AEIC SPRING 2016 MEETING MINUTES

April 19-20, 2016

Hosted by BASF

Raleigh, NC

P.L. Hunst (Bayer), AEIC Secretary

The 2016 AEIC Spring Meeting was held April 19-20 at the Embassy Suites Hotel Brier Creek, Raleigh, NC. Arno Krotzky (BASF) welcomed the group on behalf of BASF as the host company and gave a short overview of BASF. Dr. Krotzky was the recipient of the ACS/AEIC award for immunochemistry for chemicals in the mid-1990s. BASF creates chemicals and chemical solutions. In 2015, BASF had $10 billion in sales. Globally, BASF has sites on all contients with +112,000 employees and 10,000 of these employees are engaged in research in bioscience, process and chemical engineering, advanced materials and systems. BASF focuses on agriculture, health/nutrition, energy/resources, construction/housing and consumer goods. BASF spends $2 billion/year on research. The bioscience research engages 1600 employees, with headquarters in RTP, NC, who work on projects in crop protection, plant science, white biotechnology (microbes) and experimental toxicology. BASF has a joint project with Cargill to produce in plants high quality long chain fatty acids (EPA and DHA). They have engineered a complete, de novo PUFA pathway into canola which is enable via novel technology. BASF conducts the trait research and regulatory aspects and Cargill is responsible for the commercial product development. A long-standing research agreement with Monsanto has resulted in several traits such as DroughtGard maize. BASF has recently re-organized to take on new challenges in food/nutrition, environment/climate and quality of life.

AEIC BUSINESS MEETING

Secretary's Minutes of Fall 2015 Meeting (P. Hunst): A motion was made, seconded and voted positive to accept the minutes as posted on the website.

Treasurer's Report (D. Layton):

Item	Projected ($)	Actual/Projected ($)
Beginning balance	32757	32757
Dues	9500	8434
CD interest	250	
TOTAL	**9750**	**8434**
Expenses		
Scientific Paper	2000	
DE Franchise Tax	25	25
ANSI/ISO/TAG	2900	2900
Board Meeting	700	85
Spr Meeting 2016	4500	7529
Website maintenance	2000	

Credit card processsing	596	169
Fall Meeting 2016	4500	
Graphic design		
Brochure reprints		
Subscriptions	100	
Misc	100	
TOTAL	**17421**	**10708**
Total balance + CD	**25087**	**30482.67**

A motion was made, seconded and voted positive to approve the Treasurer report.

Membership Update (D. Layton): A synopsis of the current membership is given in the table below:

Member Type	Number	Projected Dues ($)	Actual Unpaid Paid Dues ($)
Large Companies	21	10500	2000
Small Companies	14	3500	2000
Associate	2	100	50
Individuals	5	500	300
TOTAL	**42**	**20000**	**4350**

Updating of Website Content (D. Thiede): A team needs to form again and start meeting to discuss the updates. Volunteers for the team are Ray Shillito, Denise Thiede, Penny Hunst, David Syme, Guomin Shan.

2016 Fall Meeting (C. Pick): EPL Bioanalytical will host the meeting in Decatur, IL. The group decided the dates of Oct 4-5 would be acceptable and not conflict with other industry meetings such as AOCS and AACCI. Travel to Decatur, IL can be via Chicago, St. Louis or Indianapolis (3 hr drives) or regional airports in Springfield or Bloomington, IL (1 hr drives). Possible topics were discussed as follows:

- Crop: soybean; breeding, seed production, where do soy products go, specialty soy (Cargill, ADM)
- New detection technologies
- Update on new traits/products (CLI update on traits)
- Gene editing: challenges in regulatory; how to detect single point mutations; what is done for gene editing in livestock
- Meat speciation and contamination
- NGS challenges
- Consequence of driving detection limits down and impact on regulatory thresholds
- Verifying botanical products via sequencing

ISO/TC34 Update (R. Shillito, Bayer): Ray gave the group an update on the U.S. TAG to ISO/TC 34/SC 16 of which he is the chair. A standard is a speciation that establishes a common language, and contains technical specifications or other precise criteria and is designed to be used consistently, as a rule, a guideline or a definition. ISO is an independent non-governmental organization (NGO) found in 1947 and has 162 members and 281 technical committees. ISO develops standards to facilitate and expedite trade. Standards are to safeguard consumers and make their lives simpler. ISO cooperates with other

standard developers, such as Codex. The Technical Committee (TC) 34 is for Food Products, under which resides the the Sub-Committee (SC) 16 for biomolecular markers. One reason for industry to be involved in the TC and SC is to counter the use of ISO standards by various countries to restrict the development and use of agricultural biotechnology. Participation in TC34/SC16 has prevented a separate sampling standard for GMOs, gained input into standards for PCR and protein methods for GMOs and influenced the way that sampling, PCR and protein methods are used. The 6th Plenary Meeting was held in March, 2015 in Shanghai, China. Items discussed included microarray analysis, varietal identification, qualitative method validation, operation of plant pathology labs, terms and definitions, methods for 35S PAT, pNOS screening methods, preparation of DNA, analysis of meat, DNA quantitation and data integration. In the ISO process, countries are represented by a single nation standards organization such as ANSI in the U.S. TAG membership is open but members are expected to be active and help develop consensus for the U.S. The workflow for a standard in ISO is as follows:

Plenary meetings are held approximately every 18 months with the next meeting occurring in 2017 in the U.S. The following are some of the working groups (WG) and their projects:

- WG 3 Varietal Identification: This group has a new convener and recently issued two reports on SSR analysis of sunflower and maize. The group has also proposed a document on multiplexed SSR analysis for varietal identification of Basmati rice and has a proposal for a general document on varietal identification.
- WG 4 Plant Pathogens: This group works on the development of the standard ISO 13484 for adapting molecular biomarker technologies to plant disease molecular diagnostic labs. There was a presentation at this meeting on this activity.
- WG 6 Guidance for Method Submission: Considering a document on preparation of DNA for specific purposes (DNA quality).
- WG 8 Meat Speciation: This group started with the submission of a proposed standards by Iran and is now working on general requirements and definitions for animal materials, including contamination.

Groups and/or individuals can influence or be a part of the ISO process via submission of new proposals or be a convener or a TAG member. The hot topics for standardization are digital PCR, isothermal DNA methods and NGS use for varietal determination, meat speciation and plant pathology.

Update from Composition Working Group (N. Gillikin, Bayer): The Composition WG is discussing method optimization and harmonization. The ELLA method for lectin analysis has gone out for vote for official method status. The group is working on a method for cyclopropenoic acids. There is also an allergen sub-group, however, this sub-group has not met too frequently yet. The WG is looking at new methods for inositol and trypsin inhibitor as well as conducting reviews of existing methods for analytes to ensure they are fit for purpose.

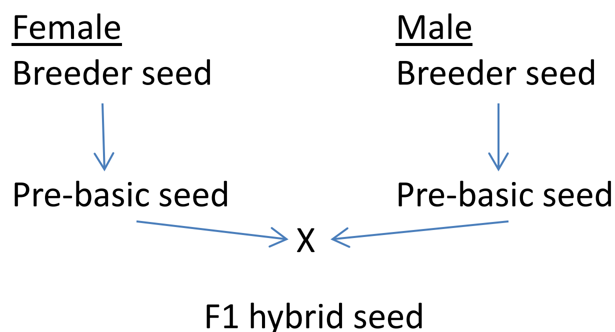
AEIC Goals and Activities (D. Levin, Covance): The group was asked to consider what AEIC may want to do to further our mission. A suggestion was to continue supporting international workshops such as those conducted through AACCI. For China, the group may want to be involved in discussions for screening methods for GM products. A collaborative study has been proposed so AEIC members may want to participate. Another suggestion was fill the void left by the absence of ILSI Taskforces to address scientific questions around detection. Several members agreed to discuss the formation of a Gene Editing TF (Denise Thiede, Ryan Johnson, Brian Beecher and a Bayer person (to be named)).

The business meeting was adjourned by D. Levin, AEIC President.

INVITED TALKS

Certified Canola Seed Production (M. Bunney, Bayer): Canola differs from rapeseed as it has <2% erucic acid and <30 uM glucosinolates. It is derived from *Brassica napus or Brassica rapa*. There were 18.5 million tons of canola produced by Canada and the U.S. in 2015. Canola oil is the third most widely used cooking oil and canola meal is primarily used as an animal feed. Canola contributes more than $19.8 billion to the Canadian economy. The U.S. imports 65% of Canadian exported canola oil and 95% of the exported meal. Canola is mainly grown in western Canada and North Dakota, USA. Herbicide-tolerant GM canola (Roundup Ready and LibertyLink) was introduced in 1995 and was rapidly embraced by growers. Bayer has >46% of the canola seed market.

Canola is breeding is outlined below:



Canola breeding stations begin the process with the creation and selection of lines. Lines are selected for yield, agronomics, combining ability, disease tolerance, maturity, stress tolerance, etc. The selected lines are grown in greenhouses. The breeder seed and pre-basic seed is grown in British Colombia to avoid contamination from canola production areas and is also grown on land that has never had canola

grown on it previously. Female plants are grown in highly isolated regions in 10x20m cages. The male plants are produced in 6x6m cages in regions separate from the regions where female plants are produced. Each plant is tested via PCR for quality at the 4-5 leaf stage. Growouts of plants are performed after harvest. Basic seed production occurs in areas with a minimum of 30m isolation from other pollen sources. Female fields are 2-10 acres in size whereas male fields are 0.5-3 acres in size. Fields are manually rogued for off-types.

Certified seed production occurs in southern Alberta province under irrigation. Some contra-season production is carried out in the U.S. The biggest threat in production are volunteer plants and cross-pollination from commercial crops. Certified seed is produced on fields that must be 4 years removed from canola growing. Fields are inspected by the third parties for quality and isolation (must be 1 mile isolation from other foreign pollen sources). Volunteer plants are manually rogued from production fields. Weeds and non-herbicide tolerant plants are controlled with herbicide sprays. Honeybees and leafcutters are used for pollination. Harvest is completed under optimal seasonal conditions for seed quality. Accepted seedlots have samples that are grown out before sale during the contra-season. The growout process is designed to look for phenotypic charactertistics, sterility, herbicide tolerance, etc.

Most canola seed companies focus their efforts on breeding and seed production. There are a few companies that are vertically integrated to capture value throughout the entire process (Cargill, Dow, DuPont).

Variety registration occurs through the Western Canada Canola Rapeseed Recommending Committee (WCCRRC). The committee includes representatives from government, industry and canola growers. Important traits in canola include Sclerotinia resistance, blackleg resistance, club root resistance and pod shatter resistance.

Seed companies and trait providers utilize intellectual property (patents) protection for enforcement. The grower license is for a single use of the planted traited crop. Possible F2 (bin-run) fields are tested using sales tracking data. Plant tissue samples may be taken and tested using lateral flow strip (LFS) tests. Third party monitoring and enforcement is used to protect R&D investments.

Canola Breeding and Traits (R. Fletcher, Cargill): Cargill only breeds canola for specialty seed and oil to create and maximize consumer value. Cargill's process is:

Market analysis → breeding/scale-up→ contract growing → crushing → refining of oil → applications of R&D → blending and packaging → sales, marketing and distribution

Cargill and Bayer have a partnership for Cargill Victory hybrid canola and Bayer InVigor health canola to produce Clear Valley oil and IngreVita EPA/DHA Omerga3 oil. A survey has found that 46% of consumers say that saturated fat content impacts their food purchasing decisions. Cargill has removed 1.1 billion punds of tran-fats for McDonald's via the low trans-fat canola blend for frying. For 2017, the vision is low saturated fats (<4.5%).

Yield is critical to the success of specialty crops. Between the years of 2006-13, the total acres of specialty canola needed to meet McDonald's increased oil needs dropped by 20% due to increased yield. Oil costs were reduced year on year during an inflationary food period. Cargill was awarded McDonald's sustainability award in 2014.

Hybrid canola is the first generation from a cross of two distinct individuals. In canola there is limited amount of genetic diversity from *Brassica rapa and B. oleracea*. The Tower canola line widdled down diversity more resulting in large regions of the genome showing no diversity. Breeding is working on creating genetic diversity to be able to develop and identify the best inbreds. These inbreds are then used for the hybrids which are screened to determine the best hybrids. Cargill has developed SPRINTER canola (SPRing growth habit, winter *B. napus*). The breeders started with winter type *B. napus* and crossed to rapid-cycling *B. napa.* These plants were then backcrossed to winter type *B. napus* and a spring type was selected. The result was a nearly 100% winter type with a spring growing habit (Cargill patent U.S. 8,759,608). SPRINTER shows genetic diversity between parents. In the future, plans are to remake *B. napus* by re-synthesis of diversity.

Molecular and Biochemical Analysis of Canola (D. Syme, Bayer): Canola is one of two cultivars of *B. napus or B. rapa* and is derived by conventional breeding from rapeseed in Canada. However, canola is not rapeseed due to its low erucic acid and glucosinolate content.

For variety registration, a minimum of 11 trials over 2 years is need. The WCCRRC recommends the registration of new varieties. Biochemical and molecular analysis tools are important for breeding selections and quality control. Canada Grain Commission runs the annual lab proficiency program for biochemical analysis.

Biochemical analysis tools include NMR, capillary gas-LC and near-infrared spectroscopy. NMR can analyze 1-20g of seed with <1 min analysis time. Capillary gas-LC is used for analysis of fatty acids and glucosinolates. Run times are typically <3-10 min with up to 200 samples per day analyzed. Near infrared spectroscopy measures the protein, oil glucosinolates, fatty acids, chlorophyll and moisture. It is non-destructive seed analysis.

Molecular analysis tools include protein (LFS) and DNA (PCR). Protein LFS are useful for checking volunteers and brown bag seed. PCR methods include gel-based, endpoint Taqman, qPCR and droplet digital PCR. Gel-based PCR is used for zygosity testing. Endpoint Taqman include event specific methods and are used for plant selection and quality control. The qPCR methods are also event specific and can be qualitative, semi-quantitative or quantitative. Droplet digital PCR is chip-based analysis that provides absolute quantitation with no reference material or standard curve. Digital PCR has a greater tolerance to inhibitors and it is possible to have multiplex methods.

Development of the Standard ISO 13484 for Adapting Molecular Biomarker Technologies to Plant Disease Molecular Diagnostic Labs (G. Dennis, USDA PPQ): The document was developed through the ISO TC 34/SC 16. It was first introduced by AFNOR (France) and assigned to WG 4. The scope of the document is general principles for the use of molecular procedures used for detection, identification, quantitation or to confirm the presence of plant pests in plant materials. Principles are applicable to plant parts and aliquots but not for GMOs, animal pathogens or human pathogens. The intended use is for comparable processes for preventing contamination and for testing such as storage issues, separation of workstations, process flow, cleaning equipment, etc. The scope of SC 16 is nucleic acids, amino acids, volatile compounds, lipids and polysaccharides. Some of the assays include: citrus greening RT-PCR method, *Fusarium oxysporum* sp. *Vainfectum* isothermal PCR from Agdia, general confirmatory sequencing techniques such as Sanger and NexGen (NGS), antibody-based detection of banana bunchy top virus via ELISA and plum pox virus via ELISA. The document does include a comparison of 13484 and 17025 sections to elucidate necessities for labs moving towards a management system stand (MSS) and does supplement the 17025 guidelines. It does not act as

technical standard for detailed requirements for specific methods or conflict with 17025 or include specific requirements for validation studies or extended validation terminology. Section 4 is lab prerequisites and some of the challenges include whether NGS can be used in routine diagnostics, what unusual circumstances contribute to volatile samples. Section 5 is diagnostic assays. Section 6 deals with development of assays such as development plan needed, study monitoring and traceability of experiments. Section 7 is the additional requirements for development and diagnostic assays. In this section describes non-conforming results as they relate to development, statistical analysis of some form being required for conformance assessments. Challenges to results interpretation include what should be positive and negative controls in NGS and digital PCR and what does root cause analysis look like for NGS or volatile compounds. Ultimately, it is all about the process: process control, process flow, process integrity and process development.

Molecular Diagnostics in the Palm of Your Hand (H. Cai, Mesatech): Mesatech was founded in 2009 as a spin-off company from the Los Alamos Labs. It is now located in San Diego and has 30 employees and an additional testing lab in Santa Fe, NM. Product focus is rapid nucleic acid diagnostics.

In the health field, point of care (POC) methods include lateral flow strips (LFS) and lab molecular testing. Molecular testing is complex, has a higher cost and takes more time for diagnosis of the patient problem. Mesatech wants to create a POC molecular test which is rapid, accurate, cost effective and easy to use, improves existing workflow and has immediately actionable results for the physician.

Mesabiotech has developed test cartridges (LFS) which use familiar sample collection, test procedure with a visual readout. The method is PCR-like amplification and sequence-specific hybridization-based detection. Qualitative results are available in 30 min and the cartridges can be stored at ambient temperatures. The biotech docking station essentially powers the heaters by either battery or a plug-in adapter to an electrical outlet. FDA considers the dock as just a glorified heating block because the test is the LFS. The cassettes consist of the sample chamber→reaction chamber→viral lysis→real-time PCR reagents→real-time PCR→amplification reagents→DNA amplification. The DNA amplification is an oscillating amplifying reaction mean there is exponential nucleic acid amplifying strategy that accomplishes PCR at a reduced absolute temperature using a higher annealing and lower denaturation temperature. The amplification product is bound to a latex particle conjugated to a probe oligo. The LFS captures the oligos. The reader has wireless transmission. The test can detect femtomoles and the reagents are stabilized via lyophilization (enzymes and particles).

The test has broad application in many markets such as human health, agriculture, food and animal health. In collaboration with USDA, a method was developed for detecting citrus greening. Leaf pieces were mashed in plastic bags with buffer. Forty microliters of the buffer extraction were loaded into the LFS device. Qualitative results were read in 40 min—test has 2 lines if the test is positive (control line and result line).

In the future, Mesatech plans to improve the manufacturing process and integrate sample preparation. Plans are also to develop a multiplex assay for 12 pathogens. Mesatech would like to collaborate and form partnerships to explore agricultural applications.

Plants in the Fourth Dimension: How Time Shapes Response to Abiotic Stress (C. Doherty, NC State University): How do plant make decisions about their environment? They have to integrate environmental signals and evaluate. Stress responses vary in rate of induction and kinetics, amplitude, duration, etc. Plants know how to integrate signals to give the appropriate response. This know-how is

all encoded in the plant DNA. Poor decision makers are removed from the gene pool. The optimal evolutionary response is not necessarily the optimal agricultural response. Environmental conditions in which plants have evolved is changing. The goal of the lab is to understand whether stress response networks can be altered to optimize agricultural beneficial responses.

Plants take advantage of the sun rising in the east every day and reset themselves each day to continue to take advantage. A question was posed as to what happens if a stress is imposed at a different time of the day. For example, in Arabidopsis, if the plant thinks it is night, the looper insects can eat freely unlike if the plant thinks it is day, the loopers cannot eat freely. The circadian clock regulates all plant activities and thus, if we can understand this, it may be possible to use this to improve yield. Time of day is integrated in plant molecular activities through transcriptional regulation. For instance, it was found that 49% of molecular transcripts were higher at dawn and 40% of molecular transcripts were higher at dusk. This is referred to as transcipriptional gating, i.e., differential molecular response depending on the time of day. Transcriptional gating response could be used to understand the underlying regulatory network such as the classic salt response. Salt response genes are highly morning responsive. Membrane damage varies depending on when the salt shock occurs.

Thermocycle plants respond to nights being cooler than the days. 95% of their genomes will continue to cycle. A small heat increase at night is more detrimental to these plants than a heat increase during the day. Thus, the impact of global warming to plants—night temperatures are gradually increasing. In a rice experiment, it was found that high night temperatures resulted in a decrease in yield due to a delay in photosynthesis during the day. It was also found that it made a difference as to what time of day plant are harvested. The major point of impact was the plant TCA cycle. High night temperatures occur when the plant least expects it which causes a decrease in amplitude of the thermocycle which then impacts the plant circadian clock.

Cross-species Exprssion QTL Mapping Reveals Novel Insights into Plant-Parasite Interactions (D. Nielsen, NC State University): Parasites need to enter the host plant and evade host responses. There is molecular signaling between species which coded at the genome level. The goal was to develop methodology for identifying hos-parasite interactions using gene expression and gene mapping.

The eQTL tool connects variations in DNA to difference in gene expression levels. Identifying self-regulating genes is cis-QTL and identifying loci that regulate each other via trans-QTL. *Meloidogyne hapla* (root knot nematode; RKN) infects *Medicago truncatula* and at these sites galls develop which interfere with the uptake of water and nutrients by the plant. Gene expression levels were monitored via RNA. Loci were identified in the RKN genome that influenced the expression in the plant. The results showed huge signals for the AGAMOUS and serine acetyltransferase indicating that RKN genome has an effect on the plant. The RKN has 19 genes: 8 genes display DNA sequence variability between parental lines and 3 genes display moderate to high expression in offspring lines. The question was whether the effect was conserved across plant species. If the expression changes are induced in other plants, this provides evidence there is a functional effect of the parasite and the interaction is not specific to just *Medicago.* The follow-up experiment was done with tomato in which 8 plants were infected with one of the parasite parental lines. The plants were then tested for differences in expression. It was found that the parasite interaction was conserved across plants as TF genes were identified in the tomato replicate.

Cross-species eQTL mapping is effective at identifying candidate genes. DNA polymorphisms were identified in the parasite that influence host genes and the reverse is also feasible.

Where are we on GM Public Education? (C. Tutino, Syngenta; F. Castle, BASF): NGOs are using social media consistently for disseminating negative messages about GM technology. There are effective in playing off emotions of consumers. Mainstream media is not accurately covering scientific information. For industry, opposing state-by-state labeling changes is unsustainable and untenable. NGOs are well funded ($2.4 billion annually) to villianize plant sciences.

Industry has acknowledged consumer skepticism about the technology and developed GMO Answers (www.gmoanswers.com) to show that industry has nothing to hide. The 5 core principles behind GMO Answers are:

- Respecting people globally and their right to choose healthy food,
- Welcoming and answering question on GMOs,
- Making GMO information, research and data easy to access and evaluate,
- Supporting farmers as they work to grow crops, and
- Respecting farmers' rights to choose the seeds that are best for their farms.

GMO Answers is supported by the Big 6 agricultural technology providers, the Council for Biotechnology Information (CBI), independent experts and supporting partners (American Farm Bureau, ASTA, ASA, Minnesota Crop Production Retailers, etc.). GMO Answer audience was intended to focus on policy makers, however, consumers from all over have been using it. The website is community-based for engaging in conversations. The community votes on the questions which keeps the anti-groups in check. There are sections on the basics of GM science, studies and educational resources. GMO Answers is also active on Pinterest, Facebook and Twitter. There are approximately 80-90,000 visitors per month, of which 15-20% are return visitors. The website provides more balance and the NGOs are gradually losing credibility as target audiences are beginning to understand benefits. The website is going global as translations are being rolled out in Portuguese, Spanish, Chines and Vietnamese languages.

Following this presentation, the AEIC group engaged in a panel discussion moderated by L. Privalle (Bayer) and panel members C. Tutino (Syngenta), F. Castle (BASF), J. Spurgat (Bayer) and A. MacMullen (NC Dept of Ag.).

New AEIC Member: Avazyme (V. Bornemann): Avazyme is a CRO provider to bridge the gap between food production and consumer safety. Avazyme provides services for toxin identification, food quality, nutritional analysis, agrosciences, food authenticity, pesticide residue trials and yield trials. Lab testing capabilities include mass spectroscopy, chromatography, automated extraction, immunoassays, etc. Avazyme provides solutions all along the food chain. Their clients include craft breweries, retailers, restaurants, agricultural chemical companies and agricultural biotech companies.

Endogenous Allergen Quantitation by ELISA Technology (T. Geng, Monsanto): Allergenicity potential for GM products is assessed to a) determine allergenicity potential of the introduced protein(s) and b) assess whether the endogenous allergenicity of an already allergenic crop is increasing. Currently, endogenous allergenicity is assessed by comparative assessment and/or human sera assessment.

In 2013, EU EFSA implementing regulation went into effect which indicates that comparative analyses must be conducted of soybean individual allergens. The soybean OECD document lists 15 proteins as potential allergens from soybean. To determine which of these to measure and assess, Monsanto decided to only measure those that have solid clinical evidence of allergenicity from the public literature. Monsanto submits data on 5 of the 7 allergens since the remaining 2 allergens are putative allergens. Also, trypsin inhibitor is measured in compositional analyses.

There are several ways to measure endogenous allergens: gel separation, mass spectroscopy, ELISA. Gel separation methodology employs 1-D and 2-D gels. Mass spectroscopy is a new approach that is expensive and is not real high throughput. The sandwich ELISA is amenable to high throughput, costs less and Monsanto felt it was the best approach. Monsanto has developed ELISA methods for Gly m 4 and Gly m 6 (glycinin) and has generated ELISA data which has been submitted in dossiers to the EU. To date, there have been no questions. Monsanto has enabled a CRO (Covance) to conduct the ELISA methods in order to accelerate GLP studies of soybean allergencity. Monsanto has completed 12 soybean allergen studies in 2 years which have yielded 36,000 data points.

Monsanto has also conducted studies to gain understanding of the natural variability in soybean endogenous allergen levels. They have looked at the variability of 5 soybean allergens in 624 conventional soybean seed samples (41 different varieties). These were grown over 6 seasons at 26 different field locations in the U.S. and South America. The results indicated that the allergen levels are highly variable and comparison to literature values is difficult since it depends on the locations, number of locations, environmental conditions, etc. The difference in allergen levels between GM and conventional soybeans is much smaller than the natural variability. In their studies, Monsanto found no significant difference between the GM soybean and the control.

Multiplexing LC/MS Technology for Allergen Quantitation (T. Oman, Dow): Compositional analyses of soybean is affected by numerous factors such as environment, nutrient stress, breeding methods, etc. Endogenous allergen quantitation has relied most on antibody-based techniques such as ELISA or immune-blotting. The pros to these methods are that they are simple and provide a low level of detection. The cons are that they require specific antibodies, multiplex assays are challenging, poor selectivity, limited scope of target proteins (globular, fibrous, membrane proteins are a challenge), and matrix interference is a problem. Measuring plant protein is challenging due to protein abundance, physiochemical properties, matrix effects and sample variability.

Mass spectroscopy (MS) measures individual molecules that have been converted to ions, thus, the mass:charge ratio is measured. A wide range of masses can be detected and MS is very sensitive, fast and reproducible, quantitative and provides information on the molecular weight and structural analysis. Generated ions are separated in the mass analyzer, digitized and then detected by the ion detector. Approximately 10,000 proteins can be analyzed in 2 hours using 1ug of complex material. MS can be coupled to further separation techniques such liquid chromatography (LC) or gas chromatography (GC).

To analyze proteins, the protein is cleaved into smaller pieces using a protease and then the fragments are quantitated from the digestion. A unique peptide is identified for each allergen in order to quantitate the allergen. Signature peptide quantitation is a stoichiometric relationship. The peptides are then quantified with LC/MS where LC provides the temporal resolution of analytes and MS provides the selection of peptides. The SRM scan is the initial mass-selection of the ion of interest formed in a source followed by dissociation of this precursor ion in the collision region of MS and then mass-selection and counting of specific product. The instruments scans constantly for particular peptides. A reference standard is used to determine protein levels based on a curve.

The Dow LC/MS method is called Plextein and has been licensed to Critical Path Services. The method has been validated by assessing sensitivity/quantitative range, matrix effects, extraction efficiency,

digestion efficiency and accuracy. Using the Plextein technology, GM proteins can be quantitated across growth stages and tissue types and is a high throughput technology.

Comparison of MSD and ELISA for Protein Quantitation in Stacked Trait Maize (J. Smith, Syngenta): ELISA is the current "gold standard" methodology for protein quantitation. However, stacked trait products require numerous ELISA methods to characterize and analyze the stacked trait samples for protein levels. Syngenta wanted a strategy to deliver future data requirements with the current resources.

The Meso Scale Discovery (MSD) technology is an antibody-dependent, multi-array technology. The basic assembly is the electrode surface + streptavidin + biotin + biotin-labeled antibody + host cell protein + sulfo-TAG-labeled antibody. An electrical signal is sent through the electrode surface which causes the sulfo-TAG to emit light. A single well (96 well format) has multiple ELISAs per well. There is no multiple sample dilutions needed with a 4-5 hour run-time. One plate with 5 proteins/well costs $1.26 per sample. To break even on cost, a minimum of 3 protein multiplexing is needed. Protein selection is based on antibody selection. Fusion proteins are a challenge for specificity. In studies with maize tissues, it was found that the MSD requires a higher dilution factor than ELISA. MSD has a broader quantitation range and an increased LLOQ. The sensitivity varies the protein and the matrix.

Unlike spiked buffer data, when using spiked maize extracts it was found that MSD is not consistently more sensitive than ELISA even when the same antibodies are used. In analysis of stacked trait protein samples, not all samples agree between ELISA and MSD. It was found that ELISA was quantitating higher than MSD. Even though ELISA is higher, it is predictable factor.

MSD is high throughput, economical and meets or exceeds single-plex ELISA.

Application of Bioinformatics in Assessment of Transgenic Proteins for Potential Allergenicity (P. Song, Dow): The principles of risk assessment for GM traits is to avoid the transfer of known allergens; assume genes from allergenic sources encode allergen until proven otherwise; assess the allergenic potential of all introduced proteins; and where allergens are identified, consider alternative gene sources or technical strategies to reduce risk or discontinue the product development.

The weight of evidence approach is followed for introduced proteins. This approach is based on the following questions:

- Is the newly introduced protein, including novel ORFs, structurally related to an allergen?
- Is the newly introduced protein heat labile?
- Is the newly introduced protein pepsin labile (digestible)?
- Was the newly introduced protein/gene isolated from a known allergen source organism?
- Is the newly introduced protein present at high concentrations in food?
- Is the newly introduced protein glycosylated?

To perform bioinformatics analyses of proteins and allergens a dedicated allergen database is needed along with criteria for entries into the database and algorithms to analyze. The E-value is influenced by the database size. The database should contain all known food allergens, aero-allergens and dermal allergens. Criteria need to be established to define what is an allergen such as protein sequence, data to indicate that individuals are allergic to protein, protein can be purified or cloned from test material and test IgE binding, supportive publications for allergenicity and sequence similarity to known allergens. The database should quality control and expert panel reviews and the database construction should be

transparent. The database should also be fully curated and updated at least once per year and be publically accessible.
Currently, the AllergenOnline database is used by industry for allergen bioinformatics comparisons. However, ILSI HESI will be rolling out the COMPARE (COMPrehensive Allergen Resource) database soon.

Prediction of potential allergen is assessed by linear epitope identification and prediction. Short contiguous peptide matches are looked for and the epitope is identified using protein physiochemical properties. Allergen specific motifs and sequence similarity searches are also used along with 3-D structure modeling to identify conformational epitopes.

The benchmark method is prediction of allergen by the use of a local alignment using sequence as a criterion. In 2001, WHO/FAO recommended a FASTA search of a database using a 80-mer sliding window and searching for matches of 6 contiguous amino acids. For the 80-mer search, more than 35% identity in the amino acids of the protein is needed. The problem was this has a high false positive rate. The whole sequence search based on >35% identity over 80 amino acids gives a slightly lower false positive rate. However, two proteins could have less than 35% identity but have 100% similarity and a significant E-value. The E-value is a statistical measure of the likelihood that the observed sequence similarity score could have occurred by chance. Regulatory authorities are reluctant to replace identity criteria in the current public environment. The standard bioinformatics tool which incorporates an E-value is superior to the WHO/FAO and Codex identity criteria. Availability of whole genome sequencing by NGS will enable a thorough bioinformatics analysis of all food proteins.

Primer on Testing in the Regulatory World (C. Devorshak, USDA APHIS PPQ): The Science and Technology Unit under PPQ is based in Raleigh, NC. The unit is concerned about pests not here (quarantined) or regulated pests. Initial detection of pests occurs by notification at the ports of entry, through cooperative agriculture pest surveys (APHIS funded), state departments of agriculture, industry representatives and consultants, university diagnosticians and academic researchers.

Testing is conducted to make decisions about the entry of a commodity, identify organisms found during an inspection, identify organisms found during surveillance, make a decision about a domestic detect, support programs like ‘clean stock’ or for export certification. USDA is legally accountable for any resulting decisions. Positive test results may lead to regulatory actions such as rejection or destruction of commodities at ports, confiscation, destruction of crops, plants, products or establishment of quarantine areas and prohibition of movement of materials. Any commodity entering the U.S. is tested and any product found to harbor pests during inspection or surveillance. Specific surveillance is targeting a specific pest, i.e., know what is being looked for. General surveillance is looking for any pest which sometimes results in not being able to identify an organism. Testing occurs throughout the whole process from pre-entry to post-entry. Testing is often determined by trade models which are getting complicated due to the movement though several countries during different stages.

USDA Confirmatory Process for Regulatory Pest and Pathogens (P. Shiel, USDA PPQ): PPQ has 75 port and domestic identifiers. Most of these do not have diagnostic labs but have the expertise. The National Seed Health System (NSHS) does phytosanitary certification. The National Seed Health Accreditation Program (NHSAPP) is a model for voluntary system of testing seed imported into the U.S. for pathogens. The National Plant Protection Lab Accreditation (NPPLAP) evaluates labs for use in making decisions, establish state of readiness when needed by PPQ in emergency situations. A potentially actionable sample (PASS) is identified and forwarded to USDA for confirmation. Samples are from areas not previously part of USDA quarantine or regulated zone or the sample not found previously

on a particular plant. There is increased participation in the program and proficiency tests are being developed.

Cotton Inc. Introduction (T. Wedegaertner): Cotton Inc. was founded when cotton was declining due to polyester clothing surgence. Cotton Inc. has check-off programs, research and marketing. It does no lobbying. It has a facility for textile research and for field research. There are programs to stimulate the demand for cotton. The analytical lab focuses on coatings for moisture management and other finishes. The color lab participates in the industry selection of what colors will be popular each year. The process begins 2-3 years in advance of a particular season.

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Organizations Attending the Spring Meeting:

Romer Labs
SGS North America
Eurofins Nutrition Analysis Center
Eurofins GeneScan
Eurofins BioDiagnostics
Bayer
Syngenta
Simplot
BASF
Primera
USDA GIPSA
USDA PPQ
North Carolina State University
Agdia
Covance
EnviroLogix
Mesa Biotech
Neogen
DuPont Pioneer
Monsanto
Dow AgroSciences
AOCS
Critical Path Services
OMIC USA
ThermoFisher
EPL Labs