POSTER 1

PEPTIDOGLYCAN HYDROLASE ANTIMICROBIALS ENGINEERED TO REDUCE RESISTANCE DEVELOPMENT.

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Multi-drug resistant superbugs are a persistent problem in modern health care, demonstrating the need for a new class of antimicrobials that can address this concern. Phage endolysins (peptidoglycan hydrolases) are a novel class of antimicrobials that having co-evolved with their host may be better suited to avoid resistance development. LysK, a staphylococcal bacteriophage endolysin from phage K, is one such enzyme with two lytic activities (an N-terminal D-alanyl-glycyl endopeptidase domain and a mid-protein *N*-acetylmuramyl-L-alanyl amidase domain) that can lyse *S. aureus* and coagulase-negative staphylococci (CoNS) and thus is a potent staphylococcal antimicrobial. Lysostaphin is a peptidoglycan hydrolase (glycyl-glycine endopeptidase) secreted by *S. simulans* that has been shown to be a potent lytic agent effective against drug-resistant staphylococci and staphylococcal biofilms. However, resistance to lysostaphin has been reported, reducing its long-term potential as a stand-alone antimicrobial. We have demonstrated that together lysostaphin and LysK target three unique bonds in the peptidoglycan of *S. aureus*. When used in combination, these proteins act synergistically to kill S. *aureus,* including MRSA USA300. We have created fusion proteins of the parental LysK and lysostaphin that retain all three catalytic activities and exhibit increased molar specific activity as compared to the parental enzymes in multiple in vitro lytic assays against *S. aureus* and CoNS. We have demonstrated that these constructs are able to disrupt *S. aureus* biofilms, and nanomolar concentrations are active in whole rat blood, eliminating an inoculum of 3×10^3 *S. aureus* cfu/ml in less than 90 min. We have demonstrated that the triple acting lytic enzymes are less prone to resistance development over multiple rounds of selection as compared to the parental enzymes LysK and lysostaphin. Rats colonized with *S. aureus* showed a 35-fold reduction in colonization following a three-day treatment with a peptidoglycan fusion protein. We propose that our triple-acting peptidoglycan hydrolase fusions are a novel class of potent staphylolytic antimicrobials that, when compared to single and double domain native parental lysins, are less subject to selecting for resistance development in a population.

POSTER 2

BALANCING AGRICULTURAL DEVELOPMENT RESOURCES: ARE GM AND ORGANIC AGRICULTURE IN OPPOSITION IN AFRICA?

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Organic agriculture has been promoted vigorously by many civil and donor organizations engaged in agricultural development in many parts of Africa in recent years. Certified organic products are being grown in more than half of African countries, targeted mainly towards export to Europe and US markets. In contrast, adoption of GM agriculture has been met with skepticism in much of Africa. There are currently only 3 African countries producing legally approved GM agricultural products. In this paper, we have made an attempt to examine whether African countries are implementing organic agriculture at the expense of GM and discern the major economic factors that explain African countries' adoption of both GM and organic agriculture. We have empirically tested several factors that may explain African attitudes toward GM and organic agriculture by a newly generated dataset on agriculture, trade, and development indicators for a subset of African countries. For example, African countries' openness to GM agriculture is significantly predicted by variables for wealth, organic agricultural area, colonial legacy, past rejection of GM, and the percentage of the county under land protection. Interestingly, our analyses reveal that openness to GM agriculture is positively correlated with the abundance of organic agriculture. This implies that GM and organic agriculture are currently able to coexist within African countries. This is furthermore evidenced by South Africa being the largest producer of GM crops and the third largest producer of organic crops by raw area in Africa. This relationship seems to indicate that countries which are serious about agricultural development are serious about it regardless of the technology being employed. We find it heartening to have evidence that at the national level, many African governments are willing to engage in pragmatic thinking about agriculture and are not completely dominated by any particular agricultural ideology.

POSTER 3

SOIL BIODIVERSITY AND TILLAGE IN MARYLAND CROPPING SYSTEMS

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Entomopathogenic nematodes (EPN) are widespread, potent generalist predators that can control outbreaks of soil insect pests. Their population distributions and their ability to manage pest populations in Maryland corn and soy have not been explored. No-till acreage has dramatically increased across the state in recent years. No-till farming and the use of seasonal cover crops can improve soil integrity and organic matter, and minimize nutrient leaching and run-off. However, as tillage destroys crop residues that may facilitate overwintering and directly kills some crop pests, the potential for crop pest outbreaks may increase. This potential problem may be particularly acute if natural enemies in the soil ecosystem, such as EPN, are disrupted by the tillage and cannot recolonize as quickly as their arthropod prey. Our study was designed to measure abiotic variables, EPN species distribution and prevalence, and insect host abundances under experimental till and no-till in soy and corn across Maryland. The overall goal was to improve our understanding of the potential role of EPN in integrated pest management.

Over three years (2009-2011) of field trials in replicated 30X30m plots at five Maryland Agricultural Experiment Station facilities (CMREC-Beltsville, CMREC-Upper Marlboro, LESREC-Salisbury, WMREC-Keedysville, WREC-Wye), we demonstrated higher prevalence of EPN, higher soil arthropod diversity, higher soil moisture and lower mean temperatures in no-till systems compared to conventional till. Although we did not observe differences in production yield, we argue that the conditions created by no-till agriculture – reduced soil erosion, increased organic matter and soil moisture, and increased persistence of plant-available soil nutrients – also results in greater persistence and diversity of beneficial insects and nematodes. This may encourage more stable agroecosystems that are less prone to pest outbreaks and the need for chemical inputs.

POSTER 4

UTILIZING RAMAN MICROSCOPIC ANALYSIS TO IDENTIFY SOURCES OF PM10 DOWNWIND OF AGRICULTURAL OPERATIONS

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Emission of particulate matter (PM) from animal feeding operations (AFOs), including open beef cattle feedlots and dairies, is a major air quality concern. Particles with equivalent aerodynamic diameter of 10 μm or less (PM_{10}) can penetrate deeply into the lungs and cause serious health problems. This work is aimed at the development of spectroscopic techniques to determine the distribution of source materials contributing to PM_{10} collected downwind from agricultural operation. Potential source materials (dust from unpaved road, pen surface material, and cattle feeds) for PM_{10} were collected and analyzed with Raman microscopy. A spectrum library of 1000 spectra from these source materials was constructed and incorporated in a multivariate statistical analysis model. In the model, cluster analysis was used to classify the library spectra and to build up a training group for subsequent discriminant analysis to classify unknown spectra. Principle component analysis was applied at different levels for data extraction. Internal cross validation of the model resulted in 99.7% correct classifications of the model spectra. Initial analysis of filter samples of PM_{10} suggests that pen surface material accounts for about 65% contribution of the emission while road dust and feeds account for 15% and 20%, respectively. Ongoing analysis of samples with large sizes can reveal a more complete characterization of the source distribution, providing important insights to producers, extension agents, and scientists seeking to improve management practices and protect air quality.

POSTER 5

GENERATING FUNCTIONAL MEMORY CYTOTOXIC T LYMPHOCYTES THROUGH REPETITIVE PEPTIDE BOOSTING

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Immunological memory is the foundation for vaccination, which is the most effective tool for fighting infectious diseases. Activated $CD8^+$ T cells, or cytotoxic T lymphocytes (CTLs), are capable of memory and play a critical role in the control of intracellular pathogens and cancer cells. The memory CTL population, derived from naïve T cells after activation, is long-lived and able to generate a rapid and heightened response upon reencountering pathogens. However, induction of functional memory CTLs remains a major challenge for vaccination due to limited vectors for immunization and poor immunogenicity of antigen. Confronted with the challenges of developing vaccines to prevent diseases including HIV and malaria, as well as anti-cancer treatments, devising methods for generating memory CTLs is a top priority in the field of immunology. This study investigates the effects of peptide and adjuvant on the induction of a functional memory CTL population. Using the OT1 adoptive transfer system, we found that intravenous peptide boosting with adjuvant can progressively induce high levels of functional memory CTLs in animals primed with a live vector. The resulting memory CTLs are not immune senescent as they are functional and able to provide protection against pathogen challenge. In addition, repetitive boosting drives the differentiation of memory CTLs to a unique and long-lasting effector memory phenotype characterized by decreased interferon-γ but increased granzyme B production. These data have important implications in the design of T cell vaccines and indicate that repetitive intravenous boosting with peptides may be one useful option for the induction of a large number functional memory CTLs in vaccination.

POSTER 6

GENOMIC REGIONS SHOWING COPY NUMBER VARIATIONS ASSOCIATE WITH RESISTANCE OR SUSCEPTIBILITY TO GASTROINTESTINAL NEMATODES IN ANGUS CATTLE

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Genomic structural variation is an important and abundant source of genetic and phenotypic variation. We previously reported an initial analysis of copy number variations (CNVs) in Angus cattle selected for resistance or susceptibility to gastrointestinal nematodes. In this study, we performed a large scale analysis of CNVs using SNP genotyping data from 472 animals of the same population. We detected 811 candidate CNV regions, which represent 141.8 Mb (~4.7%) of the genome. To investigate the functional impacts of CNVs, we created two groups of 100 individual animals with extremely low or high estimated breeding values (EBVs) of eggs per gram of feces (EPG), and referred to these groups as parasite resistant (PR) or parasite susceptible (PS), respectively. We identified 297 (~51 Mb) and 282 (~48 Mb) CNV regions from PR and PS groups respectively. Approximately 60% of the CNV regions were specific to the PS group or PR group of animals. Selected PR or PS specific CNVs were further experimentally validated by quantitative PCR (qPCR). A total of 297 PR CNV regions overlapped with 437 Ensembl genes enriched in immunity and defense, like *WC1* gene which uniquely expresses on gamma/delta T cells in cattle. Network analyses indicated that the PR specific genes were predominantly involved in gastrointestinal disease, immunological disease, inflammatory response, cell-to-cell signaling and interaction, lymphoid tissue development and cell death. By contrast, the 282 PS CNV regions contained 473 Ensembl genes which are overrepresented in environmental interactions. Network analyses indicated that the PS specific genes were particularly enriched for inflammatory response, immune cell trafficking, metabolic disease, cell cycle, and cellular organization and movement.

POSTER 7

COPY NUMBER VARIATIONS RELATED TO REPRODUCTION TRAITS IN *HOLSTEIN* CATTLE

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Daughter pregnancy rate (DPR) is one of important reproduction traits that affect overall profitability in dairy industry. However, historical selection for production and conformation rather than reproduction has resulted in a decline in cow fertility. Genomic structural variation including copy number variation (CNV) is an important and abundant source of genetic variation. In this study, we investigated CNVs associated with DPR. Based on array comparative genomic hybridization and quantitative polymerase chain (qPCR), we performed a CNV analysis using 40 Holstein bulls with either high or low predicted transmitting ability of DPR (referred as HDPR or LDPR group). We identified 685 (16.4 Mb) and 656 (17.8 Mb) CNV regions from HDPR and LDPR groups respectively. The HDPR CNV regions overlapped with 376 Ensembl genes, while the LDPR CNV regions contained 405 Ensembl genes. A total of 56% of the CNV regions were specific to either HDPR or LDPR group. It is intriguing to notice that Ingenuity pathways analysis (IPA) based on the CNV associated genes revealed several significant networks related to reproductive system development and function, and embryonic development in HDPR. We also discovered the genes which over represented in both HDPR and LDPR for molecular function of reproductive system and embryonic development, including pregnancy specific beta-1-glycoprotein (PSG), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), phosphodiesterase 4D (PDE4D), protein kinase C, alpha/beta (PRKCA) and serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5). In addition, the common “loss” CNV events at gene progesterone receptor (PGR) could reflect the high selection pressures on milk production traits in Holstein cattle. These results indicate that some CNV associated genes may be associated with reproduction trait such as DPR.

POSTER 8

INDIVIDUALIZED CATTLE COPY NUMBER AND SEGMENTAL DUPLICATION MAPS USING NEXT GENERATION SEQUENCING

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Copy Number Variations (CNVs) affect a wide range of phenotypic traits; however, CNVs in or near segmental duplication regions are often intractable. Using a read depth approach based on next generation sequencing, we examined genome-wide copy number differences among five taurine (three Angus, one Holstein and one Hereford) and one indicine (Nelore) cattle. In placed chromosomes, we identified 1265 CNV regions comprising ~55.6 Mbp sequence and 476 of which (~38%) have not previously been reported. We validated this sequence-based CNV call set with aCGH, qPCR and FISH, achieving an 82% validation rate with an 8% false positive rate. We further estimated absolute copy numbers for genomic segments and annotated genes in each individual. Surveys of the top 25 most variable genes revealed that the Nelore individual had the lowest copy numbers in 13 cases (~52%, chi squared test, p value < 0.05). In contrast, genes related to pathogen and parasite-resistance such as *CATHL4* and *ULBP17* were highly duplicated in the Nelore individual relative to the taurine cattle, while genes involved in lipid transport and metabolism including *APOL3* and *FABP2* were highly duplicated in the beef breeds. These CNV regions also harbor genes like *BSP30A* and *WC1*, suggesting that some CNVs may be associated with breed-specific differences in adaptation, health and production traits. By providing the first individualized cattle CNV and segmental duplication maps and genome-wide gene copy number estimates, we enable future CNV studies into highly duplicated regions in the cattle genome.

POSTER 9

UTILIZING THIN-FILM SPE METHODOLOGY TO ASSESS DDT AND DIELDRIN BIOAVAILABILITY TO EARTHWORMS

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DDT and dieldrin have been banned in most countries but heavily contaminated agricultural sites still exist in many areas. A historical orchard located in Beltsville, MD that received routine DDT and dieldrin applications more than 40 years ago is being assessed for potential *in situ* remediation with organic carbon amendments. The remediation goal is to reduce bioavailability of pollutants to earthworms to reach standards set by US EPA. In order to rapidly estimate bioavailability of these organic pollutants to earthworms and to assess remediation options, a thin-film solid-phase extraction assay has been tested. A recent study has shown that the thin-film polymer can mimic biological membranes and concentrations in the polymer were strongly correlated to residue uptake by mussels in sediment. In this experiment, DDT- and dieldrin-contaminated soil from the orchard site and freshly-spiked control soil were analyzed using this thin-film methodology, and results were compared to concentrations found in native earthworms. Preliminary results show that aged soil 4,4'-DDT reached equilibrium with the film in 10 days, while its metabolite, 4,4-DDE, reached equilibrium only after 110 days and dieldrin in 64 days. We expect that equilibrium times will be shorter for freshly spiked soil. The orchard soil was mixed with 5 different organic carbon amendments, and the change in bioavailability of the residues relative to the unamended soil was determined. Amendments included biochar, composted biosolids, limed biosolids, and two composts. This approach may provide a useful, rapid and easy assessment of bioavailability of organic pollutants in aged contaminated soils.

POSTER 10

IMPROVING TURFGRASS DISEASE DIAGNOSTICS USING MOLECULAR BASED TECHNOLOGIES

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Turfgrasses are important components of ecosystems around the world. They increase aesthetic value, are used for a number of recreational activities, and serve an important role in erosion prevention and groundwater recharge. As the demand for reduced pesticide use and fertilizer inputs continues to increase, environmental stewardship and sustainability of turfgrass systems has been drawn into question. Improving and implementing sustainable management practices for disease prevention and control in turfgrass is becoming a necessity. Accurate pathogen identification is key for proper management of damaging, difficult to control fungal diseases like anthracnose (*Colletotrichum cereale*), dollar spot (*Sclerotinia homoeocarpa*), and rust (*Puccinia* spp.). Traditional symptom- and sign-based diagnostic protocols are important indicators of disease; however, these methods can be time consuming and may result in misdiagnosis if pathogen morphology is ambiguous and taxonomy is confusing. In this research, our objectives were to improve turfgrass pathogen diagnosis by expanding genomic resources for anthracnose, dollar spot, and rust disease of turfgrass, and use this data for taxonomic and phylogenetic verification, as well as developing quick, efficient DNA-based diagnostic protocols.

Using multi-copy and single copy genetic markers, real-time PCR primers and probes were designed for three common *Puccinia* species and two *C. cereale* subspecies, respectively. The extreme sensitivity of the assays allows for both pathogens to be detected in less than 4 hours, as well as to be identified directly from infected tissue, eliminating the need for culturing in *C. cereale* and facilitating high-throughput genotyping of these pathogens. Sequence data was also generated from *S. homoeocarpa,* to reevaluate the currently inaccurate taxonomic placement of this pathogen. Ancient herbarium specimen DNA provided an enormous, diverse resource that spanned several decades, allowing connections to be made between modern and historical collections of the dollar spot fungus and closely related species. The successful inclusion of herbarium specimens in this study provides a new, powerful tool for evaluating taxonomy of turfgrass pathogens. Improving turfgrass disease diagnostics through the molecular-based technologies described has allowed for faster, more efficient, and broader evaluations of numerous samples from a variety of hosts, locations, and time periods. Additionally, these sequence-based tools have increased the accuracy of disease diagnosis, ensuring that proper disease management and control options are implemented in an effective, timely, and responsible manner.

POSTER 11

ANTIMICROBIAL ACTIVITY OF CINNAMALDEHYDE AND SPORAN AGAINST ENTERIC PATHOGENS ON ICEBERG LETTUCE

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Cinnamaldehyde and sporan alone or in combination with acetic acid (20%) were evaluated for their antimicrobial effects against *E. coli* O157:H7 and *Salmonella* on lettuce. Iceberg lettuce leaves were cut into pieces of approximately 2 x 3 cm and inoculated with 50 µL (5 droplets of 10µl) cocktail of five strains of *Salmonella* or nalidixic acid resistant strains of *E. coli* O157:H7 (7 log cfu/ml). The inoculated leaves were air dried for ca. 30 min, washed with 800ppm cinnamaldehyde in 0.5% Tween (800T), 800ppm and 1000ppm of cinnamaldehyde and sporan alone (800C, 1000C or 800S, 1000S), or in combination with 200ppm acetic acid (1000CV, 1000SV) for 60 s. Treated leaves were spin dried for 60s, packed in a ziplock bags, and analyzed during 14-day storage at 4ºC. Inoculated leaves washed with water were used as control. The samples were plated on XLT4 and CTSMAC-NA agar for *Salmonella* and *E. coli O157H7*, respectively. Color (Hunter L, a, b color classification) and texture (TA-XT2 texture analysis) characteristics of un-inoculated, antimicrobial-treated leaves were analyzed to determine the effects of these natural antimicrobials on produce quality. Treatment with 1000SV and 800T significantly reduced *E. coli* O157:H7 and *Salmonella* populations compared to control, 800C, and 1000CV on Iceberg leaves. Combination of acetic acid with sporan further reduced the pathogens; however, the additive effect of acetic acid was not significant. *E. coli* O157:H7 was the sensitive pathogen to these antimicrobials compared to *Salmonella*. Bacterial population decreased significantly during storage. Overall, the texture of treated Iceberg leaves was not significantly different from control except for the leaves treated with 800C. The color of Iceberg leaves treated with antimicrobials was similar ($P > 0.095$) to control leaves with the exception of lightness of the leaves treated with 800C and 1000C. Results indicate that these natural oils could be used to reduce *E. coli* O157:H7 and *Salmonella* on Iceberg lettuce without affecting the texture and the color of lettuce leaves.

POSTER 12

E. COLI O157:H7 IN DUAL BIOFILMS FORMED WITH RESIDENT BACTERIA ISOLATED FROM FRESH PRODUCE ENVIRONMENT

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In produce processing plants, biofilms may potentially provide a supporting environment for pathogenic bacteria, which can result in enhanced resistance to cleaning and sanitizing efforts. The objective of this study was to examine the potential of bacteria strains isolated from a produce processing plant to co-exist with *E. coli* O157:H7 in dual species biofilms.

Bacteria were recovered and isolated from cutting boards, cutting/peeling tools, conveyor belts, surfaces of pre-cut produce, and floor. Ninety-four of the isolates, including 15 species belongs to 9 genera, were identified. Most of the identified isolates were phytopathogens. One isolate of each genus was selected for further study. Each isolate was allowed to form a monoculture biofilm in 96-wells microtitre plate. The total biomass of biofilms was measured to evaluate biofilm-forming capacity of each isolate. After inoculated on soft agar, the spreading distance of each isolate was also measured to determine motility. To investigate the interaction between isolates and E. coli O157:H7, E. coli O157:H7 was co-cultured with a 1 day old biofilm formed by each of the resident bacteria isolates. This 1 day old biofilm was used for simulating the naturally occurring biofilms in processing environment. *E. coli* O157:H7 was found be able to co-exist with resident bacteria. To verify the co-existence in co-cultured biofilm, fluorescence microscope was conducted. In two out of nine combinations, more *E. coli* O157:H7 cells were counted in co-culture compared to when *E. coli* O157:H7 was cultured alone.

Based on our results, the biofilm formed by each of the tested bacteria recovered from a produce processing plant demonstrated the potential to provide a microenvironment for *E. coli* O157:H7. This microenvironment may protect *E. coli* O157:H7 cells from the sanitizing treatment, thus it becomes a potential source of cross-contamination in produce processing plant.

POSTER 13

SALIVARY GLAND TRANSCRIPTOME ANALYSIS OF THE POTATO LEAFHOPPER,(*Empoasca fabae*)

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The potato leafhopper, *Empoasca fabae*, is an important pest of alfalfa, *Medicago sativa*, on which it causes damage known as hopperburn. The symptoms of hopperburn include yellowing of leaves, stunting of plants, and a decrease in gas exchange (photosynthesis and transpiration). This study seeks to understand the genes that are active in the salivary glands of this pest through the creation of a sialotranscriptome. The salivary glands of 250 potato leafhoppers were dissected, and RNA was extracted from the resulting tissue. Roche 454-pyrosequencing was used to create the sialotranscriptome, which was then compared to known sequences in GenBank using BLAST. The majority of the sequences (77.94%) showed similarity to other insect species in (77.94%), including the red flour beetle (*Tribolium* castaneum), fruit fly (*Drosophila melanogaster*) and pea aphid (*Acyrthosiphon* pisum). Sequences homologous to the known insect salivary proteins endo-beta-glucanase, alpha-amylase and chitinase were detected, and Pfam analysis showed the presence of cellulase and carboxylesterase proteins domains. In addition, KEGG analysis showed the presence of pathways involved in thiamine and purine metabolism, drug metabolism, secondary metabolite biosynthesis and lysine degradation. This is the first known report of the sialotranscriptome of *E. fabae*. Knowledge of which genes are active in the salivary glands of *E. fabae* will increase the understanding of the composition of this economically-important pest's saliva and the impact that it has upon the physiology of its host plants.

POSTER 14

ENGINEERING PLANT DEFENSES TO BROADEN DISEASE RESISTANCE IN SOYBEAN (*Glycine max*)

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Soybean (*Glycine max*) is a major commercial crop, cultivated in more than 78 million acres in the U.S., generating important revenues every year. Nevertheless, the yield is severely reduced by the effect of pathogens like the soybean cyst nematode (SCN), *Heterodera glycines*, and fungi, such as *Phakopsora pachyrhizi* or *Phytophtora sojae*, among others. Chemical control of pathogens has proven not only very polluting for the environment, but also economically inefficient; therefore efforts have been directed mainly to enhance plant genetic resistance as the means to avoid pathogen effects. The defense mechanism in plants is regulated by the interaction of the salicylic acid (SA) and jasmonic acid (JA) pathways. These pathways have been well studied in the model plant *Arabidopsis thaliana*. Our strategy consists of complementing *A. thaliana* mutants for genes involved in the SA or JA pathway with the soybean homolog, followed by the overexpression in Soybean of those that show to have an effect on cyst nematode or disease resistance. The first step will be cloning the bacterial gene nahG gene, which converts SA to catechol, into a soybean variety demonstrated to be resistant to SCN. This will help us establish the influence SA has on SCN resistance, and will be the basis to determine the effect of the overexpression of other genes from the SA pathway, as well as to determine the role of JA in the resistance mechanism. The soybean homologs will also be silenced to inhibit their expression. The main contribution of this study will be the transfer of knowledge from the model organism, as it is expected that the results will help broaden disease resistance in economically important crops.

POSTER 15

SOIL DENITRIFICATION POTENTIAL AND GENE DIVERSITY OF NIRK, NIRS AND NOSZ ACROSS FIELD SCALE COMPARISONS OF LOW INPUT SUSTAINABLE AND BUSINESS-AS-USUAL AGRICULTURAL SYSTEMS.

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The Farming Systems Project (FSP) in Beltsville, MD. is a long term cropping systems experiment comparing a wide diversity of organic and conventional corn/soybean/wheat production systems. Ecologically, the systems in the FSP represent a gradient of disturbance and plant species diversity and encompass conventional tillage, no-tillage and a range of Organic production practices. We have recently shown that in farming systems with linked carbon and nitrogen inputs (green and animal manures, e.g. Organic systems) soil nitrogen mineralization rates and carbon use efficiency are higher than in conventional tillage, no-tillage systems. What is still unknown is whether the nitrogen liberated during mineralization is re-immobilized by microbes and plants or if it is lost from the system through denitrification and leaching. Determination of the fate of mineralized nitrogen on annual cycles, across this diversity of farming systems is critical for understanding how land management influences agricultural nitrogen use efficiency. We determined the denitrification potential and gene diversity of nirK, nirS and nosZ across farming systems using a combination of quantitive PCR, targeted gene cloning and sequencing. Analysis of functional diversity of the microbial community was coupled with in field greenhouse gas sampling twice a month. Farming systems varied in denitrification potential, in general organic systems showed greater denitrification potential as determined by quantity of nirK, nirS and nosZ copy number. Although denitrification potential did not always result in higher greenhouse gas flux as determined by empirical field measurements. We using this data to develop models that consider soil biogeochemical and physical conditions as well as the functional diversity of soil microbial comminutes to better understand agricultural greenhouse warming potential and nitrogen use efficiency.

POSTER 16

DEVELOPMENT OF AN AUTOMATED GEOSPATIAL TOOLKIT TO EVALUATE THE EXTENT OF WINTER GROUND COVER ON AGRICULTURAL FIELDS.

Kusuma Prahabkara (UMD Geography), W. Dean Hively (USGS-EGSC), and Gregory W. McCarty (USDA-ARS-HRSL)

Wintertime occurrence of vegetated groundcover, including cover crops and small grains, can prevent soil erosion and reduce post-harvest nitrate runoff into waterways such as the Chesapeake Bay. This project uses moderate resolution multispectral satellite imagery (Landsat-5) to map wintertime ground cover on agricultural fields, combines that information with ancillary geospatial datasets depicting crop species distribution (NASS National Cropland Data Layer) and distribution of fields enrolled in the Maryland Department of Agriculture cost share programs for winter cover crops, and produces summary reports that can assist land managers to attain watershed conservation goals. Tabular and map outputs were calculated for three USDA-NRCS 'Showcase Watersheds' that have been targeted for intensive conservation implementation (Conewago Creek, PA, Upper Chester River, MD, and Smith Creek, VA), and for Talbot County, Maryland. For these target areas, satellite imagery was calibrated and converted to normalized difference vegetation index (NDVI), and vegetation thresholds were specified for zero, low, medium and high biomass. Summary statistics were then calculated for the intersection of biomass and land use categories, yielding winter groundcover estimates for agricultural areas. The results depict significant differences in winter groundcover depending on crop type, and increased ground cover on fields enrolled in the Maryland cover crop program. Results have implications for adaptive targeting, implementation, and management of agricultural conservation programs. The project is a collaboration among the USDA-ARS Hydrology and Remote Sensing Laboratory, the UMD Department of Geography, and the USGS Eastern Geographic Science Center.

POSTER 17

IMPACT OF TREATMENT WITH ESTROGEN PRODRUGS ON COGNITIVE PERFORMANCE IN A MURINE MODEL OF ALZHEIMER'S DISEASE

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There is evidence that estrogens are neuroprotective; thus estrogenic compounds have become likely candidates for therapeutic interventions, including those to potentially treat Alzheimer's Disease (AD). However, the positive effects of estrogens may be offset by negative impacts of stimulation of estrogen sensitive-malignancies and risk of venous thrombosis. In AD, over-expression of human mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1) in the double-transgenic mouse, and the addition of overexpressing Tau in the triple-transgenic mouse model, have been used to examine the progression of the disease pathology. In this study, female APP/PS1 double-transgenic mice and non-transgenic litter mates (6-8 months) were treated with either the vehicle (propylene glycol), estradiol (E2; 2ug/day), or E2-quinol (2ug/day). Treatments were administered over 8 weeks using Alzet osmotic pumps. Mice were assessed for cognitive function, using a three-day radial-arm water maze (RAWM). Function of the osmostic pumps was verified by increased serum estradiol levels. Two amyloid-beta forms (1-40 and 1-42) were measured as correlates of AD. Results showed the ovariectomized APP/PS1 female mice exposed to E2-quinol made fewer mistakes locating the hidden platform in the RAWM compared to APP/PS1 non-ovariectomized mice treated with E2-quinol. Amyloid-beta levels were reduced in the E2 treated wild type and double-transgenic females. These results suggest a possible cognitive benefit for the APP/PS1 mice treated with E2-quinol and decreased amyloid burden for the APP/PS1 mice treated with E2.

POSTER 18

CHEMICAL COMPOSITION AND ANTI-PROLIFERATIVE AND ANTI-INFLAMMATORY EFFECTS OF THE LEAF AND WHOLE-PLANT SAMPLES OF DIPLOID AND TETRAPLOID *GYNOSTEMMA PENTAPHYLLUM*

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Ethanol extracts of different genotypes (diploids *vs* tetraploids) and different parts of *Gynostemma pentaphyllum* (GP) were investigated and compared for their chemical compositions, their anti-proliferative and anti-inflammatory effects. The highest level of total flavonoids and phenolics were obtained from the fresh botanical of diploid (2L3) at 36.84 mg rutin equivalents/g and 41.15 mg gallic acid equivalents/g, respectively. The whole botanical of tetraploids (4L3) had the highest total saponin contents of 227.1 mg saponin equivalents/g. The anti-proliferative effect was investigated in prostate cancer cells. All samples showed time- and dose-dependent effect. The fresh botanical of diploid had the strongest inhibition on cell growth at a concentration of 250 μg botanical/mL media, followed by the whole botanical of tetraploid. The fresh botanical of diploid showed the strongest inhibitory effects on mRNA level of TNF-α, IL-6, and PTGS2 expressions at final concentrations of 0.2 and 1 mg botanical/mL media. The results from this study will be used to develop new products from *Gynostemma pentaphyllum*.

POSTER 19

ANTI-INFLAMMATORY ACTIVITIES OF ENGELETIN AND ASTILBIN IN J774A.1 MACROPHAGE CELLS

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Engeletin and astilbin were two flavonoid compounds isolated from traditional Chinese medicine Engelhardia *roxburghiana*, and the chemical structures of which were confirmed by ^{1}H and ^{13}C NMR and MS spectra. The anti-inflammatory activity of the two compounds was studied in lipopolysaccharide (LPS)-stimulated mouse J774A.1 macrophage cells. J774A.1 cells were treated with 10 μM or 50 μM engeletin and astilbin for 2 hours and then stimulated with LPS at final concentration of 0.5 μg/mL. After 24 hours, total RNA was extracted and Real-time PCR was performed to detect expression level of several cytokines. Results of this study showed that LPS induced inflammatory state in macrophage cells and increased mRNA expressions of cytokines were observed. Engeletin and astilbin exhibited remarkable inhibitory effects on interleukin (IL)-1â and IL-6 mRNA expression, significant inhibition of LPS-mediated mRNA expressions were also seen in LPS receptor toll-like receptor (TLR)-4, pro-inflammatory cytokine tumor necrosis factor (TNF)-á, IL-10, chemoattractant monocyte chemotactic protein (MCP)-1, and cyclooxygenase (COX)-2 genes. The reduced expression of these cytokines may alleviate immune response and reduce inflammatory activation, which indicated that engeletin and astilbin can be considered as anti-inflammatory food ingredients or drug candidates.

POSTER 20

MONITORING BOVINE (*BOS TAURUS*) FETAL FIBROBLAST REPROGRAMMING UTILIZING A BOVINE NANOG PROMOTER-DRIVEN GFP REPORTER SYSTEM

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The transcription factors Nanog and Oct4 are central regulators of pluripotency in embryonic stem cells (ESC) and have been well studied in human and mice. However, in large domestic species, including cattle, fully validated ESC lines have not been established. Induction of pluripotent stem cells (iPSC) may provide an alternative approach for establishing pluripotent cell lines in domestic species. *Oct4* and *Nanog* promoter-driven green fluorescent protein (GFP) has been used successfully for monitoring pluripotency of mouse, human and porcine stem cells. Although these pluripotency related genes are highly conserved, differential regulation of murine and bovine Oct4 promoters have been demonstrated. Therefore, in order to more effectively monitor pluripotency in bovine cells, we have designed a bovine pluripotency reporter gene consisting of a nuclear-localized, enhanced GFP driven by the bovine *NANOG* promoter (*bNANOGP-GFP*). We have transfected the linearized *bNANOGP-GFP* construct into bovine fetal fibroblast (BFF) cells to test its functionality following reprogramming of these somatic cells. BFFs were fused to non-transfected human teratocarcinoma cells (hNTERA2). Since hNTERA2 cells make an abundant amount of NANOG protein as well as other pluripotent determining transcription factors, fusion of hNTERA2 cells to BFFs induced nuclear reprogramming and expression of GFP. Non-fused transgenic BFF did not express GFP. These results indicate that the *bNANOGP-GFP* transgene can be used to monitor nuclear reprogramming of BFFs. In addition, we constructed 7 different lengths of the bovine *NANOG* promoter in the PGL3 vector: 315bp (-134/+181), 446bp (-265/+181), 1100bp (-919/+181), 1870bp (-1689/+181), 2632bp (-2451/+181), 3853bp (-3672/+181), and 4944bp (-4763/+181). Analysis of promoter activity using a dual luciferase report assay system in undifferentiated mESCs helped define the highest activity promoter fragment, which is 3853bp (-3672/+181) in length. This promoter fragment is down-regulated appropriately with differentiation. In future studies, we will attempt to reprogram BFFs using several different approaches including transfection of BFF cells with retroviral vectors encoding the four transcription factors (Oct4, Sox2, Klf4, and cMyc). The *bNANOG*-promoter driven GFP will be a useful tool for monitoring BFF derived iPSC and to further define the regulatory mechanisms controlling NANOG expression in cattle.

POSTER 21

ADDRESSING URBAN TRENDS IN AGRICULTURE WITH PEST MANAGEMENT USING BIOLOGICAL CONTROL AND PHEROMONES

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Under the project "Insect Management Systems for Urban Small Farms and Gardens," our group is pursuing environmentally-friendly pest management for key vegetable pests with an emphasis on the most important insect threats to small-scale, diversified vegetable production. Increasing small-scale urban production of vegetables calls for innovations in organic pest management and similar alternatives to pesticide-based control, but growers lack adequate controls for a number of key pests. Behavioral controls based on semiochemicals, including pheromones and other attractants and repellants, in combination with biological and microbial controls, hold great promise. Our main foci are cole crops (harlequin bug, lepidoptera caterpillar complex, root maggots) and cucurbits (cucumber beetles and squash bug). Research also addresses generalist pests such as brown marmorated stink bug, and generalist natural enemies such as predatory stink bugs and lacewings. This poster profiles six areas of research now underway:

- Baculovirus selection and selectivity against lepidopteran caterpillar pests;
- *Chromobacterium subtsugae*: a novel microbial control recently approved by US EPA for commercial application;
- Digging deeper into *Bacillus thuringiensis* (Bt) diversity: can it be a true biological control?
- Pheromones for generalist native natural enemies: novel synthetic chemistry plus biological control for applied pest management are now commercialized for small farms;
- Targeting and developing pheromones of key vegetable pests for use in organic and sustainable management: Aggregation pheromones of Harlequin bug and striped cucumber beetle;
- Tools to address invasions: Brown marmorated stink bugs and more.

POSTER 22

H3K27 TRIMETHYLATION PATTERNS IN SELECTED CLUSTERS OF CO-EXPRESSES GENES DURING INFECTION OF *A. THALIANA* BY *CUCUMBER MOSAIC VIRUS.*

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We performed a computer analysis of the chromosomal distribution of genes associated with response to pathogens in *Arabidopsis thaliana.* This revealed numerous functionally-related, non-homologous and co-expressed genes that were co-localized in close proximity to each other. These clusters of genes, whose co-regulation may depend on infection with a variety of plant pathogens, were distributed among all chromosomes of *A. thaliana.* Experimental assessment of computer prediction in *A. thaliana* ecotypes Col-0 and C24 during infection with the yellow strain of *Cucumber mosaic virus*, demonstrated that co-regulation of the neighboring gene clusters may be affected by the presence of resistance (*R*)-genes. To examine if detected gene clusters share similar epigenetic properties that could have a direct influence on their co-regulation and transcription, we also studied posttranslational histone modifications (PTM) in two arbitrarily selected clusters. PTMs represent one of the mechanisms involved in epigenetic control of gene expression and play a critical role in processes affecting chromatin structure and chromatin-mediated epigenetic regulation of transcription in plants. Using chromatin immunoprecipitation (ChIP), we showed that methylation of the lysine residue at position 27 of histone H3 (H3K27me3) plays a major role in the regulation of some of the clustered genes in response to virus infection in both susceptible and resistant ecotypes.

23. BACTERIOPHAGE ENDOLYSINS FOR THE CONTROL OF BACTERIAL CONTAMINATION OF FUEL ETHANOL FERMENTATIONS.

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Fuel ethanol is not produced under aseptic conditions and contamination of the fermentations are expected. Recent studies on pilot-scale cellulosic ethanol fermentations report reduced yields due to infections by LAB, with *Lactobacillus* sp. generally being the predominant contaminants, suggesting that bacterial contamination will continue to be a problem for the cellulose fermentation ethanol industry. Antibiotics are used to prevent and treat contamination, but prophylactic dosing is expensive, and the emergence of antibiotic resistant strains is undesireable. Bacteriophages (phage) produce peptidoglycan (PG) hydrolases that hydrolyze the major bacterial cell wall structural component causing cell lysis. The phage endolysin naturally lyses the host cell (lysis from within) during phage replication allowing new progeny to escape and infect new host cells. When Gram-positive bacteria are exposed to purified phage PG hydrolases, externally, they experience exolysis (lysis from without). Thus these lytic enzymes have the potential to be utilized as antibacterial agents. Our lab has shown that three lytic domains fused into one protein is more effective and reduces the risk of bacterial resistance development, therefore a triple fusion will be constructed of multiple active lytic domains and a cell wall binding domain. However, the use of purified antibacterial enzymes would likely be too expensive for large scale fermentation applications. Therefore our approach is to develop fermentative yeast strains that will express on the surface or secrete triple-acting PG hydrolase fusion antimicrobials to control lactobacilli contamination in ethanolic fermentations.

POSTER 24

BACTERIOPHAGE ENDOLYSINS FOR CONTROL OF *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is a Gram-positive pathogen relevant for both human and livestock health, with multi-drug resistant strains (methicillin-resistant *S. aureus*; MRSA) becoming increasingly prevalent. It is the major causative agent of bovine mastitis, which results in annual losses between $1.7 billion and $2 billion in the United States alone. While conventional treatment of mastitis by broad range antibiotics is often not successful and contributes to antibiotic resistance formation, bacteriophage endolysins present a new promising source of antimicrobials against these pathogens.

In this work, we comparatively characterized nine staphylococcal peptidoglycan hydrolases representing five homology groups and four stand-alone proteins. All enzymes displayed lytic activity against staphylococcal cells in zymograms, turbidity reduction and plate lysis assays, and most of them were active against a comprehensive set of strains including surface mutants, MRSA strains, mastitis isolates, and coagulase negative strains. Various lysins also proved effective in removing staphylococcal biofilms from polystyrene surfaces in a biofilm plate assay.

Chimeric proteins consisting of an endopeptidase domain from the streptococcal phage λSA2 endolysin and a *Staphylococcus* specific SH3b cell wall binding domain from either the phage endolysin LysK (λSA2-E-LysK-SH3b) or the bacteriocin Lysostaphin (λSA2-E-Lyso-SH3b) were shown to kill staphylococci in cow milk, reducing cell numbers by 2 to 3 log units within 3 hours when used at a concentration of 100 µg/ml. Furthermore, both chimeras acted synergistically with Lysostaphin in killing *S. aureus*, as demonstrated by a plate lysis checkerboard assay.

In a mouse model of bovine mastitis, 25 µg of either λSA2-E-LysK-SH3b or λSA2-E-Lyso-SH3b per murine mammary gland reduced intramammary *S. aureus* concentrations by 0.5 to 1 log units, compared to 2 logs for Lysostaphin. When applied in combination, λSA2-E-LysK-SH3b and Lysostaphin (both at 12.5 µg/gland) caused a ~3 log reduction in bacterial numbers.

Overall, our results demonstrate the high potential of bacteriophage endolysins as sources of antimicrobials for the treatment of staphylococcal infections.

POSTER 25.

WEED AND INSECT MANAGEMENT IN ORGANIC ROTATIONAL REDUCED-TILL

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POSTER 26

TRANSFERRING BASIC KNOWLEDGE FROM *ARABIDOPSIS THALIANA* TO THE FIELD

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Soybean cyst nematode (SCN), an obligate parasite of plants, is the most damaging pathogen of soybean, causing $469 to $818 million in soybean yield losses annually in the United States. However, there are no soybean cultivars available that are resistant to all SCN populations. To achieve this goal, we cloned Arabidopsis genes proven to be important to the defense response and over expressed them in soybean. For example, a large number of genes involved in jasmonic acid (JA) and salicylic acid (SA) signaling, production and response play a crucial role in basal and R gene mediated resistance to biotrophic pathogens. Mutant plants compromised in JA and SA biosynthesis and signaling exhibit decreased resistance to biotrophic pathogens and fail to trigger the expression of PR genes in response to pathogen attack. In this work, we over expressed an *Arabidopsis thaliana* Gene A in soybean roots using PRAP15 as vector. This vector was designed to express enhanced green fluorescent protein (eGFP) so that transformed roots could be recognized easily. We tested this vector by insertion of the gene encoding Red Fluorescent Protein (RFP) into the cloning site and transforming onion cells using the biolistic methods. The Gene A construct was transformed into soybean roots using an *Agrobactrium rhizogenes* based transformation system. Transformed roots were challenged with SCN and root-knot nematode (RKN). The number of the mature SCN females were counted after 35 days after inoculation and number of galls formed by RKN were counted after 30 days after inoculation. Reduction in the number of the nematodes will indicate that Gene A may be useful for engineering resistance in soybean to SCN.

POSTER 27

REMOTE SENSING OF WINTER VEGETATION TO PROMOTE ADAPTIVE CONSERVATION MANAGEMENT ON CHESAPEAKE BAY FARMLANDS

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For six years, scientists with the USDA Choptank River Conservation Effects Assessment Project have been working in partnership with Federal and State agencies to increase our knowledge of agricultural impacts on water quality in the Chesapeake Bay watershed. The use of winter cover crops has been identified as a key conservation management practice for reducing the loss of nitrogen and sediment from agricultural lands. However, the effectiveness of this practice varies widely depending on landscape, climate, and agronomic management. This project uses satellite imagery and on-farm sampling to provide accurate distributed measurement of vegetation abundance on agricultural fields, and obtains site-specific knowledge of agricultural management practices through partnership with conservation programs. This combination of data sources allows a field-by-field evaluation of winter cover crop performance that can be reported back to farmers, Soil Conservation Districts, and conservation program managers, leading to practical insights into conservation practice performance in the working farm landscape. Wintertime maps of agricultural vegetation can also be combined with ancillary data sources such as the National Cropland Data Layer to identify cover crop niches within crop rotations, to map voluntary (non-cost shared) winter cover crops, and to identify areas of exposed soils. Geospatial toolkits are being developed to automate these calculations, and provide access to useful information that can be used to inform adaptive management of conservation practices.

POSTER 28

“Removing External DNA Contamination from Arthropod Predators Destined for Molecular Gut-Content Analysis”

Matthew H. Greenstone, Donald C. Weber, Thomas C. Coudron, Mark E. Payton, and Jing S. Hu