AEIC 2018 Spring Meeting Minutes

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AEIC Spring 2018 Meeting Minutes

April 25-26, 2018

Portland, Oregon

P.L. Hunst, Secretary

The 2018 AEIC Spring Meeting was held in Portland, OR on April 25-26. OMIC USA hosted the group at the Doubletree Hotel. T. Nagasaka, President of OMIC USA, welcomed the group to Portland on behalf of OMIC USA. Mr. Nagasaka explained that "OMIC" stands for "Overseas Merchandise Inspection Company" which was founded in Japan over 60 years ago. OMIC is a commodity testing company. OMIC USA was established in 1993.

Heather Flynn (OMIC USA) gave a brief overview of the company. OMIC has a global network of labs with its headquarters in Tokyo. In 2018, OMIC USA completed a large expansion to its labs which includes separate pressure and temperature-controlled labs and sample prep room to minimize contamination. OMIC USA supports the food industry by conducting GMO tests, nutrition panels, pesticide residue analysis, etc. The company employs a QMS system.

AEIC BUSINESS MEETING

Approval of 2017 Fall Meeting Minutes: A motion was made, seconded and voted positive to approve the minutes as posted on the AEIC website (www.aeicbiotech.org).

Treasurer Report (D. Layton): The 2018 budget is as follows:

2018	Proposed	Actual	Comments
Balance (Jan 1, 2018)	21474	21474	
Dues	17000	8750	Assessments still coming in
Spring Meeting Registration	2000	1785	$50/registrant
Fall Meeting Registration	2500		
Total Revenue	**21500**	**10535**	
Science paper	2000		
DE franchise	25	25	
ANSI/ISO	2900	2900	
Board Meeting	800	637	
Spring Meeting Expenses	5000	4325	Up to actual meeting
Fall Meeting Expenses	3000	521	
Website Maintenance	600		

Credit Card Processing Fees	4000		
Reprints	3000		
Subscriptions	100		
Misc	100	6	
Total Expenses	**21525**	**8413**	
Balance	**21449**	**23596**	

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

Membership Update (D. Layton); Current membership is as follows:

Type of Membership	Number	Potential dues $	Unpaid	$ unpaid
Large	14	14000	10	10000
Medium	13	6500	7	3500
Small	8	2000	3	750
Associate	2	100	1	50
Individual	2	200	1	100
TOTAL	**39**	**22800**	**22**	**14400**

AEIC Mission Statement Review (R. Shillito): A discussion was undertaken to affirm that AEIC is doing what the mission statement says. The Composition Working Group fulfills the "bioanalytical" portion through its work on compositional tests and bringing the ELLA lectin method forward as an official method. Members are diverse, ranging from multi-national agricultural companies to associate and individual members. AEIC has educational programs which includes the available slidesets on the website, numerous publications which have influenced international bodies such as Codex, and member participation on ISO Standards bodies such as the TC 34/SC 16 (Food and all food products) and TC 276 (Biobanking, DNA primers, etc). Members were encouraged to keep reaching out and working on committees to further bioanalytical methods and education.

DNA/Protein Comparison Paper (C. Pick): Charles gave an update from the authors that the paper has received reviewer comments and these are now being addressed.

Composition Working Group (C. Maxwell): The CWG also reviewed their mission statement during their meeting on April 24 in Portland. The fatty acid method for cotton has been recommended as a practice for AOCS and there has been discussion to make it an official method. The group is also looking at the isolation of crude fat from seed. The trypsin inhibitor activity assay has been tabled by the group for the immediate future. Endogenous allergen measurement has been in the CWG but may move to the Protein Working Group (PWG) in the future.

Protein Working Group (M. Cheever): The PWG is a proposed new working group which came as a suggestion from the breakout sessions at the Fall 2017 Meeting. The initial meeting was held on April 24 in Portland and discussion was held on a number of topics such as transgenic protein analysis by LC/MS/MS, guidelines for the validation of multiplex analytical methods, assessing whether AEIC 2006 protein paper should be updated, etc. M. Cheever (Bayer U.S.) and L. Liu (Monsanto) were appointed as the co-chairs. The group will draft a mission statement for the AEIC Board to review and approve.

Update on Breakout Sessions from Fall 2017 (B. Johnson): Unfortunately, the notes from these sessions are missing and attempts are being made to track them down. Abbreviated notes are available in the Fall 2017 Meeting Minutes on the website.

Fall Meeting 2018 (B. Johnson): The Fall Meeting 2018 will be held in Portland, Maine on October 10-11 and will be hosted by EnviroLogix, Inc. The Composition Working Group and the Protein Working Group will meet on October 9. More information on logistics will be available on the AEIC website. The membership was asked for suggested topics for the meeting:

- Non-GMO Project speaker
- KnowGMO speaker
- Speaker from organic industry
- Update on food authenticity testing
- Update on the industry COMPARE allergen database (suggested speaker>ILSI PATC)
- Crop session (possibly corn?)
- ASTA speaker
- Interaction of value chain and block chain
- USDA labeling (speaker from GMA)
- Adventitious presence testing for movement of seed/grain
- Breakout sessions
- Update on next generation sequencing > methods, acceptance, etc
- ILSI Composition Database challenges
- How should AEIC interface with CropLife International?
- EnviroLogix tour

Locations for upcoming 2019 meetings (B. Johnson): F. Spiegelhalter indicated that Eurofins GeneScan would host the group for the Spring 2019 Meeting in New Orleans. L. Liu (Monsanto) will check if the group can be hosted in St. Louis in the Fall of 2019.

Website Content Update (R. Shillito): The content on some of the website pages needs updating. This includes the slidesets and brochure. N. Djuranovic (EnviroLogix) agreed to head up a committee to look at the content and provide changes. Volunteers for the committee include R. Shillito (Bayer U.S.), L. Liu (Monsanto), P. Hunst (Bayer U.S.), C. Metzler (BASF).

Adjournment: A motion was made, seconded and voted positive to adjourn the business meeting.

INVITED TALKS – DAY 1 MORNING

Introduction to Seed Quality in Grasses (D. Stimpson, Oregon State University): Seed quality for grasses embodies two attributes: is it what it claims to be and is it alive. What it claims to be: This is varietal and physical purity. One way to determine varietal purity is via varietal fluorescence level such as in annual ryegrass vs perennial ryegrass. Annual ryegrass roots fluoresce which affords the capability of stating how much annual vs perennial ryegrass is in a seedlot. For physical purity, what contaminants are not allowed is based on where the seed is going to. Is it alive: This is determined by germinative quality which is defined as the emergence/development from the seed embryo of those essential structures which are indicative of the ability to produce a normal plant. Vigor, defined as seed properties which determine the potential for rapid, uniform emergence, is also determined. Seed quality requires good production practices. The Williamette Valley has the perfect climate for grass seed production since the weather pattern is similar to the northern Mediterranean. Inherent quality in seed, which is species specific, is also important. The OSU lab is the official certification lab for Oregon. It receives about

13000 samples/year and issues 30000 reports. Approximately 45% of the samples are for certified seed and 10% of all the samples are for ISTA certificates. Almost all seedlots submitted for ISTA certificates pass as they have all been previously tested. The challenges for a seed quality system include a) shortage of analysts, efficiencies (automation), varietal purity. The appearance of GMO grass seed soon will present even more needs.

Turfgrass Breeding (L. Brilman, DLF-Pickseed): Organized breeding of turfgrass began in the 1920s with the selection of a superior plant from the USDA/USGA-Arlington Turf Gardens (now the site of the Pentagon). In 1947, Merion Kentucky bluegrass was released and in the 1970s, bluegrasses were protected by plant patents. In 1970, the Plant Variety Protection law was passed. In the U.S., there is public/private cooperation in turfgrass breeding. Private companies cooperate in selecting for seed yield and other traits. There are many different environments in the U.S. and seed production areas are the not the primary turfgrass growing areas in the country. All turfgrass species are wind pollinated. For breeding, it is necessary to know the ploidy level and how to pollinate. Most species have high ploidy levels which makes it harder to determine genetics and fix genes. Inbreds are difficult to develop. The U.S. and EU have public and private breeding programs whereas in Australia/New Zealand, there are few public/private programs. Most breeding is focused on forage. In the U.S., most private breeders are located in the Pacific Northwest. The emphasis is on species and market segment. In the U.S., dark green color, fine texture and density is preferred. Wear tolerance and recover is important for sport locations. Winter is the active growth period in the Pacific Northwest and California. Production goals are high seed yields, early heading, disease resistance, stem rust resistance and stress resistance. Turfgrass goals include high density, late heading, disease resistance, wear tolerance and stress resistance. The process of breeding starts with selection of parent in source nursery through screening process for desirable characteristics. The breeding material is then identified. Controlled hybrids are used to study species realtionships. Variability is obtained from existing varieties or breeding populations, collections and/or related species. The selected parents are then crossed with the polycross being the most common. Breeding is a numbers game. Drones are now being used to image plots and use aerial imaging analysis. Root imaging, thermal imaging, DNA profiling, fingerprinting, marker-assisted selection, CRISPR are all used. Genome-wide selection, patterned after use in cattle, is also employed. Production takes 3 years and is in competition with wheat for land acres. Hazelnut production is replacing grass seed production in Oregon.

GM Plants for Degradation of Pollutants: From Mutations on Training Ranges to Indoor Air Pollutants to Greenhouse Gases (S. Strand, University of Washington): Munitions by-products are RDX and TNT which are toxic/recalcitrant to degradation. There is large scale contamination of high explosives in manufacturing and storage facilities. There are over 10 hectares of land contaminated by munition constituents. RDX and TNT concentrate in living plants and then move into the ground when the plants senesce. RDX degrading bacteria are being isolated from some sites. The cytochrome P450 enzyme can break down the RDX molecule with the XPLB enzyme assisting in the degradation. The U. of WA lab has produced transgenic *xplA* switchgrass. Swtichgrass is useful on training ranges since it is durable. The transgenic switchgrass plants remove RDX and then can use them as fertilizer. Field trials are now being conducted in upstate NY. Organic pollutants in the home air come from air fresheners, cooking, particle board, solvents, fuel sources and second hand smoke (benzene). Previous studies suggested that houseplants could remove some of these pollutants. Removal rates vary by several orders of magnitude between studies for the same plants and same pollutants. Soil bacterial use the pollutants as carbon/nitrogen sources. The U. of WA lab has used over-expression plant formaldehyde dehydrogenases (bacterial genes). Cytochrome P450 2E1 oxidation was successfully transformed into tobacco. Pothos plants have also been transformed using the *Agrobacterium* system. Pothos is difficult to regenerate but transformed plants do remove benzene by transforming to phenol and then lignin. Since plant are GM, they cannot be released without approvals from the U.S. agencies. Phytoremediation is not

under FDA or EPA. USDA is concerned about weediness potential. Pothos does not flower so transgene transmission into wild populations is impossible, however, it is grown outside in Florida, therefore, it is regulated. Canada has approved since frost occurs all over the country. Another challenge is that the plants have to be attractive to the consumer. A visual cue is needed to indicate the plant is unique such as putting the green fluorescent protein gene in marbled pothos plant. All functional gene insertions are not patentable except acrolein dehydrogenase. PVP protection is applicable outside the U.S. To prevent competitors from appropriating clones, have encoded poem into the third generation plants which is copyrighted. The lab has also explored phytoremediation of greenhouse gases by looking at methane monooxygenase (MMO) in designed and developed stacked vectors. These did not work even though the proteins were produced but did not assemble appropriately. Nitrous oxide reductase was also used for N_2O and engineered into plants. Multiple proteins from the same gene were assembled and nosZ was active under anaerobic conditions. The next step is to target mitochondria.

Business of Cannabis Testing (A. Stevenson, Eurofins Cannabis): There are many products from Cannabis: inhalable, edible, powder, etc. Testing is done for cannabinoid potency, terpene, residual solvents, microbiota, pesticide residues. Standardized testing is lacking. Since California became a recreational Cannabis state, $9.2 billion will be spent on Cannabis products. Cannabinoid is a non-psycho active compound. It is marijuana or hemp derived. In hemp, the level must be less than 0.3% THC in order to be classified as hemp and sold into industry channels. About 10-14 cannabinoids are tested. Terpene supplies the smell, aroma and flavor of Cannabis. Terpenes occur in many plants: myrcene (earthy aroma), limonene (citrus), carophyllene (pepper), linalook (floral), a-pinene (pines aroma). Residual solvents can occur in Cannabis concentrates such as crumble, butters, shatter, distillate, crystalline, dry sift, rosin, bubble and hash. Solvents are used to make these concentrates and currently, GC is the testing method. Pesticides are a growing concern for consumers and states are starting to adopt requirements for Cannabis testing. Cannabis is susceptible to molds, bacteria, viruses, root rots, powdery mildew. Breeding solutions are being sought for resistance. Mycotoxin testing is required in California since there could be increased levels in Cannabis distillates. Eurofins is building a knowledge exchange to advance public health and safety. There is a great need for cultivation and breeding practices. Various government amendments have occurred but alignment is needed between state and federal regulators since it affects methods standards.

Cannabis Production: Propagation, Analysis Needs, Regulatory Environment (T. Shipley, Canopy Growth): Canopy Growth was established in 2013 and is located in a former Hershey plant in Ottawa, Canada. The company has grown from 5 employees to 820 and is a $5.6 billion publicly traded company which has locations globally. The company has also licensed locations in Vancouver for production. Cannabis production starts with a pre-production process which includes identification of mother plants. This is challenging since stability has been bred in medical varieties. Milling and homogenization have produced unfavorable organoleptic properties. Strict control on genetics and environment are required. Approximately 500 plants are grown out and judged for smell, phenotype and chemotype. Unique profiles are desirable. The next phase if production of clones to expand master to seed to produce working seed. Mother plant are immortalized by maintaining in vegetative growth phase. The number of passages are kept to a minimum. Genetic sequencing of Cannabis models has demonstrated a strong prevalence of transposable elements and retro-transposons (activation may cause virus symptoms). The next phase is the rooting of clones for 1-3 weeks in rooting substrate. Challenge in this phase is possible introduction of pathogens via the substrate (peat moss), introduction of harmful plant growth regulators (PGR) and possible carryover of disease from mother plants. Rooted clones are grown in pots which are tagged with serialized identification. The plants are maintained under vegetative growth for a defined period which is optimized. Flowering is induced by changing light cycle to a short day length. The risks at this phase are pathogens, heavy metals, pesticides and fertilizers. The next phase is harvesting and trimming of the plants. Plant may be trimmed wet or dry. Consistency and process optimization are

introduced at this phase. The risks at this phase are chemical contamination from incidental contact and metal filings (hazard for inhalable products). The drying and packaging phase occurs in humidity and temperature controlled drying rooms. Accelerated drying at same endpoint creates a less favorable smell. The risks at this phase are poor organoleptic properties, bacterial and mold growth. The last phase is lot release testing. Specifications are the same as for herbal products and must be performed according to validated methods. Testing is done for potency (HPLC), microbiological limits, heavy metals and aflatoxins. Currently testing is conducted for 95 pesticides at the 0.01ppm limit. Micronutrient fertilizers must be registered with CFIA (Canada). In Canada, recreational use of marijuana will be legal in mid-August, thus, there is significant expansion in the variety of products. Distribution is regulated at the provincial level, similar to alcohol regulations. Cannabinoids will be delisted and sale will be permitted as Natural Health Products which must contain <10ppm THC. This allows hemp produced under Industrial Hemp Regulations to be processed and sold under Cannabis Act. Production follows GMP requirements (documentation, testing of incoming materials, cleaning validations, process validations). GMO marijuana would follow same definition as applied to other agriculture crops, i.e., assessed as a novel trait.

Growth of Industrial Hemp (D. Sinning, State of Colorado): Industrial hemp is legal at the Federal level but no marijuana. Colorado allows 6 marijuana plants/household to be grown but finds it illegal to grow hemp. States struggle with many interpretations:

- What does delta9 THC concentration mean?
- How do you sample and what material is included?
- What testing method to use?
- Can non-institutions of higher education grow hemp?
- What does marketing research allow?
- Who regulates products at what THC level?
- Is cannabinoid regulated by states?

There is a lack of a uniform interpretation of the Farm Bill, i.e., Bureau of Reclamation does not allow use of appropriated water in West to grow hemp. USDA seed label will not enforce Federal Seed Act, thus, becomes a dumping ground for low quality seed from other countries. USDA will authorize phytosanitary certificates for export but the DEA does not allow export. Industrial hemp is defined as having <0.03% THC on a dry weight basis. This limit was arbitrary set by the UN office on Drugs and Crime, US Farm Bill and Canada. Most international trade agreements generally recognized a limit of 0.3%, however, the EU uses 0.2% THC. The 0.3% level gives the hemp industry operating space as it has psychoactive potential. Hemp and marijuana cannot be distinguished phenotypically since they are the same genus and species. The only difference is the level of THC. Hemp has 25000 different uses including paper, textiles, biofuels, graphene for batteries, car parts, insulation, building products, etc. The U.S. started producing hemp in 2014 but imports have spiked. Consumer use has outpaced production. In 2017, there were 25000 production acres (9800 acres in Colorado). The size of individual growing sites has increased and moved to more traditional agriculture areas. Market areas are cannabinoid and seed production, fiber and biomass production. For the seed industry, 2014 was the first year of legally produced hemp, in 2016 the first certified seed program was established and in 2017 the first U.S. bred variety of industrial hemp was launched. For grain and seed, equipment manufacturers are testing equipment to mechanize harvesting. New uses of oils are being explored as well as use as an animal feed. The industry segment is expanding rapidly as consumer demand continues. However, the fiber/biomass segment has been slow to emerge. Culturally, hemp is produced in rows like corn or drilled like wheat. Grasshoppers, spider mites, powdery mildew are common pests. Few pesticides are labeled for use on hemp. Hemp does tolerate poor quality soils and uses less water that corn or soybeans. Timing is very important for harvesting as a late harvest could increase the THC limit, and affect the terpenes. If the THC level is above 0.3%, the crop is no longer considered industrial hemp. Most states then require disposal or destruction. Most states, however, will allow for alternate purpose before plant is mature.

Most states take samples within 30 days of harvest and usually only sample female flower. Compliance is increasing (91% in 2017). Hemp production is a blip on the scale vs commodity crops but may increase due to its lower water demands and nematode suppression.

***Cannabis sativa* Genome and Crop Development (M. Holmes, Phylos Biosciences):** Cannabis is projected to the 3rd largest agricultural crop. Currently, it is genetically where corn was 100 years ago. There has been small trait improvements but these are just "stirring the pot" in genetics. People have shared varieties, some of which are now above 30% THC vs the previous 3-4%. This doesn't work for consumers yet but the chronic pot smokers like it. Marijuana is medically valuable but only adapted to most expensive growing environments. It is expected the specialized varieties will be bred that will be optimized for outdoor growing, automated harvesting and minimal water use, high carophyllene, high limonene, disease resistance and 4% THC. The barriers to crop development include a) advanced breeding requiring vast genomic data resources; b) commercial and academic scientists cannot touch it; c) Cannabis producers cannot do it themselves; d) nothing can be moved across state lines; e) intellectual property landscape is a total mess. Phylos has worked with the American Museum of Natural History to collect data on Cannabis. Cannabis was domesticated in Asia and the conquistadors brought it to the Americas. It was moved to North America in the 1900s where it was found in many products in pharmacies. In 1937, it was deemed illegal. In the 1960s, seeds were brought to California from all over the world. Trying to determine the clusters of populations is a mess. Phylos is selling genetic test for a) plant sex test and b) plant genotype test (use cotyledon or stem tissue scrapes). The big data approach has been used to develop markers by using chemical, agronomic and patient data. Plants may receive patents and PVP in certain countries. Trade secrets are a viable option in industry to protect the genetics via licensing and for internal production.

INVITED TALK – DAY 1- AFTERNOON

Challenges and Benefits of Being a Science Communicator (L. Katiraee, Science Moms): L. Katiraee worked in Illumina for 2 years in Canada and then moved to the Bay Area of California. She moved to Integrated DNA Technologies and recently has gone back to school for a master's degree in bioinformatics. She started a blog on GMOs when her son was a toddler. Her Facebook page was inundated with stories on GMOs and rat tumors. She took a trip to Cambodia and was impressed about the guide telling her about rejuvenating Cambodia agriculture with GM crops. After this trip, she came back and started to learn more about GMOs. Initially, she wrote under a pseudonym on her blog and relaying information, both pro and con, about GMOs. She moved to Twitter and became acquainted with Biology Fortified who wanted to publish her article on dengue and use of GM mosquitoes. She had to think about the consequences since she would need to publish under her name but in the end decided to publish with them. Layla then started using her blog to tackle complex topics on GM crops. Celebrity moms against GMOs published a letter and she teamed up with other science moms and wrote a letter from Moms for GMOs. One of the members decided to make a movie on "Science Moms" which was launched in 2017 and is available for download. After the 2016 elections in the U.S., it became clear that "silos" needed to broken down so Science Moms started publishing on a wide variety of topics (guns to food) on science for parents. This has been successful. Science Moms receives some funding from Biology Fortified and rest comes from the members. Industry scientists need to be more vocal as academics are overloaded and underfunded. More financial security is needed to do the initiative on information. Layla has found that participating in Science Moms has made her a better scientists who reads more critically and has more effective writing skills. Parting lessons: a) there are mean people out there and they cannot be avoided; b) join an existing platform; c) choose a field you are passionate about and interested in; d) share the "why"; e) listen; f) science communication is not just writing; g) be honest and transparent; h) be honest with your employer about your activities; i) talk with your family because it will impact them; j) reach out to others whose style you admire; and k) if you cannot do, then support others.

INVITED TALKS – DAY 2- MORNING

Genetic Purity: Past, Present and Future (F. Ghavami, Eurofins BioDiagnostics): Plant grow-outs have been historically relied on in the field for purity testing which is dependent on visual identification. Protein-based markers were then employed such as isozymes, iso-electric focusing and SDS-PAGE. Isozymes are separated on starch gels based on the protein size and charge. Eight different stains are needed to evaluate isozymes from 16 loci. Iso-electric focusing (IEF) separates proteins based on their isoelectric points. The variability of IEF banding patterns is high and changes between labs. The number of isozymes to test is limited. Both methods are not high throughput but labor intensive. Neither can separate sister lines. DNA-based markers include RFLPs, RAPDs, CAPs, AFLPs, SSRs, SNPs, DArT, etc. Simple sequence repeats (SSRs), called microsatellites, have variant number of tandem repeats of 1-4 nucleotide motifs in the genome. The flanking sequence is used as PCR primers to show polymorphism between members of a species. Single nucleotide polymorphisms (SNPs) occur between sequences of different members of a species. They are abundant, easy to find, easy to detect and easy to analyze (automate). Genotype by sequencing is restriction enzyme mediated genotyping. Hybridization and amplicon are based on a targeted sequence. The process is: quantify genomic DNA → normalize, add enzyme, adapters, ligase → pool samples → PCR, clean, size → sequence. The number and distribution of molecular markers matter which is why SNPs are popular. A panel is designed by high density genotyping of foundation lines. Then a chip array is selected. Markers are selected with high minor allele frequency and the most informative markers are selected. A minimum number of markers are selected that can separate 95% of lines. Array-based markers or SNP sequence is converted to PCR-based markers and then the best markers are selected that have best quality for genetic purity panel. The panel is validated *in silico* and on random lines to find its efficiency. As an example, using 5400 SNP markers in wheat could differentiate all 546 lines. In corn, a 16 SNP panel separates 90% of 517 public lines and using an 18 SNP panel separates 95% of the lines. A one marker difference in genetic purity panel should be translated to a bigger difference in genome. Detection of sister lines requires more markers.

Varietal Identification Testing for Rice (P. Kahn, OMIC USA): Rice is the second largest produced cereal in the world. It is primarily produced in western and eastern Asia and covers 9% of the global arable land. *Oryza sativa* and *Oryza glaberrima* are main species. There are two varietal groups in O. sativa – Indica and japonica. The rice genome is 430 mbp on 12 chromosomes. The Indica and japonica genomes have been published. Rice is bred for crop quality and productivity, giving rise to short, medium, long-grain, glutinous and aromatic. There are economically and ecologically important phenotypes. The rice genebank contains over 100,000 accessions. Varietal identification is done to a) detect adulteration of premium varieties; b) cross-contamination along the process line; c) detect cross-breeding (intentional or unintentional); d) verify varieties for financial security and quality control. Basmati is an aromatic variety which is a tall stature plant and does not respond to fertilizer. It has been crossed with high yielding long grain varieties but the quality has gone down. It is necessary to differentiate between India traditional basmati from the cheaper cross-bred basmati. The EU has a lower import tax on traditional basmati varieties. The UK indicates that the level of non-basmati cannot exceed 7%. Identification is done by morphological inspection and physiochemical traits and biochemical markers. DNA-based methods are gene specific and can be small scale to large scale. There are few markers to whole rice genome. Many SSR markers occur in non-coding regions which cannot be selected against. Mutations accumulate over generations and cause increasing variability. There is a long history of SSR research. SNPs have a greater distribution across the genome and SNP rice assay collections are available. OMIC rice identification began in the mid-2000s with the method being developed in-house with Oregon State University. The SSR marker database currently has 10 markers and will be expanded to 16 markers. In the future, more markers and varieties will be added. There will be a supplementation

of the SSR database with SNP markers which will be useful for difficult varieties. There will also be broad scale screening of Indica vs japonica for contamination by other sub-populations. There was a ring test conducted by Taiwan University with the goal to have universal varietal identification system with results being directly comparable among labs. The testing used SSR high polymorphism with 12 markers uniformly distributed across the chromosomes. The results indicated that the data between labs was different due to relative allele sizes across platforms.

Novel Application for DNAble: Zygosity Determination of Crude Soy Leaf on Various Equipment (S. Tapley, EnviroLogix): There is a need for a more rapid tool for event identification and zygosity determination. In soy, the progeny can have two copies of any gene from parents. A high throughput, rapid kit was developed based on DNAble technology. The results were available in 15 minutes. The conventional assay spans the insertion site whereas the trait assay span the junction of the conventional genome and insertion site of the transgene. A simple preparation protocol was developed using a leaf punch. The method was validated for Roundup Ready 2 soy. The MON89788 zygosity kit was the first one developed. This kit/method showed 96% accuracy and shows the capabilities of DNAble in determining zygosity in fresh or lyophilized leaf tissue.

GMO Testing by PCR: Testing Difficult Matrices (F. Spiegelhalter, Eurofins GeneScan): Most testing done by Eurofins GeneScan is for no-GM confirmation. Products teste include food ingredients, plants, seeds, etc. For event testing, techniques focus on DNA (PCR – qualitative or quantitative) and ELISA for proteins. Challenges for PCR include inhibitors which are co-extracted with the DNA and prevent the test from working. These often occur in matrices with high salt, soy, canola, sunflower and seeds, if treated. Usually an endogenous control is used in the test to determine inhibition. Another challenge is compromised DNA/low concentration DNA/no DNA. Processing of sample can contribute to this. Detection limits are moving targets which is the same for LODs. A large genome will give less DNA. Foreign material and botanical impurities are challenges. Soy left in barges which are now being filled with corn can make the corn appear GMO when it may not be. Soy has more DNA/weight. Wheat flour tends to always be GM positive due to foreign material. It is important to remember that "GMO" is not an analyte. %GMO is typically ambiguous and poorly defined and in finished food cannot be verified by testing. The limitation is intrinsic to the product's DNA.

Problems Associated with DNA Testing in Challenging Matrices (C. Singsit, OMIC USA): Detection of DNA from challenging matrices is used for product verification and protection, authentication, non-GMO verification and for certification. In cotton, differentiation between the hirsutum species vs the barbadense species is desirable since there is a premium price for long strand barbadense fiber. Thus DNA from the fiber is needed. For extra virgin and virgin olive oil, DNA needs to be extracted from the oil to distinguish. Quality DNA is key to a quality outcome in PCR analysis. Highly fragment or degraded DNA is not acceptable. And inhibitors may block the PCR assay. DNA integrity is determined by measurement of the 260/280 ratio for concentration. Gel electrophoresis will tell if the DNA is fragmented or degraded. PCR requires the use of unique primers and probes to facilitate distinguishing species. PCR facilitates improved cultivar identification and is central to establish genetic traceability. For oils, it is a challenge to obtain sufficient PCR quality DNA in crude oil. Standard curve is not based on DNA from oil. Exposure to extreme temperatures and chemical treatment is deleterious to DNA. Other challenging matrices to obtain DNA from include raisins, chickpea puree, pacifier sanitizer spray, toothpaste, pullulan capsules, marinara sauce, fruit juice kim chi, spring rolls, supplements, etc. A high throughput platform with emphasis on SNP detection is needed. Also investigating non-nuclear DNA (i.e., chloroplast).

Meeting Attendees:

	Name	Organization
1.	Allen, Ann	Romer Labs
2.	Beecher, Brian	USDA
3.	Bednarcik, Mark	Syngenta
4.	Brilman, Leah (Speaker)	DLF-Pickseed
5.	Brune, Phil	Syngenta
6.	Brustkern, Sarah	DowDuPont
7.	Cheever, Matt	Bayer U.S.
8.	Cummings, Simone	Syngenta
9.	Djuranovic, Nevena	EnviroLogix
10.	Emmitt, Robert	Agdia
11.	Fisher, Ashley	Simplot
12.	Flynn, Heather	OMIC USA
13.	Ghavami, Farhad (Speaker)	Eurofins BDI
14.	Gillikin, Nancy	Bayer U.S.
15.	Gillikin, Jeff	North Carolina State Univ.
16.	Haudenshield, James	Merieux NutriSciences
17.	Holmes, Mowgli	Phylos Bioscience
18.	Houchins, Donna	Romer Labs
19.	Hunst, Penny	Bayer U.S.
20.	Johnson, Bradley	AgReliant Genetics
21.	Johnson, Brenda	Eurofins BDI
22.	Kahn, Peter (Speaker)	OMIC USA
23.	Katiraee, Layla (Speaker)	Science Moms
24.	Kouba, Kristen	DowDuPont
25.	Layton, Dean	EnviroLogix
26.	Levin, David	Covance
27.	Liu, Kai	Eurofins Nutrition
28.	Liu, Zi Lucy	Monsanto
29.	Lupean, John	OMIC USA
30.	Maxwell, Carl	DowDuPont
31.	Metzler, Chelsie	BASF
32.	Miller, Anthony	AOCS
33.	Motyka, Shawn	BASF
34.	Muschinske, Luke	Covance
35.	Nagasaka, Terry	OMIC USA
36.	Persons, Keith	Eurofins Nutrition
37.	Pick, Charles	SeqID
38.	Roberts, David	BASF

39.	Schuld, Brian	Eurofins Nutrition
40.	Shillito, Ray	Bayer U.S.
41.	Shipley, Tom (Speaker)	Canopy Growth
42.	Shippar, Jeffrey	Covance
43.	Singsit, Chong	OMIC USA
44.	Spiegelhalter, Frank (Speaker)	Eurofins GeneScan
45.	Stevenson, Austin (Speaker)	Eurofins Cannabis
46.	Stimpson, Dave (Speaker)	Oregon State University
47.	Strand, Stuart (Speaker)	University of Washington
48.	Tapley, Susan (Speaker)	EnviroLogix
49.	Zhao, Qiang	Bayer U.S.

Compostion Working Group Minutes

AEIC Composition Working Group

April 24, 2018

Participants: Keith Persons, Kai Lu, Brian Schuld, Dave Roberts, Matt Cheever, Luke Muschinske, Nancy Gillikin, Anthony Miller, David Levin, Lucy Liu, Jeff Shipper, Phil Brune, Donna Houchins, Ann Allen, Ray Shillito, Tao Geng (phone)

Fatty Acids in Cottonseed—Luke Muschinske (Barb Mitchell)

1. Cyclopropenoic fatty acids
    a. Originally analyzed separately from other fatty acids
    b. Now all fatty acids can be combined into one method
    c. AOCS recommended practice
2. AOCS official method
    a. Would require some parameters to be changed and repeat of ring trial
    b. Concentration range needs to be expanded
        i. Maybe a concentration series achieved by diluting with other oils
    c. Optimization of crude fat extraction from whole seed
        i. Probably most important contributor to variability
    d. Helium versus hydrogen as GC carrier gases
        i. Likely less important
    e. Possible harmonization around GC column
        i. Likely less important
    f. Regulators seem to be okay with our methods and the way we present them
        i. Usually look at one study at a time
    g. The method is validated and published as a recommended practice

        i. Probably not worth the extra work to take to official method status
        ii. Additional work around crude fat extraction/analysis on the front end would be helpful

Trypsin Inhibitors—Luke Muschinske

1. Recommended pulling from active list of projects
    a. Making slow progress through Covance
        i. Placed on Covance internal method development list for evaluation
    b. Can wait and see how EFSA handles the Kunitz Trypsin inhibitor allergen

Proximate Methods—Keith Persons

1. See attached slide presentation
2. Generally, methods in the ILSI Crop Composition data base are suitable
    a. The main difference in methods is crude fat
3. Methods have been updated in the ILSI CCDB but still need further work on differentiating types of methods
    a. Focused on presenting data
4. Difficult to find original references anymore
5. Crude Fat
    a. Need to have high level discussion among the CRO's about what method they are using and why.
    b. Could work on a crude fat method for canola
        i. Explore if CRO's would be willing to work together on this
        ii. Not an AOCS method for canola
6. Kjeldahl versus Dumas (combustion) nitrogen determination for crude protein
    a. Probably 90% of data in ILSI CCDB is Kjeldahl
    b. Dumas is safer, faster, cheaper than Kjeldahl
    c. Would require outreach to Regulatory agencies to encourage acceptance
        i. Need to make a case to AEIC about getting behind this
            1. Research existing literature
            2. Make a case for why change is needed.
            3. Design a study to demonstrate advantage of change

Allergens—Tao Geng

1. Review from last meeting
    a. 10 soybean allergens for EFSA
        i. ELISA and LC-MS/MS both accepted
    b. Proposed Gly m 7 for harmonization of an LC-MS/MS method
2. A book chapter is being written on methodology for endogenous allergen assessment in association with the American Chemical Society
    a. Reviewing methodology
        i. Geng, T., D. Stojšin, K. Liu, B. Schaalje, C. Postin, J. Ward, Y. Wang, Z.L. Liu, B. Li and K. Glenn. 2017. Natural Variability of Allergen Levels in Conventional Soybeans: Assessing Variation across North and South America from Five Production Years. Journal of Agricultural and Food Chemistry 65:463-472.

ii. Hill, R.C., B.J. Fast and R.A. Herman. 2017. Transgenesis affects endogenous soybean allergen levels less than traditional breeding. Regul Toxicol Pharmacol 89:70-73.

b. Emphasizing the 10 allergens required by EFSA

3. Will move endogenous allergens to protein working group once formally organized
   a. Keep a place-holder in the composition working group for reporting on allergen activities

Proximate Slides:

ILSI Proximate Data

- Draft Conclusion?
  - Original question: Are these methods suitable?
  - General answer: Yes.
- Suggest categorization via principle with (or in place of) reference method
- Discourage use of instrument manuals as reference method
- Avoid use human nutritional analytes
  - Carbohydrates by difference
  - Calories by Atwater calculation

Proposed Projects

- Crude Fat in Canola method
  - Most commercial oilseeds have specific crude fat methods from AOCS
  - Extended extraction seems necessary.
- Kjeldahl versus Combustion Protein
  - Potential for acceptance for regulators
  - Interest in comparative study

<u>Protein Working Group Minutes</u>:

<u>AEIC Protein Working Group - Meeting Minutes</u>

Introduction

Matt Cheever (Bayer Crop Science) provided a brief introduction on how the Protein Working Group (PWG) was formed during the breakout session at the 2017 Fall Meeting. The following topics were discussed in more details.

1. Endogenous allergen analysis

Tao Geng (Monsanto) reviewed EFSA's request of allergen data and how analytical methods have evolved in recent years. Shawn Motyka (BASF) proposed that samples be submitted to third-party labs for analysis. Lucy Liu (Monsanto) brought up the in-country method requirement by China. Attendees agreed that technical aspects of endogenous allergen activities should transfer from the Composition Working Group and PWG will consider development of harmonized methods, possibly starting with the most recent allergen guideline by EFSA.

2. Alternative analytical platforms

David Levin (Covance) reviewed alternative analytical platforms to ELISA. Mark Bednarcik (Sygenta) shared the Multiple Reaction Monitoring (MRM) platform based on LC-MS/MS. It measures analytes directly, shows similar sensitivity to ELISA but wider dynamic range and higher specificity to distinguish closely related proteins and is ideal for multiplex assay format. Potential drawbacks include low throughput and use of heavy peptides as standard. Several participants also raised additional considerations from regulatory perspective, such as that some countries only accept immunological methods and how new data would compare with historical data based on ELISA methods. PWG will develop a rationale for AEIC's role that is aligned with the AEIC mission statement, work towards international acceptance of, but not requirement for, these alternative methods, and look into intellectual property and freedom to operate.

3. Protein characterization and allergenicity assessment

Lucy Liu led the discussion on protein expression and characterization with emphasis on post-translational modifications (e.g., glycosylation, acetylation, hydroxylation, methylation, etc.). Tao Geng mentioned specific requirements in the most recent allergen guideline by EFSA such as 9-aa peptide sequence as well as the lack of clarity on how to implement it under GLP conditions.

4. Validation of multiplex protein methods

Kristen Kouba (Corteva Agriscience) led the discussion on different platforms being used, regulatory considerations and validation criteria. Attendees supported the concept of end point not particular methodology and agreed that the PWG should work on a publication with guidelines that could subsequently be used to draft an ISO or Codex standard.

5. Intractable proteins

This topic will be postponed till the next meeting. It was suggested that we invite Barry Schafer (Corteva Agriscience), who is one of the co-authors on the Bushey, *et al*. paper, to come present to the PWG on this subject.

At the end, David Levin made a motion and Matt Cheever and Lucy Liu were selected as PWG Chair and Co-Chair respectively. Matt was tasked with preparing a draft mission statement for the PWG and presenting this as part of an update during the AEIC business meeting.

Draft Mission Statement for the AEIC Protein Working Group

The mission of the Protein Working Group is to leverage the collaborative expertise across the agricultural biotechnology and analytical industries to:

- Support the development and adoption of high quality and scientifically sound protein analytical methods, particularly for new techniques, applications or modernization of existing methods.
- Seek standardization of protein analytical methods, where beneficial, for increased efficiency and international acceptance.
- Produce and publish scientific literature and standard documentation to support protein analytical methodologies.

Attendees

Name	Company
Anthony Miller	AOCS
David Roberts	BASF
Shawn Motyka	BASF
Matt Cheever	Bayer
Ray Shillito	Bayer
Qiang Zhao	Bayer
Penny Hunst	Bayer
Nancy Gillikin	Bayer
Sarah Brustkern	Corteva Agriscience (DowDuPont)
Kristen Kouba	Corteva Agriscience (DowDuPont)
Carl Maxwell	Corteva Agriscience (DowDuPont)
Luke Muschinske	Covance
Jeff Shippar	Covance
David Levin	Covance
Nevena Djuranovic	Envirologix
Susan Tapley	Envirologix
Brian Schuld	Eurofins
Farad Ghavami	Eurofins Biodiagonstics
Keith Persons	Eurofins NAC
Brenda Johnson	Eurofins US
Kai Liu	Eurofins US
Zi Lucy Liu	Monsanto
Tao Geng	Monsanto
Jeff Gillikin	North Carolina State University
Ann Allen	Romer
Donna Houchins	Romer
Ashley Fisher	Simplot

Simone Cummings	Syngenta
Mark Bednarcik	Syngenta Crop Protection
Phil Brune	Syngenta Crop Protection

Slides used in PWG meeting:

Draft PWG Mission Statement

The mission of the Protein Working Group is to leverage the collaborative expertise across the agricultural biotechnology and analytical industries to:

- Support the development and adoption of high quality and scientifically sound protein analytical methods, particularly for new techniques, applications or modernization of existing methods.
- Enable standardization of protein analytical methods, where beneficial, for increased efficiency and international acceptance.
- Produce and publish scientific literature and standard documentation to support protein analytical methodologies.

Protein Working Group (PWG)

- Technical aspects of endogenous allergen activities will transfer from the Composition Working Group to the PWG
  - Considering development of harmonized methods, possibly starting with most recent allergen required by EFSA
- Explore the topic of transgenic protein analysis by multiple methods
  - Develop a rationale for AEIC role that is aligned with the AEIC mission statement
  - Work towards international acceptance of, but not requirement for, these methods (drive towards focus on endpoint not method)
  - Consider questions around freedom to operate
- Develop and publish guidelines for validation of multiplex analytical methods
  - Potentially work towards an ISO or Codex standard in the future
  - Support the concept of endpoint and not method (if validated)
- Assess whether the 2006 Grothaus publication on analysis in agricultural biotechnology could be updated (possibly as an output from multiple methods study).

- Matt Cheever (Bayer) and Lucy Liu (Monsanto) selected as PWG Chair and Co-Chair, respectively