**AEIC FALL MEETING 2010**
**MINUTES**

**October 6-7**
**Lincoln, NE**
**Hosted by: Neogen/GeneSeek**

*P.L. Hunst, AEIC Secretary*

The 2010 AEIC Fall Meeting was held on October 6-7 in Lincoln, NE and was hosted by Neogen/GeneSeek. There were +50 attendees from industry, government and academia.

Jason Lilly and Jeremy Walker (Neogen/GeneSeek) welcomed the attendees to Lincoln and gave an introduction to Nebraska and Neogen/GeneSeek. Little known facts about Nebraska include:

- Hastings invented Kool-Aid,
- Morton found Arbor Day in Nebraska City,
- The state insect is the honeybee,
- The Lied jungle, located in Omaha, is the world's largest indoor rainforest,
- Nebraska is the birthplace of the Reuben sandwich,
- Spam is produced in Fremon, NE,
- Nebraska has the largest aquifer in the U.S.,
- Nebraska has more miles of rivers than any other state,
- Lincoln is home to the University of Nebraska and on days of home football games, it becomes the third largest city in the state (90,000 people),
- Nebraska has the lowest electric rates in the U.S.,
- The Lied Center for the Performing Arts is one of the top venues in the Midwest.

Neogen's mission to be the dominant company in food and agricultural diagnostics. It is headquartered in Lansing, MI and was founded by James Herbert (former Dow Chemical CEO) in 1982. Neogen has 600 employees globally and in 2010, it was ranked #82 on Fortune's 100 fastest growing companies list. Neogen is expanding their market share with internally developed products and growth acquisitions. Neogen has expanded to international markets with 41% international sales. There is a changing food safety environment which includes tougher regulations, greater food security concerns, globalization and farm gate security. Neogen is meeting the demands via diagnostic products and intervention products such as vaccines, pharmaceuticals, supplements, vitamins and wound care.

GeneSeek is the market leader in genomic analysis for agricultural biotech. With 36 employees, GeneSeek provides genomic solutions for high content SNP profiling and marker assisted selection, identity management, veterinary and clinical diagnostics. GeneSeek works with its customers to target desired areas of interest. Its primary technology platforms include Illumina's Infinium. GeneSeek is an active partner to its

customers for proprietary development, marketing and service. They prefer to be a passive service provider to their customers.

**AEIC BUSINESS MEETING**

**Approval of 2010 AEIC Spring Meeting Minutes:**

A motion was made, seconded and voted to approve the 2010 Spring Meeting Minutes which are posted on the AEIC website (www.aeicbiotech.org).

**AEIC Treasurer Report (D. Layton):**

| | Projected | Actual |
|---|---|---|
| Beginning Balance | 20564.00 | 20564.00 |
| 2010 Membership Dues | 8000.00 | 8500.00 |
| **TOTAL** Revenue | 8000.00 | 8500.00 |
| | | |
| Expenditures | | |
| Scientific Paper | 4000.00 | |
| Wire Transfer Fees | | |
| DE Franchise Tax | 25.00 | 25.00 |
| ANSI/ISO (AOCS – ISO TAG) | 2900.00 | |
| Board Meeting | 100.00 | |
| Spring Meeting | 1500.00 | 1093.00 |
| Website | 500.00 | 128.00 |
| Bank Service Charge | | 6.00 |
| Fall Meeting | 1000.00 | |
| Reprints | | |
| Subscriptions | 300.00 | |
| Miscellaneous | 100.00 | |
| **TOTAL** Expenditures | 10425.00 | 1252.00 |
| **TOTAL** Balance Checking | 18139.00 | 27812.00 |
| | | |
| Cert. of Deposit | 11396.00 | 11396.00 |
| Interest | 150.00 | 75.00 |
| **TOTAL** CD | 11546.00 | 11471.00 |
| **TOTAL** BALANCE | 29685.00 | 39283.00 |

**Membership Update (D. Layton):**

Membership of AEIC is as follows:

| Type of Membership | Number |
|---|---|
| Large Companies | 13 |
| Small Companies | 10 |

| | |
|---|---|
| Associate | 2 |
| Individual | 3 |
| TOTAL | 28 |

Four companies are still outstanding on dues payments. AEIC would like to have more members from the grain industry, processors and academia.

**AEIC Brochure:**

D. Layton has copies of the brochures available upon request. A PDF of the brochure is also available on the AEIC website.

The brochure will need to be updated prior to upcoming Global Conference on GMOs in 2011. Members are asked to provide any inputs before the end of 2010.

**AEIC Website:**

The AEIC website is located at www.aeicbiotech.org. Members were asked to take a look at the website content and let the Board know if any changes or updates are needed.

**2011 AEIC Spring Meeting:**

The membership discussed possible dates and suggested April 13-14. Guomin Shan volunteered Dow AgroSciences to host the meeting in Indianapolis, IN.

Suggested topics for the meeting included:

- Day on allergens; applied aspects of detection
- Regulatory process for GM products – challenges of registering a product
- Labeling and the role of detection
- GM animals
- Mycotoxin testing

**Board Election:**

The election for the office of Vice President will be held in early November. The membership was asked for nominations during the meeting and may forward any other nominations to the AEIC Secretary by October 31.

Nominations from the floor:

- Clara Alarcon (DuPont/Pioneer)
- Yelena Dudin (Monsanto)

Both nominees accepted the nominations.

**AEIC Activities Update:**

Paper on Protein vs DNA Methods (R. Shillito)
The group is still gathering input and a conference call will be held soon.

Paper on Testing for Commercialization of a New Biotech Event (L. Privalle)
The group working on this project has an outline and a partial draft and will be meeting during this meeting to discuss further plans.

Paper on Quantification by Sub-sampling (B. Kaufman)
It was mentioned that this could be a new work item in the TAG, however, nothing has been submitted as a proposal. There has been a lot of discussion on this topic and now it is ready for items to be put into action. D. Grothaus volunteered to assist, along with K. Remund.

D. Layton indicated that Virginia Pantella (VIP Consulting) is available to work with any or all of the groups to help in editing/finishing the papers.

There was also a suggestion that the members should take a look at the current AEIC publications and determine if any of them need updating.

**INVITED TALKS**

**ADM Bean Processing (J. Rayapati):**

ADM has 29,000 employees, 240 processing plants and operates in 60 countries and earns $62 billion in revenue. ADM started as a flax processor. Today, ADM processes a variety of oilseeds – 3.1 million bushels per day. ADM's mission is to serve vital needs in the processing area by adding value after harvest and fostering sustainable growth.

ADM's core processing competencies include extractions, distillation, hydrogenation, and enzymatic conversions. Advanced processing competencies include chromatography, membrane separations and fermentation technology. In R&D, ADM has 250 scientists in Illinois, Wisconsin and Kansas whose mission is to develop superior products through advances in research and technology.

Soybean processing yields many products such as phytosterols, vitamin E, lecithin, etc. Soybean is 15% soluble carbohydrates and 18% oil. For oil, the greatest need is stability of the oil following extraction.

ADM is also involved in plant breeding of edible beans such as navy beans, pinto beans and black beans. The intent of breeding is increase the fiber in breakfast and snack foods. The interest is in underground traits such as nitrogen fixation, nitrogenase, leghemoglobin and PEP carboxylase as well as biomass and its impact on yield components (pods/plant, pod bearing nodes). Tolerance to plant diseases such as root rots is also important.

ADM utilizes FTIR of whole seeds to look at moisture, protein, fiber, minerals, fatty acids and starch as well as protein digestibility, energy digestibility, lysine in feed and barley malting vs. feed traits. Value is captured by dividing into super commodity (no need to separate) and minimal IP (risk of mixture: allergens (high) and fatty acids (low).

**Challenges in Soybeans (R. Wilson, United Soybean Board)**

The United Soybean Board has 68 farmer leaders who oversee the investment of the national soybean check-off. Funding in the 2010 research program went to biobased products (9.3%), biotic stress (49.5%) and cultural practices.

There are several prominent soybean diseases which includes Phytophthora, sudden death syndrome, cyst nematode infestation, stem canker, white mold and soybean mosaic virus (SMV). Phytophthora rot is kept in check with the use of 14 resistance genes in breeding. The cyst nematode is parasistic on roots and decreases yields. There are currently 3-4 resistance genes in use. Stem canker is caused by a Diaporthe fungus. Although there are 2 resistance genes, southern stem canker is still a problem.

The sudden death syndrome is caused Fusarium which destroys the vascular system. The fungus infects cyst nematodes and enters the plant via the nematode. White mold is caused by Sclerotinia and infection of soybean plants is dependent on weather conditions (wet and cold being favorable). There is one known resistance gene. SMV affect seed quality and is spread by the soybean aphid. There at least 2 resistance genes in use. Bean pod mottle virus (BPMV) is spread by a beetle and there are no resistance genes known.

Heliopthora is the causal organism of brown stem rot and is currently controlled by 3 resistance genes. Cercospora leaf blight fungus produces cercosporin which destroys cell walls. The fungus infects the seed which causes a purple staining. The disease is controlled via crop rotation. Charcoal rot is a widely distributed disease and often occurs when plants are under heat and/or drought stresses. The disease is caused by the fungus *Macrophomina phaseolina*. Frog eye leafspot is mainly a disease found in the southern soybean growing areas. There are currently 3 known resistance genes. Asian soybean rust is a foliar disease which causes significant yield losses. It was found in the US in 2004, however, it does not survive cool, dry winters.

Soybean has several major insect pests. These include soybean aphid (a virus vector), green cloverworm, stem borer, spider mite, longhorned weevil, Japanese beetle, seed corn maggot, soybean looper, thistle caterpillar and the white fly.

With the introduction of glyphosate-tolerant soybeans, weed control was made much easier for growers. However, glyphosate-resistant weeds are now becoming more of an issue in soybean areas. Common weeds of soybean include Palmer amaranth, waterhemp, pigweed, lambsquarter, Johnsongrass, common ragweed, giant ragweed and morning glory.

In the coming years, US farmers will be expected to produce more crops on the same number of acres. Soybean and corn yields are projected to remain constant over the next decade. Corn yield in 2010 is expected to be 185 bushels/acre and soybean at 51.1. bushels/acre. Soybean will be relied on more in the future to meet projected oilseed demands. Soybean varieties are selected based upon yield, disease resistance, maturity group, grain composition, height and lodging.

Qualisoy is a roundtable representing key members of the soybean value chain. Some of the members include Monsanto, Cargill, Latham, Pioneer Hi-Bred, Bunge, ADM, ASA, Akey, USB, Syngenta, USDA ARS. Soybean product pipelines include high-stearic soy, omega-3 soy, mid-oleic/low-saturated oil, increased energy/low-phytate and improve amino acid levels.

Soybean meal and protein provide advantages in digestibility and energy content of food and feed. Soy meal is relatively expensive and poultry/swine producers are cutting back on the amount used. RNAi has been used to suppress major soy storage protein genes, however, the protein concentration has been found to remain the same whether the genes are suppressed or not. There is as yet no adequate explanation for this. Due to the silencing of storage proteins, anti-nutritional proteins have also been removed. The Bowman-Birk trypsin inhibitor makes up 3.5% of the soy protein and is difficult to get rid of during processing. The gene belongs to a large gene family. If an extra glycine is inserted at the active site, the molecule becomes susceptible to the serine protease inhibitor and thus the animal gut can process it.

**Evaluation of Novel Input/Output Traits in Soybean (T. Clemente, UNL)**

At UNL, the plant transformation core facility was opened in 1996 and *Agrobacterium* transformation is performed here. UNL also has a dedicated field site (25 acres with irrigation) and will soon have an additional site at North Platte which presents a different environment. UNL has dedicated equipment for extrusion and the process under BQMS.

*Agrobacterium*- mediated transformation involves using competent cells to receive the DNA. The embryonic axis is removed from a germinated seed and inoculated with *Agrobacterium*. The *bar* gene is present in the genetic construct which allows the use of glufosinate herbicide to select those cells which have been transformed. The transformed cells are then regenerated into plants (4-6 months). Progeny of the regenerated plants are screened with glyphosate herbicide to determine which are the most tolerant of the herbicide.

T. Clemente's lab is working on input traits (dicamba, nematode, fungal, viral, abiotic, aphids and photosynthetic) and output traits (high oleic, steridonic, ricinoleic, palmitoleic and astraxanthum). Virus resistance is also an area of research. Vruses are usually held in check by the use of insecticides which control the insect vectors. For SMV, AMV and BPMV, an RNAi approach is being investigated. A triple hairpin has been made to target all 3 viruses and was transformed into soy. In the greenhouse, the regenerated plants have absolute immunity to the 3 viruses. Field tests will be conducted in 2011.

For improving photosynthetic capabilities, a gene from the Calvin cycle and a gene from ICTB have been transformed into soy. The regenerated plants were screened to obtain those with a simple insert and then were looked at for expression and phenotype. In small plot field tests in 2009, no increase in yield was observed. Larger plots were put out in 2010 and that data is currently being collected.

For drought tolerance, the gene(s) need to be regulated, i.e., not on at all times. *Arabidopsis* promoters have been screened to find those that would be adequate, however, it was difficult to a non-leaky promoter. The rice *hrd* gene with induction promoter was transformed into soy.

For low saturate oil, genes were down-regulated to increase oleic acid and reduce palmitic. A gene from mango was used to increase stearic acid along with oleic acid.

A project has been developed to displace fishmeal/fish oil in aquaculture feeds with soy protein. A marker-free event was developed containing delta-6-desaturase. This was stacked with delta-15-desaturase which gave an additive effect of both genes. Trout could then metabolize the STA soybean oil. The STA oil was also tried with Kamachi fish. Initially, the fish would rather die than eat soybean oil, i.e., they could not metabolize the long chain fatty acids. Crnivorous fish are somewhat like cats in that they need taurine. When this was added to the feed, it made a dramatic difference for the fish in that they achieved weight gain with soy/fish oil and STA/fish oil.

Another project was to look at feeding salmon soy. In order to attain the orange color, salmon need to have carotenoid in their feed. Three genes were stacked in soy which resulted in the soybeans appearing pink due to carotene and other carotenoids. This raises the possibility of using these soybeans to feed salmon.

**Development of Dicamba-Tolerant Soybeans (D. Weeks, UNL)**

Dicamba is a benzoic acid derivative used to control broadleaf weeds in grassy crops. Maize is tolerant to dicamba. The goal of the research project was to convert sensitive broadleaf crops into dicamba-tolerant crops by inserting an enzyme gene to deactivate the dicamba molecule. The dicamba deactivating enzyme is dicamba o-demtheylase which was identified from bacteria (*Pseudomonas* maltophilia) which degrade the molecule in soil. The bacterial use dicamba as a sole energy and carbon source. The dicamba o-demethylase is a 3 gene enzyme system consisting of a reductase, ferredoxin and oxygenase. It was found that only the oxygenase gene was needed (dicamba mon-oxygenase).

Soy was transformed with the mono-oxygenase gene under the control of the strong constitutive promoter from peanut chlorotic streak virus. A chloroplast transit peptide was upstream of the oxygenase gene. *Agrobacterium*-mediated transformation was used to insert the construct. The transformed plants had dicamba tolerance up to 5 lbs/ac a.i. (10X the normal rate).

The dicamba mono-oxygenase, when crystallized, occurs as diamond-shaped crystals. Electrons are transferred from ferredoxin to the iron-sulfur. The mono-oxygenase is actually a trimer structure which facilitates electron transfer.

Wild type plants were affected by 0.001 lb/ac a.i. whereas plants containing the gene in the nuclear genome could tolerate >25lb/ac a.i. and those with the gene in the chloroplast could tolerate >50 lb/ac a.i. In the greenhouse, the transgenic soybean plants tolerant to 1 and 5lbs/ac and in the field, they were tolerant to 2.5 lbs/ac.

Monsanto has stacked dicamba mono-oxygenase with RR2Yield and it has shown good tolerance. The product should help to combat weed resistance to glyphosate. Monsanto has also stacked with the *bar* gene for glufosinate tolerance.

The dicamba-tolerant plants are examples of many projects that may languish in universities and small companies due to the prohibitive cost and time needed to obtain approvals for transgenic crops. Monsanto currently projects that it costs ~$100 million to bring a product from gene discovery to market. There needs to be a fundamental change in the regulatory system such as being able to register a trait rather than registering events.

**Allergy to Soybeans, Allergens and Cross-Reactivity of IgE in Legume Allergy (R. Goodman, UNL)**

Currently, the Allergenonline database resides at UNL. This database is used by most companies to perform homology searches on allergens to their potential product proteins. The Food Allergy Research and Resource Program (FARRP) was established in 1995 between UNL and seven founding members.

There has been a lot learned about soybean allergies in the past 20 years but there are still a lot of “holes” in the knowledge base. Many adverse reactions to foods are mistaken for allergy. These adverse reactions are often caused by bacterial food poisoning. Enzymatic deficiencies also mimic food allergy such as lactose intolerance.

IgE-mediated reactions are in response to parasites, etc. whereas non-IgE-mediated reactions are reactions such as celiac disease or protein-induced enterotitus. Allergy is a reproducible reaction which occurs in the same person with the same food producing the same symptoms. Allergic reactions may progress from dermatitis or hives to vomiting, asthma or anaphylaxis. Few people outgrow allergies to peanuts, lactose, tree nuts, etc. Thirty percent (30%) of people have allergies to inhaled antigens. IgE-mediated allergies are the most common. Eight foods account for 90% of food allergies and requie labeling. These include peanuts, eggs, crustaceans, milk, fish, tree nuts, wheat and soybeans. However, allergies to to wheat and soybeans are not as severe as was once thought. In the EU, celery root, mustard and sesame seeds are added to list and Asia adds buckwheat.

Allergy prevalence varies with age. In children, ~6% have food allergies whereas in adults the prevalence is ~3.7%. Food needs to be ingested by individuals in order to be

exposed to allergenic proteins. It cannot be predicted which individuals will have a reaction. Proteins cause allergic responses due to the IgE binding epitopes.

Diagnosis of allergies is difficult. *In vivo* diagnostic methods include compiling a clinical history, engaging in an elimination diet to pinpoint the offending food, skin prick tests with antigens and food challenges. *In vitro* diagnostic consists of specific IgE measurements.

Allergenic proteins in foods classified as allergenic vary with the food. Peanuts have 3-5 major allergenic proteins (2S albumins, 7S and 11S globulins), 5-7 minor allergenic proteins in a total of 10-40,000 genes. Soybeans contain 3-5 moderate allergenic proteins and corn has one major allergenic protein (LTP).

There are several IgE test methods available. These include dot blot (microarray), immunoblot, ELISA, RAST, EAST. The RAST (radioallergosorbent test) or EAST detects specific IgE antibodies to suspected or known allergens from blood samples via radiolabelled antibodies. There is a lot of "bad" data available due to the loose selection of subjects, quality of skin prick test reagents, lack of challenge tests and low specific IgE binding. For IgE tests, the specificity of the anti-IgE detection antibody needs to be characterized to avoid false results.

The food allergy labeling act was enacted to protect those allergic to common allergens in food. Humans learned to eat safely through experience such as knowing that legumes must be cooked to inactivate lectins and trypsin inhibitors. Allergic individuals must avoid the offending foods.

For GM products, the weight of evidence approach has been utilized for allergy assessment. This includes determining whether the protein of interest is derived from an allergenic source, assessing the sequence homology to known allergens and the *in vitro* digestibility through testing in simulated gastric fluid. Regulatory agencies also want evidence that the genetic modification did not change the endogenous allergenicity of the plant, i.e., the endogenous allergen levels in soybean are no greater after the genetic modification.

Dr. Goodman has published an article on the interpretation of the 2003 Codex allergenicity assessment (Goodman, RE, Vieths, S., Sampson, HA, Hill, D, Motohiro, E, Taylor, SL, and van Ree, R. 2008. Allergencity assessment of genetically modified crops—what makes sense? Nature Biotechnology 26: 73-81).

**High-Throughput Absolute Quantitation of Soybean Seed Allergens using MS (J. Thelen, U. of Missouri):**

The project has been funded by International Life Sciences Institute (ILSI) HESI Protein Allergenicity Technical Committee (PATC). The objective of the project was to endeavor to understand the range of variability of endogenous allergen expression in non-genetically modified soybeans. The information will be useful for determining the

tolerable level of endogenous allergen expression and could be useful for developing guidelines for genetically-modified (GM) crops.

The first task was to quantify ten (10) protein allergens from nineteen (19) commercial soybean accessions. The protein preparation from dry seed was optimized (method published) and then the assay was developed for relative and absolute quantitation of the allergens using mass spectrometry (MS) from the soybean isolates.

The developed method utilizes label-free proteomics—quantitation and identification re coupled (inexpensive and fast). The method employs spectral counting using ion current (peak) integration. Spectral counting monitors the frequency of peptide fragments during liquid MS/MS. Soy samples were spiked with an internal standard (BSA) and then analyzed via LC MS/MS. The MS data was mined against the translated soybean genome resulting in identification of 100 proteins, including all 10 allergens. The spectral counts of the unique peptides were matched to the allergens. The technical variation was 11%. Glycinin G1 and G2 showed lower variation ranging 8-11% whereas glycinin G4, Gly m 6 and Gly m 5 showed higher variation than BSA. The last set of allergens exhibited much variation of 18-31%.

For quantitation, AQUA (absolute quantitation) peptides were made which contained a single stable isotope-labeled amino acid. The labeled amino acid contained 98% $C^{13}$ and 98% $N^{15}$ isotopic content. The amino acids labeled were either arginine or lysine. The labeling increased the molecular weight by 10 thus there is a difference in mass between the AQUA and native peptides. This difference allows the quantitation of the native peptides.

The linear range of quantitation of the soybean matrix for each peptide was determined. This was dependent on the size of the column and the type of mass spectrometer. The results showed a narrow window for all the allergens to overlap. The linear quantitation range for the AQUA peptides in the soybean matrix was also determined. This range was much broader which allowed for a robust multiplex assay.

The results indicated that the highly abundant proteins were the least variable. The moderate abundant proteins were highly variable in expression with the Kunitz inhibitor being extremely variable. The low abundant proteins had the highest variability. Relative and absolute quantitative data showed similar patterns.

In summary, synthetic peptides (15) were prepared for 10 allergens. The LC MS/MS was optimized for 13 of the AQUA peptides. The relative and absolute quantitation showed similar data patterns. The quantitation between different peptides of the same allergen may be due to alternative cleavage sites. The variability between allergens is not consistent and the total amount of allergen per soybean variety is similar.

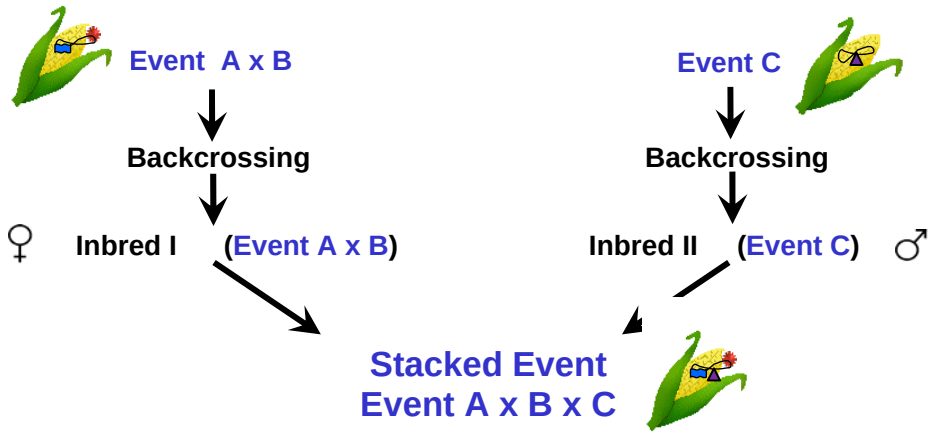
**Review of ILSI Taskforce 9 on Stacked Events and Safety Assessment Recommendations (N. Weber, Pioneer/DuPont):**

Stacked trait products (or combined event products) may be made via molecular methods (inserted DNA contains two or more genes/traits), transformation methods (approved event is transformed with additional trait(s)) or stacking (combining two or more events in a plant via traditional breeding methods). Gene stacking is not new since multiple conventional traits have been combined in plants for decades.

A molecular stacked trait product and those created by transformation of existing approved events are treated as a new events and must be approved or authorized by regulatory authorities. Stacks resulting from conventional breeding are also regulated by certain countries although the data package may be quite small (bridging data to parental events) to quite extensive (molecular, expression, composition on stacked product). Regulations vary across countries and can result in asynchronous approvals.

Molecular stacks generally are a single locus insertion whereas stacks made via transformation of an approved event, the insertion may be at multiple loci. In breeding stacks, each insertion is at a separate locus. Each loci is detected individually by PCR.

An example of breeding a stack with 3 events is as follows:




Breeding may become more complex with more events.

Stacks are important to the grower and consumer since the products will target specific consumer needs. It allows the crossing of events to create custom varieties. Global usage of stacks will continue to increase. In 2009, the global commercialization of GM events was as follows:

| **Trait** | Corn | Soybean | Cotton |
|---|---|---|---|
| Herbicide Tolerance | 2 | 1 | 3 |
| Insect Resistance | 7 | | 8 |

In 2010, stacks comprise ~50% of the acreage for corn and 60% of cotton acreage. By 2015, new events in the regulatory process and R&D pipelines is projected to be:

| **Trait** | Corn | Soybean | Cotton | Rice |
|---|---|---|---|---|
| Herbicide Tolerance | 2 | 10 | 2 | 3 |
| Insect Resistant | 5 | 3 | 13 | 8 |
| Disease Resistant | | | | 4 |
| Crop Composition | 6 | 3 | | 2 |
| Abiotic Stress | 2 | | | 2 |

The entrance of these new events will result in an exponential increase in the number of new stack products, i.e., theoretical 3-way stacks = >2000 and 4-way stacks = >10,000.

In the future, there will be increasing global cultivation and development of GM traits/events which will increase the frequency of stacked event submissions to regulatory authorities.

CropLife International (CLI) is one of the organizations working on issues with the increasing development of stacked products. CLI represents the plant science industry (BASF, Bayer CropScience, Dow AgroSciences, Monsanto, Pioneer/DuPont and Syngenta). CLI encourages understanding and diaglogue about agricultural technology and promotes agricultural technology in the context of sustainable development. CLI leads and coordinates a global network of regional and national associations. The Combined Events Project Team (CEPT) is under the Plant Biotech Regulatory Steering Committee.

The International Life Sciences Institute (ILSI) is a nonprofit, global organization whose mission is to improve public health and well-being. It is a tri-partite neutral forum of academic, government and industry scientists. ILSI is funded by industry members, governments and foundations. The ILSI International Food Biotechnology Committee (IFBiC) is funded my industry members (BASF, Bayer CropScience, Dow AgroSciences, Mars, Monsanto, Pioneer/DuPont, Proctor & Gamble, Syngenta). IFBiC supports the development and harmonization of science-based regulations around the world for biotechnology-derived food products. IFBiC addresses scientific issues related to the development, distribution and safety of such food and food products and disseminates

science-based assessment of biotechnology-derived food products globally. The ILSI IFBiC Taskforce 9 is considering the safety assessment of stacked event products. The project scope is stacks combined through conventional breeding techniques with the individual events already assessed for food and feed safety. The taskforce is considering two questions: Does stacking affect the stability of the inserted transgenes? How can potential trait interactions be assessed in a stacked event product?

For genomic stability, the baseline knowledge is that:

a) the plant genome is dynamic,
b) genetic variation occurs naturally in all plants through processes such as transposon movement, recombination and DNA mutation,
c) genomes evolve
d) new varieties are developed.

The baseline knowledge about transgenic events is that the insertions are stably integrated into the genome which is demonstrated from the breeding programs and demonstrated for the safety assessment. No situation surrounding stability is unique to stacks (repeated elements, gene silencing, changes to proteins). Thus, genomic analysis of stacked event products would not contribute to the safety assessment of the products.

For interactions, the baseline knowledge is:

a) potential interactions considered in the context of breeding,
b) scientific baselines of the single transgenic events are the prior safety assessment and the understanding of transgene function,
c) consideration specific to the crop species.

Interactions in conventional crops are universal, extremely important, underlie the phenomenon of heterosis, result in superior varieties, the mechanisms are not understood nor are the interactions testable. To evaluate interactions in stack products, the evaluation should be based on what is known and focus on the introduced traits. Considerations for the interaction evaluation include compartmentalization, expression patterns and molecular/biochemical activity. If there is a potential for the events to interact based on prior trait knowledge, this could require targeted food/feed assessment of the stack. If there is no reasonable expectation for interaction, i.e., no hypothesis for hazard exists, then the food/feed safety assessment of the single events are sufficient to assess the stack.

**Challenges in Interpretation of PCR Results for Biotech DNA (F. Spiegelhalter, Eurofins GeneScan):**

GM crops are generally tested via PCR, ELISA and lateral flow devices (LFD) Screening tests generally test for common elements of events such as the 35S promoter.

There is considerable acreage of conventional corn grown in the US which is co-mingled with other corn. At elevators, the trucks unload and the grain is tested via LFDs. For barges, the tests are LFDs and PCR. Usually about 1% biotech corn is in conventional corn. Testing for common elements, such as 35S, covers most of the GM corn. Other events may require special tests to detect them. Stacked events look the same as the single events in bulk samples and thus, can cause an over-estimation. In 2009, Japan published their sampling method. A 500g samples is ground and tested for 35S presence. If the result is close to the 5% threshold, then a single kernel test is conducted. If less than 2 kernels are positive for GM, the samples is considered non-GM.

The same sample analyzed by the same method and yielding the same data can yield different results? This may be caused by the choice of the method/analyte, the sub-sampling conducted, PCR set-up, calibration and the instruments used. Data interpretation is the biggest source of variability. Some labs use relative quantitation and oher labs use absolute values. Methods should cover everything—including data interpretation. In reality, this is not the case.

Quantitative PCR can be event-specific such that the PCR primer is targeted to the junction of the event insert and the genome DNA. Percent biotech DNA is determined from the following formula:

$$\frac{\text{Biotech DNA} \times 100}{\text{Species DNA}} = \%\ \text{biotech DNA}$$

The ratio is not an absolute amount although it is treated that way. Relative PCR methods have a chance if applied to GM products.

**The Power of One: the Importance of Isothermic Amplification for Agri/Industrial Diagnostics (J. Lilly, Neogen/GeneSeek):**

DNA testing is the one of the fastest growing sectors. PCR came about in 1980-1990 and all patent protection has not expired. There have been many advances but no dogmatic shifts. PCR will continue to be the dominant diagnostic platform for lab testing, however, diagnostics are moving away from the lab. The trend is toward field-based tests with low resource settings.

Why isothermal amplification? The specificity of molecular diagnostics allows for SNP-based resolution. Fluorescent probes are decreasing in price as well as the fluorescent readers. Thermocycling requires more hardware and the enzymatic reaction coupled with heating/cooling leads to matrix effects. The drivers for isothermal amplification include lower entry costs, simplicity, portability and speed (10-30 min). Isothermal amplification is basically PCR but with the use of a variety of enzymes. Detection in isolathermal amplification can be by an istrument-free lateral flow cassette or real-time fluorescence.

The workflow of isothermal amplification is to collect the sample, prepare the DNA, incubate the DNA and reaction mixture, read results. The faster the reaction, the greater the difficulty to quantitating.

There are several systems on the market. The Gen-Probe system is thermal mediated amplification in that the target is captured and the amplification, followed by fluorescence detection. NASBA is nucleic acid sequence-based assay. RPA is recombinant protein amplification and RT-LAMP using RNA loops.

Isothermal amplicfication is truly the next generation of molecular diagnostics due to its low resource environment, easier, faster, cheaper with plenty of options.

**DNAble: Rapid On-Site DNA Detection Test Kits (T. McFadd, EnviroLogix):**

DNAble is:

- Easy to use
- Composed of a 3-step amplification
- Reagents are stable at room temperature
- Portable instrumentation
- Two detection formats
- Rapid qualitative test (14 min)
- Self-contained amplification products
- Specificity down to a single nucleotide base
- Sensitive detection of DNA

There are traits on the market or coming to the market that will have very low protein expression and/or no novel protein expressed. There is a need for diagnostics for these products.

DNAble workflow is: sample preparation, rapid isothermal nucleic acid amplification (NEAR), fluorescence detection. In NEAR, the primer design is the first level of specificity. The primers incorporate a nicking sites. For every 1000 bp there is a nicking recognition site. The polymerase binds and extends at these sites and this happens over and over. The products can become targets again. Amplification is carried out at 56C. Detection is via fluorescence. A molecular beacon binds to targets and fluoresces. There is a separate beacon for the internal control. Results are qualitative—yes or no. The detection may also be a lateral flow device (LFD). The strip is in a closed cartridge and functions as a traditional LFD with capture and control lines. Samples may be used from crude extracts.

The kit has been validated with sensitivity and specificity in conjunction with sample preparation. Reproducible LLOD, ULOD, negative results with conventional matrices, and negative results with other matrices have been generated. Robustness had also been determined. Real-time analysis is the goal—qualitative, initial step to quantitation, 96 well throughput, multiplex reactions and RNA detection. Another goal is to develop custom assays in 3-4 months.

A pathogen kit has been demonstrated and a GM event kit should be ready in Q1, 2011. Currently, the technology has worked in potato, corn, soybean, cotton, rice and tomato matrices.

**Detection Tools for Agricultural Biotechnology (R. Shillito, Bayer CropScience):** The general process for developing a biotech crop is to a) identify the trait/crop, b) isolate the gene, c) transform plant cells, d) select the cells that contain gene of interest and regenerate into plants, e) confirm the functionality/efficacy of the transferred gene/trait and f) select the commercial event. Analytical methods used in this process include herbicide spray, protein-based, DNA presence, DNA quantitative and protein quantitative methods. Many events are produced but only a few are chosen to go into development. Events are sorted based on their molecular complexity, expression of the protein of interest, agronomic characteristics, efficacy, etc.

When should detection methods be developed? A key inflection point is when a single event is chosen. The technology providers generally develop the DNA-based detection methods for their events around this time.

As the product nears the market, methods and kits are validated by external laboratories and government authorities. External testing laboratories and government agencies may also develop methods. For this reason, it is desirable to minimize the number of methods out there for specific traits/events.

The level of a detection method specificity is high for an event specific method and low specificity for a gene-specific method. Quantitative vs. qualitative tests—when to use?

- Qualitative tests are sufficient for the initial identification of an event and for breeding purposes,
- Qualitative tests are used in sub-sampling approaches or for single seed testing,
- Quantitative and/or qualitative tests are required by some government agencies as part of the approval process,
- Quantitative tests can identify low level presence in seed, and
- Quantitative cannot be used to do purity testing.

CropLife International Detection Methods Project Team (CLI DMPT) advocates for the implementation of harmonized/practical laws, regulations or polices for the development, validation, utilization of detection methods for plant biotechnology products. CLI DMPT have committed to make detection methods and reference materials available to meet regulatory requirements, consumer preference and to facilitate stewardship of products. CLI will publish detection methods, associated information and internet links to the sources of reference materials after approval of the product and generally at the time of commercial sales. On the website, those requesting the methods must agree to the terms contained in the drop-down information box.

The benefits of this program are that it will a) enable other companies to test for adventitious presence in their seed without additional legalities, b) establishes clarity of

the ownership of methods, c) provides transparencey, stewardship and goodwill, d) questions and inquiries about the methods will come directly to the trait providers. The databse is projected to be available prior to the end of 2010.

**A Rapid, DNA-Based Field Detection Technology (D. Grothaus, Monsanto):**

As more biotechnology products are brought to market, there is a need for rapid detection of these products in the field. The current state of rapid field detection is protein-based (e.g., lateral flow devices). The current state of DNA detection is lab-based, complex methods which are slow to perform, costly and low volume sample processing. Monsanto has first developed rapid DNA detection to support the Roundup Ready 2 Yield soybean product.

Monsanto is using the technology of Twist Dx, a biotech company in Cambridge in the UK. They are working directly with the inventors of the RPA technology whose core focus has been in the medical diagnostics area. Monsanto has an exclusive agreement with Twist Dx for agricultural biotechnology applications.

The RPA systems is an isothermal system which works at 39C and takes about 10-15 min. The specificity of the system is down to a single nucleotide. The workflow of the system is a) grinding of the seed sample and b) placing in lysis buffer, c) transfer of 1 bacterial loop of lysate and pelleting by centrifugation and then to d) the rehydration buffer, e) the mixture is incubated at 39C and then f) detection is carried out via fluorescent monitoring. The test can be in real-time or may be carried out on an instrument-free platform. Under perfect test conditions, 0.1% RR2Y soybean can be detected in RR1 soybean.

There is no kit available yet but 8-10 assays have been developed. Monsanto has a need to launch the technology within the next 2 years due to the launch of RR2Y in South America and the need to distinguish it from RR1 soybeans. Monsanto's goal is to have a semi-quantitaive dipstick available in 2014 but an instrument-free system in 2012. Monsanto is open to partnering with other technology providers in order to keep one consistent platform out in the public domain. They are currently in negotiations with two other agricultural biotechnology companies.

**Multiplex GMO Screening: A Unique 4-Target RT-PCR (C. Harzman, BIOTECON Diagnostics):**

BIOTECON Diagnostics Gmbh is located outside of Berlin in Potsdam. The company was founded in the 1980's and is now partnered with Merck (EMD). The company has products for the food industry for pathogen detection and spoilage organisms (particularly in beer products). They also have products for hygiene screening such as for the Staphylococcus organisms. The GMO screening test kit was introduced in 2010 and is used for food, beverages, feed and cosmetics. The company is ISO certified and authorized to work with pathogenic organisms and GMOs (requirement in Germany).

The 4-target RT-PCR kit has flurorescence for real-time detection and is compatible with most open platform RT-PCR instruments. The kit can detect 95% of the GMO products

currently on the market. It is not even-specific and relies on the detection of 35S, NOS, *bar*, FMV and other common elements. It is a qualitative test with 128 reactions to accommodate 64 samples (duplicate reactions/sample). The kit also contains positive and negative controls and uracil-N-glycosylase (UNG) which degrades old amplification products. Twenty-one (21) GM crops have been tested and 50 different food products. The LOD is 10 initial template copies.

There is a publication which describes the kit at:

Dorres, H, Remus, I, Gronewald, A, Gronwald, C, Berghof-Jager, K. 2010. Development of a qualitative, multiplex real-time PCR kit for screening of genetically modified organisms (GMOs). Anal Bioanal Chem (2010) 396: 2043-2054.

**********

The meeting was adjourned and those who were interested took a tour of UNL's Beadle Center. The Beadle Center was named in honor of Nebraska native George W. Beadle, a Nobel prize winner in genetics for his and Edward Tatum's contribution to biochemical genetics by delineating the idea that a gene could only give instructions (information) for the production of a particular chain of amino acids (proteins) that would become the specific enzyme (protein) that would enable the production of a certain physical trait.