AEIC Fall Meeting 2015

October 20-21

South Bend, IN

Secretary's Minutes

P.L. Hunst, Secretary

AEIC BUSINESS MEETING (OCT 20):

The AEIC Business Meeting was called to order by David Levin, Vice President.

Secretary's Minutes of AEIC Spring Meeting 2015 (P. Hunst): A motion was made and seconded to approve the minutes as written. The motion was voted positive by the members present to accept the minutes.

Treasurer Report (D. Layton):

2015 Budget/Actual	Projected	Actual/Projected
Beginning balance	$34592	
2015 Membership dues	8000	$12800
CD interest	250	250
TOTAL	8250	13050
Expenditures		
Scientific paper	2000	
DE Franchise Tax report	25	44
ANSI/ISO	2900	2900
Board Meeting	700	766
2015 Spring Meeting	2500	3595
Website Re-design	2500	2583
Credit card processing	527	548
2015 Fall Meeting	2500	5055
Graphic design (brochure)		
Subscriptions	100	
TOTAL Expenditures	13752	15491
TOTAL Balance	28990	32150

A motion was made, seconded and voted positive by the membership to accept the Treasurer report.

Member Update (D. Layton):

Type	Number	Potential Dues ($)	Unpaid Dues ($)	Amount Unpaid ($)
Large	21	10500	2	1000
Small	14	3500	6	1500
Associate	2	100	0	0
Individual	5	500	3	300
TOTAL	42	14600	11	2800

Website Update (D. Theide): The Board has decided to disconnect from Pulatech and spend $1000 to clean up the website and transition to the Board for maintenance.

Spring 2016 Planning: The Spring Meeting will be held in Research Triangle Park, NC and will be hosted by BASF. Proposed dates will be communicated in the next couple of months.

The Fall Meeting will be held in Decatur, IL and will be hosted by EPL Laboratories.

The group was asked to brainstorm on suggested topics for the meeting. The list is as follows:

- Non-GMO testing methods: are they appropriately used and validated.
- Food Safety Modernization Act: what it entails, especially for testing
- Legislative landscape for labeling
- Focus on canola
- USDA APHIS development of standard for plant phytopathology labs: adapting PCR and NGS
- Mass spectrometry for proteins: what is being done

Manuscript Update: Critical Path Services (P. de Lisio): Manuscript has been developed and circulated to the authors. Comments have been received and will be addressed.

AEIC Goals and Activities (D. Levin/D. Theide): M. Petty (Monsanto) gave an update from the Composition WG meeting (preceded AEIC meeting). The Composition WG had a good discussion. The ELLA method for lectins is out for a vote to become an official AOCS method. This process should take about 2 months. For fat soluble vitamin multiplexing, the group found that UPLC works better than HPLC. The Allergen Sub-Team of the Composition WG has had several group calls and surveyed the group as to what methods are being used. Covance is using an ELISA method and other labs are using mass spectrometry methods. The sub-team hopes to have more data exchange. For cyclopropenoic fatty acids, these are being combined with the traditional fatty acids. The protocol for the method has been completed and the samples have been distributed to the participating labs. Cotton Inc. purchased the standard for the protocol. Morgan is transitioning to a new role within Monsanto. Thus, Nancy Gillikin (Bayer) and Carl Maxwell (DuPont) will co-chair the Composition WG. The WG will begin to harmonize analyte methods and will start with proximates methods.

Detection method training workshops are still being held by AACCI. The Board will reach out to R. Shillito to provide an update.

A question was posed as to whether the PCR paper (from 2005) needs to be updated since there are new methods such as isothermal PCR and digital PCR. The membership felt that there was not enough to warrant an update to the paper.

Vice President Nominations: Nominations were received at the meeting as follows:

- Yelena Dudin (Monsanto)
- Charles Pick (SeqID)
- Dave Rambow (Agdia)
- Donna Houchins (Romer Labs)
- Gina Clapper (AOCS)

P. Hunst will confirm with each nominee that they will accept the nomination. Additional nominees should be sent to the Secretary by Nov 1. The ballot will then be distributed in early Nov for members to vote.

ANSI/ISO Update (G. Clapper): The Quality Method Validation paper is ready to go out for a committee draft vote. There is also a guidance document/QMS for plant pathogen laboratories and a new work item proposal on meat speciation. In the near future, it is expected that there will be work items on isothermal methods. The plenary meeting will be in March and will be hosted by China. Only 5 people will be able to attend from the group and this will be the people running the topics.

INVITED TALKS

Evolution of Corn Breeding (J. De Leon, Dow AgroSciences): Maize was domesticated by the Mayans who converted teosinte into the modern maize. Modern maize has one stalk with one ear. Yields have continually risen since 1930s due to plant breeding and management practices by growers. There is currently more selection for stress tolerance occurring in the newer hybrids. Corn will be adapted in the coming years to sub-optimal soils and environmental conditions (such as flooding). Current trends in corn breeding include several areas. The first is genetic disease tolerance such as in Brazil for northern leaf blight and Puccinia polysora tolerance in China. For drought tolerance, epicuticular wax (EW) is being looked. EW is one of the most important traits for plant survival in a stress environment. EW is prevalent in sorghum. Two options would be to replace corn with sorghum (more sustainable crop) in a drought area or make corn imitate sorghum by producing more EW as a protection barrier. For flood tolerance, a below ground trait is needed. Corn which is flooded produces a predominance of adventitious roots. The goal is to have female-male synchrony under soil water flooding conditions. Brown mid-rib corn is a recessive trait which gives the corn less lignin and a higher digestibility for animals. Work is continuing to make corn lines with better agronomic characteristics. Another area for improvement is considering the source vs sink. Modern corn has one stalk and 1-2 ears/stalk. The upper ear predominates over the lower ear. The ear is the only sink for all sources and thus may be grain yield limiting. If the number of sinks can be increased, grain yield may be also be increased. Approaches to this are having twin ears, increased ear size or bifurcated stalk in which each stalk develops an ear. The bifurcated stalk trait has been found in South America and may be controlled by extra chromosome. More work is continuing with this trait. The future of corn may be a new era with native traits predominating.

Syngenta Integrated Strategy and Trait Pipeline (S. Herrero, Syngenta): Farmers need technology to increase value. There is continued grower demand for traits and multiple technologies are needed to

meet the demand. The trait market potential is $900 billion which is split for biotic stresses (weeds, insects, etc) being $300 billion and abiotic stresses (climate change, change in input factors, major pest shifts) being $600 billion. Weed and insect resistance are becoming more problematic. In the U.S., 58% of the farmers have been affected by weed resistance to glyphosate herbicides. Overall, 21 glyphosate resistant weeds have been identified globally. Controlling lepidopteran insect pests is a $2 billion market. However, lepidopteran resistance has developed to GM insect resistance crops in Brazil. Viptera is now the only insect resistance trait which controls fall armyworm.

Yield improvement remains as an imperative goal due to the population increasing. The yield increase rate cannot keep up with the population growth. Agricultural land use will be less in the future. In 2050, 25% of the world food production may be lost to climate change and land degradation.

Syngenta has an innovation life cycle which utilizes chemistry, breeding and genetically modified traits. The chemistry pipeline is rich. Syngenta currently has breeding programs in 8 different crops. For GM traits, there are 12 leads in late research potential, 10 leads in late research. In corn, traits are being worked on for above and below ground insects. Syngenta has a native trait for drought called Artesian. There are several molecular stacks in pre-launch. The next traits are for corn rootworm, a broad spectrum lepidopteran resistance trait, new herbicide tolerance, soybean rust resistance, sucking insect resistance, and drought/yield trait. Syngenta licenses out its traits through their Greenleaf program to independent seed companies and directly to other major seed/trait providers.

Syngenta is leveraging technology in genetics, chemistry and computational science for new products. The mode of action is being used to drive innovation by actively monitoring for the first signs of herbicide resistance. The task is then to understand how the shape of the target has changed and adapt the herbicide to work again.

Syngenta has four waves of innovation: corn trait revenue, scaling seed production capability (hybrid wheat), new phase of GM traits (replacement of insect and disease control) and GM traits for abiotic stresses (carbohydrate yield).

Corn Seed Production Lab vs Field: The Battlefield of Seed Purity (J. Schweigert, GroAlliance):

GroAlliance was founded in 1941 in northern Wisconsin based on hybrid corn. In 1977, the company passed to the next generation and the name was changed to NorthGro Seeds and was relocated to southern Wisconsin. The company produced seed corn, soybeans and also dealt in small grain retail. In 2004, NorthGro Seeds became GroAlliance and exited the retail area to focus 100% on contract seed production. At this time, the company also purchased a site in Mt. Pulaski, IL. In 2013, GroAlliance launched MySupply LIVE which allows for real-time production data sharing internally and with clients.

GroAlliance is the largest independently owned contract seed corn business in the U.S. It has a diverse client base, producing organic seed corn, GM seed corn, popcorn and also provides nursery services. The headquarters are in Cuba City, WI with sites in Mt Pulaski, IL and Howe, IN.

To make hybrid corn, the female must be detasseled so pollen from the designated male plants will fertilize the ear. This is done by planting 4 rows of the female inbred and one row of the male inbred. The female and male inbreds do mature synchronously, therefore, the male is planted at two different times which gives a long pollination window. Off-type plants are scouted and rogued. Generally, off-types which can be identified in the field are due to height differences and other phenotypic differences.

To catch all off-types, a total of 21 billion plants would need to be looked at. GroAlliance is constantly looking for ways to reduce the labor intensiveness of rogueing and scouting. Drones are currently not useful since the cameras are not precise enough. Robots are not cost-effective. Quality testing of inbred seed does give some off-type potential reading. Grow-outs are sometimes difficult to interpret.

Detassling occurs in 3 steps. First, mechanical cutting removes 1/3 of the tassel. A roller is then used to pop out the tassels. The 10-20% of the remaining tassels are removed by hand.

The male row is removed prior to harvest so only the female rows are harvested for seed. The size of corn seed is determined by where on the ear it was located. The biggest kernels are at the bottom of the ear (near the stalk) and the small rounds are at the tip of the ear. Pollination of the ear starts at the butt of the ear and proceeds to the tip. Most growers prefer the flat seed because they feel the germination quality is retained, they don't like small seed and feel that big seed has more energy (myth).

There are multiple levels of seed purity that the company is trying to obtain. Multiple isolation systems are used to accommodate organic, GM, and the milling industry constraints. Isolation distances are usually supplied by the customers and testing agencies. The highest standard is 660 feet. For non-GM, farmers are using LFS to test their planting seed. Non-GM is a challenge for the supply chain since millers are now pushing back arbitrary levels. Standards are arbitrary and on a sliding scale.

In the packaging operation, the first step is cleaning to remove cob pieces and non-seed debris. The seed is then color sorted which is done by a camera. Sizing is accomplished through the use of screens (round screens to sort for size and slotted screens to sort flat from round). The gravity table is used to sort by relative density of the seed. Those seed without germ would be lighter. Following sorting, the seed is treated with fungicides and insecticides. There are 30 different combinations of chemcials used on corn seed. Refuge-in-a-bag (RIB) blending is also done for certain customers. There are two methods: instream dribble method (dribbling in non-GM seed as bag is filled) and blending at the bag which involves weighing the amount of non-GM seed for each bag. Most RIB bags are done by the weight method.

GM seed production made up 90% of seed corn production in 2014. In 2015, 65% of seed corn production was GM. Use of conventional seed has increased in northern Iowa and Dakotas mainly due to light disease, light insect infestations and generally good weather. If 2016 growing season is different, farmers using conventional seed could experience big losses.

Overview of Dow AgroSciences Seed Quality Testing (N. Zhou, Dow AgroSciences): Seed quality testing is important because seed products are valuable, are alive and the quality is regulated (ASTA, ISTA, USDA, OECD, ETS, EPA). There are rules that dictate seed quality testing from AOSA (Association of Official Seed Analysis), U.S. Federal Seed Act, ISTA guidance, CFIA (Canadian Food Inspection Authority).

The quality of seed analysis is only as good as the sampling done. Geography specific rules are available through AASCO, ISTA, AOSA. AOSA rules describe purity analysis, plant germination testing, evaluation of the germination test, interpretation of the results and the reporting of results. Factors that impact testing are genetics, segregation and other variation in inbred/hybrid samples, seed age, unwanted pollination (self-pollination or unintended pollination), weather, processing (incorrect labeling, identifying seed mixtures). Germination testing determines the specific seeding rate since the seed vigor (seed's potential for rapid/uniform emergence) and purity (confirmation of genetic/trait purity) is

determined. Germination testing must follow AOSA/ISTA procedures. A rolled towel or creped cellulose is used with a defined amount of water, number of seeds, specified temperature and specified time period. Results are reported as normal, abnormal or dead seed. Cold germination is done in the same manner with a lower temperature (10C). Accelerated aging is accomplished by a pre-treatment of 72 hours at 43C, 100% humidity. The objective is to determine viability after long term storage.

Purity is the percent of the population that contains the intended trait. Purity can be determined by immunoassay, DNA-based tests and bioassay. Immunoassay (ELISA, LFS) complements genetic purity results and provides the capability for simultaneous trait protein detection. The methods can also be automated, however, the tests cannot distinguish hybrid varieties with the same trait. For herbicide tolerance, the bioassay method is cheaper to use. This method is a rolled towel method using 200 seeds at 25C for 7 days with light for 24 hours. The test determines that the target trait is present and expressing. Zygosity testing is a qualitative test for the presence of the trait. It is usually done by a Taqman-based assay using endpoint or real-time PCR. Physical purity is the percent pure seed, percent inert material, percent weed seed and percent other crop seed. A weed examination is done to determine if noxious weed seed is present. Genetic purity is done to determine trueness of type. The methods used include isoelectric focusing (fingerprint of storage proteins), isozymes and molecular markers (SNPs, endpoint PCR to identify homozygous and heterozygous based on fluorescence). DNA-based testing is preferred since DNA does not change with the environment (unlike protein expression), the process is highly automatable and scoring is automated.

Use of SNP Technology for Genetic and Trait Purity Testing (D. Hemphill, DuPont Pioneer): Genetic purity is determined by isozymes, SNPs, endpoint PCR or grow-outs. Trait purity is determined by ELISA, bioassay or endpoint PCR. Trait confirmation is done by endpoint PCR or real-time PCR and zygosity screening is done by endpoint PCR or real-time PCR. LLP (low-level presence) and trait detection are also done via LFS or endpoint PCR. The common method throughout all of these is PCR. DNA determines the genetics and is not influenced by environment as is protein expression.

The DuPont Pioneer lab utilizes DNA testing so that hybrids and traits can be looked at together. Also, the lab staff are trained on one common technology. The advantages for DNA-based testing include increased resolution/reliability, automation (never faster than humans), objective scoring/interpretation, decreased cost per informative data point, trait purity/genetic purity can be determined at the same time, applicable for all crops in all regions and single inbred profile database can be used globally. SNPs have replaced isozymes testing for genetic purity since scoring is less subjective. They provide more informative data points, the platform is scalable (manual to automation), reagent availability from several vendors and markers are available (MaizeGBD.org). ISTA rules are followed in the lab for testing.

Science, Industry and Perception: Engaging the Public on Agricultural Biotechnology (K. von Mogel, Biology Fortified): Biology Fortified started as a group blog and incorporated as a non-profit organization in 2012. It is a contributor-based blog (www.biofortified.org).

The public has a perception that GM is scary which is fostered by the opponents to the technology. In 2010, a survey by Reuters indicated that the higher education public understood GM more. The survey also indicated that the majority of respondents were unsure of the safety of GM. A similar Pew survey found that 88% of AAAS members said GM was safe while the general public was at 37%. 18% of the general public also indicated that scientists do have a clear understanding of GM technology.

One anti-GM activist is Jeffrey Smith who is a swing dancer from Iowa who also worked for GeneticID. He claims that GM foods cause allergies, inflammatory bowel disease, obesity, asthma, cancer, smaller testicles, auto-immune disease, liver disease, etc. He has posted on his website an interview which blames GM crops and Roundup for the Boston bombings. He says GM crops are inherently dangerous and industry funded and has also appeared on the Dr. Oz show. He is an influencer against GM technology as are Dr. Oz, Vandana Shiva, Adams (sells food supplements), Mercola (sells food supplements) and Vani Hari (the "food babe"). All operate under the premise that there is money to be made in manufacturing risk for the public. They all hijack or create venues that appear scientific. They also publish via predatory publishers that allow publication of work that lacks scientific rigor. Why are they successful? May be due to a lack of outreach and engagement by the GM industry.

GENERA is a program to make science accessible. There is a database for studies conducted on GM products (http://genera.biofortified.org). GMOanswers is an industry effort to engage the public to answer their questions about GM technology. They have just topped 1000 questions answered. The anti-GM activists are now harassing public scientists who answer the questions. They attack scientists for studies that are politically inconvenient to their NGO cause.

What is the GM technology debate about? It is about science—equivalence, safety, environment and efficacy. It is also about non-science factors such as culture, philosophy, ethics, economics, aesthetics, politics. The activists make people believe GM products are "unsafe" and then do not address philosophical beliefs. If facts don't convince, what should industry do? Industry should show that science supports their values. The public, however, views science as geared to sell products for money and this is the only motivation. Industry needs to engage in "story-telling" such as the story of the virus-resistant papaya in Hawaii that not only saved the papaya industry but also affected many papaya farmers' lives. Okanagan is very open and interactive about their GM apples and eagerly tell their story.

Biology Fortified is involved in repeating the "squirrel/GM corn experiment" that is actively shown on activist web pages in which the squirrels have apparently only eaten the "non-GM" corn and left the GM corn (https://experiment.com/projects/elppsxasaqyqkcmdppkd).
They have secured GM corn from Monsanto (SmartStax and its isoline) and will send it to people who wish to participate in the experiment. They will collect the data and hope to publish the findings.

Biology Foritified (Karl) hopes industry will join the conversation and tell stories and the importance of our work. Karl also hopes we all think more creatively on outreach and training for communication and explain the risks and how they are managed. Karl can be contacted at karl@biofortified.org.

GM Detection in Brazil (R. Melo Sartari Coelho, Ministry of Agriculture, Brazil): Brazil has safety norms and inspection mechanisms that involve GMOs. Forty-nine events have been approved in the country which includes single events and stacks of events. To import seed into Brazil, a previous authorization is required and importers must declare GMOs. Inspection are conducted for authorized events (research, exclusion, co-existence areas, food/feed labeling) and non-authorized events (field, seed imports, food/feed). Isolation distances in the field are 100 meters between GM and no-GM without border rows and 20 meters with border rows. There are two official labs for GMO testing – Lanagro GO and Lanagro MC. Both use PCR methods with are validated for authorized events. Lanagro GO had 17 validated methods in 2014 for use. Accreditation is required for GMO labs. The demand for GMO testing has increased with 17000 analyses conducted to date in 2015. The challenges for the future in Brazil include a) detection of 3rd generation products (near intragenics – no element for screening) and

4th generation products (cisgenics, intragenics) which are considered GMOs; b) availability of certified reference material; c) lack of proficiency programs to check labs' performance; d) use of digital PCR to qualify reference material; and e) use of sequencing to identify unauthorized GM events. Brazil has not yet decided if genome edited crops will be considered GMOs.

Aspects of Development and Use of GM Trees (J. Zhang, FuturaGene): FuturaGene was originally founded at Purdue University (U.S.). The parent company is now located in Brazil. In 2014, FuturaGene submitted a dossier to CTNBio for eucalyptus which was approved in 2015. FuturaGene vision is to be the leader in biotech for plantation forestry, biopower and biofuel markets. The trees involved in this are eucalyptus, poplar and some desert species. The key geographies are Brazil, China and U.S. In Brazil, forestry plantation involved 5.5 million hectares (2013). Eucalyptus has a slow development (7 year life cycle) and is not used for food. Its main uses are in paper, steel and charcoal. For Brazil, eucalyptus is an exotic species imported from Australia which is not expected to impact ecological interactions and has no target or vulnerable organisms. It is clonally reproduced so it is difficult to prevent its propagation.

FuturaGene's eucalyptus has an inserted enoglucanase gene (cel1) from *Arabidopsis* which has been found to increase biomass – 20% yield enhancement compared to conventional eucalyptus. The process for approval in Brazil took 7-8 years to complete. Studies that were needed included gene flow, honey bees (and honey), susceptibility to disease and pests. The dossier is available on the internet (www.ctnbio.gov.br/index.php/view/12386.html. Even though FuturaGene requested a hearing at CTNBio for transparency, there are still problems with NGOs against the technology. Although the approval was received earlier in 2015, planting of the trees was delayed until August due to the activist activities at the plantation sites. Value capture is done by planting the trees at the company's own plantation in Brazil.

In China, trees are #7 for GM crops (cotton is the largest GM crop). There have been no cultivation in China since 2002. Public acceptance and awareness of GMOs is a problem and can delay activities for 3-4 years. The China government wants to shorten the process since it is still too expensive and too long even for non-food crops. FuturaGene set up a lab in China in 2011 to work on transgenic poplar (insertion of cel1 gene). No other company is doing GM trees in China.

Safe Consumption of dsRNAs (M. Petty, Monsanto): Small RNAs include dsRNA from viruses, endogenous dsRNA from plants and transgene dsRNA. The pathway of action for dsRNA from viruses is now well known. Small RNAs are a common phenomenon in nature and are not new to agriculture. Examples are chalcone synthase suppression of anthocyanins in soybean (seed coat color), maize (stalk color) and petunias (star pattern), RNAi mediates low glutelin in rice, plant virus resistance (papaya), soybean oil composition changes, amylopectin potato, Simplot innate potato, Okanagan apple and reduced lignin alfalfa from Monsanto.

There are barriers to the uptake of ingested dsRNA. The systemic barriers include saliva, stomach acid, pancreatic nucleases, intestinal epithelium, vascular endothelium, blood and systemic nucleases. Cellular barriers include the plasma membrane, endosomal sequestration and lysosomal degradation. In summary, dsRNA would need to get through membranes and blood.

Barriers have precluded the oral use of RNA drugs. Without delivery agents, oral uptake of dsRNAs is less than 1%. Oral RNA drugs need to be given via direct injection in a formulation with stabilizing

modifications. None of these are employed in biotech crops. Even with the formulation and stabilizing agents, dsRNA drugs are broken down and eliminated within minutes. dsRNA drugs have been safely injected into humans at high doses—much higher doses than would ever be anticipated to be expressed in dsRNA crops.

dsRNAs have been safely consumed for millennia as human diets contain plant and animal dsRNA. Small RNAs and long dsRNAs with identity to human and animal transcripts are safely consumed in staple crops. Plant virus-derived small RNAs with identity to human transcripts are present in fresh market produce. Rice small RNAs match many genes and transcripts in consuming animals and animals have no problem in eating rice.

In summary, dsRNAs are natural regulators of gene expression. Dietary RNA has limited potential for impact on human or animal health due to systemic and cellular barriers.

Detection of RNA can be done via northern blots, however, this technology is labor intensive, low throughput but can be qualitative or quantitative. Real-time PCR is high throughput but RNA is challenging to reverse transcribe and sequence amplify. Quantigene technology (from Affymetrix) is high throughput, uses signal amplification, quantifies expression but is not feasible to detect multiple dsRNAs.

QuantiGene and ViewRNA: Versatile Solutions for Gene Expression and RNAi Analysis (S. Menon, Affymetrix): Affymetrix was founded in 1992 and has acquired several companies since 2005. In 2015, it acquired Eureka Genomics. The technology is based on branch DNA (bDNA) which was first developed by Chiron.

RNAi is used for crop improvement such as for disease and pathogen resistance, male sterility and for engineering metabolic pathways (soybean oil modifications). The challenges for its use are the selection of the target gene and target region within the gene and the length of the dsRNA. QuantiGene can quantify dsRNA directly from the source. Samples can be collected, stored and then homogenized for detection. Purified nucleic acid can also be used. It is a single-plex assay (chemiluminescent) or multiplex (fluorescence). The assay components include a 96 well plate, universal capture probes coated in each well, probe sets for the target and amplification reagent. To conduct the assay, the RNA sample is added to the well followed by the addition of probes. The capture probe immobilizes the RNA to the well. The label extender is then added and blocking probe fills gaps not covered by capture probe or label extender. Amplification involves building the trunk DNA, adding branches (have alkaline phosphatase) and then luminol. The light is measured to give the results.

In the multiplex assay, microspheres are used with red and infrared dyes. Each microsphere has a unique spectral signal. Antibodies or oligos are coupled to the beads to capture specific targets. Beads are read by red and green lasers—red laser tells what the bead is and the green tells how much. To conduct the assay, beads with unique oligos are added to each well. RNA molecules are then added, followed by capture extenders for each RNA. Amplification involves building the branch structure with streptavidin used for the signal.

Both assays save time since no purification of samples is necessary. There is a fast turnaround, i.e., custom panels can be designed in 2 weeks. There is no bias since no enzyme is involved and subtle changes can be precisely quantitated. Key applications include *in situ* assays for biomarker validation

and expression heterogeneity, expression profiling and gene signatures, heterologous expression of transgenes and gene silencing (detection of dsRNA in plants), testing RNAi efficacy in insects (mosquitoes resistant to pyrethroids—trying to regain pyrethroid activity by identification of the ion channel). Another application is the ecological risk assessment of dsRNA in the environment. Monsanto has looked at the fate of dsRNA in different soil types with the QuantiGene assay (Dubelman, et al., 2014. Environmental fate of dsRNA in agricultural soils. PLoS One 9(5): e93155.

In summary, the assays are quick and accurate for analysis of gene expression. They allow the ability to detect nucleic acid directly from lysates and lysate compatible. They have an ELISA-like workflow which is scalable and can be used for *in situ* detection of gene expression for spatial and temporal analysis.

DNAble Amplification Systems (D. Shaffer, EnviroLogix): DNAble 1.0 was known to generate high levels of background and non-specific amplification products. Non-specific amplification products consume reactants before the specific product is amplified. Designs were limited to those that could out-compete the noise. The assay was limited to endpoint detection and had a very low success rate and limited utility. The fundamentals of the system are:

Target → primers →molecular beacon → light is measured → cut → extend → displace → repeat

There is a lot of stuff going on behind the scenes. The challenge was to not generate background products and to perform better than the standard reaction. Also wanted to provide a means to predictably modify the reaction to yield conditions conducive to duplexing and establish uniqueness.

DNAble 2.0 resulted from converting the assay to be more sigmoidal and cleaning up the reaction. The reaction is continuous and has no pauses like qPCR. The non-specific background amplification was eliminated, a homogenous assay mastermix was used, tunable amplification, compatibility with automated reaction set-up, expanded application profile and streamlined the candidate assay screening.

The next challenges were to have a reaction that performed better than DNAble 2.0, enable quantitation with tight replicates, use a quantitative algorithm with kinetics, make it rapid, simple proprietary sample preparation method and use a non-competitive internal control. DNAble 3.0 is an endpoint or real-time assay which is clean, fast for use in discovery or development. DNAble Plus has been enhanced algorithmically. It utilizes empirically-derived rules/assay performance. It has a proprietary combination of computational algorithms and provides the capability to design, screen and optimize assays. Assay design can be done in minutes with active assays in hours and full development in weeks.

In summary, EnviroLogix has solved the isothermal amplification mystery and made a product that has better, faster, easier assay development. The assay development is predictable and streamlined with rapid sample preparation, rapid assay setup and rapid results.

Evolution and Use of DNAble Isothermal DNA Amplification Technologies (B. Parker, EnviroLogix):
Isothermal technology has a tainted reputation due to noisy backgrounds from non-specific amplification, intolerance to inhibitors, the inability to control or tune reaction, inability to multiplex reactions and difficulty in providing quantitative answers.

DNAble allows crude sample preparation A simple grinding of seed or leaf with the sample buffer, flick the tube, add an equal volume of sample buffer II and equal volume of mastermix. The assay has good specificity with different crops. EnviroLogix has a patent pending for use of an internal control to check for integrity of the reaction without competing in the assay. Axxin is an AOAC approved method for use with the DNAble salmonella kit which gives qualitative results. AmpliFire can be used in the field for on-site testing. It can be run by non-laboratory personnel in the field. For example, soy leaf punches in a tube or soy seed crushed in a tube can be used as samples. The Roundup Ready 2 assay is event specific to allow distinguishing RR2 from RR1 samples. There is also a SNP assay for soybean disease which uses two beacons to distinguish spores. This allows the grower to determine which fungicide to use. It can also be used for adventitious presence testing using a multi-trait GMO test strip. The Nexar array tape allows the use of crude preparations. A high throughput assay using crude sample preparation allows the detection of Cry1 and Cry2 proteins.

Development and Application of AmplifyRP Isothermal Amplification Detection (P. Russell, Agdia):
AmplifyRP is based on a recombinase-polymerase methodology which uses a mixture of enzymes, reagents in a lyophilized pellet. It allows rapid detection of DNA or RNA. Samples can be extracted using the sample extraction buffer, transferred (1 ul) to the reaction pellet and mixed. In the RPA cycle, the recombinases bind with the probes and primers. The polymerase starts synthesis. The results are the same as qPCR. There are two formats: XRT and Acceler8. XRT is basically qPCR and Accerl8 is a nested PCR. XRT is monitored in real-time and can use real-time PCR machines. Acceler8 uses an endpoint detection immunostrip in an amplicon detection chamber. A labeled streptavidin is used to capture the biotinylated 3' nucleic acid. XRT tube contents may be put on Acceler8 strips. Agdia has products for pathogens (Phytophthora, Fusarium viruses, viroids) and some are commercial now. XRT may be quantitative and it is possible to identify 2X differences in concentration. XRT can also detect a single target such as 35S or the NOS terminator.

In summary, the system is robust, fast, can be done on-site, can detect a wide array of pathogens from plants, can detect single copy genomic targets, is a sensitive assay which is repeatable and can be validated as a quantitative assay.

New Member: ABC Labs (J. Rhodes, ABC Labs): ABC Labs is based in Columbia, MO and has two locations there. It was founded in 1968 by Dr. Charles Gehrke from the University of Missouri. Dr. Gehrke worked with NASA to test moon rocks for amino acids in the 1970s. In 2015, the company was acquired by Evans Analytical Group which is a large global testing organization (7000 clients). Evans acquired a core group of companies for agchem – PTRL West and PRTL EU, Wildlife International, etc. ABC Labs brought now only agchem analysis but also pharmaceutical analysis to Evans.

ABC Labs capabilities include environmental fate work, animal and plant metabolism, analytical chemistry and product chemistry, physical chemistry, ecotoxicology. ABC Labs can address >80% of the agchem testing requirements. ABC Labs is also involved in biotech crop development by being able to provide protein expression analysis (ELISA, MS, Orbitrap), feeding and exposure studies, ecotoxicology and non-target organism testing, environmental fate of degraded proteins, amino acid analysis.
****

Composition Working Group Minutes

October 19, South Bend, IN

Attending in Person: Morgan Petty, Nancy Gillikin, Jane Sabbatini, David Levin, Kai Liu, Keith Persons, Anders Thomsen, Jeff Klucinec, Fred Claussen, Carl Maxwell
Phone: Barb Mitchell, Elisa Leyva-Guerrero, Karen Launis

Actions (also in the body of the Minutes)

Method Harmonization

- David Levin: Distribute information about approaches infant formula industry is taking for method harmonization for discussion as he receives it.
- Nancy Gillikin: Work with Laurie Bennett (ILSI) and pull information from ILSI database (last 3 years?). Format the data for review by and distribute to the sponsors, leaving a spot for comments and notes. Organize a phone conference/discussion of the information.
- Sponsors: Provide information by matrix of what methods they are using in support of Nancy Gillikin work with the ILSI Database; start with proximates. Need to find a good place to house this.

Allergens

- CWG members also on the ILSI DB Group: To discuss inclusions of allergen information into ILSI Database.

New Method Proposals

- Kai Liu: Raffinose
- Fred Claussen: Inositol
- Jane Sabbatini: Trypsin inhibitor

CWG Administration

- David Levin: Ask the AEIC Board about space for the CWG on the AEIC website for group access to minutes, presentations, actions to support continuity (understanding that the CWG minutes are rolled into the AEIC meeting minutes).

2:00 PM Welcome and Introductions / Antitrust Statement

Morgan Petty

2:15 PM Review Notes from Spring 2015 Meeting (Morgan Petty)

ELLA Status:

- Method went out for a vote; not sure of results.
- Gina to have feedback on lab participation for a 2nd publication.

Cyclopropenoic acid: Gina was successful in getting funding from Cotton, Inc. for standards
Gossypol: moved forward to collaborative phase in AOCS.
Allergen Subgroup: meetings continue. Working to reach agreement on level and approach to discussion.
Mission Statement: review planned for today's meeting.

2:45 Updates

ELLA Status (Morgan Petty)

The Collaborative study is completed.
Initial method publication was approved and available on AOCS website.

Cyclopropenoic Fatty Acid in Cottonseed method (Barb Mitchell)

Single Lab Validation

Single lab validation was published (AOCS)

Collaborative Phase

Method was moved into collaborative phase with samples shipped out in July

- Waiting on at least two labs to complete analysis before performing statistics
- Data looks promising; a few issues with resolution but able to work through these with good information to put into the method.
    - What can we learn from this and include in the final version of the method?
        - Preferred column manufacturers; some worked less well than others. Could indicate this in the notes for the method.
        - Will be able to add information on carrier gas (two awaiting results running with Helium).
- Challenge: left fat extraction method open to the preferred method for the lab. Seeing the most variation based on that extraction method.
    - Will continue to move forward because fat extraction was not part of the method.
    - The longer you extract, the better your numbers will be. Acid hydrolysis will also impact the results (+2x range of minimum values)
        - Soxhlet method (16 h with pentane) is a standard; microwave assisted, Soxtec, various Soxhlet methods also used.
    - Will look at the data to see if there are variations in what is extracted (after normalization) based on the amount of lipid extracted.
        - Preliminary data viewed.
        - High RCs with small peaks.
        - Higher variation with Malvalic is observed.
        - 4-6% RSD when outliers are eliminated; looks promising.
- Review of raw data from standards (Sterculic)
    - Response = peak area/IS*Concentration. Variation in response; also observed with other fatty acids.

Application of Lectin Method learnings:

- Even with a good method, the publication process takes time. (Methods board may have had some issue with high RSD with lectin method)
- Do not anticipate that this will be a method in a short time.

Fat-Soluble Vitamin Multiplex - Single Lab Method Advances (Fred Claussen)

Switch from HPLC to UPLC

- Better resolution of critical pair (beta and gamma tocopherol)
- Repeatable, reproducible, on old and new columns
- Good linearity with standards

System and Parameters

- Detailed shared in the presentation (Fred willing to share these)
- Waters UPLC with Waters Acquity TQ (triple-quad) with ESI source

- Phenomenex Kinetix PFP - reversed phase; sub 2.0 micron particle size
- 35°C column temp
- Strong flushing solvent (isopropanol)
- Mobile Phase A: 0.01M TFA in water (water alone does not work)
- Mobile phase B: Methanol
- 6.5 min run with 2 min re-equilibration, with some gradient changes being non-linear.

- Evaluated APCI - same ion generation pattern; stayed with ESI - easier to work with.

Chromatograms

- Reduced runtime ~2x with better separation with conditions given.

Linear Range

- 1-200 ng/mL for all target analytes based on previously proposed extraction (0.05 to 10 mg/kg). Some tocopherols will need dilution.
- Approximate LOQ of 0.05 mg/kg (important for K1 and beta-carotene in soy)

Proposed extraction protocol

- Not doing anything to remove the extracted triglycerides.

Reviewed matrices

- Soybean Seed - well covered low range, with need for dilution with some tocopherols (based on ILSI database data review)
- Canola - beta-carotene not required.
- Maize grain - vitamin K not required.

Precision

- Previous methods for testing included a clean-up SPE step. Updated the proposed method to remove the clean-up step.
- Soybean: concentrations increased, standard deviations decreased, and RSDs improved.
- Comparison to historical data (with soybean QC check with historical data from validated method): Some loss compared to previous data.
  - Suppression in Mass spec?

Collaboration and Support from CWG

- Discussed collaborative review at other CROs.
  - Fred feels good about sharing methodology and working together at this time.
  - Break-up across matrices
    - Eurofins: canola
    - Covance: maize
    - EPL: soybean
    - Methods may need to change based on matrix (extraction and any needed clean-up). Expectation is that the methods will be broken up by matrix.
  - Challenges of working on non-revenue generating projects.
  - Fred happy to coordinate next steps.
- Framework: SMPR from AOAC
  - Standard Method Performance Requirements (AOAC)
    - AOAC Website
    - SMPR Guidelines
  - Scope of development work and objectives
    - Accuracy? Precision? Matrices? …
  - Document will help with prioritization and maintenance of timelines.
- Cost sharing and acceleration of method. (Driver of most method improvement is reduced cost and acceleration of achieving results to Sponsors).

- Cost benefit analysis - always make some assumptions with investment in method development vs. benefit of method in use.

Differences to previous collaborative projects

- This is method development from scratch (vs a method a Sponsor or CRO has as a putative shared method); longer term and more complicated.

Next Steps

- Keep on agenda for future discussion.
- Consider look at Infant Formula as a model for harmonization of methods
  - Many new players in infant formula industry.
  - David Levin will distribute information about approaches infant formula industry is taking for method harmonization for discussion as he receives it.

CLI Methods Flexibility Paper (Morgan Petty)

Crop Life International - White Paper

- Development of a white paper making arguments that multiple and different analytical methods may all be accepted for a given analyte, anticipating a potential situation where a regulator may be proscriptive with a method(s).
- The Scope of the white paper is not restricted to composition.
- Morgan has provided feedback to CLI
  - Application of multiple valid methods has historically been the case for composition methods.
  - Provided example of ELLA lectin method.
- Morgan willing to talk in more detail if you are interested.

FOCUS ON GM SAFETY ASSESSMENTS

- Most important is a comparative assessment with a valid method within a study.

Questions

- Who or what agency prompted this initiative? Morgan could inquire for more information.

Opportunities for additional method harmonization

Starting point: can all participating groups lay out what methods groups are using?

- Allergens Unique: restrictions in freedom to operate that are not observed for compositional analysis.
- Start with information ILSI database
  - Nancy Gillikin to work with Laurie Bennett (ILSI) and pull information from ILSI database (last 3 years?). Format the data for review by and distribute to the sponsors, leaving a spot for comments and notes. Organize a phone conference/discussion of the information.
- Sponsors to provide information by matrix of what methods they are using; start with proximates. Need to find a good place to house this.
- **This exercise will help with identifying new targets for future work.**

3:45 Allergens: Next Steps (Morgan Petty)

Since the last meeting

- A number of meetings have been held. There are challenges with harmonization discussions.
- Those participating in meetings are different than those in the CWG.

Method Camps

- ELISA: Monsanto; not interested in making changes.
- Mass-Spec: Others; not interested in moving to ELISA.

- Traps with mass spec - need quantitative detection with peptides.
- Freedom to Operate - DOW has patented some technology. May have softened the discussion.

Starting point for continued discussions

- Establish a basis for understanding variability and differences.
  - May be due to methods or due to matrix or other factors? Not sure (not enough information to day).
- Not focused on differences within natural variability.
- Maybe developing: may go into the ILSI database (depending on view of ILSI and Sponsors).
- CWG members also on the ILSI DB Group: To discuss inclusions of allergen information into ILSI Database.

  Can use the framework of the ILSI database to house this information?

4:00 Next Projects

New method Proposals

Matrices

Corn

- Kai Liu: Raffinose (by electrochemical detection?, GC?)
  - No official method; all journal articles.
  - Eurofins has an aqueous extraction; extraction is a challenge depending on modifiers (alcohol) and enzymatic reactions.
- Fred Claussen: Inositol (could use harmonization)
  - Total? Free?
  - No differentiation in ILSI database.
- Jane Sabbatini: Trypsin inhibitor (uses soy method that says applies to soy-corn mix). Corn vs. Soy TI activity and relationships to concentration and binding.
  - Mass spec vs. ELISA-type method
  - Current method does not use a purified standard and looks at difference with an impure standard.
  - Low amount in corn, but is listed as an analyte.

Cottonseed

- Gossypol: needs a white paper to provide evidence that there is no value. Mike Dowd for feedback and support.

For those bringing methods:

- Bring best analytical method(s) you can find.
- USE SMPR format to define group needs (at AOAC website and with a template and instructions for development).
- If not sure about method, at least define performance parameters.

Suggestions and Recommendations for foci

- Start with proximates
  - Ash; propose change in time for a complete combustion
- Vendor comparative studies.
- Methods with any evidence that suggests that they are not accurate?
- Methods that were not optimized for crops.
- Validation of new technology against old methods.
- Cost-benefit-scientific validity.

- Identification of what Regulators are looking for (sometimes only guidance is OECD).

Application of Effort

- Referencing if ILSI database data in submissions reference all sorts of methods and results - inconsistency and ambiguity.
- Significant investment in products; everyone wants the most robust data possible.
- Easier justification for changes if there is alignment amongst the group.
    - Need sponsors to say that they want CROs to use the new method.
    - Sponsors are ultimately accountable to regulators.

4:30 Future Chair/Leadership of the CWG (Morgan Petty, All)

- Morgan has shifted roles in Monsanto - not touching composition work any longer.
- Carl and Nancy expressed interest in co-chair role.
- Starting term: 18 months
    - Defined Commitment term.
- Maintaining continuity.
    - Review roles at the next meeting. May consider chair/vice-chair roles in the future.
    - David Levin to ask the AEIC Board about space for group access to minutes, presentations, actions to support continuity (understanding that the CWG minutes are rolled into the AEIC meeting minutes).

5:00 Adjourn

AEIC Meeting Attendee List:

Charles Pick	SeqID
Patricia deLisio	Critical Path Services
Ning Zhou	Dow AgroSciences
Doug Miller	Illinois Crop Improvement
Dean Layton	EnviroLogix
Penny Hunst	Bayer CropScience
Jeff Klucinec	BASF
Anders Thomsen	Eurofins
David Levin	Covance
Angela Umthun	Stine Biotechnology
Joe Hudson	Bayer CropScience
Ann Black-Fendley	Bayer CropScience
Dave Rambow	Agdia
Jason Lilly	Neogen Corp
Nancy Gillikin	Bayer CropScience
Gina Clapper	AOCS
Frank Spiegelhalter	Eurofins GeneScan
Jeff Gillikin	NC State University
Matt Cheever	Bayer CropScience
Amanda Ver Helst	SGS North America
Keith Persons	Eurofins Nutrition Analysis Center
Kai Liu	Eurofins Nutrition Analysis Center
Sonia Herrero (speaker)	Syngenta
Morgan Petty (speaker)	Monsanto
Brenda Johnson	Eurofins Biodiagnostics
S. Menon (speaker)	Affymetrix
Jose de Leon(speaker)	Dow AgroSciences
Chris Culkin	Agdia
Tim Goldy	Agdia
Josh Kuipers	Agdia
Jim Schweigert (speaker)	GroAlliance

Denise Theide	Eurofins Biodiagnostics
James Zhang (speaker)	FuturaGene
D. Hemphill (speaker)	DuPont Pioneer
Gene Hookstra	Eurofins STA
Breck Parker (speaker)	EnviroLogix
Trisha Scott	Covariance Biosciences LLC
Jane Zollinger	Covance
Joseph Nicholl	Agdia
Qiang Zhao	Bayer CropScience
Daniel Shaffer (speaker)	EnviroLogix
Carl Maxwell	DuPont Pioneer
Natae Daniels	Dow AgroSciences
Huihua Fu	BASF
Ryan Sizemore	Monsanto
Wenjin Yu	Syngenta
Karl Haro von Mogel	Biology Fortified
Dean Hemphill (Speaker)	DuPont Pioneer
Emily Dierking	ICIA
Heather Chambers	Agdia
Nathan McOwen	Agdia
Andrea Harness	Agdia
Paul Russell	Agdia
Mary Lou Mountain	Agdia
Jon Rhodes (speaker)	ABC Laboratories