Fundamental Plant and Animal Systems

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V(A). Planned Program (Summary)

1. Name of the Planned Program

Fundamental Plant and Animal Systems

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
201	Plant Genome, Genetics, and Genetic Mechanisms			25%	
206	Basic Plant Biology			15%	
301	Reproductive Performance of Animals			25%	
302	Nutrient Utilization in Animals			15%	
304	Animal Genome			10%	
305	Animal Physiological Processes			10%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of professional FTE/SYs expended this Program

Year: 2008	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.0	4.2	0.0
Actual	0.0	0.0	5.7	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c 0	1890 Extension 0	Hatch 514769	Evans-Allen 0
1862 Matching 0	1890 Matching 0	1862 Matching 774723	1890 Matching 0
1862 All Other 0	1890 All Other 0	1862 All Other 863419	1890 All Other 0

V(D). Planned Program (Activity)

1. Brief description of the Activity

Conduct research; publish / present results

Research involving fundamental systems in animals is to increase our understanding of reproductive, nutritional and general physiological systems and processes. Practical problems addressed include embryonic mortality in sheep and cattle, performance limiting amino acids in animal rations, health and disease resistance in poultry. In plants, program emphasis varies from determining functions of ubiquitin and other polypeptide tags, to understanding basic mechanisms of flower senescence and cold shock adaptation, to combating the impacts of phytophthora and Chestnut blight, to defining and eliminating negative effects on grazing animals of ergot alkaloids produced by fungi symbiotic with pasture grasses.

The long-term goal of a current research project is to establish models of oxidative stress in birds and determine the efficacy of manipulating plasma uric acid concentrations to modulate oxidative stress, thereby increasing our understanding of how oxidants exert their specific affects on tissues.Allopurinol is a xanthine oxidase inhibitor that has been recently proposed as a therapy to reduce oxidative stress in different human tissues. The effects of xanthine oxidase on oxidative stress are complex, since it forms uric acid (a potent antioxidant) but also catalyzes the formation of reactive oxygen species. These studies investigated the effects of allopurinol on plasma uric acid (PUA) concentrations and oxidative activity in broilers. Feeding allopurinol decreased feed intake and significantly reduced body weight compared to that measured in the control group. As expected, allopurinol reduced PUA at each wk although there was no effect of allopurinol on measurements of oxidative stress over the first 3 wk of study, but at wk 4 oxidative stress measurements were greater than that measured in control birds. The general lack of effect of allopurinol on measurements of oxidative stress would indicate that the increase in oxidative stress caused by the reduction of PUA could have been overcome by a reduction in the formation of reactive oxidative species.

Studies have shown a positive correlation between age and the accumulation of pentosidine in the breast skin of birds. However, previous research has only analyzed skin from deceased birds. The major objective of these studies was to determine if pentosidine concentrations can be determined from skin biopsy collections of living birds.Patagial tags have been placed on many wild birds without serious side effects, which makes the patagium a potential site for skin collection in living birds. Patagial and breast skin samples from deceased Double-crested Cormorants (Phalacrocorax auritus), Monk Parakeets (Myiopsitta monachus), and living Black Vultures (Coragyps atratus) were provided by USDA Wildlife Services in Gainesville, FL and Starkville, MS. These samples were analyzed for pentosidine concentrations according to the procedure published by Iqbal et al. Initial comparisons of Double-crested Cormorants and Monk Parakeets revealed that there were no significant differences in pentosidine concentrations between the breast and patagium for either species. These results indicates that age curves generated from the analysis of breast skin can be reliably used to age birds with skin collected from the patagium. The collection of patagial skin from Black Vultures indicated that skin samples can be collected safely from living birds.

Nucleoplasmin 2 (nPM2), a maternal gene, is essential for early embryonic development, but must be silenced to allow activation of the embryonic genome. Bovine nPM2 has 561 bp, a predicted length of 176 amino acids and 55% homology with Homo sapiens nPM2. Expression was greatest at the GV and MII stages, decreased to the 2-cell stage, remained constant until the 16-cell stage, and was barely detectable thereafter. Protein of nPM2 was constant through the 16-cell stage, but barely detectable in blastocysts. A shift in molecular weight of nPM2 at MII was presumed due to phosphorylation. One possible microRNA site, bta miR181a, showed perfect complementarity with nPM2 in the first 7 bases of pairing (seed region). Real time PCR for miR181a revealed that miR181a was expressed in the oocyte and embryo. Nucleoplasmin 2 and miR181a were cloned and ligated into PcDNA3.1 expression vectors. The microRNA was stably transfected into HeLa cells. Nucleoplasmin 2 was then co-transfected with the stable cells for 24 h. Control cells were transfected with nPM2 only. Co-transfection of miR181a and nPM2 decreased nPM2 expression. Apparently nPM2 is targeted by miR181a for degradation or silencing. This finding may contribute significantly to better understanding embryonic loss in cattle.

Additionally, microarray analysis identified 180 differentially expressed genes ($P<0.05$) in d4 and 10 CL, confirmed by real-time PCR. Functionally, these genes are involved in cell signaling (13%), metabolism (10%), protein degradation (5%), RNA processing (15%), protein biosynthesis (19%), extracellular matrix and cytoskeletal (6%), DNA replication (2%), antioxidant (3%), miscellaneous (17%), and unknown (10%). Prostaglandin F2alpha increased expression of a guanine nucleotide binding protein (G protein), beta polypeptide 1 (GNB1) and the calcium/calmodulin dependent kinase kinase 2 beta (Camkk2) in day-4 and -10 CL, respectively. Thus GNB1 and Camkk2 are candidate genes for roles in acquisition of luteal sensitivity to PGF2 alpha. Experiments have been initiated in regard to endothelin in CL. One tests the hypothesis that Endothelin 1 through its receptor type A regulates expression of genes involved in structural luteolysis in the ewe during induced regression of the corpus luteum. A second tests whether endothelin receptor type A antagonist prevents luteolysis by endogenous PGF2alpha in sheep and increase length of estrous cycle, as shown by progesterone secretion pattern and CL morphology. The third tests, using an oxytocin receptor antagonist, whether oxytocin might be involved in endothelin-independent actions of PGF2alpha.

During the most recent reporting period we have continued to collect samples of placental and endometrial tissue from late pregnant (day 70, 90 and 110 of gestation) gilts in a project designed to evaluate variation in reproductive efficiency. We have begun to assay those samples for the expression of a variety of nutrient transporters and stain samples from each feto-placental unit so that we can estimate the density of blood vessels both in the placenta and adjacent endometrium. We also collected blood samples from ewes treated with growth hormone around the time of breeding, a treatment we have previously demonstrated increases lamb birth weight, weaning weight and reduces lamb response to a growth hormone releasing hormone challenge. These samples were collected every week from treatment (week 0) until week 8 of gestation. We then assayed these samples for IGF-I as a proxy for growth hormone to estimate the duration of the growth hormone treatment effect. We have also continued feeding dairy cows diets designed to elicit divergent insulin secretion. From these cows we have collected liver biopsies to estimate the activity of progesterone catabolic enzymes and used continuous infusions to

estimate the metabolic clearance rate of progesterone and liver blood flow in response to our dietary treatments.

The impact of these research projects has been significant. Relative to placental function in the pig, we have detailed the expression of a variety of nutrient transporters in the placenta and adjacent endometrium from each feto-placental unit in litters collected from gilts on days 70, 90 and 110 of gestation. Real-time PCR was used to determine mRNA expression of nutrient transporters, including: rBAT-1, FAT-1, FAT-2, yLAT-1, CAT-1, ASCT-1 and GLUT-3. On day 90, ASCT-1 was found to be more highly expressed in the endometrium associated with feto-placental units having a higher placental efficiency. On day 110, individual feto-placental units having a lower placental efficiency tended to have a higher endometrial mRNA expression rBAT-1 and FAT-1. Relative to the duration of the periconceptional treatment of ewes with growth hormone, we determined IGF-I concentrations (as a proxy for growth hormone). For weeks 1, 2, 3 and 4 of gestation, treated ewes had an increased IGF-1 concentration compared to control ewes (3, 5, 4 and 2.5-fold, respectively). Periconceptional treatment with GH causes IGF-1 to remain elevated for the first half of gestation, potentially altering the uterine environment during a crucial developmental period.

Additionally, gilts were randomly assigned to ovario-hysterectomy on day 70, 90 or 110 of gestation. Fetal, placental, liver and heart weights and placental efficiency were recorded for each fetus. Endometrial and placental samples were collected and used for determination of the mRNA expression of nutrient transporters by real time RT-PCR. Placental, fetal, heart and liver weights, as well as placental efficiency increased with gestational age. Placental and fetal weights were positively correlated with heart and liver weights for day 70, 90 and 110. Heart and liver weights exhibited a positive relationship for day 70, 90 and 110. Placental efficiency was negatively correlated with heart and liver weights on days 70 and 90. On day 70 heart weights were negatively correlated with placental expression of yLAT-1 and CAT-1 as well as endometrial expression of CAT-1. Liver weights were negatively correlated with the placental expression of CAT-1 and the endometrial expression of CAT-1 and rBAT-1. The endometrial expression of yLAT-1 and placental expression of CAT-1, ASCT-1 and rBAT-1 showed a positive relationship with heart weight on day 90 while the endometrial expression of ASCT-1 exhibited a negative correlation. Positive relationships were observed on day 90 for liver weights and the placental expression of yLAT-1 and ASCT-1 and the endometrial expression of yLAT-1. Negative relationships were present for the endometrial expression of ASCT-1 and FAT-1. On day 110, both heart and liver weight exhibited a positive correlation with the endometrial expression of GLUT-3. Liver weight and placental expression of GLUT-3 exhibited a negative relationship. The relationship of heart and liver weight to nutrient transporter expression during a critical developmental period may have resulted in altered placental function resulting in an altered uterine environment, which may possibly explain the negative relationship of heart and liver weight to placental efficiency during gestation. In late gestation, during the time of greatest fetal growth, endometrial expression of glucose transporters and heart and liver weights exhibited a positive relationship, while the placental expression of GLUT-3 exhibited a negative relationship with liver weight. Though no relationship was observed between placental efficiency and heart or liver weight, a negative relationship did exist between the endometrial expression of GLUT-3 and placental efficiency.

Relative to the nutritionally mediated regulation of progesterone catabolism, holstein dairy cows (n = 11) were randomly assigned to a high starch or high fiber diet in a cross-over experimental design consisting of two 14 d periods. Dry matter intake, milk yield and milk lactose yield were not different between the two diets. Insulin concentrations were increased by 22% in cows fed the high starch diet, and both cytochrome P450 2C and cytochrome P450 3A activities were decreased by 50%. Liver blood flow was not different between the two diets. Metabolic clearance rate of progesterone tended to be lower in cows fed the high starch diet versus the high fiber diet. The half-life of progesterone was increased in cows fed the high starch diet versus the high fiber diet. In addition, twenty-two Holstein cows were trained to acquire feed from the Calan gate system and randomly assigned to either a high corn starch or high fiber diet in a cross-over experimental. Dry matter intake was similar for the high starch and high fiber diet, as was milk yield and milk lactose yield. Insulin concentrations at the time of liver biopsy were elevated by 47% in cows consuming the high starch diet versus the high fiber diet. Cytochrome P450 2C activity was decreased by 45%, while cytochrome P450 3A activity tended to be lower by 34% in cows consuming the high starch diet. Insulin was elevated by 19% in cows consuming the high starch diet. The half-life of progesterone tended to be longer in cows fed the high starch diet versus the high fiber diet. In summary, cows with elevated insulin concentrations and lower enzyme activity showed a decrease in progesterone clearance without any changes in liver blood flow, dry matter intake or milk yield. This relationship between insulin and enzyme activity may be a useful approach to decrease high rates of progesterone clearance during pregnancy

A proteomic analysis of expression patterns in cryopreserved semen of bulls of known low fertility identified three seminal plasma proteins: 1) Seminal plasma protein PDC-109 precursor, MW 15.4 kDa, pI 4.76, 2) Spermadhesin-1 precursor, MW 15.0 kDa, pI 4.93, and 3) Spermadhesin Z13, MW 13.4 kDa, pI 5.50. Low fertility of dairy bulls was associated with higher expression of Spermadhesin Z13 isoforms in accessory gland fluid (Moura et al., 2006). Therefore, identification of that protein and its precursor in seminal plasma of low fertility bulls strengthens its potential use as a marker of low fertility in bulls.

Nuclear proteins, including transcription factors and chromatin remodeling proteins, are required for initiation of transcription in early embryos prior to embryonic genome activation.Full-length cDNA sequence for a novel oocyte transcript with sequence similarity to the importin-alpha family of genes. Full-length cDNA sequence for this gene was obtained by RT-PCR and 5'RACE. Sequence analysis revealed that the cDNA (1817 bp) has an open reading frame encoding a protein of 522 amino acids. A search of the Pfam protein database revealed that the protein contains a conserved importin β binding domain (IBB) and 7 armadillo (ARM) motifs, typical characteristics for importin α proteins. RT-PCR analysis revealed that the novel gene is predominantly expressed in ovarian tissues and mature oocytes. Quantitative real time PCR analysis demonstrated that the expression of this novel importin α gene in GV stage oocytes is over 30 to 2000 times higher than other known importin α genes. Analysis of temporal expression of the novel gene during early embryonic development showed that the transcript is abundantly present in GV and MII stage oocytes as well as in 2-cell, 4-cell, and 8-cell embryos, but barely detectable in morula

and blastocyst. Western blot analysis using antibodies against this novel protein showed a similar expression pattern at the protein level. A GST pull down assay revealed that the novel improtin α protein has a much stronger binding affinity with a nuclear protein, Nucleoplasmin 2, compared to other bovine importin α proteins. RNAi experiments demonstrated that knockdown of the expression of the novel importin α in bovine embryos results in a decrease of the proportion of embryos developing to the blastocyst stage. We suggest that this novel importin α might be responsible for nuclear transport of certain key transcription factors and/or other essential nuclear proteins required for activation of the embryonic genome during early embryogenesis. Recently, with the updating of the bovine genome database, we were able to identify a predicted bovine transcript that matches the partial JY-2 cDNA we isolated from the bovine oocyte library. Cloning of the full-length cDNA for this gene was therefore successfully completed by overlapping RT-PCR. The cDNA codes for a protein of 507 amino acids. Analysis of the predicted protein sequence revealed that the novel protein has conserved coiled-coil domains, a functional motif typically found in proteins involved in the regulation of gene expression such as transcription factors.

A project recently completed investigated the association between male age and the frequency of sperm with de novo structural chromosomal abnormalities. Semen specimens collected from two groups of 10 healthy, nonsmoking men, aged 22-28 and 65-80 years were analyzed with the use of a multicolor fluorescence in situ hybridization assay for detecting breaks, segmental duplications and deletions, and aneuploidy and diploidy involving chromosome Significant increases in the frequency of sperm carrying breaks and segmental duplications and deletions of chromosome 1 among older men compared with younger men. Older men carried twice the frequency of sperm with segmental duplications and deletion of chromosome 1. The frequency of sperm carrying breaks within the 1q12 fragile-site region nearly doubled in older men. In contrast to female gametes, there was no effect of age on the frequency of sperm with numerical chromosomal abnormalities.Our findings suggest that advancing male age is associated with a gradual and significant increase in the risk of fathering children with various chromosomal defects such as segmental aneusomy syndromes.

Experiments were conducted to determine the effect of dietary lysine on indices of lysine metabolism in the chicken.Focus was on establishing whether or not the initial enzyme of saccharopine-dependent lysine degradation, lysine alpha-ketoglutarate reductase (LKR), is regulated by post-translational processes.Results showed that decreases in dietary lysine reduce LKR activity and that although there was a small decrease in LKR mRNA, it appears as if the regulation of enzyme activity is largely mediated through post-translational processes.We also developed procedures to measure messenger RNA levels for mitochondrial transport proteins involved in basic amino acid transport in mitochondria.Over the last year this has been complicated by the finding of two different transporters that may contribute to the process.

Two different ergot alkaloid-eliminating gene knockouts inserted in the perennial ryegrass endophyte Neotyphodium sp. LP1, were compared with respect to their effects on nematode reduction, mammalian feeding deterrence and satiety, insecticidal effects, and seed transmissibility.One of the mutants carried a disruption in dmaW, the first gene in the ergot alkaloid pathway, and accumulated absolutely no ergot alkaloids. In the second mutant, the gene lpsA which encodes a later pathway step, was knocked out. The lpsA knockout accumulated clavine alkaloids, which are intermediates from the early part of the ergot alkaloid pathway, but not ergovaline or other lysergic acid derivatives. Perennial ryegrass plants infected with either of these two mutants, the wild-type fungus (producing all ergot alkaloids), or no fungus (endophyte-free) were compared in a variety of trials. In the absence of ergot alkaloids, endophyte infected grass (containing the dmaW mutant) was favored by rabbits (used as a model mammalian herbivore) over endophyte-free grass. Accumulation of clavine ergot alkaloids as in lpsA mutant, and to a lesser extent in the wild type fungus, counteracted the endophyte appeal and deterred rabbit feeding. In satiety tests, consumption of ergovaline (the ergot pathway end product) but not clavine-type ergot alkaloids reduced the appetite of rabbits. Tests with black cutworm (Agrotis ipsilon), performed in collaboration with Dr. D.A. Potter (University of Kentucky), showed that ergovaline and lysergic acid derivatives which accumulated in grasses containing the wild-type fungus were effective feeding deterrents and caused larval mortality, whereas clavine ergot alkaloids (which accumulated in the lpsA knockout) were ineffective against this insect. Collectively the data showed that ergot alkaloids from different parts of the pathway are required for anti-mammalian and anti-insect properties. In experiments with lesion nematodes (Pratylenchus scribneri), nematode population growth on perennial ryegrass roots was greatly inhibited by wild-type endophyte presence. However, elimination of ergovaline and lysergic acid derivatives (as in the lpsA mutant) or all ergot alkaloids (as in the dmaW mutant) did not significantly reduce the endophyte-associated population suppression. These data indicate that ergot alkaloids can be eliminated from grass-endophyte symbioses without compromising lesion nematode resistance. Factors other than ergot alkaloids are responsible for the documented effects of Neotyphodium sp. endophytes against lesion nematodes. Similarly, seed transmissibility of endophytes was approximately 100% in plants infected with either mutant and in wild-type infected plants, indicating that vertical transmission of endophytes is unaffected in the mutants. In further basic studies on the ergot alkaloid pathway, knockout mutations in the ergot alkaloid-producing fungus Aspergillus fumigatus showed the involvement of four additional early pathway genes. Biochemical functions for three of the genes were deduced by chemical analyses. Homologues of each of the four genes have been identified in endophyte genomes.

Previous work with *Enterobacter sakazakii*, an opportunistic pathogen, demonstrated its ability to endure prolonged stress in aquatic environments. We have continued our studies on the survival, detection, recovery, and physiological adaptations of this pathogen. Part of this effort was directed toward an evaluation of the recovery efficiency of various media modifications to a commercially available selective/differential medium for detection of *E. sakazakii*. Our current studies show that the commercially available culture medium is relatively ineffective in permitting detection of the target bacterium. However, we were able to modify the standard medium by addition of certain compounds and other adjustments in the routine isolation procedure to achieve substantially enhanced recovery of *E. sakazakii* while coincidentally inhibiting nontarget antagonists. Ten strains of potentially antagonistic bacteria as well as two strains of the target microorganism, *E. sakazakii*, were subjected to these newly modified media. The most effective culture conditions which simultaneously inhibited the growth of unwanted potential

competing organisms and permitted detection of the target opportunistic pathogen was a medium formulated with 0.25 molar sodium chloride, 0.1 percent pyruvate, and 10 milligrams of vancomycin per liter. This newly developed medium was then used in a modified membrane filtration procedure (0.22 micrometer porosity filters) to reliably and successfully isolate *E. sakazakii* from water samples containing antagonistic bacteria that could possibly confound and compromise detection of the target opportunistic pathogen. Our newly modified procedure requires rigorous field testing in the future to determine its suitability as a reliable technique to detect environmentally stressed E. sakazakii.

In a related study, we examined the influence of desiccation on subsequently thermally stressed E. sakazakii. Possible physiological changes associated with the desiccation of E. sakazakii were monitored with the BIOLOG microplate system, a substrate utilization profile testing system (panel of 95 different potential carbon sources). Desiccation resulted in increased thermotolerance of survivors compared to non-desiccated cells. In addition, notable differences in utilization of specific substrates were observed between desiccated and non-desiccated E. sakazakii. These findings should provide valuable information on the possible public health significance of pathogenic E. sakazakii in aquatic and food-associated environments. Equally important, the identification of key substrates required for the detection of desiccation-stressed E. sakazakii with the BIOLOG microplate system suggests the possibility of modifying the currently available growth medium to enhance the sensitivity and selectivity of the detection procedure, thereby leading to a better understanding of the ecology and etiology of this opportunistic waterborne pathogen. Successful completion of the objectives and goals of the project should contribute to improvement in the quality of surface water and ground water.

In vitro cell callus lines of *Panax quinquefolius* (American ginseng) have been successfully established. The three tissue types (root, leaf and in vitro callus) for generating the three cDNA libraries of *Panax quinquefolius* have been prepared for generating cDNA libraries. The cDNA libraries will be generated as well as expressed sequence tags (ESTs) established at the University of Arizona under the direction of Dr. David Gang. This will be completed early in 2009. A protocol has been developed for utilizing flow cytometry for estimating the size of the *Panax quinquefolius* genome. Genome size estimation will be completed early in 2009.

Understanding flower senescence at a molecular level will enable future efforts to increase the economic value and productivity of agricultural crops through genetic engineering. Delaying flower senescence will lead to higher crop productivity through the increased window of opportunity for flower pollination and increase their economic value as shelf life of floriculture crops increases. Inducing full or partial flower senescence may lead to more efficient breeding efforts by alleviating emasculation of flowers and may be used to decrease the invasiveness of crop and ornamental plant species.Our efforts have focused on elucidating the trigger of flower senescence and on providing evidence for a possible regulatory and/or functional role of CEBP, a nuclear-encoded chloroplast protein, expressed throughout flower development and senescence. We also wanted to more closely examine changes in chloroplast number and chlorophyll content in petals of carnation flowers. Since limited information is available on chloroplast development and function in petals, measuring and possibly correlating these changes with previous results on CEBP expression and cellular localization in petals should lead to a better understanding of flower senescence. In addition, we expect that results of these experiments will impact our hypothesis that chloroplasts have a central role to play in flower senescence. In our current work we showed that chlorophyll accumulation, and the number of chloroplasts decreased throughout development and senescence in epidermal cells in the lower part of the petals, in a pattern reminiscent of CEBP accumulation and localization.

We have constructed a rainbow trout high density oligo-array representing 37,394 unique TCs using Agilent's SurePrint technology. Performance of the array has been evaluated by analyzing expression of genes associated with vitellogenesis-induced muscle atrophy in rainbow trout. To add single nucleotide polymorphisms (SNPs) to the growing number of genome tools in rainbow trout, we have evaluated multiple bioinformatic pipelines for their ability to detect SNPs from expressed sequence tag data. Candidate SNPs were evaluated and 9 SNPs representing 7 genes were successfully genotyped on the broodstock panel from the NCCCWA selective breeding program. To identify miRNAs that might be important for early embryogenesis in rainbow trout, we constructed a miRNA library from a pool of unfertilized eggs and early stage embryos. Sequence analysis of random clones identified 14 miRNAs. Distinct expression patterns were observed during early embryogenesis and some miRNAs showed up-regulated expression during embryonic genome activation.

Trap cultures from eight grassland sites (160 total) have been harvested and over 20 sporulating arbuscular mycorrhizal fungal (AMF) species have been identified and have been started as monospecific cultures. Representative species of all genera of AMF except *Pacispora* were recovered from pot cultures. *Glomus* species dominated, and most putative undescribed species were found in this genus.

A rhizohyphatron was invented which consists of mycorrhizal plants growing in opposite chambers connected by a slide chamber containing agar-coated slides (onto which only hyphae will grow from plants at both ends). This system is being used to test the hypothesis that gene flow occurs within asexual AMF species via hyphal anastomosis. Ideal conditions for hyphal development have been achieved and criteria for visually distinguishing vegetative compatible versus incompatible reactions have been defined. So far, self-anastomosis has been characterized in isolates of ten AMF species, and species showing no self-anastomosis under more artificial conditions did so with the highest frequency in the rhizohyphatron. The beta-tubulin gene of *Gigaspora gigantea* and *Glomus clarum* was the first full-length tubulin gene to be cloned and sequenced from AMF. The gene in both species was organized into five exons and four introns, for former encoding a 447 amino acid protein. The amino acid sequence was most similar to that of fungi in the *Chytridiomycota* but nucleotide sequences indicated greater phylogenetic affinities with other fungal phyla. Unique to AMF β-tubulin genes was the absence of any intron before codon 174. Using an 800 bp region of the beta-tubulin gene, a phylogeny was reconstructed from 50 isolates of 45 AMF species. This phylogeny was concordant with that of the 18S rRNA gene at the family and species level, but it was not informative at the order level.

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Paralogous sequences in *Glomus* species likely contributed to phylogenetic ambiguity. Trees generated using different fungal phyla as outgroups yielded concordant topologies. The 25S rRNA gene sequences from 77 AMF species and additional isolates (5 per selected species) generated a phylogenetic tree that correlated well with morphology at the genus and species levels. These data complemented morphological characters in taxonomically placing nine ambiguous or undescribed species with closest relatives and providing evidence for publishable descriptions. Intraspecific sequences clustered within the boundaries of morphological species as well. A collaboration with Dirk Redecker resulted in identification of haplotypes of *Glomus intraradices* from a global sampling of isolates in our culture collection (INVAM) and elsewhere. Primers to selectively amplify intron regions of beta-tubulin, ITS, FOX2 and TOR2 genes were designed to genotype single spore isolates of two AMF species to be used in anastomosis studies of paired isolates.

The ubiquitin/26S proteasome pathway of selective protein degradation is essential for proper plant growth and development. It is responsible for the degradation of many short-lived regulatory proteins including those involved in hormone responses, light perception, and cell cycle progression.Efforts in this project have focused on the ubiquitin-specific proteases (UBPs), the enzymes involved in dismantling polyubiquitin chains and detaching ubiquitin from target proteins. We and others have shown that these enzymes are essential for proper ubiquitin pathway function. Whereas, the general functions of the UBPs in the ubiquitin pathway are known, the specific roles performed by each of the 27 UBPs in Arabidopsis remain largely unknown. We have identified mutant plants that do not synthesize specific UBPs and have compared their phenotypes to wild-type plants. We have assembled a collection of UBP mutants in Arabidopsis that we distribute upon request. We concentrated our efforts on the UBP3/4, UBP12/13, and UBP15/16/17/18/19 subfamilies because mutant plants display aberrant phenotypes.

This year, we continued to focus our attention on the ubiquitin-specific protease subfamilies UBP3/4, UBP12/13, and UBP15/16/17/18/19. Single gene and higher order mutant plants were compared to wild-type plants at the phenotypic level. The UBP3/4 subfamily was found to be essential for pollen transmission and important for pollen mitosis II in the male gametophyte. We have assembled several modified UBP3 expression constructs to investigate the role of N-myristoylation and nuclear localization on UBP3 function. Some of these constructs have been introduced into Arabidopsis and analysis is forthcoming. UBP12/13 double mutant pollen appears to be less successful than UBP12 or UBP13 single mutant pollen at fertilizing wild-type ovules. Ovule transmission appears to be normal. UBP12/13 double homozygous mutant plants are dwarfs, never set seed, and produce abnormal flowers. Introduction of a UBP12 cDNA construct expressed under the control of the Cauliflower Mosaic Virus 35S promoter restored seed production in double homozygous mutant plants. A genetic analysis of the five member UBP15/16/17/18/19 subfamily has been complicated because many double homozygous mutant combinations are possible and a UBP19 T-DNA insertion mutant is unavailable. UBP15/16 double homozygous mutants produce perhaps one or two viable seeds per plant, have irregular, elongated leaves, and produce very little pollen. The phenotype of these mutants have been analyzed and documented carefully with regards to seed yield, silique length, leaf shape, and flower morphology. We are in the process of generating a quadruple homozygous mutant in which UBP19 is the only UBP of this subfamily that can be expressed. The ubiquitin conjugating enzymes (UBCs) are also an important class of enzymes in the ubiquitin pathway. Earlier, we attempted to disrupt the expression of UBC subfamilies UBC4/5/6 and UBC15/16/17/18. We had great hope for success because of the availability of T-DNA insertion lines for all seven of these genes. We generated triple homozygous UBC4/5/6 mutants and quadruple homozygous UBC15/16/17/18 mutants. However, we observed no phenotype in either case. We discovered that one or more of the T-DNA insertions did not disrupt the expression of the desired gene.

Work continued on the molecular characterization of cold shock proteins (CSP) in Arabidopsis.We observed the first described putative localization of plant cold shock domain proteins to P-bodies in the moss model system. We also confirmed that plant CSPs are targets of sumoylation. Using site-directed mutagenesis, we were able to identify the critical target residue for sumoylation. We confirmed that rice CSPs are regulated on a gene and protein level in a very different manner than plants that are capable of cold acclimating. We also showed for the first time that CSPs are seasonally regulated in an extremely freeze tolerant perennial woody plant (red-osier dogwood). These correlative data suggest that CSPs might be involved in the process of cold acclimation in woody plants.

2. Brief description of the target audience

Primarily researchers; professional practioners, regulators, some producers

V(E). Planned Program (Outputs)

1. Standard output measures

Target for the number of persons (contacts) reached through direct and indirect contact methods

	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Year	**Target**	**Target**	**Target**	**Target**
Plan	0	0	0	0
2008	0	0	0	0

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year	Target
Plan:	0
2008:	0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

	Extension	Research	Total
Plan	0	5	
2008	0	17	17

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

Research presentations

Year	Target	Actual
2008	7	8

Output #2

Output Measure

Refereed scientific manuscripts

Not reporting on this Output in this Annual Report

V(G). State Defined Outcomes

O No.	Outcome Name
1	Reduced embryonic mortality in cattle and sheep %
2	Increased efficiency of amino acid utilization in ruminants %
3	Identify genes coding for cold shock proteins in plants
4	Identify and map genes affecting flower senescence
5	Decrease mortality in poultry production %
6	Develop ergot alkaloid deficient grasses with wild-type vigor
7	Successfully develop and employ hypovirus as a biological control agent for Chestnut blight

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Outcome #1

1. Outcome Measures

Not reporting on this Outcome for this Annual Report

2. Associated Institution Types

3a. Outcome Type:

3b. Quantitative Outcome

Year	Quantitative Target	Actual

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

What has been done

Results

4. Associated Knowledge Areas

KA Code	Knowledge Area

V(H). Planned Program (External Factors)

External factors which affected outcomes

Natural Disasters (drought,weather extremes,etc.)
Appropriations changes

Brief Explanation

Measurement of outcomes # 1, 2, and 5 are not supported by data collection and will be replaced in future plans of work.

V(I). Planned Program (Evaluation Studies and Data Collection)

1. Evaluation Studies Planned

Before-After (before and after program)

Evaluation Results

Key Items of Evaluation