

AEIC Spring Meeting 2011
 April 13-14
 Indianapolis, IN

P.L. Hunst, Secretary

The AEIC Spring Meeting 2011 was held April 13-14 in Indianapolis, IN and was hosted by Dow AgroSciences. Twenty-two companies and organizations were represented.

Dow AgroSciences Welcome (K. Clayton, Dow AgroSciences): Dow AgroSciences provides science solution for agriculture. The company provides products in several areas including plant genetics and seeds, biotechnology, crop protection, urban pest management, vegetation management, range and pasture management. Dow AgroSciences (DAS) is wholly-owned subsidiary of the Dow Chemical Company. DAS' roots were the Dow Chemical Agriculture Business (1950-1989) which united in a joint venture with Eli Lilly Elanco to become DowElanco. From 1990-1996, DowElanco was headquartered in Indianapolis and made acquisitions such as Mycogen. In 1997, DowElanco was renamed Dow AgroSciences when Dow Chemical acquired 100% of the joint venture. Since then, DAS has continued to grow by acquisitions of several U.S. seed companies such as DairyLand. DAS' markets are diverse, ranging from solutions for row crops to right-of-ways to protection of buildings and other structures. In 2009, global sales totaled \$4.5 billion. North America accounts for 42% of the sales, followed by Europe (23%), Latin America (22%) and Asia (13%). Herbicides (55%) account for the bulk of the sales, followed by insecticides (15%) and seeds/traits (15%). DAS has an active R&D pipeline for both chemicals and biotechnology. R&D is carried out in over 40 countries and includes agreements with World Wide Wheat (Arizona), Chromatin, Exelixis and NemGenix (Australia). DAS is focused on strategic growth worldwide through targeted innovation.

AEIC Business Meeting

The AEIC Business Meeting was brought to order by President Laura Privalle.

Secretary's Minutes of Fall 2010 Meeting: A motion was made, seconded and received a positive vote to accept the minutes as posted on the AEIC website.

Treasurer' Report (D. Layton):

2010 Budget—

| | Proposed | Actual |
|-------------------------|-----------------|---------------|
| Beginning balance | 20564.00 | 20564.00 |
| Membership dues | 8000.00 | 9350.00 |
| vgTotal revenue | 8000.00 | 9350.00 |
| | | |
| Expenditures | | |
| Scientific paper | 4000.00 | |
| DE Franchise tax report | 25.00 | 25.00 |
| ANSI/ISO | 2900.00 | 2900.00 |
| Board meeting | 100.00 | |
| Spring meeting expenses | 1000.00 | 1093.00 |
| Website | 500.00 | 128.00 |
| Bank service charge | | 6.00 |
| Fall meeting expenses | 1000.00 | 2198.00 |
| Reprints | 300.00 | |
| Subscriptions | 100.00 | |

| | | |
|------------------------|----------|----------|
| Miscellaneous | 100.00 | |
| Total expenditures | 10125.00 | 6360.00 |
| Balance | 18539.00 | 23563.00 |
| | | |
| Certificate of Deposit | 11396.00 | 11396.00 |
| Interest | 150.00 | 152.00 |
| Total | 30084.00 | 30111.00 |

2011 Budget—

| | Projected | Actual |
|-------------------------|-----------|---|
| Beginning balance | 25653.00 | 25653.00 |
| Membership dues | 8000.00 | |
| Total Revenue | 8000.00 | |
| | | |
| Expenditures | | |
| Scientific paper | 4000.00 | |
| Wire transfer fees | | |
| DE Franchise tax report | 25.00 | |
| ANSI/ISO | 2900.00 | |
| Board Meeting | 100.00 | |
| Spring Meeting expenses | 2500.00 | |
| Website | 500.00 | |
| Fall Meeting expenses | 2500.00 | |
| Graphic design expenses | | |
| Reprints | 300.00 | |
| Subscriptions | 100.00 | |
| Miscellaneous | 100.00 | |
| Total Expenditures | 17025.00 | |
| | | |
| Balance | 14538.00 | 25653.00 |
| Certificate of Deposit | 11548.00 | 11581.00* (withdrawn and put into interest bearing account) |
| Interest | 120.00 | |
| Total Assets | 26206.00 | 35144.00 |
| | | |

A motion was made, seconded and voted positive to accept the 2010 budget and the proposed 2011 budget.

Membership Update (D. Layton):

| Member | Number |
|--------------------|--------|
| Large Companies | 13 |
| Small Companies | 13 |
| Associate Members | 2 |
| Individual Members | 3 |
| TOTAL | 31 |

Solae joined as a member, however, they are owned by DuPont so their dues are covered by DuPont's membership.

It was suggested to contact Randy Giroux to see if another Cargill person would consider joining.

2011 Fall Meeting (C. Alarcon):

Pioneer/DuPont will host the meeting in Des Moines, Iowa. A date will be determined soon.

The following were suggested topics by the membership for the meeting:

- a) animal biotechnology
- b) crop focus—wheat, alfalfa
- c) sampling—ongoing issues at ISO by regulatory agencies
- d) export commodities—issues
- e) testing frequency based on country of origin, i.e., EU
- f) new food safety bill and mycotoxin testing—ask an FDA person to speak?
FDA invited labs last year to participate in allergen testing so it would be good to invite Organizers to speak on it.
- g) ILSI IFBiC workshops—share more in depth information on workshops
- h) economic fraud/authenticity testing in food

Spring Meeting 2012:

Eurofins GeneScan has offered to host the meeting in New Orleans, LA.

Update on AEIC paper drafts:

Protein vs. DNA (D. Layton, T. McFadd, R. Jenkins, G. Shan, T. Scholdberg, C. Alarcon, B. Kaufman): The paper will look at the pros and cons of each method for each application such as grain, food, etc. Case studies will also be included. The group had an outline and each member has been assigned a section to draft.

Quantification by sub-sampling (B. Kaufman, K. Remund): This paper will be based largely on statistics so Kirk and other statisticians will be working with Beni.

Commercialization of a new biotech event (L. Privalle, C. Zhong, J. Chen, G. Clapper, P. Hunst, F. Spiegelhalter): The group has put together an outline and sections have been assigned for drafts. Most of the draft sections have been received. It was decided to work with a consultant to bring all the sections together and make it a cohesive, edited paper. Laura has received a quote from Virginia Pantella at VIP Consulting so negotiations are in progress. The timeline is 6 months to complete the paper. Several journals have been identified such as GM Crops and Nature Biotech to submit the paper to when completed.

AEIC Brochure (D. Layton):

To facilitate member access to the brochure, a PDF will be posted on the AEIC website for download and printing. Dean has printed brochures available so member just need to request them.

UPDATES

ILSI Workshops (D. Grothaus): Mini-workshops are planned for Korea and Singapore. A full workshop will be held in Brazil in September, 2011. India has also requested a workshop but plans are not set at the moment. There was discussion of holding a workshop in North Carolina, however, the ILSI group has been struggling to define who the audience would be, i.e., US agencies and if so, why do they need a workshop? The group currently has 17 different modules that have been used for the workshops and some of these have recently been updated.

GMO Conference: The GMO Conference 2011 has been postponed until May or June, 2012. The conference will most likely be held in Italy (home to JRC who is organizing the conference).

ACS Symposium (L. Privalle): BASF is organizing a symposium to be held Aug. 28 – Sept. 1 at the ACS Meeting in Denver. This will be an opportunity to have AEIC brochures there to increase awareness of the group.

CLI Detection Team website launch (D. Grothaus): The CropLife International (CLI) Detection Methods Team has launch a website on detection methods (www.detection-methods.com) which contains detection methods from the technology providers for their products. Methods can be downloaded from the site, however, fee-for-service labs will need a license from each individual company prior to downloading.

ISO/TC 34/SC 16 (G. Clapper): The 3rd Plenary Meeting will be held in the U.S. on October 25-27 at the George Washington Carver Center in Beltsville, MD. Everyone is invited to attend as observers. There are new work items on topics such as microsattellites and pathogen detection.

Bio Trade Status Database (P. Hunst): BIO has launched a database (www.biotradestatus.com) to provide public users an easy-to-use and updated source of information on the global authorization status of commercialized agricultural biotech events and products for food, feed and/or cultivation. The contributors to the database are BASF, Bayer CropScience, Dow AgroSciences, Monsanto, Pioneer/DuPont and Syngenta.

ILSI Crop Composition Database: The ILSI Crop Composition database (www.cropcomposition.org) has recently been updated via the release of a new version of the database.

New AEIC Goals/Activities: D. Layton brought up a suggestion for AEIC to take over the method verification testing of kits. GIPSA had been performing these but they will now only do for items that they have directives for. A suggestion was to have an organization such as Illinois Crop Improvement (which has lab facilities) perform the verifications on behalf of AEIC. Another suggestion was to use a lab(s) within AEIC membership. AOCS indicated that they may be able to administrate the program for AEIC. Method verification is not proficiency testing. There is a need for method verification in the marketplace. Dean will explore further the feasibility of AEIC engaging in this and report back to the membership.

INVITED TALKS

Use of Quartz Crystal Microbalance Technology to Directly Measure Molecular Interactions on the Cell Surface (S. Fogarty, Tecan): Tecan acquired Attana Biosensor Technology (www.attana.com) which was established in 2002 in Stockholm, Sweden. Attana has a presence in the EU, U.S. and Asia and provides label-free detection methodology. In quartz crystal microbalance, an AC-potential is applied to a piezoelectric quartz crystal. The crystal then oscillates at its resonance frequency. Adding or removing molecules from the surface of the crystal alters its frequency, thus the crystal is used as sensitive microbalance. Cells or other bioreceptors may be coated on and then an antibody is injected. When the antibody binds to the bioreceptor, the frequency change is monitored. Buffer is then added and the antibodies dissociate and this dissociation can also be measured. The measurement of changes in mass is independent of cell size attached to the biosensor surface. Biosensor chips are coated for a range of biochemical and cellular purposes. The chips are the size of a microscope slide and can be coated with cells, carboxyl molecules, biotin, gold, etc. Attana has several models of instruments to support the biosensor chips. There are single channel, dual channel (for dirty material) and cell-based (dual channel optimized for cell binding). The capacity is 2 x 96 deepwell blocks and replicate injections can be made from single samples. The sample throughput is determined by the binding properties of the specific ligand.

For cell chips, the cells are dispensed and allowed to bind overnight in the cell. Cells such as red blood cells and bacterial cells as well as mammalian tissue culture cells can be used. The cells bind to the polystyrene surface. The goal is to have 60-80% confluency across the surface. Cells are fixed with formaldehyde and incubated with a fluorescent dye (such as DAPI). High cell numbers cause the frequency to go down due to the access to the cell receptors being reduced. The cell surface can be regenerated but varies by cell type. Over time, the signal will go down due to the cell receptors becoming damaged. Live cells can also be used, however, they are harder to handle and the regeneration process does kill them off.

Several applications have been tried with the quartz crystal microbalance including metastatic cancer biomarker screening (MD1 target), target immunotherapy (tumor specific monoclonal antibody to kill cancer cells) and determination of the glycosylation profile of cancer cells.

In summary, the system bridges the gap between traditional biosensors and cell-based assays; enables the study of binding kinetics directly on the cell surface; and biologically relevant data can be obtained at an early stage in the evaluation process.

Bio-layer Interferometry on the Octet Platform for Label-Free Crude Sample Analysis (S. Kumaraswamy, ForteBio): Bio-layer interferometry is a label-free, real-time assay based on the Octet platform. There is 16-channel simultaneous monitoring on 96-well and 384-well microplates with a throughput of 1000 or more samples in an 8-hour day. Assay development is rapid and the disposable biosensors reduce the cost of testing. The biosensors are affordable and the ability to regenerate them drops the cost to \$0.05 - \$0.80/well. Re-racking of the biosensors will further reduce the costs. Complex samples are well tolerated in the system.

Interferometry involves a white light source going through fiber-optic channels. The optical layer reflects the reference signal with a second reflection from the binding surface of the biosensor. The analyte changes the thickness of the bio-layer which is measured at the detector. Unbound molecules have effect on the signal. The 'dip and read' technology requires no labels, has direct measurements and is fast and easy. Real-time monitoring in a microplate-based platform. The system is microfluidics-free. Crude samples may be analyzed which reduces the sample preparation.

The Octet system can perform quantitative assays such as direct and sandwich ELISA (mg/ml to sub-ng/ml). For kinetics, the system is label-free and analysis can be done on proteins, peptides, oligos and small molecules. The system can be used for function testing, rank ordering, epitope binning (determining if the same antibody clones share the same epitopes or recognize different regions of the target protein) and isotyping.

The Octet instrument has an optics box which moves sensors to the samples. The 8-channel system provides medium to high throughput. The samples are in a 96-well plate on a temperature-controlled orbital shaker. Samples remain inside the microplate so it is non-destructive testing. All systems (except the Octet QK) allow re-racking of the biosensors in the sensor tray. Converting ELISA to the Octet system results in a reduction in the number of solutions and reagents, a reduction in quantities of reagents, no capture plates, reduction in hands-on-time required by the analyst, reduction in total assay time from 6 hours to 1.5 hours, improved accuracy/precision and less assay failures. An example was shown of using crude corn extract containing a protein X (proprietary). The direct binding assay used crude extract and biosensors loaded with specific antibodies. One dip in the samples can provide a standard curve in ~300 minutes. Concentration of samples is calculated directly from the standard curve. Another example was the ability to delineate binding kinetics/equilibrium of small molecules and the ability to do a binding pre-screen of a compound library.

The assays for the Octet system are 1-step (direct binding), 2-step (2 incubation steps) and 3-step (similar to most ELISAs in format). The 3-step assay is faster than an ELISA since it is automated and no-washes minimizes the handling. The Center for Disease Control (CDC) uses the Octet system in influenza surveillance. The assays could detect HA antibodies in serum and plasma with high sensitivity and minimal sample processing. The disposable biosensors reduced cross-contamination and there is a long shelf life of the HA-coated biosensors. The real-time, label-free method allows the kinetic characterization of HA protein to antibodies.

In summary, the Octet system enables assay development, productivity improvements and has cost benefits. The assays are direct, high throughput assays allowing for small molecule kinetics, affinity and screening. Low affinity interactions may also be detected as well as cell signaling protein interactions monitoring. The system is easy to use, microfluidics-free, reliable and robust instrument platform.

EXZACT Precision Technology (S. Berberich, Dow AgroSciences): The EXZACT technology is a toolkit which allows adding (single gene traits, stacking of traits, pathway engineering), deleting (gene/sequence removal/knockout) and editing (changes in sequence). The technology is based on zinc finger (Zn finger) technology from Sangamo. Zn fingers are transcription factors which bind to specific domains on DNA. Cleavage is the work-horse of the technology. The dimeric FokI nuclease domain

induces double-stranded breaks which invokes the natural DNA repair systems in the cell. The repair system uses the donor DNA provided by the Zn finger.

For the “add” function, the mechanism is analogous to classical gene conversion. This function allows re-targeting a transgene insert site and for stacking genes. The endogenous loci act as landing pads for the targeted insertion of the multiple trait genes. The donor DNA of the Zn finger is the template. Dow has successfully added a herbicide tolerance trait to a specific location within the corn genome (described in Shukla, et al., 2009. *Nature* 459 (7245): 437-441). This was a single copy, targeted, hemizygous or homozygous transgene integration with no concomitant integration of the Zn finger.

The “delete” function is targeted mutagenesis and/or DNA excision. It is based on non-homologous end-joining which is an imperfect ligation-like reaction. There is no foreign DNA present in the genome. Dow has shown precise, heritable transgene removal *in planta*. The target (marker) and Zn finger loci segregated independently. Thus, it is a genetically induced, targeted removal of a transgene.

The “edit” function is based on homology-directed repair resulting in pre-specified, direct allele modification.

The EXZACT technology reduces the probability of unintended genome effects; increases the technical probability of success for trait development; delivers time/cost savings during research and product development. The “add” function results in more efficient event creation. The “delete and edit” functions result in no foreign DNA being integrated and the end-product is distinguishable from traditional breeding, i.e., a more efficient mutagenesis/selection method.

In the ZmIPK1 corn project, the locations of mutations were known or pre-determined. No genetic material was introduced into the genome. The outcome (plants) was identical to existing products currently exempt from regulation. Thus, USDA APHIS concluded that the Zn fingers and the resulting outcomes (deletion-mutant corn lines) “posed no plant pest risks” and thus no permits were required for field testing. Discussions are currently occurring in the EU on these types of technologies and whether they should be regulated.

More information on the EXZACT technology may be viewed at www.exzactprecisiontechnology.com.

Improving and Enhancing Weed Control in Herbicide Tolerant Crops: Developing a New Family of Herbicide Tolerant Traits (T. Wright, Dow AgroSciences): The use of broad spectrum, non-selective herbicides in row crops has changed the focus of weed management. There is a greater emphasis on post-emergent weed control, an increase in no-till farming practices, greater use of burndown herbicide applications and reduced reliance on residual soil applied herbicides at planting. The advantages of the non-selective herbicide use is convenience, simplicity, flexibility, excellent weed control efficacy, reduced environmental impact, allows technology systems approach (combining insect resistant and herbicide tolerant traits) and allows growers to farm more acres with the same labor units. The challenges include the declining diversity in tools used, an over-reliance on a single mode of action, residual herbicides used less frequently and a decline of mechanical weed control practices. There has also been an increase in weed resistance, especially to glyphosate. Glyphosate-tolerant crop adoption continues to mature and is a platform for corn, soybeans and cotton. The rates of glyphosate (“rate creep”) continue to rise as the number of resistant weed species increases. New herbicide tolerant traits are needed to address this issue.

The Enlist herbicide tolerant trait provides proven power of 2,4-D combined with other weed control systems. Depending on the crop, Enlist also provides multiple modes of action: tolerance to 2,4-D in corn, soybeans and cotton; tolerance to glufosinate in soybean and cotton; and FOP tolerance in corn. 2,4-D is a common mixing partner for a lot of herbicides and it is not usually used alone. The *aad-1* gene is a plant-optimized gene from *Sphingobium herbicidovorans* which encodes aryloxyalkanoate dioxygenase-1 (AAD-1). When present, AAD-1 cleaves the aryloxyphenoxypropionate family of grass-active herbicides and is stereospecific for R isomers of phenoxy auxins and FOP herbicides. The protein is expressed in the cytosol and is a non-heme iron tetramer. Plants containing the AAD-1 protein display robust tolerance to pre- and

post-applications of 2,4-D in monocots and robust tolerance to applications of all commercially available FOP herbicides (propionic acids are preferred substrates).

The expression of the AAD-1 protein in corn protects the crop from 2,4-D damage such as goosenecking, brittle snap and deformed brace roots. Quizalofop is generally lethal to conventional corn, however, GM corn expressing the AAD-1 protein are protected. Dow is now using the *aad-1* gene as a selectable marker. A USDA petition for deregulation has been submitted for an event for use in corn.

Another gene, *aad-12*, produces the AAD-12 protein which has the same mode of action as AAD-1 but is stereospecific for S isomers. The herbicide tolerance spectrum includes phenoxy auxins (2,4-D, MCPA) and pyridoxyl auxins (triclopyr, fluroxypyr). Plant expressing the AAD-12 protein exhibit robust tolerance to pre- and post-applications of 2,4-D as well as applications of triclopyr and fluroxypyr. An event in soybean has been submitted to USDA for deregulation.

In summary, the Enlist herbicide tolerance improves glyphosate activity by allowing the use of 2,4-D and FOP herbicides to provide a broad spectrum of weed control and the use of burndown and pre-applications. Dow is promoting responsible use to sustain the viability of the technology and the compatibility with other crops/cropping systems and is providing distributors, retailers, growers and applicators comprehensive guidance and support to comply with state and federal regulations.

To go along with the Enlist herbicide tolerance, Dow has improved the formulation of 2,4-D (called Colex-D). Colex-D is a 2,4-D choline salt which reduces drift and has improved compatibility and lower odor (due to the manufacturing process).

Dow plans to launch Enlist herbicide tolerant corn in 2013. Enlist herbicide tolerance in other crops (soybean and cotton) will follow.

Method Improvements and Automation for Detection of Mycotoxins (B. Poepping, Eurofins): In the EU, aflatoxin levels have been determined by the CONTAM panel at 4-10ppb which should not be exceeded. Groundnuts and other oilseeds must comply and the following have been added—almonds, pistachios, apricot kernels, hazelnuts, brazil nuts and treenuts. Foodstuffs from Brazil must be sampled at 100%, from China at 20% and from Iran at 50%. For T2 and HT2 toxins in the EU, oats, barley, malt, maize and rarely wheat are at risk. The presence derives from a *Fusarium* organism. There are no maximum levels currently set for T2 and HT2.

Sampling and sample analysis for mycotoxins is done as follows. Three hundred (300) gram samples is split into 100 lots of 30g each. Each 30g samples is split into 3 ten gram samples. Therefore, the sub-sample is 50-75 grams of the original sample. Crude samples are filtered and cleaned up by utilizing immunoaffinity columns. The “clean” samples are tested using various methods such as ELISA, microarray biosensors, etc. The only reference method is a HPLC method. Recently, a LC-MS/MS multimethod has been developed to detect several mycotoxins. Robot supported mycotoxin analysis has been found to provide high reproducibility in analyses, however, the robot must be validated prior to use.

Canola: From Modest Beginnings to a Major Crop (L. Sernyk, Dow AgroSciences): Canola is from the genus *Brassica* and species *napus*, *rapa* and *juncea*. Oil from rapeseed had been used in Asia for thousands of years. It was found that rapeseed oil with high levels of erucic acid adhered to steam engines and thus, was a better lubricant than other oils. Prior to WWII, the EU and Asia supplied the North American demand for rapeseed oil as a marine lubricant. In 1936, canola was grown in Canada and showed that it was well adapted to western Canada. WWII promoted the need for rapeseed oil. T.M. Stevenson of the Canadian Department of Agriculture acquired seed from sources within Canada to plant and over the years, the acreage increased to 12,500 acres. In 1945, the first crush plant was opened in Moose Jaw, SK. After WWII, the acreage reduced to 500 acres due to the removal of blockades and the decreasing use of steam engines. William White started a rapeseed breeding program in SK and Henry Sallans of the NRC Oilseeds Lab in SK worked on the chemistry. Together, White and Sallans made the first variety, Golden, in 1954. This dedicated group of scientists believed in the potential of rapeseed as a crop and continued finding alternate uses for the oil. Acreage in Canada increased from 6000 to 760,000

acres (currently). The application of gas liquid chromatography for analysis of fatty acid profiles provided a breakthrough for breeders to be able to make improvements in the oil. Eventually the half-seed method was developed which allowed fatty acid analysis on one-half of a seed and then the other half of the seed could be planted.

In the 1970s, studies with high erucic acid suggested a link with fat accumulation around the heart. Health Canada promptly called for a switch to low erucic acid oils. Also, glucosinolates were also discovered to be goitrogenic. Keith Downey (SK) and Baldur Stefansson (Winnipeg) were at the forefront of canola breeding and had a friendly competition to develop the first low erucic acid variety. Thus, they became the fathers of canola. Downey introduced the first low erucic acid variety, Oro, in 1968. In 1974, Stefansson introduced Tower, a low erucic acid/low glucosinolate variety. The term “canola” is from “Canada” and “oil” and was trademarked in 1978. The name and trademark were transferred to the Canola Council in 1980. The long-term acreage projection in Canada is 20 million acres. In the U.S., canola is a newer crop and is grown mainly in North Dakota. Canola oil is healthy, light and versatile. It is low in saturated fats and is a source of omega-3 and vitamin E. Canola oil performs well in cooking, baking, salads and marinades, however, it has not traditionally been good in frying. In October, 2006, canola oil was granted a qualified health claim in regards to reducing the risk of heart disease. Canola meal is used as a source of protein in animal feeds. It is best in ruminant animal feeds since provides higher fiber as compared to soy meal. It can also be used as an organic fertilizer.

Spring canola is planted in May and harvested in September. Spring varieties fit well in cropping systems in the north central prairies in rotation with spring cereal grains. Although winter canola yields more, it is too cold in the northern U.S. to plant it. High yielding, disease tolerant varieties have been a key factor in the success of canola as a crop. Initial breeding focused on developing open pollinated varieties using classical breeding techniques. The development of anther and ultimately, microscope culture techniques in the 1990s gave canola breeders the ability to rapidly make fixed lines from F1 crosses. In the 1980s, the possibility of F1 hybrids in canola was investigated. Significant levels of heterosis were found for yield in hybrids produced by hand crossing. A number of genetic mechanisms were identified that had the potential to make hybrid seed, e.g., cytoplasmic male sterility. The first hybrids were launched in 1989-1991 and were based on the Polima cytoplasmic male sterility from China. It was challenging to find females with high levels of male sterility and it required the use of leaf cutter bees to transmit. The Ogura cytoplasmic male sterility system was developed by INRA in France. The male sterility on the females with this system is excellent. The system is from radish so there has been a challenge to move the restorer gene from radish to canola. The Ogura system is main cytoplasmic male sterility system utilized today. Hybrid adoption is up to 80-90% in Canada. Microspore culture has been found to be a very powerful breeding tool since it accelerates the production inbred genotypes and reduces population sizes. Molecular marker technology is now a routine tool for breeding new varieties.

In 1996, there was a shift to herbicide tolerance traits for weed control. As a result, in 2010, only 1% of the canola grown was conventional canola. There are 3 herbicide tolerances—Clearfield, Liberty Link and Roundup Ready. Liberty Link and Roundup Ready dominate the market. Insect resistant traits have not caught on since the insect pressure is not always there and thus, it is difficult to justify the return on investment.

Output traits have focused on the oil. Since the oil contains 10-12% linoleic acid, it is not well suited to frying applications. Canola oil had to be partially hydrogenated to reduce the linoleic acid but this resulted in the production of trans-fatty acids. Omega-9 oils have high stability, high omega-9 content, low linoleic acid and low saturated/low trans-fatty acids. Dow has omega-9 varieties (www.omega-9oils.com) marketed under Nexera varieties. There are over 100 users of omega-9 oil such as Taco Bell, Olive Gardent, Pop Weaver, etc. For the future, DHA omega-3 fatty acid oil is being developed. DHA has been shown to provide cognitive/brain function benefits as well as cardiovascular benefits. Dow has a collaboration with Martek, a manufacturer of DHA via algal fermentation, to transform their proprietary gene into canola.

Biochemical and Molecular Analysis of Canola (G. Clapper for D. Syme, Bayer CropScience): Variety registration of canola is done by Western Canada Canola/Rapeseed Recommending Committee

(WCC/RCC). The committee is made up of representatives from government, industry and growers. The committee reviews data from private and public trials (minimum of 11 trials conducted over 2 years in Canada) and make recommendations for registration based on agronomics and quality. The Canadian Food Inspection Agency (CFIA) is responsible for the approval and registration of the varieties which is based on the recommendations of the WCC/RCC.

NMR is used to measure the total oil content and uses whole seed (1-20g sample). The seed must be dried for CW models but pulse model instruments correct for residual moisture. Analysis time is <1 minute. Capillary gas LC is used for fatty acid analyses and glucosinolate analyses. Glucosinolates in canola cause the spicy flavor of mustard which is why animals do not like canola meal if too much of it is incorporated into their feed. Runtimes are typically <3 to 10 minutes and up to 200 samples/day can be analyzed. High throughput gas LC will perform fatty acid analysis in <1 minute. Glucosinolate analysis requires 2 days due to the sample preparation. Near infrared reflectance spectroscopy measures protein, oil, glucose, fatty acids, chlorophyll and moisture. It is non-destructive, single seed analysis and takes <1 minute.

There are lab accreditation and proficiency programs for the methods. These are run through the Canadian Grain Commission and the AOCS LLP.

Due to unforeseen circumstances, David was unable to attend and will be back at the next meeting to talk about the molecular aspects.

MoniQA Milk Allergen ELISA Validation Study and Recent Multi-Screening Developments to Allergen Detection by MS (B. Poepping, Eurofins): MoniQA is a program funded by the EU Commission within the 6th Program. There are 33 partners in the program which is running over 5 years with a funding of 12.3 euros. The program covers microbiological analysis, contaminants, food allergens, food authenticity, food additives and socio-economics. The key analysis aspects of the food allergens working package is validation (collaboratively trialed) using reference materials (produced materials of baked cookies spiked with NIST milk powder at 5 different levels). A key learning was what can go wrong in such trials. For instance, 3 labs used an extraction method that was inappropriate for the matrix. Some labs miscalculated the standard curves and some generated false positives. Seven labs failed to use the 50ppm standard despite clear instructions to do so. Three labs were unable to find milk with one commonly used kit in both the cookies and the soy-based infant formula. But despite these problems, the trial worked pretty well. All the kits performed well. Kit A was always lower than the others resulting in underestimates. Three of the 5 kits had a LOQ >1. At the 50ppm level, there were multiplication and dilution errors but overall, results were consistent across the kits.

For detection of allergens, it is difficult to run four or more different ELISAs to detect all allergens and at the appropriate levels. For example, detection of barley in beer by ELISA resulted in all readings below the LOQ. Use of mass spectrometry (MS), however, showed substantial amounts of gluten present.

In the U.S., the “big 8” is the allergen list but in the EU is the “big 14”. Thus, there are 26 protein targets and it would be advantageous to detect all of these with one method. An MS method was tested on bread that was baked containing 7 allergens (27 transitions/peptide and 4 peptides/protein). The MS detected the allergens but the sensitivity was not quite good enough. Another instrument was used which gave a 7X improvement in sensitivity. The results are being published in the Journal of Chromatography. ELISA kits were also used in the experiment. Three of the kits did not detect 1000ppm of egg. These results are being published in the AOAC Journal (April 15). In summary, in raw materials, both ELISA and MS perform equally well. In processed material, ELISA misses the detection targets whereas MS will still find the targets. More information on MoniQA can be found at www.moniqa.org/allergens.

Innovations in Portable Mass Spectrometry Systems (Z. Ouyang, Purdue University):

Miniaturization of mass spectrometry systems requires the simplification of the current process. To simplify the process, ambient sampling by desorption is utilized. Three methods have been looked at— DESI, LTP and paper spray.

DESI creates charged droplets of the sample analyte. Air is injected to create a spray of sample, the particles are charge and then go the MS instrument. The images are recorded using a methanol-water spray. LTP (low temperature probe) electrode discharges gas which cause a spray of the analyte, The particles are charged and then go to the MS instrument. This method can detect pesticides at the ppb level. The paper spray utilizes a paper loaded with sample. Voltage is applied to the paper along with water to create charged droplets which then go to the MS instrument. This method allows the wiping of a surface to sample such as wiping fruit to detect pesticides. This has also been used in therapeutic drug monitoring by placing a drop of blood on the paper and allowing it to dry. Methanol and water are added to create charged particles which can be analyzed by the MS. Quantitation can be achieved down to 0.1ppb. The use of the paper will eventually lead to the building of a cartridge for the sample which can then be put into a MS instrument.

Dr. Ouyang's lab has also found that a chunk of tissue can be put on the paper, apply methanol/water to create charged droplets and analysis is done by MS. Recently, plant parts have been used directly (such as leaves) to apply voltage to with methanol/water to create charged particles for MS analysis. Glucosinolates in brussel sprouts have been analyzed using this technique.

Dr. Ouyang's lab has also built a mini-MS instrument which weighs about 10 pounds. The key was to shrink the mass analyzer to be able to use lower voltage. Also, the use of a discontinuous atmospheric pressure interface (DAPI) avoid the necessity for a huge pumping system (seen on conventional MS instruments). The mini-MS instrument has the full function capabilities of a large instrument. The goal is to eventually get the mini-MS instrument down to the size of an iPod.

Advances in Rapid Measurement of Mycotoxins in Grain and Grain By-products (A. Davis, EnviroLogix):

The major mycotoxins analyzed for are aflatoxin, ochratoxin A, vomitoxin, fumonisin and zealarone. Mycotoxins are secondary fungal metabolites which affect animals by being hepatotoxic and carcinogenic. There are regulatory and guidance levels in place in various countries. In the U.S., total aflatoxin can be 20ppb for cattle and 300ppb for feedlot beef. For vomitoxin, the levels are 5ppm for swine feed and 10ppm for cattle feed. For fumonisin, 5ppm in horse feed and 60ppm in breeding cattle feed.

Mycotoxin testing facilities can vary between small crude labs in grain elevators to more sophisticated labs. Assays must have rapid turnaround times, give accurate/precise determination of levels and be a facile technology (minimal equipment, limited steps, data traceability).

Assay technologies include HPLC, EIA, immunoaffinity, fluorometry and lateral flow devices (LFD). HPLC will miss masked mycotoxins (those bound to proteins) and EIA tests are multi-step assays. LFDs are rapid and may be either visually read and qualitative or reader-based, quantitative assays.

Mycotoxins are small molecules with limited antigenic sites and have little similarity in structure. There are also isotypes which can be detected by immunologic means yet are refractory to HPLC determination resulting in discordance between the methods. Action or regulatory levels differ by three orders of magnitude from ppm to ppb.

The competitive LFD employs an immobilized mycotoxin and an antibody control line. The reagents are dry and thus, the LFD is stable. The QuickScan system is a flatbed scanner, PC with software, optional printer and a software key. The system allows for rapid assay times and flexibility (simultaneous processing of single assays or multi-strip combinations). The system is accurate, precise and provides traceability. Data reports are continuously updated and can be archived for trend analysis. The reports can also be printed for inclusion with individual lot analyses.

Quantitative assays are developed by first preparing a carrier protein derived with the mycotoxin. Animals are then immunized and antibodies produced and screened. The assay is optimized for extraction, strip reagent levels, buffers, etc. Calibration curves are developed and robustness tests are performed. QC is performed during production. The assay performance is validated for sample types.

In summary, quantitative LFDs meet customers' needs for rapid, easy, reliable, accurate tests for mycotoxins.

The meeting was adjourned and the group was transported to Dow AgroSciences for a tour of the facilities.

Participant Companies/Organizations at the 2011 Spring Meeting

Acadia BioScience LLC
Agdia
AgReliant Genetics
AIT Bioscience
AOCS
BASF
Bayer CropScience
BioAgilytix
BioDiagnostics
BIOTECON
Dow AgroSciences
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OMIC USA
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Purdue University
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SGS Brookings
Stine Biotechnology
Syngenta
Tecan
United Soybean Board