit Board Applications

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Department of Materials Science and Engineering

An effort is underway to replace the standard solvent-based epoxy resin systems with water-based systems for use in epoxy/glass electronic circuit boards. While the elimination of organic solvent-based systems presents environmental and health benefits, water-based alternatives pose several potential challenges; chief among these are moisture resistance and electrical performance. Cured water-based epoxies contain residual surfactant from the original suspension. This surfactant is believed to promote moisture uptake which can pose problems for electrical performance. The current study of both neat resins and epoxy/cloth composites found that, following moisture exposure, the microwave frequency electrical  $\tan \delta$  in the water-based systems was significantly greater than that of solvent-based systems. Mechanical performance of the neat water-based vs. solvent-based epoxy resins are also under investigation to determine moisture and aging effects. The durability of the water-based epoxy/copper interface, critical in circuit board construction, is being studied for both adhesive and electrical performance.

## 29 Stress Rupture of Unidirectional Polymer Matrix Composites in Bending at Elevated Temperatures

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Departments of Materials Engineering and Science  
and Engineering Science and Mechanics.

A new predictive method for stress-rupture of specimens in bending has been developed and used to describe the behavior of unidirectional polymer matrix composites. The method is based on a simple end-loading method which produced coherent results. These results have been modeled using the theory of large deflection of buckled bars (elastica) to predict, with good accuracy, the strain at each point of the deformed specimen. The failure process has also been experimentally characterized. The formation and propagation of inclined bands (microbuckles) leads to a compressive failure. Based on the elastica and classical lamination theory, a model for the distribution of the stiffness along the length of the specimen has been established. Three different micromechanical models have been applied to analyze the time-to-failure versus strain behavior at two temperatures - one below and one above the glass transition. The first micromechanical model considers the nucleation of the microbuckles as the main cause of failure. In addition, the stiffness and stress distributions at any time before failure are calculated based upon the rotation of the fibers in the damaged regions. The second and last models, based upon a Paris Law and energy considerations, respectively, relate the time-to-failure to the propagation of the main microbuckle. For this last model, a good correlation between experimental and theoretical data has been obtained. Finally the influence of the temperature on these models has been investigated.

## 30 <sup>13</sup>C CP/MAS NMR Characterization of the Wood - Phenol Formaldehyde Bondline

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Department of Wood Science and Forest Products

Neat resole phenol-formaldehyde was cured under a variety of conditions and evaluated by thermomechanical analysis, swelling studies and <sup>13</sup>C CP/MAS NMR. Swelling tests showed that this system has a polymer-solvent interaction parameter in the range of 0.92 - 0.99. Results indicated that correlations exist between glass transition temperature and proton rotating frame spin-lattice relaxations as well as crosslink density and proton rotating frame spin-lattice relaxations. In the systems examined, sufficient contribution from spin-lattice relaxation processes occurs so that relative estimates of network rigidity can be made from  $T_{1\rho}^H$ , even when complicated by spin diffusion processes. The use of deuterium enrichment of the resin provides a more sensitive estimate of motional processes. A <sup>13</sup>C labelled resole phenol formaldehyde resin was synthesized using 99% <sup>13</sup>C paraformaldehyde. Wood flake sandwich composites were manufactured using the resin and characterized by <sup>13</sup>C CP/MAS NMR. The effects of cure time and temperature were assessed. Conversion of adhesive in the cured state as a function of cure schedule was determined by monitoring the corrected relative intensity ratios of the methylene : hydroxymethyl peaks. Proton spin-lattice relaxation rates allowed qualitative estimates of resin network mobility to be made. Variable temperature experiments revealed that increasing cure time or temperature increased the mean correlation time of the resin protons. No difference in the mean correlation time of the wood protons was noted as a function of cure schedule.

## **Social Sciences Category**

### 31 Modified Postnatal Social Experience Alters Intersensory Responsiveness of Bobwhite Quail Chicks (*Colinus virginianus*)

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Recent work with several precocial avian species indicates that sensory stimulation provided by the young chicks' social environment can influence perceptual responsiveness during both prenatal and early postnatal development. The present study examined the sensory features of postnatal social experience bobwhite quail chicks (*Colinus virginianus*) require to maintain species-typical patterns of responsiveness to the audible and visible attributes of a bobwhite hen. Chicks were reared in one of three experimental conditions following hatching, including: (a) altered tactile experience, (b) altered auditory experience, or (c) altered visual experience with siblings. Findings revealed that altered tactile, auditory, or visual experience during the first 72 hrs following hatching delayed chicks' preferential responding to species-specific maternal auditory and visual cues. Furthermore, altered sensory experience in any modality presented during the first 36 hrs of postnatal development was sufficient to alter normal patterns of perceptual responsiveness. Altered tactile or auditory sensory experience during later periods of postnatal development also disrupted chicks' perceptual development, whereas altered visual experience during later periods of postnatal development failed to affect species-typical responsiveness to maternal cues. These findings indicate that normally occurring sensory experience derived from early social interaction is important for species-typical perceptual development in bobwhite quail chicks and suggest that timing of postnatal visual experience can be an important variable in affecting early filial responsiveness to maternal cues.

### 32 The Honey Pot

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The Honey Pot is the result of a problem which was to create a garment inspired by Benjamin Hoff's book, *The Tao of Pooh* (1982). The garment is a metaphor for dipping into one's honey pot. The inside of the honey pot reflects our Inner Nature. Subtle value changes were created on the polyester satin lining through discharging. The lining was then marbled to create rich, warm swirls in brown, black, and yellow hues. The vessel shaped exterior of the garment supports the fragments of our self that we communicate to the outside world. These fragments are represented by the colorful kaleidoscope of thousands of tiny cotton fabric squares sewn to the garment form. The form was piece dyed to provide color harmony and then machine embroidered in an abstract zig zag with four thousand yards of cotton covered polyester and metallic threads. The research process included reading, contemplating, and discussing. *The Tao of Pooh*; journal writing and sketching; experimenting with form, fabric application, thread application, and marbling; and a weekly dialogue was maintained between designer and adviser for discussion and input. Hoff, B. (1982). *The Tao of Pooh*. New York: E. P. Dutton. The Honey Pot was awarded "Graduate Student Best in Show" at the International Textiles and Apparel Association conference 1996.



### 33 Scanlon Plans and Section 8(a)(2) of the NLRA: Productivity in the Balance

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Department of Management

Innovative programs that include worker participation, intended to enhance productivity and profitability, have been found illegal in certain situations. The worker participation element is often the most important component of these innovative programs. The protection offered to employees through the National Labor Relations Act (NLRA) has, in many ways, become an impediment to participation. Overcoming the obstacle of retaining the basic nature of participatory programs and remaining in compliance with the law is a challenge presently facing many business organizations. Two tests have been developed to examine sections 2(5) and 8(a)(2) of the NLRA. When Scanlon plans are examined with these tests, they frequently fail, implying that they would be ruled by the courts to be labor organizations which are unlawfully dominated by the employer. Possible solutions to this finding include changing the programs or changing the related legislation. Neither seem particularly likely, since changing the Scanlon plans themselves negates many of their benefits, and many recent calls for legislative change have gone unanswered. Fortunately, Scanlon plans have not yet been directly addressed by the courts, but the evidence supports the conclusion that when they are, they will be found to be in violation, and, at present, there seems no viable alternative to that predicament.

### 34 Constructing Identity Through the Production of Place

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Environmental Design and Planning

The built environment influences human behavior. Such is the argument presented by scholars in the Environment and Human Behavior studies. In such a relationship between people and their environment, people are represented as passive receivers of information from the environment regarding how to behave. It is more appropriate to visualize an interactive relationship between the built environment and people.

People both influence and are influenced by the built environment they create. Studies in place-identity and material culture literature show that people internalize a concept of self that is closely related to the representation of the material world, be it a place or material possession (Proshansky et al., 1983 and Belk, 1988). Self identity is closely linked to place or material possession if ownership is involved (Belk, 1988). Participation in the design and construction of place is one type of ownership. Thus, participating in the production of place is actively constructing an identity for self and the group.

This study proposes to understand how Asian Indian immigrants to the US construct their identity through the Hindu temples in the Chicago Metropolitan Area.

Preliminary data gathered on site suggest that temples do play an important part in the lives of the Asian Indians in the Chicago area. Temple activities include social and religious activities. Social activities include parties, potlucks, cultural classes, and language classes. Preliminary data also suggest that the temple allows the Asian Indian community to express themselves as Asian Indians.

### 35 Analysis of U.S. Textile Exports in the 1980's

Mikyung Lim \*

Department of Clothing and Textiles

The U. S. textile industry in the 1980's experienced changing macroenvironments, surging imports and competition, industrial restructuring, increasing capital intensity, and product differentiation. This study investigated U. S. textile exports for 1979-81, 1982-84, 1985-87, and 1988-90. The constant-market-share (CMS) analysis decomposes change in a country's exports into the effects of world market, industry-structure (commodity or geographic markets), and competitiveness under the assumption of constant market shares. CMS analysis revealed a positive work market effect for U.S. Textile exports in the 1980s. The U.S. fared less well than competitors, however, given that the U.S. share of world textile exports fell from 7.8% to 5.1% during the 1980s. The U.S. lost market share to other countries within all its export markets. CMS analysis showed a positive industry-structure effect for U.S. textile exports in the early 1980s, but negative in the late 1980s, probably due to industrial restructuring and move toward capital-intensive and differentiated products which showed low growth rates in the world market. Trade data show increasing and higher U.S. export shares, thus greater specialization, in yarns and special fabrics than for competitors. U.S. export destinations also shifted as the ODC outran the EC as the major market. The patterns may reflect the growth of diverse countries' textile production and of intra-regional trade encouraged in part by protectionism. U.S. textile exports were competitive in 1979-81 and 1988-90, but not in 1982-84 and 1985-87. The overvalued dollar and restructuring of U.S. textile industry probably influenced the negative and positive competitiveness.

### 36 Constant-Market-Share Analysis of Textile Exports of the EC, Far East, and Emerging Textile Exporting Countries

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Department of Clothing and Textiles

This research examined the factors influencing changes in textile exports of the European Community (EC), Far East, and Emerging Textile Exporting countries (EMERG) in 1979-81, 1982-84, 1985-87, and 1988-90. The Constant-Market-Share (CMS) analysis was used to decompose changes in a region's exports into the world trade effect, the commodity / market effect, and the competitive effect, under the assumptions of constant market share and price as a key factor in determining a region's export competitiveness. According to the results, the lowest growth and the negative competitiveness of EC textile exports and the positive competitiveness of Far East and EMERG textile exports in the 1980's indicate that the advantage of low labor costs exceeds the advantage of high labor-capital substitution in world textile trade. The rapid growth and competitiveness of Far East exports suggests the importance of efficient utilization of accumulated factor endowments in manufacturing and marketing systems with the advantage of relatively low labor costs. Meanwhile, the slow growth of EMERG exports indicates the region's lack of sufficient factor endowments and efficient utilization. The formation of the single European market and the North American Free Trade Agreement (NAFTA) are expected to promote tendencies of intra-regional trade within Europe, North America, and Asia. Meanwhile, the phase-out of MFA is likely to counteract the effects of those regimes and improve the competitive position of Far East and EMERG textile exports in developed markets, suggesting more intensive world competition.

### 37 Comparing Infant Directed and Adult Directed Pitch Contours: Infants' Attention to Adult Speech

Jason McCartney\* and Robin P. Cooper

Department of Psychology

The purpose of the present study was to investigate the role of pitch contours in directing infant attention to adult speech. Several studies have shown that infants from a few days old to 9 months of age prefer infant-directed (ID) over adult-directed (AD) speech. Moreover, 4-month-olds have been shown to prefer pitch contours that simulate ID speech, suggesting that the exaggerated pitch contours are necessary for infant attention. The current study investigated this attentional preference utilizing ID and AD pitch contours in a fixation-based preference procedure. Results from the first experiment failed to show a similar preference for the ID pitch contours. Because a lack of preference could have been due to a failure to discriminate, a habituation study was also conducted. The results from the second experiment showed that 4-month-olds can discriminate the ID and AD pitch contours. From these results, it is argued that the pitch contour may be but one of many possible prosodic characteristics that attract infant attention, but this attention may occur only within a language context. It is suggested that future studies investigate ID speech using a more context-dependent procedure, where natural or more complete speech samples are utilized.

### 38 Application of the Open Space Planning Methodology for Roanoke County

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Department of Landscape Architecture

The population of Roanoke County during the last decade grew by ten percent. The recent growth has changed the countryside. The agricultural lands in the county has been replaced by sprawling housing subdivisions, and strip commercial development. The growth in Roanoke County has also affected neighboring counties. Roanoke County and its surrounding areas now face problems of traffic congestion, visual and environmental degradation due to commercial and residential growth. Roanoke County needs an innovative open space plan which preserves the natural character and unique qualities of the place.

There has been parallel progress in the field of landscape ecology and landscape planning for better understanding of our environment. The primary goal of this thesis is to use concepts from both of these fields with an intent of developing a methodology which will help both planners and landscape architects to plan and design for open space in a way that will help meet regional needs and concerns. This thesis is an attempt to develop a process whereby an optimum spatial pattern for Roanoke County (one that maintains biodiversity, protects natural and cultural resources, improves water quality, protects soils, and increases productivity) is derived.

Spatial attributes of the landscape were targeted and analyzed to provide a foundation for an open space plan. The management priorities were then established for protecting and enhancing agricultural lands, forest lands, wetlands, and streams. The tools of preservation proposed for the open space plan were education, community involvement, and government regulations.

### 39 The Thought Processes of Machiavellians: Connecting Machiavellianism and Inhibition

Daniel Russell\* and Trina Doran  
Department of Psychology

The present study attempted to show a connection between Machiavellianism and the ability to inhibit potentially distracting internal and external stimuli. It was hypothesized that high-Machs would be better able to inhibit potentially distracting internal and external stimuli which would allow them to be more effective manipulators in social interactions. This study used the Mach IV instrument developed by Christie and Geis (1970) to identify high-Machs and show a difference between these participants and low-Mach controls on written and performance measures of inhibition ability. Individual's self-reports on the Thought Occurrence Questionnaire (TOQ) and the Cognitive Failures Questionnaire (CFQ) were used as written measures of distractibility and the Speed and Accuracy task including the conditions of No Distractors, Visual Distractors, and Auditory Distractors was used as a performance measure of inhibition ability. No differences were found between the groups on any of the Speed and Accuracy tests (baseline,  $F(39,1) = .038$ , n.s.; visual distracter,  $F(39,1) = .294$ , n.s.; auditory distracter,  $F(39,1) = .002$ , n.s.). However, when the combined CIQ/TOQ was factor analyzed, five factors were obtained. The factors were interpreted as: 1) emotional disruption, 2) time and external factors, 3) task based factors, 4) excitability, and 5) separating details from background. A significant relationship was found between Machiavellianism and Factor 1; emotional disruption ( $r = .1681$ ,  $p < .05$ ). The results indicate that high-Machs are only better able to inhibit internal, emotional disruptions as assessed from their self-reports. It shows that while high-Machs self-report an enhanced ability to inhibit distracting emotional material, this ability does not carry over to an actual better performance on speeded tasks.

## **Life Sciences Category**

#### 40 Cloning and Expression of *Brucella Abortus* Stress Proteins by Baculovirus Recombinant

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The live strain 19 and killed strain 45/20 vaccines against brucellosis protect only about 70 % of vaccinated cattle and the identity of the protective antigens is not known. Improved understanding of the protective antigens is needed for better understanding the pathogenesis of and the immunity against the disease. Objectives of this study include determining expression of *Brucella abortus* three recombinant proteins and analyzing mouse humoral and cell mediated immune responses against these recombinant proteins. Baculovirus is proposed to use as an alternate eukaryotic cloning and expression system supplementing our *B. abortus*/vaccinia virus recombinant efforts in terms of the synthesis of large quantities of proteins. The BAC-TO-BAC Baculovirus Expression System is based on site-specific transposition of an expression cassette of the recombinant plasmid pFASTBAC1 into a baculovirus shuttle vector (bacmid). *B. abortus* molecular chaperon GroEL, GroES, and heatshock protein HtrA, presenting a central role in the metabolism of bacteria have expressed in Baculovirus system. The bacmid recombinants have identified by Southern blots, then they have transfected using cellfection and infected into *Spodoptera frugiperda* cells. The expression of GroEL, GroES, and HtrA recombinants have confirmed by Western blots, and kinetic studies have done in order to determine the condition for the best expression. Humoral, cell mediated immune responses and protection study will be done with the immunization of each recombinant protein using mice model.

#### 41 Hydrophobic cellulosic surfaces engineered by surface migration

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Materials of biological origin are prime candidates for biomedical implants due to their biological compatibility and biodegradability. However, the implant material has to meet characteristic surface requirements in order to prevent thrombosis. Implants with a low critical surface free energy (hydrophobic) seem to cause less thrombosis. Although cellulose derivatives are already established in the biomedical field as dialysis membranes, broader use has eluded them due to their hydrophilic surface character. The present study examines the engineering of cellulosic films with hydrophobic surfaces by surface migration. This is achieved by blending cellulose propionate with a small amount of a fluorinated cellulose propionate. Two types of F-containing derivative are being examined, a mixed ester with several F-containing groups randomly distributed along the backbone, and a terminated oligomer with exactly one F-group. Also, different types of F-containing groups are being investigated, having either a CF<sub>3</sub> or a CF<sub>2</sub>H terminus. Preliminary result of contact angle measurements show a strong dependence of contact angle on both, architecture and terminus. Altogether, the terminated oligomers are not as effective as random copolymers in reducing the surface free energy. This is explained with micelle formation with the fluorine end in the core and a corona of cellulose propionate.

## 42 Bifurcation Analysis of a Model of the Frog Egg Cell Cycle

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Fertilized frog eggs (and cell-free extracts) undergo periodic oscillations in the activity of "M-phase promoting factor" (MPF), the crucial triggering enzyme for mitosis (nuclear division) and cell division. MPF activity is regulated by a complex network of biochemical reactions. Novak and Tyson, and their collaborators, have been studying the qualitative and quantitative properties of a large system of nonlinear ordinary differential equations that describe the molecular details of this system as currently known. Important clues to the behavior of the model are provided by bifurcation theory, especially characterization of the codimension-1 and -2 bifurcation sets of the differential equations. To illustrate this method, I have been studying a system of 9 ODEs that describe the frog egg cell cycle with some fidelity. I will describe the bifurcation diagram of this system in a parameter space spanned by the rate constants for cyclin synthesis and cyclin degradation. My results suggest either that the cell cycle control system should show dynamical behavior considerably more complex than the limit cycles and steady states reported so far, or that the biochemical rate constants of the system are constrained to avoid regions of parameter space where complex bifurcation points unfold

## 43 Tooth Lesions and Fluoride Content in Teeth and Bones of Mule Deer

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Mule deer (*Odocoileus hemionus*) from the US Air Force Academy (USAFA) at Colorado Springs, Colorado have shown antler fractures and tooth lesions. Brittle bones and tooth lesions have been associated with ingestion of excess fluorides. The present study compared prevalence and severity of tooth lesions with fluoride content in teeth and bones of mule deer from the USAFA with deer from two areas in Colorado. Samples of deer older than 1.5 years were collected during fall/spring 1993-1995 from the USAFA, (N=189), Pinyon Canyon Maneuver Site (PCMS, N=23) in Model, and from several game management units (GMU's N=16) from the northwestern area of Colorado. Tooth lesion severity was determined with a modification of the Shupe et al. (1963) classification system. This method uses a scale from 0 to 5 (0 indicates no lesions on the enamel, 1-very light white spots, 2-generalized white spots, 3-generalized spots with increase in wear, 4-generalized mottling and severe hypoplasia, and 5-abnormal wear, generalized mottling and severe hypoplasia). Though the worst lesions were only encountered at USAFA, the distribution of tooth lesions among areas did not differ. Median fluoride concentrations were higher ( $P<0.001$ ) in second incisors and bones of mule deer collected at the USAFA [763.7  $\mu\text{g/g}$  d.w., N=188 incisors (I), 869.6, N=189 bones (B)] than in deer from PCMS [509.1 (I), 494.4 (B), N=23] and GMU's [426.2 (I), 446.2 (B), N=16]. Fluoride concentrations found in deer tissues at the USAFA were considerably higher than that found in uncontaminated natural and control areas from other studies.

#### 44 Specific associations between three core enzymes of flavonoid metabolism

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One of the hallmarks of the living cell is the ability to catalyze thousands of specific chemical reactions in a spatially- and temporally-regulated fashion. Although the *in-vitro* kinetic and catalytic properties of hundred of enzymes have been characterized over the past several decades, our knowledge of how cells spatially organize all these catalysts in the bulk cytosol remains unclear. Two-hybrid analysis has identified interactions between the *Arabidopsis* chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol reductase (DFR), the first, second, and fourth flavonoid biosynthetic enzymes, respectively. When CHS, CHI, or DFR are fused to the DNA-binding domain of GAL4, these proteins specifically interact with DFR, CHS, or CHI trans-activation fusions, respectively. Furthermore, affinity chromatography techniques have shown that immobilized CHI is capable of selectively purifying CHS, CHI, and DFR from *E. coli* cell lysates via specific protein-protein interactions. This work extends previous immunocytochemical and cell fractionation studies suggesting that the flavonoid biosynthetic pathway exists as an enzyme complex associated with the endoplasmic reticulum. This model explains how the cell directs the timing, abundance, ratio, and subcellular deposition of diverse flavonoid end-products by channeling intermediates through dedicated biochemical circuits.

#### 45 Screening of lambda gt11 expression libraries of Dictyostelium discoideum at amoeba, 8 h and 12 h of development with recognition site DNA

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A critical event during the life cycle of *Dictyostelium discoideum* is glycogen turnover. This process is catalyzed by *glycogen phosphorylase-2 (gp-2)*. Since *gp-2* expression is first induced during the transition from growth to differentiation, understanding how this gene is controlled may provide some insight into the process of differentiation. In order to identify the *trans*-acting factors responsible for activating *gp-2* expression, three techniques are used: 1) *in vitro* footprint analysis to identify the *cis*-acting elements; 2) generation of  $\lambda$ gt11 expression libraries for recognition site screening; and 3) southwestern analyses to confirm the feasibility of isolating developmentally regulated *trans*-acting factors from the expression libraries. For this approach to succeed, high quality cDNA libraries are essential. RNA quality and the fidelity of the time points were determined by RT-PCR using several marker genes. The cDNA was assessed for complexity and full-length synthesis by PCR and radioactive primer extension, respectively. We have generated cDNA for 3 stages of development: amoeba (when *gp-2* is not transcribed), 8h (when *gp-2* is first transcribed) and 12h (when *gp-2* is highly transcribed).  $\lambda$ gt11 expression libraries were generated for amoeba ( $2.5 \times 10^5$  and  $2.75 \times 10^5$  pfu/ml) and 8 h ( $4.75 \times 10^4$  pfu/ml). The libraries will next be screened with *cis*-acting elements from the *gp-2* promoter identified through footprint analysis. To date, we have identified a developmentally regulated footprint spanning two C-rich elements. When this region is used to probe a southwestern blot, three peptides (84, 78 and 62 kD) are recognized exclusively in nuclear extract derived from differentiated cells. This result indicates that recognition site screening may be a feasible approach for isolating the genes encoding these potential transcription factors.



## 46 Development of a Leaf Distribution Program for On-Farm Composting

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On-farm recycling of organic wastes can benefit both agricultural and suburban communities by producing a valuable soil amendment and reducing landfill burden. A joint project between Virginia Cooperative Extension (BCE) and the Rivanna Solid Waste Authority (RSSWA), was conducted to develop and document a program for distribution and composting of leaves on farms in central Virginia. Between 100 and 175 tons of leaves from the city of Charlottesville were delivered to each of six farms and nursery operations to co-compost with agricultural manures. A multi-disciplinary team of researchers and extension personnel provided guidance to the farmers and nursery operators in compost processing and assessing the economics of composting. A tractor-pulled windrow turner, provided by the RSWA, was utilized at 4 of the 6 farms during initial composting, with infrequent or not subsequent turning using a tractor-mounted bucket loader.

Field and pot studies are being conducted at two of the farms to determine compost quality as an amendment to improve plant growth and reduce fertilizer costs in the production of vegetable crops and potted perennials. A demonstration field day was held in June 1996 for local and state government officials, extension personnel, farmers and other interested persons to demonstrate the composting process and utilization of the final product at two farms.

Three VCE publications will derive from this project: 1) Farm composting Guide I: Principles, Systems, and Operation; 2) Farm Composting Guide II: Operating Planning, Siting, and Regulation; and 3) Recycling Municipal Yard Wastes to Farms: A Community Handbook.

## 47 Cloning of Dhurrinase-1 ( $\beta$ -Glucosidase) cDNA From *Sorghum bicolor* (L.) Moench and its Temporal and Spatial Expression

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*Sorghum bicolor* (L.) Moench contains two isozymes of the cyanogenic  $\beta$ -glucosidase dhurrinase: dhurrinase-1 (*Dur1*) and dhurrinase-2 (*Dur2*). A full-length cDNA encoding dhurrinase-1 (*dur1*) was isolated and sequenced from 4 day-old etiolated seedlings. The cDNA has a 1695-nucleotide-long open reading frame which codes for a 565-amino acid-long precursor and 514-amino acid-long mature protein respectively. Deduced amino acid sequence comparisons show 70% identity between maize and sorghum  $\beta$ -glucosidase precursor proteins. Multiplicity of  $\beta$ -glucosidases (dhurrinases) and their expression in different plant parts were studied using 5'- and 3'-end specific probes derived from the cloned cDNA. Southern blotting data indicated that  $\beta$ -glucosidase is encoded by a small multigene family, having at least two members. Northern blotting data indicated that the mRNA corresponding to the cloned cDNA is present at high levels in the node and the upper portion of the mesocotyl in etiolated seedlings but at lower levels in the root and only in the zone of elongation and root tip region. Light-grown seedling parts had lower levels of mRNA than those of etiolated seedlings. Immunoblotting analysis performed using maize-anti- $\beta$ -glucosidase sera detected two distinct dhurrinases in sorghum (57 and 62 kD). The data indicated that the cloned cDNA corresponds to *Dur1* (57 kD). The distribution of dhurrinase activity in different plant parts also supported the mRNA and immunoreactive protein data, indicating organ specific regulation of the *dur1* gene.

## 48 No-till Cotton Production in Virginia: A study of moisture, nitrogen, and yield

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Conservation tillage cotton (*Gossypium hirsutum* L.) practices have increased with increasing acreage during the last few years in Virginia. This trend is primarily due to the 1990 farm bill conservation requirement, and an effort to reduce operating costs. An experiment was conducted during 1996 at the Southern Piedmont Agricultural Research and Extension Center to evaluate yield benefits of different cover crops under no-till and conventional tillage systems. Cover crops (rye, hairy vetch, hairy vetch + rye, wheat, crimson clover, and lupine) were planted in the fall of 1995. In the spring of 1996, three weeks prior to planting cotton, the cover crops were burned down using various herbicides. Soil moisture, cover crop biomass, ground cover estimation, and cotton lint yield were evaluated for each treatment. All cover crops provided adequate ground cover with the exception of lupine. Cover crops and tillage systems did not have any effect on soil moisture. Above average rainfall throughout the growing season provided adequate moisture for cotton growth and eliminated the possible effect of cover crops and tillage practices on soil moisture. Total nitrogen in cover crops varied among treatments. No differences in lint yield were observed due to cover crops or tillage practices. To fully understand yield benefits from the various cover crops, more information is needed, especially under conditions of moisture stress.

## 49 THE EUROPEAN GYPSY MOTH IN COASTAL PLAIN PINE-HARDWOOD STANDS: OVERSTORY DEFOLIATION AND TREE MORTALITY

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As gypsy moth (*Lymantria dispar* L.) populations have expanded their range into the southeastern United States, outbreaks in previously undefoliated forest types have become more frequent. Recent laboratory studies have revealed that sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.) are potential hosts for the gypsy moth. Both of these species occur in pure and mixed stands throughout the southeast, therefore the potential for defoliation by advancing populations appears to be great. In 1992 we initiated a study to determine the effects of gypsy moth defoliation on the growth and mortality of loblolly pines and hardwoods growing in mixed stands. Between 1992 and 1993, 141 research plots were established in 47 mixed stands in the Coastal Plain of Virginia and Maryland. Two stand types were selected for study, pine-oak and pine-sweetgum. In both stand types, the proportion of susceptible species present at the time of plot establishment influenced the intensity of defoliation. Stands with a larger proportion of hardwoods had greater annual defoliation levels. Oaks and sweetgum were both heavily defoliated. Individual pines were observed to experience some defoliation, however, even in areas where the susceptible hardwoods had been completely stripped, large-scale defoliation of pines did not occur. Pine-oak stands lost more total basal area than pine-sweetgum stands. The majority of this mortality consisted of susceptible hardwoods, mainly oaks. Conversely, while the defoliation of sweetgum was intense, mortality was negligible indicating that this species may be less vulnerable to mortality than some of the common oak species.

## 50 The Arabidopsis Kinesin-like Calmodulin Binding Protein Exhibits Ca<sup>2+</sup>/Calmodulin Dependent Binding to Microtubules

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As one of the major components of the eukaryotic cytoskeleton, microtubules (MTs) play essential roles in organelle transport, organization of the cytosol and cell division. Many of the functions of MTs are attributed to a class of MT-associated proteins termed MT-dependent motor proteins. MT-dependent motor proteins such as the kinesin-like proteins use the energy of ATP hydrolysis to "walk" along MT tracks and in doing so transport a wide variety of cargo (vesicles, organelles, MTs) within cells. Although numerous kinesin-like proteins have been identified from a wide variety of organisms, investigation into the regulation of these proteins has been limited. Recently, a new member of the kinesin family from *Arabidopsis thaliana*, KCBP (kinesin calmodulin-binding protein) has been identified by virtue of its ability to bind to calmodulin (a calcium binding protein), suggesting that the activity of this protein is regulated *in vivo* by calcium and calmodulin. We have expressed the motor domain of KCBP in *E. coli* and have examined its enzymatic activities and MT binding properties. Consistent with its putative identification as a motor protein, KCBP was found to bind MTs in an ATP-dependent manner and exhibited MT-stimulated rates of ATP hydrolysis. Ca<sup>2+</sup>/calmodulin was found to inhibit the binding of KCBP to MTs under conditions which ordinarily induce a tight association. In addition, Ca<sup>2+</sup>/calmodulin inhibited the MT-stimulated, but not basal rates of ATP hydrolysis of KCBP. These results suggest that Ca<sup>2+</sup>/calmodulin may serve to regulate the activity of KCBP *in vivo* by regulating its activity and associating with MTs. Characterization of the regulation of KCBP will not only further define the role which this motor protein plays in plant cells, but will help to elucidate the mechanism by which a cell can temporally and spatially control a multitude of proteins in a specific and organized fashion, a behavior which is essential to the survival of an organism.

## 51 The Antioxidant Effect of Soy Isoflavones In Vitro

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Oxidized low density lipoprotein (LDL) are increasingly implicated as participants in the formation of atherosclerotic plaque. Antioxidant compounds that occur naturally in foods are of interest due to their potential to diminish oxidation of plasma lipids. There is evidence that soybean isoflavones have lipid antioxidant effects in food systems (1), however these antioxidant effects in human plasma have not been fully investigated. Recent research on the antioxidant effects of red wine polyphenolics (similar to soy isoflavones) indicated antioxidant effects on LDL both *in vivo* and *in vitro* (2). The long term goal of this study is to determine the antioxidant effects of the soy isoflavones genistein, genistin, daidzein and daidzin on plasma. Oxidation and protein content of different LDL dilution's have been assessed to determine the ideal concentrations of protein in the plasma in respect to concentrations of copper sulfate, which is the oxidizing agent. Oxidation has been determined by the Thiobarbituric Acid Reactive Substances (TBARS) method (2). This is a colorimetric method that gives a good estimate of the degree of oxidation in lipids. The protein content has been determined using the Lowery method, since the results will be expressed as nmoles TBARS/mg protein. The current focus of the research is understanding the TBAR assay and reproducing the data of the method used. In ongoing research the TBAR assay will be used to evaluate the antioxidant effects of the different soy isoflavones. Final data will be statistically analyzed using Statview 512+ (Brainpower, Inc., CA).

## 52 The Effects of Genotype and Supplementation and Supplementation of Chromium Picolinate on Quality Characteristics of Pork

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Genetics and pre-slaughter handling can affect pork quality. The objective was to evaluate the effects of halothane gene, chromium, and sex on pork quality. Halothane negative (NN) and Halothane positive (Nn) barrows and gilts were supplemented with 0 and 200 ppb chromium (96 pigs; 12 pigs per treatment). Postmortem pH decline, drip loss, and CIE L\*a\*b\* values were determined on the *Longissimus dorsi* (LD). Vacuum packaged, frozen LD samples were analyzed for Warner-Bratzler shear (WBS) and cooking loss (CL). A genotype by time interaction ( $P < 0.05$ ) was determined for pH decline. LD from Nn had a lower pH at 45 min and 3 h compared to NN but there was no difference between the two genotypes at 24 h. Nn had a higher drip loss ( $P < 0.05$ ) than NN, however genotype did not affect CL. A genotype by sex interaction was determined for CIE L\* values. Nn had higher ( $P < 0.05$ ) CIE L\* values than NN. Nn barrows had higher ( $P < 0.05$ ) CIE L\* values than Nn gilts. There was a genotype by storage time interaction ( $P < 0.05$ ) for CIE a\* and L\* values. However, there were no differences in CIE a\* values between NN and Nn at any of the storage times. CIE a\* values decreased ( $P < 0.05$ ) with storage time regardless of genotype. A genotype by day interaction ( $P < 0.05$ ) was found for WBS samples. Day 10 samples were more tender ( $P < 0.05$ ) than day 1 for both NN and Nn. There was no difference in tenderness due to genotype. Chromium did not affect ( $P > 0.05$ ) pH, drip loss, CL, CIE L\* a\* b\* values, or WBS. Sex did not affect ( $P > 0.05$ ) pH, drip loss, or CL. Chromium supplementation may be used for potential performance and carcass composition benefits with no impact on pork quality.

## 53 Isolation and Characterization of Soybean Phytase

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Much of the phosphorus in soybean [*Glycine max* (L.) Merr.] meal is sequestered as phytate (*myo*-inositol hexaphosphate). Non-ruminant animals are unable to utilize phosphorus in phytate. To meet nutritional requirements, soy-based diets are supplemented with inorganic phosphate, increasing feed costs. In addition, excreted phytate is applied to croplands in manure, and can contribute to environmental phosphorus pollution. Phytase (E.C. 3.1.3.8) is an enzyme that breaks down phytate, liberating inorganic phosphate. Commercial phytase (Natuphos®) has been added to feed to release inorganic phosphate from phytate, eliminating the need for phosphorus supplementation. Phytase occurs naturally in soybean, but high levels of activity are not detectable until seed germination. Our goal is to isolate, characterize, and modify a soybean phytase gene to ensure expression and accumulation in the seed. We have purified a major form of soybean phytase from cotyledons of 10-day old germinating seedlings by sequential ammonium sulfate precipitation, heat treatment, cation exchange, lectin affinity, and anion exchange chromatography. A partial amino acid sequence was obtained by automated Edman degradation. A BLAST search was performed against the Swiss & PIR & Translated protein database. Our sequence showed little similarity to proteins in the database. Using amino acid sequence data from purified soybean phytase, we will employ a PCR strategy to generate probes for screening a cDNA library from 10-day old germinating soybean cotyledons.

#### 54 Cloning of the Putative *Lex2b* Gene of *Haemophilus somnus*.

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*Haemophilus somnus* causes a variety of diseases in cattle, including abortion, pneumonia, and neurologic disease. Virulent strains are capable of altering the structure of lipooligosaccharide (LOS) epitopes in order to evade the host's immune response. In the human pathogen *H. influenzae* type b (Hib), phase variation of LOS epitopes is due in part to genes containing variable repeats of the DNA sequence CAAT. A change in the number of CAAT repeats appears to shift start codons in or out of frame with downstream sequences, thereby controlling whether or not a functional gene is expressed. Hybridization studies using a digoxigenin-labelled probe made of seven CAAT repeats revealed the presence of several CAAT-rich regions within *H. somnus*, including one within a 3.9 kb EcoRI fragment. The CAAT-rich region was further localized and sequenced. The region contains 31 repeats of the sequence CAAT, which contribute to a short open reading frame of only 47 amino acids. However, computer analysis shows that the addition or loss of a CAAT subunit would shift the open reading frame to encode either a 245 or 239 amino acid protein. These hypothetical proteins are nearly identical, differing only in their start codons, and show approximately 50% homology to the *lex2b* gene product from Hib. In Hib, the *lex2* genes have been shown to be directly involved with LOS expression through mutational analysis. This gene may serve a similar role in *H. somnus*.

#### 55 Dietary Carbohydrates and Fat Influence Milk Composition and Fatty Acid Profile of Mares' Milk

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The composition of mare's milk reflects the nutritional status of both mare and foal. Our objective was to compare milk concentrations of protein, fat, fatty acids, lactose, and total solids in milk of mares fed pasture and concentrates rich in sugar and starch or fat and fiber. Throughout gestation and lactation, mares maintained on pasture were fed a corn and molasses concentrate (SS), or a corn oil and fiber supplement (FF). The concentrates were isocaloric and isonitrogenous, with mineral contents balanced with the pastures to meet or exceed current recommendations. Milk was sampled at 6–12 h and 24–48 h after parturition, and at 1, 2, 4, and 6 mo of lactation. Each sample was analyzed by AOAC official methods for milk protein, fat, lactose and solids. The fatty acid profile was analyzed using gas chromatography, immunoglobulin G (IgG) concentration by radialimmunodiffusion. In colostrum sampled at 6–12 h after foaling, there was higher protein, IgG, and solids, lower lactose, and a tendency for higher fat in the milk of FF mares, as compared to the SS mares. The fatty acid profile indicated higher linoleic, lower palmitoleic, and tendencies for lower palmitic and dodecanoic acids in the FF milk. Dietary linoleic acid in FF mares' milk may reduce the risk of gastric ulcers in foals. Increased IgG content of the FF mares' milk may enhance passive immunity. Supplementation of corn oil in the pregnant mare's diet may improve health of foals.

## 56 Absorption of Free and Peptide-bound Amino Acids by Isolated Ovine Omasal Epithelium Following Ruminal Incubation of Dietary Proteins In-vitro

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Absorption of free and peptide-bound amino acids across the ovine omasal epithelium was quantified using parabiotic chambers. Substrates consisted of cell-free supernatant obtained following incubation (6 h) of soybean meal, fish meal, and corn gluten meal in a buffered (pH 6.9) ruminal fluid inoculum. Ruminal fluid was obtained from ruminally cannulated, lactating Holstein cows. Omasal epithelial tissues were collected from ewes and were mounted between the halves of parabiotic units thus creating mucosal and serosal chambers. The mucosal chambers were filled with buffer alone (pH 6.9), buffered ruminal inoculum, or the cell free substrates of ruminally incubated soybean meal, fish meal or corn gluten meal. Serosal chambers were filled with buffer (pH 7.4) and this buffer was sampled at 0, 3, 6, 12 and 24 h. Free and peptide amino acids were quantified by HPLC. The appearance of peptide amino acids in the serosal fluid increased ( $P < .05$ ) with time, and was greater ( $P < .05$ ) than the free amino acid appearance (.39 vs. 7.72 mg L<sup>-1</sup> mg<sup>-1</sup> dry tissue at 24 h). Serosal appearance of peptide amino acids was similar ( $P > 1$ ) for soybean meal and fish meal (5.04 vs. 6.39 mg L<sup>-1</sup> mg<sup>-1</sup> dry tissue) and lower ( $P < .05$ ) for corn gluten meal (1.37 mg L<sup>-1</sup> mg<sup>-1</sup> dry tissue). Free amino acid appearance in serosal fluid was not different among protein sources. These data suggest that the in-vitro absorption of free amino acids across omasal epithelium is minimum and the intact peptides can be absorbed thus providing further evidence that the omasum may be a site of peptide absorption in ruminants.

## 57 Altered Expression of T Cell Receptor and other Adhesion Molecules Correlates with the Induction of Apoptosis in Thymocytes of Mice Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD).

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TCDD is well known for inducing thymic atrophy in mice although the exact mechanism of its action remains unclear. Recent studies from our laboratory demonstrated that Fas<sup>-</sup> mice (lpr/lpr) were more resistant to TCDD-induced immunotoxicity when compared to the Fas<sup>+</sup> wild type mice. Further investigation revealed that administration of 50 µg/kg body weight of TCDD into C57BL/6 mice i.p. triggered significant apoptosis in thymocytes at 8-12 h after the treatment and was not detected later on, upto 120 h. The apoptosis was demonstrable using TdT-mediated FITC-dUTP nick end labeling method and analyzing the cells flow cytometrically as well as using the JAM test in which thymocytes from TCDD-treated mice labeled with <sup>3</sup>H-thymidine exhibited increased DNA fragmentation when compared to the controls. Recently, thymocytes undergoing natural apoptosis were shown to upregulate the T cell receptor as well as exhibit altered expression of a number of adhesion molecules. In the current study we analyzed the expression of a variety of adhesion molecules on TCDD-treated thymocytes. Interestingly, in TCDD-treated thymocytes undergoing apoptosis, there was a significant increase in the expression of CD3, αβTCR, CD45R and IL-2R and a decrease in the expression of J11D, CD4 and CD8 molecules, when compared to the control thymocytes. Thymocytes undergoing spontaneous steroid-induced apoptosis in culture were also shown to downregulate CD4, CD8 and J11D while upregulating TCR and IL-2R expression. It is striking that in the current study, we noted similar alterations *in vivo* following TCDD treatment thereby corroborating that apoptosis constitutes an important mechanism of TCDD-induced immunotoxicity.

## 58 Ncd Tail Domain Binding to Microtubules

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Non-claret disjunctional (ncd) is a kinesin related microtubule motor protein that is required for proper chromosome distribution in meiosis and early mitosis in *Drosophila* oocytes and early embryos. Ncd has two microtubule (MT) binding sites: an ATP-independent-N-terminal tail, and an ATP-dependent-C-terminal motor domain binding sites, and it moves towards the minus ends of MTs. Through the action of these binding sites ncd cross-links and bundles MTs. To understand the role of ncd motor protein in spindle assembly and formation in the process of chromosome segregation, the tail domain proteins were expressed as C-terminal fusions to thioredoxin (Trx), and ATP independent interactions of ncd with MTs were characterized. Based on sedimentation and blot overlay assays Trx alone, and Trx-NT1 (amino acids 27-63) did not bind MTs, whereas Trx-NT2 (amino acids 27-119), Trx-NT3 (amino acids 27-149), and Trx-NT4 (amino acids 27-187) bound MTs. The ncd tail domain proteins that bound MTs also showed MT bundling activity. Based on these results, ncd amino acids 1-63 and 188-204 are not required for ATP-independent binding of ncd to MTs, while the sequence from amino acid 64 to 119 contains a MT binding site. Additional MT binding sites may exist in the sequence from amino acid 119 to 187.

## 59 Critical and Threshold Level Detection: A New Approach.

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The top goal for producers of aseptically packaged products (i.e., food and medical devices) is to create technology that will enable such producers to predict package sterility through distribution to the consumer. However, influences that contribute to the loss of package sterility are poorly documented. Many companies have installed in-line package integrity inspection systems without the knowledge of the defect size that permits loss of package sterility (i.e., the critical defect size). This project poster depicts highlights of methodologies previously used to determine the critical leak size as well as an overview of research presently underway in the Department of Food Science and Technology at Virginia Tech, featured as a "New Approach". This research project is funded by; The Center for Aseptic Packaging and Processing Studies (CAMPS).

## 60 Nitrogen Fertilization of Grain Sorghum Production in Virginia

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Research with grain sorghum (*Sorghum bicolor* (L) Moench), a relatively new crop to Virginia, has shown sorghum as a more water-use-efficient-crop than corn (*Zea mays* L.). It is grown in rotation with winter wheat (*Triticum aestivum*) and double crop soybeans (*Glycine max*). However, sufficient data on N fertilization of grain sorghum in this region is not available. The objective of this study was to evaluate the response of multi-rate N fertilization on grain sorghum production. Treatments consisted of factorial combinations of four starter band, and four side-dress N rates to supply a total of ten different N fertilization rates of 11, 33, 56, 78, 101, 123, 146, 168, 190, 213 kg N ha<sup>-1</sup>. A broadcast treatment receiving 67 kg N ha<sup>-1</sup> at planting was also included. Starter band treatment was applied 5 cm to the side and below the seed at the time of planting. Side-dress N treatment was applied 35 days after emergence. Grain yield data was taken at harvest. Grain yields were regressed against fertilizer application to evaluate the response of grain sorghum to N fertilization. There was a significant effect of nitrogen fertilization on the grain sorghum yield on both soils. Grain yields ranged from 4830 to 9915 kg ha<sup>-1</sup>. There was a significant effect of nitrogen fertilization on the grain sorghum yield. Grain yield ranged from 4922 to 9140 kg ha<sup>-1</sup>. Total fertilizer N applications of 123 to 145 kg ha<sup>-1</sup> were found to be sufficient for producing maximum yields. Pre-plant broadcast N applications were as efficient as starter band-placed N in this experiment.

## 61 Neurotoxicity of the Organochlorine Insecticide Heptachlor and Its Role in Parkinsonism

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Epidemiological studies have consistently implicated pesticide exposure as a possible etiologic factor in Parkinson's Disease. Behavioral and neurochemical changes in C57BL/6 mice were investigated following subchronic doses (3 treatments over two weeks) of the persistent organochlorine insecticide heptachlor. The major behavioral effects of heptachlor include increased rearing frequency and open field ambulation. Neurotoxicity of heptachlor in vivo was expressed by a dose-dependent reduction of striatal synaptosome respiration in vitro. In addition, heptachlor increased [3H]dopamine uptake in ex vivo striatal synaptosomes prepared from treated mice, which may reflect dopamine transporter up-regulation in response to increased synaptic levels of dopamine. Neurotransmitter release studies with striatal synaptosome preparations were undertaken to ascertain any direct effects on nerve terminals that might facilitate dopamine release. These studies revealed that heptachlor epoxide, the metabolic activation product of heptachlor, as well as heptachlor itself, stimulated release of pre-loaded dopamine, serotonin, glutamate and GABA. The lack of effect by picrotoxinin and bicuculline precludes an interaction with the GABAA receptor as a mechanism for this release of transmitter. These findings suggest that heptachlor can have significant effects on the nigrostriatal tract and that exposure to organochlorine insecticides may play a role in idiopathic Parkinson's Disease.



## 62 Landscape-level Temperature Estimation for Virginia

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Temperature is an important factor in determining the distribution of communities and species. Knowledge of the temperature regime of an area is extremely valuable to researchers studying these distribution patterns. Temperature data are seldom available for study areas at an appropriate scale. The objective of this study was to produce estimates of monthly mean temperature for Virginia at a landscape level using a geographic information system. Estimates were synthesized by combining data from pixels with temperature information and the topographic characteristics of pixels without. Temperature data were obtained from 87 National Oceanic and Atmospheric Administration weather stations. Topographic data were obtained from U.S. Geological Service digital elevation models. Temperature was estimated for each pixel by adjusting the temperature recorded at the 5 closest weather stations for differences in elevation, then interpolating values for the pixel with an inverse-distance weighting algorithm. Mean monthly temperature was estimated for 12 months of the year. The accuracy of the estimates were assessed with a Wilcoxon signed-rank test comparing estimated temperature to actual recorded temperature for 16 weather stations omitted from the estimation procedure. Estimated temperatures did not differ significantly from the actual recorded temperatures. It was concluded that this method can provide accurate estimates of temperature for areas without data at the landscape level and can be helpful in research investigating community and species distribution.

## 63 Detection of *Actinobacillus pleuropneumoniae* and Identification of Serotype 5 by Multiplex Polymerase Chain Reaction

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*Actinobacillus pleuropneumoniae* (Ap) is the etiological agent of swine pleuropneumonia and is responsible for major losses to the swine industry each year. In addition to detecting the organism, the serotyping of Ap is critical to understanding the epidemiology of the disease and may be important in the development of future vaccines. Serological classification of the bacteria has determined 12 serovars based on capsular antigens with types 1, 5, and 7 being the most prevalent in the United States. Most methods of serotyping Ap have been based on serological assays and have had problems with cross-reactivity between serotypes. For instance, cross-reactions between serotypes 3, 6, and 8, 4 and 7, and 1, 9, and 11 are particularly problematic.

A multiplex polymerase chain reaction (PCR) assay was developed to detect Ap and to distinguish serotype 5 strains. Primers specific to the conserved export and serotype-specific biosynthesis regions of the capsular polysaccharide of Ap serotype 5 were used to amplify 0.7 kb and 1.1 kb DNA fragments, respectively. The 0.7 kb fragment was not amplified by any heterologous species tested, but was amplified by all Ap strains tested with one exception. In contrast, amplification of the 1.1 kb fragment was specific for serotype 5 strains. The assay was able to detect Ap and identify serotype 5 strains from samples of infected lung tissue. The sensitivity of the assay was  $10^2$  colony forming units (CFU). This PCR assay enabled us to detect Ap rapidly and to distinguish serotype 5 strains from other serotypes. The use of primers specific to the biosynthesis regions of other Ap serotypes would expand the diagnostic and epidemiologic capabilities of this assay.

#### 64 Plant Response to Phosphorus on Yard Waste-Poultry Litter Compost Amended Vance Soil: Clayey, Mixed, Thermic, Typic Hapludult.

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Land application of poultry litter or yard waste compost has been practiced for centuries to maintain and improve soil fertility. The slow composting process of yard waste compost keep the C:N ratio high and is not as efficient as it could if C:N could be lowered. A project attempt to combine yard waste compost and poultry litter was a way to lower the C:N ratio. A field study was conducted to determine a short term effect of yard waste- poultry litter compost on P availability in soil solution, P concentration in tissue, P uptake by corn, and corn yield. This soil was treated with 3 rates of fertilizer P, one rate of poultry litter, and 3 rates of yard waste poultry litter compost based on P contain. Three compost mixes, with C:N ratios of 15:1, 20:1, and 25:1 were used at all levels of P. Results showed differences among treatments in the early dry matter production, the soluble P concentration in soil, the P uptake, and the corn yield. Overall, the use of yard waste-poultry litter at the rate of 59 kg P ha<sup>-1</sup> with C:N ratio range from 20:1 to 25:1 appears to be promising for corn production on acid soil.

#### 65 Neuropathologic Effects of Phenyl-methane-sulfonyl Fluoride (PMSF) Induced Promotion and Protection in Organophosphorus ester-Induced Delayed Neuropathy (Opidin) in Hens.

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The serine/cysteine protease inhibitor phosphate PMSF has been used both to promote and to protect against neuropathic events of OPIDN in hens (Lotti et al., 1991; Veronesi et al., 1985; Pope et al., 1993). We expand upon this work by correlating clinical and neuropathological findings in these modifications of OPIDN. To provide appropriate models of OPIDN, single phenylsaligenin phosphate (PSP) dosages of 0.5, 1.0, or 2.5 mg/kg were administered to adult hens. 90 mg/kg of PMSF was given either 4 hours after or 12 hours prior to PSP administration. Clinical signs and pathologic changes in the biventer cervicis nerve (El-Fawal et al., 1988) were monitored. 2.5 mg/kg PSP alone elicited severe OPIDN (terminal clinical score 7.5 +/-1.0 [0-8 scale]; neuropathology score 2.7 +/-0.3 [0-4 scale, based on myelinated fiber degeneration]). PMSF given 12 hours prior to PSP gave complete protection (clinical & neuropathology scores of 0; p<0.0001). Signs and lesions of OPIDN were absent following 0.5 mg/kg PSP alone, but giving PMSF 4 hours after PSP potentiated its neurotoxic effects (clinical score 4.0 +/-0.0; neuropathology score 3.5 +/-0.3; p<0.0001). At the time of sacrifice, there was a correlation (r = 0.61) between the last clinical signs and the neuropathology scores (P<0.0001). This study demonstrates that the intensity of peripheral nerve myelinated fiber degeneration correlates with clinical deficits in PMSF-induced potentiation and protection in OPIDN.

## 66 Absorption of 2-Hydroxy-4-(Methylthio) Butanoic Acid by Ovine Omasal and Ruminal Epithelia

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In order to study the absorption of 2-hydroxy-4-(methylthio)butanoic acid (HMB) across ovine omasal and ruminal epithelia, tissue sheets were collected from eight lambs (BW = 67.58 kg ± 9.07) and mounted in parabiotic chambers that were repeatedly sampled throughout a 60-min incubation. The appearance of HMB (using [<sup>14</sup>C]-HMB as a representative marker) in serosal buffers increased quadratically (P < .004) with time in both tissues. More (P < .0001) HMB appeared in the serosal buffers with omasal than with ruminal epithelium. Both tissues responded similarly and, after 60 min of incubation, the accumulation of HMB in the tissues increased linearly (P < .001) as substrate concentration (.375, .75, 1.5, 3.0, 6.0, 12.0 mM) increased in mucosal buffers. As the concentration of HMB in the mucosal buffers increased, there was a quadratic (P < .001) increase in the appearance of HMB in the serosal buffer of the omasal epithelium, indicating some saturation of the system. The increase in serosal appearance of HMB was linear (P < .001) with ruminal tissue. The results indicate that there are probably multiple mechanisms involved in the absorption of HMB. Because saturation was observed in the omasum, it is likely that mediated transport accounts for at least a portion of the absorption of HMB in the omasum. Other mechanisms (e.g. diffusion and/or paracellular absorption) are responsible for the balance of the absorption. Omasal epithelium appears to have a greater capacity for HMB absorption than ruminal epithelium.

Support for this study was provided, in part, by Novus International, Inc.

## 67 Delineation of Slope Position from Digital Elevation Data and Topographic Modeling

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Slope position describes the location of a piece of land in relation to the surrounding three-dimensional area, and is related to many ecological phenomena. Ecological features such as available moisture, soil depth, soil texture, exposure to sunlight and wind, and tree growth can all be related to slope position. It is a subjective feature in landscape analysis that has not been adequately defined or standardized. Classification of slope position has traditionally been performed using paper topographic maps or through field analyses. Both methods are time consuming, and subjective. A process was devised that could increase the speed and consistency of slope classification. The first step was to define the slope positions to be delineated in such a manner that the classes could be easily interpreted and understood. The slope positions decided upon were ridge top, summit, side slope, toe slope, floodplain, and plateau. A digital elevation model (DEM) was then used in conjunction with a geographic information system (GIS), and several topographically based models, to delineate slope positions for a section of the Craig Creek area. It was possible to delineate slope positions over a large section of land in very little time. Qualitative analyses of the results indicate a high degree of classification accuracy. Accurate, rapid classification of slope position may then be used for purposes ranging from environmental impact statements, road planning, timber harvest, planting and crop selection, and habitat analyses for wildlife studies.

## 68 Land Cover of Virginia

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Department of Fisheries and Wildlife Sciences

A raw land cover mosaic has been created using Landsat Thematic Mapper imagery, circa 1991-1993. A variety of image processing techniques were employed depending on the conditions in each Landsat scene. Unsupervised classification was used in the Piedmont region. Supervised classification, with special attention to topographic shadow and brightness, was performed in the mountainous province. In the Coastal Plain supervised classification was again used, along with a haze correction procedure. A series of digital cover type maplets and 1:40,000 scale color-infrared photographs were utilized as training data during classification. The next step will be to assess the thematic accuracy of this map using over 2000 reference points. These ground points were obtained by flying aerial videography transects across the Commonwealth during the fall of 1995 and spring of 1996. This map is the first product of the Virginia Gap Analysis Project (VAGAP). The goals of this cooperative statewide effort are to 1) produce a detailed map of land cover (i.e., habitat), 2) predict the distribution of vertebrate wildlife species via the habitat map, 3) display regions of high species richness within and without protected areas (i.e., wildlife refuges, National Forests, etc.). The map discussed here will serve as the upper level in the mapping hierarchy required in objective 1. Funding and support for this project is provided by USGS Biological Resources Division (formerly NBS), Virginia Department of Game and Inland Fisheries, and The Fish and Wildlife Information Exchange at Virginia Tech.

## 69 Using Landsat and Ecological Modeling to Delineate Forest Communities in Shenandoah National Park.

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A detailed, vegetation based, land cover map is a large component the Virginia Gap Analysis Project (VAGAP). To accomplish this, VAGAP proposes a combined remote sensing/ecological modeling approach. This approach has been tested on Shenandoah National Park (SNP). LANDSAT Thematic Mapper imagery were used to delineate the land cover into an Anderson et al. (1976) Level I -plus scheme via supervised classification. Areas determined to be deciduous, coniferous, or mixed forest were separated into community types through ecological modeling. Eight cover types (black locust, pine, hemlock, red oak/ash/basswood, chestnut oak, yellow poplar, red oak, barren, and open) were outlined in digital stand maps provided by SNP personnel. Polygons representing the six vegetative cover types were randomized and divided in half. The first half were used to generate training polygons or statistical models for classification. Forest cover types for the entire SNP area were predicted through a maximum likelihood classification algorithm. Accuracy of this process was assessed quantitatively using the second set of forest cover type polygons. A qualitative assessment along with a discussion of the benefits and limitations of this approach to vegetation mapping is presented.

## 70 Taxol Modulates Tumor-Distal Macrophage Nitric Oxide Production *in vivo*.

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Tumor-induced macrophages (M $\phi$ s) mediate immuno-suppression, in part, through overproduction of nitric oxide (NO) and tumor-necrosis factor-alpha (TNF- $\alpha$ ), and perhaps through underproduction of Interleukin-12. Tumor-distal M $\phi$ -derived NO suppresses T-cell proliferation but lacks antitumor activities. Because the antineoplastic agent taxol (paclitaxel) differentially regulates normal host and tumor-bearing host (TBH) M $\phi$  NO *in vitro*, we assessed M $\phi$  NO production following *in vivo* administration of taxol. Using a murine fibrosarcoma model, taxol was administered in doses approximating human antitumor chemotherapies (30-60 mg/kg). At intervals following administration of taxol to normal host or TBH, splenic (tumor-distal) M $\phi$ s were harvested and cultured with various doses of interferon- $\gamma$  (IFN- $\gamma$ ) and the triggering agent LPS. Taxol treatment enhanced normal host M $\phi$  NO production; M $\phi$ s collected 8-24 h following taxol administration produced significantly higher levels of NO. In a clinically more-relevant scenario, taxol administration downregulated NO production by tumor-induced M $\phi$ s. In agreement with our *in vitro* findings, TBH M $\phi$ s from taxol-treated animals produced less NO than M $\phi$ s from untreated TBHs. Taxol treatment (30 mg/kg) reduced TBH M $\phi$  NO production more than 50%, following *in vitro* activation and triggering with optimal doses of IFN- $\gamma$  (10 U/ml) and LPS (10  $\mu$ g/ml). Taxol differentially regulates normal host and TBH M $\phi$  production of NO in tumor-distal locations, suggesting the success of taxol as an anticancer agent may partly derive from immunotherapeutic activities that reverse tumor-induced M $\phi$  suppressor functions.

## 71 Poly(A)<sup>+</sup> RNA From Sheep Oasal Epithelium Induces Expression of a Peptide Transport Protei(s) in *Xenopus Laevis* Oocytes

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In order to verify the research from this laboratory that sheep omasal epithelium contains mRNA encoding for a peptide transporter (s) and to determine di- to octapeptide transport capability, poly(A)<sup>+</sup> RNA isolated from sheep omasal epithelium was injected into *Xenopus laevis* oocytes. Poly(A)<sup>+</sup> RNA was functionally expressed in *Xenopus* oocytes 4 to 7 d post-injection. Peptide (5 di-, 10 tri-, 6 tetra-, 2 penta-, 1 hepta-, 1 septa-, 1 octapeptide) transport capability was measured by impaling oocytes with a microelectrode to monitor membrane potential ( $V_m$ ). Oocytes were maintained in pH 5.5 buffer. Peptide transport was identified as being expressed when, in the presence of a buffered peptide substrate (1 mM), the oocyte membrane showed persistent depolarization (a more positive  $V_m$ ). In the absence of peptide transport, the membrane became depolarized with the addition of buffered substrate, but rapidly repolarized to the resting potential. Peptide transport was expressed for some di-, tri-, and tetrapeptides. Measured depolarization ranged from about 9.6 mV to 42.1 mV. Larger peptides were not transported by the oocytes. When transport expression was measured with the substrates in a pH 7.5 buffer, no transport occurred indicating that transport was dependent on a proton gradient. The data indicate that sheep omasal epithelium contains mRNA that code for a protein(s) capable of proton-dependent di-, tri-, and tetrapeptide transport. This provides further evidence that absorption of peptides from the ruminant stomach is possible.

## 72 Dioxin Differently Affects Activated and Naïve Cells Within The Same Animal

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2,3,7,8 Tetrachlorodibenzo-*p*-dioxin (TCDD), commonly known as dioxin, is a highly toxic environmental contaminant formed as a byproduct during the manufacture of products from chlorinated phenols and from the combustion of chlorinated materials. One of the most characteristic features of TCDD toxicity is its effect on the immune system. At doses that do not cause organ toxicity, TCDD is able to cause immunosuppression; the exact mechanism by which this happens is not understood. Recent studies have shown that dioxin actually enhances the proliferation of activated T cells *in vitro*. When activated cells from TCDD-treated mice are re-challenged with the same antigen, however, they show a diminished proliferative response to the recall antigen. To date, all research has focused on activated cells, and no work has been done with naive cells from TCDD-treated animals. In our study, adult female C57BL/6 mice were injected intraperitoneally with TCDD or with oil vehicle on day 0. At the same time, both rear footpads were immunized with anti-CD3 mAbs to activate T cells in the popliteal lymph nodes. At time points day 7, day 14, and day 21, animals were sacrificed. Popliteal lymph nodes and axillary lymph nodes were harvested, and were cultured for 18, 24, and 48 hours with RPMI, IL-2, and anti-CD3 mAbs. No proliferative differences were observed between the oil-treated and TCDD-treated axillary lymph node cells, which constitute naive cells. In contrast, popliteal lymph node cells from TCDD treated animals show a markedly decreased proliferative rate in response to recall antigen stimulation. This proliferative decrease is evident at day 7, but is gone by day 14. In addition, the differences in proliferative response between the oil- and TCDD-treated popliteal lymph node cells has a kinetics aspect. Activated, TCDD-treated cells appear to have a delayed *in vitro* activation. Additionally, the surface marker expression of activation molecules, such as CD3,  $\alpha\beta$ -TCR, IL-2R, CD44, CD45R, and Fas, was examined via flow cytometric analysis. The expression of surface molecules on activated, TCDD-treated cells is dysregulated. Our results clearly show that TCDD exerts a differential effect on lymph node cells, dependent upon activation status, within the same animal.

## 73 Changes in Skeletal Muscle Sarcoplasmic Reticulum Function in Aged Rats

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The decline in physical ability during aging has been linked to reduced skeletal muscle function. It is possible that  $\text{Ca}^{2+}$  exchange by the sarcoplasmic reticulum (SR) is altered with aging and may be responsible for decreased muscle function. However, data pertaining to the actual functional changes of SR in aging are limited. Thus, this research examined  $\text{Ca}^{2+}$  uptake and release kinetics in aged muscle. Six 12 and 27 month-old Fisher 344-Brown Norway rats were used and three muscles were examined: the soleus (S), a predominantly "slow" muscle, the plantaris (P), a predominantly "fast" muscle and the diaphragm (D), the primary respiratory muscle. Rates of  $\text{Ca}^{2+}$  uptake and release were measured in a muscle homogenate fraction using the fluorescent indicator fura-2. Masses of the S, P and D were 22, 23 and 15% lower in the aged animals. However, these differences were eliminated when masses were normalized by body mass. In all three muscles examined, the rates of  $\text{Ca}^{2+}$  uptake were not significantly different between the young and old animals. Rates of  $\text{Ca}^{2+}$  release, however, were reduced by 30% in the P and D of the old animals. These results suggest that SR function is altered in "fast" muscles of the rat. It is possible that changes in SR  $\text{Ca}^{2+}$  release may contribute to diminished muscle function and lead to the decline in physical ability of the elderly.

## 74 The Effect of Phytase on Calcium Bioavailability in Weanling Pigs Fed a Corn-Soybean Meal Based Diet

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Ninety-six weanling pigs (equal barrows and gilts) were used in a 4-wk experiment to investigate the effects of dietary Ca levels and microbial phytase activity on growth performance, Ca and P digestibility, and rib shear force and ash. A 19% CP, corn soybean meal, diet low in Ca was used. Four levels of phytase (0, 167, 333, and 500 U/kg) and four levels of total Ca (.26, .32, .37, and .42%) were fed. Phosphorus was maintained at .64% in all diets. Body weight and pen feed consumption were measured weekly. During wk 4, pen fecal samples were collected twice daily for 5 d. At the end of wk 4, the barrow from each pen (n = 48) was killed for collection of ileal digesta and tenth ribs. Added phytase had positive linear effects on daily gain, (wk 3-4,  $P < .002$ ) feed efficiency (wk 3-4,  $P < .02$ ), and rib ash weight ( $P < .001$ ). Calcium total tract ( $P < .001$ ) and Ca ( $P < .001$ ) and P ( $P < .001$ ) ileal digestibility were also improved by phytase. Added Ca had positive linear effects on daily gain ( $P < .02$ ) and feed efficiency ( $P < .01$ ) during wk 3-4. Rib ash weight ( $P < .001$ ), percent ( $P < .001$ ), shear force ( $P < .03$ ) and energy ( $P < .008$ ) were linearly increased by added Ca. Total tract ( $P < .001$ ) and ileal ( $P < .001$ ) digestible Ca were linearly improved by added Ca. Based on phytase and Ca response equations for daily gain (wk 3-4), rib ash weight and shear force, and ileal and fecal digestible Ca, 500 U of phytase is equivalent to .78 g of Ca as  $\text{CaCO}_3$ .

## 75 Analysis of novel chalcone synthase mutants in *Arabidopsis*

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Mutants have been powerful tools in the characterization of the flavonoid biosynthetic pathway in petunia, snapdragon, maize, and *Arabidopsis* because disruption of flavonoid biosynthesis is non-lethal and results in visually distinguishable phenotypes such as altered flower or seed color. Flavonoids, important secondary metabolites in plants, play various roles in addition to pigmentation, including protection from ultraviolet-B (UV-B) radiation and signaling between plants and microbes. In some plants, flavonoids are also required to maintain male fertility. The first enzyme in the flavonoid pathway, chalcone synthase (CHS), is encoded by a single-copy gene in *Arabidopsis* and mutants at this locus are termed *transparent testa 4* (*tt4*). *tt4* mutants are deficient in flavonoid biosynthesis and produce yellow seed instead of brown (wild type). Five novel CHS mutants have been isolated as second-site suppressors of the UV-hypersensitive phenotype of chalcone isomerase (CHI) mutants (L.G. Landry, T-M Ou-Lee, and R.L. Last, unpublished data). These alleles are currently being characterized at the DNA and protein levels in our laboratory. The addition of these new alleles to the two previously described for *tt4* creates an allelic series that will help elucidate structural and regulatory roles of CHS. In future experiments, this allelic series will be used to examine interactions of CHS with other flavonoid enzymes as well as subcellular localization and organization of flavonoid metabolism.

## 76 Induction of Glu1 in Maize Leaf

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A maize plant has at least two isoforms of  $\beta$ -glucosidase (i.e. Glu1 and Glu2). They are found in different tissues/organs of a maize plant. A 15 day old maize leaf has just one isoform of  $\beta$ -glucosidase, Glu2. Our studies show that upon water stress, the other isozyme Glu1 also appears in the leaves. The leaves of 15-day old seedlings of two mutants, albino and rolled-leaf, also have both of Glu1 and Glu2 isozymes, instead of just Glu2 which is present in leaves of 15-day old wild maize seedling. On the other hand, exogenous application of phytohormones like abscisic acid, indole acetic acid and gibberellin do not induce Glu1 in the maize leaves. Salicylic acid and a fungal elicitor and senescence also failed to stimulate the appearance of the isozyme in maize leaves. The results suggest that  $\beta$ -glucosidase isozyme Glu1 may have a physiological role in coping with different stress condition in maize plant.

## 77 Importance of Seed Moisture on Temperature-Enhanced After-Ripening of Switchgrass Seeds.

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Newly harvested switchgrass (*Panicum virgatum* L.) seeds are often highly dormant. The dormancy declines during storage in a little-understood process called after-ripening. There is very little dormancy decrease when seeds are stored at 5 to 10 °C. More than 1 year of after-ripening at room temperature is necessary for the seeds to break 80 to 90% of the dormancy. After more than 1.5 year, seeds kept in paper envelopes at 45°C have a sharp decrease in germinability because of aging. For seeds stored in paper envelopes, which permits the loss of seed water at the increased temperatures, the  $Q_{10}$  of after-ripening was close to 1 between 20 and 45°C. When seeds were held in closed vials and water content maintained at 80 to 110 g kg<sup>-1</sup>, the  $Q_{10}$  was 2.5 between 20 and 45°C. Temperature-enhanced after-ripening appears to occur most rapidly only if suitable seed water content is maintained at the higher temperatures. To speed up the after-ripening process, it is suggested to store switchgrass seeds at a warm temperature and retain water content if possible.



## 78 Direct Evidence That Lactate Contributes to the Development of Skeletal Muscle Fatigue

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Previous studies have linked the decrease in skeletal muscle force production during fatigue to the accumulation of lactate. Unfortunately, there is little direct evidence to support this notion. These experiments sought to determine if lactate, at normal pH, directly alters force production by skeletal muscle. Mouse *m. extensor digitorum longus* (EDL) were incubated in a Ringers solution (pH 7.2) and exposed to 10, 20, 30, 50mM lactate. At 21°C, tetanic force production ( $P_o$ ) decreased to  $99.3 \pm 1.0$ ,  $97.1 \pm 1.2$ ,  $94.9 \pm 1.1$ ° and  $93.1 \pm 1.3$ %° of initial ( $X \pm SE$ , \* $p < 0.05$  vs untreated muscles). At 37°C,  $P_o$  was reduced to  $89.7 \pm 1.1$ ,  $81.0 \pm 2.4$ ,  $73 \pm 3.9$ °, and  $61.6 \pm 5.4$ %° of initial (\* $p < 0.05$ ). The next phase was to determine if the changes in  $P_o$  are due to alterations in sarcoplasmic reticulum (SR)  $Ca^{2+}$  exchange. The SR of EDL homogenates were actively loaded with  $Ca^{2+}$  and release initiated by  $25 \mu M AgNO_3$ . The rate of  $Ca^{2+}$  release was significantly reduced by 31% ( $2.48 \pm 1.21$  vs.  $1.72 \pm 0.24 \mu mol \cdot mg^{-1} \cdot min^{-1}$ ) in the presence of 25mM lactate. These results indicate that lactate, independent of pH changes, decreases force production of whole muscle, effects that are greater at 37 than at 21°C. In addition, they suggest that the depression of  $P_o$  is due, in part, to reduced SR  $Ca^{2+}$  release. These findings provide direct evidence that increased lactate during exercise contributes to the development of fatigue.

## 79 Transcriptional Regulation of the glycogen phosphorylase-2 Gene During Development in Dictyostelium discoideum

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*Dictyostelium discoideum* is a model system for the analysis of regulatory processes that control cellular differentiation and morphogenesis. These processes are known to be directed by the action of extracellular morphogens such as cAMP and DIF-1, which regulate prespore and prestalk differentiation respectively. Glycogen phosphorylase-2 (GP-2) plays an important role in *Dictyostelium* development by generating glucose precursors that are essential for cell differentiation. Transcription of the gene is non cell-type specific and is regulated by cAMP and DIF-1, suggesting that the gene is independently regulated in the two cell types. Using 5' deletion analyses, several regions within the gp-2 promoter were identified that regulate expression of the gene during differentiation. This preliminary promoter analysis was used to design site directed mutagenesis experiments, and we present here the identification and definition of a cAMP regulatory sequence element. This element contains a C-box motif that is found in regulatory regions of several cAMP-regulated genes. Mutation of this element resulted in a large drop in levels of transcription, and this mutant promoter was no longer cAMP-responsive. Expression of the gene still occurred in the correct temporal manner during differentiation; this basal activity may be pre-stalk specific and we propose experiments to test this hypothesis. Additionally, we present the definition of further regulatory sequence elements in the gp-2 promoter by site directed mutagenesis. The precise function of these elements in transcriptional regulation is not yet known and we aim to define these in future experiments. Of particular interest is the identification of a DIF-1-responsive element and other cell-type specific elements.

## 80 Activation of the Prolactin Gene by the Transcription Factor, Pit-1, in the Domestic Turkey

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Transcription factors are proteins which interact directly with the DNA promoter sequences of specific genes to regulate transcription of those genes. Pit-1 is a transcription factor, which in mammals, has been shown to stimulate transcription of the prolactin gene, however its role in regulating the prolactin gene in poultry is unknown. The turkey Pit-1 gene and three cDNA isoforms have been cloned. Alternative splicing at the 5' end of the mRNA transcript gives rise to three turkey Pit-1 isoforms, tPit-1\*, tPit-1 $\beta$ \* and tPit-1W\*, with different amino-termini. All turkey Pit-1 isoforms contain an extra 38 amino acid exon, (exon 2a), which is not found in rat Pit-1. Previous cotransfection studies with Pit-1 expression vectors and prolactin promoter luciferase reporter constructs showed that turkey Pit-1 does not activate the prolactin promoter as does rat Pit-1. The objective of this study was to determine whether the presence of the extra 38 amino acids encoded in exon 2a was responsible for the decreased ability of turkey Pit-1 to stimulate the prolactin gene promoter. Turkey Pit-1 expression vectors, which lack exon 2a, were created and then tested with the prolactin promoter luciferase reporter constructs. Results indicated that the inability of turkey Pit-1 to activate the prolactin promoter is not due to the presence of the extra 38 amino acids of exon 2a.

## 81 Effect of Post-Exercise Macronutrient Intake on Metabolic Response to Eccentric Resistance Exercise

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The purpose of this study was to examine the effect of carbohydrate (C), carbohydrate-protein (CP) and placebo (P) beverages on muscle damage and inflammation following eccentric resistance exercise. Twenty-six untrained males consumed a controlled diet for 9 d. On d 4 evening subjects cycled for 40 min at 70% of  $VO_{2peak}$  followed by 5 sprints. On the morning of d 5 subjects performed 100 eccentric leg flexions at 120% of 1-RM. The subjects drank either C (1.25 g C $\cdot$ kg $^{-1}$ ), CP (0.875 g C $\cdot$ kg $^{-1}$ , 0.375 g protein $\cdot$ kg $^{-1}$ ), or P following exercise and 2 h later. Fasted blood samples were obtained on d 4-10 with additional samples on d 5 immediate, 3 h, and 6 h post-exercise. Isokinetic quadriceps peak torque at 60 $^{\circ}$ s $^{-1}$  decreased 24% ( $P < 0.01$ ) on d 6 across all groups from baseline and remained depressed by 21% on d 8. Muscle soreness peaked at 3.8  $\pm$  1.6 out of 10 across all groups on d 7 ( $P < 0.01$ ). Serum creatine kinase (CK) increased ( $P < 0.01$ ) over d 5 ( $\Delta$  CP 24.6  $\pm$  19.1,  $\Delta$  P 39.2  $\pm$  71.6,  $\Delta$  C 70.8  $\pm$  60.4 U $\cdot$ L $^{-1}$ ) with a trend towards interaction between groups ( $P < 0.08$ ). There was also a trend ( $P < 0.10$ ) for increased CK over the a.m. values with peak CK of 1108  $\pm$  2710 U $\cdot$ L $^{-1}$  on d 8 and no interaction between groups. Interleukin-1 showed no changes. IL-6 had no change in a.m. values but rose ( $P < 0.01$ ) on d 5 and peaked at 6 h ( $\Delta$  CP 0.80  $\pm$  2.48,  $\Delta$  C 0.83  $\pm$  1.79,  $\Delta$  P 1.61  $\pm$  1.66 pg $\cdot$ ml $^{-1}$ ) with no interaction between groups. Tumor necrosis factor fell ( $P < 0.01$ ) at 6 h on d 5 ( $\Delta$  CP 0.77  $\pm$  1.5,  $\Delta$  C 0.93  $\pm$  0.57,  $\Delta$  P 0.77  $\pm$  0.7 pg $\cdot$ ml $^{-1}$ ) with no interaction. At 3 h on d 5, serum insulin was higher for CP (24.6  $\pm$  15.5  $\mu$ IU $\cdot$ ml $^{-1}$ ) than C (17.2  $\pm$  10.9  $\mu$ IU $\cdot$ ml $^{-1}$ ,  $P > 0.05$ ) and P (5.3  $\pm$  0.4  $\mu$ IU $\cdot$ ml $^{-1}$ ,  $P < 0.01$ ). Serum glucose at 3 h was higher for CP (4.73  $\pm$  0.9 mmol $\cdot$ L $^{-1}$ ) than P (4.64  $\pm$  0.4 mmol $\cdot$ L $^{-1}$ ,  $P > 0.05$ ) and C (4.27  $\pm$  0.7 mmol $\cdot$ L $^{-1}$ ,  $P < 0.05$ ). In summary, CP>C>P for most metabolic indicators of recovery 3-6 h after eccentric exercise in that CP tended to have highest insulin, glucose, and lowest changes in CK and IL-6.

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82 Significance of Age Dependent Appearance of Cytotoxic Double Negative T Cells and Their Ability to Constitutively Produce Cytokines in the Regulation of Immune response in Mice Exhibiting Defects in *Fas* and *Fas Ligand (FasL)* Expression

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Mice homozygous for *lpr* or *gld* mutations exhibit deficiencies in the expression of *fas* and *fasL* respectively and develop an age dependent lymphoproliferative disease characterized by the appearance of CD4<sup>+</sup>CD8<sup>-</sup> (double-negative, DN) T cells. We have earlier shown that such DN T cells from *lpr* mice are cytolytic cells that express perforin constitutively and mediate spontaneous NK-like cytotoxicity (*J. Exp. Med.*, 178: 2225). In the current study, we noted that unlike *lpr* DN T cells, similar cells from *gld* mice failed to exhibit cytolytic activity despite constitutive expression of perforin. The cytolytic activity of the DN T cells was dependent on *Fas-FasL* interaction. These data suggested that the DN T cells, although cytolytic in nature, cannot exhibit this function *in vivo* due to defects in the expression/function of *fas* and *fasL*. The DN T cells were also found to constitutively express a variety of cytokines including IFN- $\gamma$ , TNF, TGF- $\beta$  and IL-10. The constitutive expression of such cytokines suggested that the DN T cells represent activated T cells unable to undergo apoptosis. Also, such cytokines may contribute towards induction of autoimmunity. Thus, defects in the expression of *fas* or *fasL* could lead to accumulation of activated T cells secreting a variety of cytokines which may induce dysregulation in the immune response.

Notes:

