



AEIC 2019 Spring Meeting Minutes



New Orleans, Louisiana



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AEIC Spring 2019 Meeting Minutes

April 10-11, 2019

New Orleans, Louisiana

P.L. Hunst, Secretary

The AEIC Spring 2019 Meeting was held in New Orleans, LA on April 10-11. Eurofins GeneScan hosted the group at the Maison Dupuy Hotel. John Reuther, Eurofins, welcomed the group to New Orleans.

AEIC BUSINESS MEETING

Approval of 2018 Fall Meeting Minutes: A motion was made, seconded and voted positive to approve the minutes as posted on the AEIC website.

Treasurer Report (L. Muschinske): The 2019 budget was presented as follows:

Item	Projected (\$)	Actual (\$)
Balance 1 January 2019	21515	21515
2018 Dues	1000	1000
2019 Dues	15000	9750
2018 Fall Meeting Fees	2250	2350
2019 Spring Meeting Fees	2250	
TOTAL revenue	20500	13100
Scientific paper	2000	
DE Franchise Tax	225	225
ANSI/ISO	2900	2900
Board Meeting	800	179
Spr Meeting Expenses	5000	2329
Fall Meeting Expenses	5000	
Website	2000	372
Credit Card	600	258
Graphic Brochure		
Marketing	1500	
Subscriptions	100	



Misc	100	100
TOTAL Expenses	20225	6382
Balance	21790	28253

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

Membership Update (L. Muschinske): The following table depicts the current membership composition of AEIC:

Category	Number	Projected Dues (\$)	Unpaid Dues (number)	Dues Amount unpaid (\$)
Large Companies	9	9000	5	6000
Medium Companies	10	5000	7	3500
Small Companies	12	3000	9	2250
Associate Members	3	150	3	150
Individual Members	2	200	1	100
TOTAL	36	17350	25	10400

AEIC Website Update (B. Johnson): There has been little progress on the slideset posted on the website. The Protein Working Group has agreed to work on the protein portion of the slides. Updated pictures and graphics are needed, however, the graphics are editable in the posted pdf. J. Haudenschild has offered to assist on the DNA portion and will be included in sub-team calls.

AEIC Trifold Handout (B. Johnson): Brenda presented a mock-up of the trifold brochure. The membership was asked to review and send any edits/suggestions to the Board.

Scientific Paper (R. Shillito): The Protein vs PCR Methods paper was published. The citation is:
 Alarcon, C., Shan, G., Layton, D.T., Bell, T.A., Whipkey, S. and Shillito, R.D. 2019. Application of DNA- and protein-based detection methods in agricultural biotechnology. J. Agric. Food Chem. DOI: 10.1021/acs.jafc.8b05157.

Composition Working Group (C. Maxwell, Corteva Agriscience): The CWG has been discussing the use of the Dumas method for crude protein instead of the Kjehldahl method. In order to convince regulators, comparative work needs to be done. EPL Labs has written a proposal which will be discussed. EPL Labs also has written a multiplex method for fat soluble vitamins which is being reviewed by the CWG.

Protein Working Group (M. Cheever, BASF): The PWG elected Kristen Kouba as a co-chair to replace Lucy Liu who has transitioned to the AEIC Board. The PWG will work on the protein slides in the slideset on the website (V. Messmer, B. Matthews). The PWG has three project workstreams. The first is the multiplex protein method validation workstream. The workstream is putting together the literature for standard procedures and validation parameters and acceptance criteria for singleplex and multiplex methods. Recommended steps for adding traits to a validated multiplex method are also being formulated. The second workstream is for mass spectrometry for protein quantitation. The workstream has finished their mission statement. They have also begun a scientific review paper on the value of ELISA and MS to quantify proteins. The paper will not include all antibody methods, however. The third workstream is on allergen analysis. This workstream has also finalized their mission statement and has recruited six more members. The workstream has discussed a database of allergen natural variability and initiated a paper to review a MS approach for in vitro digestion. A sharepoint site has also been established to facilitate document sharing.

Upcoming AEIC Meetings (L. Liu): The Fall 2019 Meeting will be hosted by Corteva Agriscience in Des Moines, IA. The following topic suggestions were made by the membership for the general theme of seed testing:

- Assay throughput
- Use of different chemistries
- Use of bar coding and NGS
- Adulteration (Nantes meeting proceedings)
- How to feminize hemp seed
- Point of use detection
- Impact of tariffs on agriculture
- International seed shipping (disease indexing)
- Production of seed for regulatory submissions
- Production of certified reference material
- Uses of hemp fiber
- IP protection for gene edited products and stewardship around this.

By-Laws Amendment (P. Hunst): Suggested by-law amendments had been sent out prior to the meeting. Since there was not a member quorum at the meeting, no vote could be held. After review and discussion, it was decided that a sub-group (Kalyn Brix, Frank Spiegelhalter, Ray Shillito, Penny Hunst) would look at all the by-laws and make suggested revisions.

The Business Meeting was adjourned.

INVITED TALKS

Research and promotion activity for uses of cotton co-products (including oils)—T. Wedegaertner, Cotton Inc.: Cotton Inc. has been working on gossypol content for a

number of years. Gossypol comes from the pigment glands in cotton leaves, seeds, stems and flower buds. About 60 years ago, a plant was discovered which was glandless and thus, had no gossypol. It was highly susceptible to predation since without gossypol there was no deterrent compound to predators.

Cotton Inc. and Texas A&M have developed a biotech low gossypol cotton in which the gossypol production is in the foliage but not in the seed. This maintains the predation protection but reduces gossypol in seed to less than 200ppm. The market being targeted for this product is aquaculture. Cottonseed can provide the necessary protein via low gossypol cottonseed meal. Low gossypol cottonseed meal has been successfully substituted for at least 75% of fish meal. The low gossypol cotton trait has been deregulated by the USDA and the consultation with FDA is expected to finish soon. A submission to Mexico will be made after finishing the FDA consultation. Another project which is being worked on is developing plants that utilize phosphite instead of phosphate. Forty-five tons of phosphate fertilizers are used every year. These fertilizers may get into the water and runoff and can cause algal blooms. By developing plants that use phosphite, weeds would be suppressed since phosphite would be applied and weeds would have no phosphate. This is only effective in fields that are very low in phosphate. Otherwise, weeds will flourish.

In the non-woven market, Johnson & Johnson has introduced baby wash, shampoo and lotion that contain cotton fiber. The addition of the fiber retains moisture. Also, cotton fiber is being used in 3-D printing products such as toothbrush handles and travel mugs.

Cottonseed oil in the diet improves health more than olive oil. Participants in the study who consumed the cottonseed oil-rich diet consumed 80 calories less/meal. The fatty acid DHSAs is a novel in cottonseed oil and appears to survive the processing of the oil. A company is being engaged to conduct clinical trials to fully understand the DHSAs impact. The majority of cottonseed (70%) goes directly to incorporation into dairy feed. Only 30% is further processed for the oil.

Improving cotton fiber through molecular and genomic approaches—D. Fang, USDA

ARS: The USDA center in New Orleans a) develops a broad base of knowledge on fundamental cell biology; b) develops approaches to improve cotton fiber. The current projects are a) fiber QTL identification, validation and utilization; b) elucidation of fiber development; and c) introgression of superior fiber traits from exotic germplasm into upland cotton. For fiber QTLs, bi-parental mapping can be done. This is simply doing crosses and determining the segregation. The QTLs can then be identified and then be validated in different cotton lines. The MAGIC population method uses random mated population involving six cycles of mating. The fiber quantitative loci are then identified. These are then validated so that they can be used in breeding.

Fiber mutants are available for use in studying fiber elongation. The Li_1 gene causes the actin to not function correctly resulting in a short fiber. The Li_2 gene blocks the elongation function. If these genes can be blocked, then the fiber will elongate. For fiber thickness, the im locus works with mitochondria in the cells. If mutated, the energy diversion is inhibited resulting in immature fiber.

Introgression of exotic germplasm may result in developing stronger fiber. Gossypium genus has over 50 species and is very diverse. Diploid species with no fiber



have been crossed with fiber species and the result is stronger fiber. More work is needed to understand.

Potential for low gossypol cotton—M. Dowd, USDA ARS: Low gossypol cotton has the potential for a wide variety of applications in food. These include protein fortified drinks, peanut butter substitute and edible food film packaging. Cotton protein is highly water soluble which makes it applicable to these applications. For use as a nut, the challenge is in maintaining the kernel size. Seed cracking and de-hulling techniques need to improve as well as the separation of the hull from the kernels. Low gossypol cottonseed oil makes the color set go away and eliminate the miscella refining. Crushing plants could produce a crude oil product that could go to dedicated refining operations.

Several food grade protein ingredient products are possible from low gossypol cotton. The current standard is 41% protein which has a lot of hulls. Defatted protein flour has 50-54% protein and protein concentrate has 56-63% protein. A 97% protein base isolate is possible by base solubilization. High oleic cottonseed oil would need to be 40% high oleic. The USDA center surveyed the national accessions and found 40, of which 3 had high oleic. All happened to be *Gossypium barbadense*. GB173 is a wild photoperiodic cotton that is also resistant to reniform nematodes. This was crossed with a line resistant to root knot nematodes. These lines were also high oleic lines.

For seed quality, the seed to fiber ratio has fallen to 1.41 from 1.7. The percent seed linters has decreased from 12% to less than 10%. There has been little change in the percent of oil or protein. Conditioning of seed is important. Acid delinting has little effect on the hull strength. Small seed may be due to the intense breeding efforts for fiber quality.

Organic cotton breeding challenges and native trait targets—J. Dever, Texas A&M:

Native traits are characteristics existing in a species genome which may or may not be phenotypically expressed. They are generally not targets for GM research and are usually multigenic traits. Native traits include nematode resistance, blight resistance, fiber quality, abiotic stress response, etc.

Organic cotton production is a system of farming which uses no synthetic pesticides and is certified by a third party. There were approximately 20800 organic cotton bales produced on 20680 acres in the US. Organic upland fiber and seed is about twice the price of conventional cotton. Organic pima fiber is about 1.7 times the price of conventional cotton. Organic cottonseed is primarily used as organic dairy feed. The need for organic cotton is uniform cultivars instead of heterogeneous populations. Organic cotton requires the same distribution of water management as conventional cotton. The difference between organic cotton and conventional cotton growing is weed management practices, required crop rotation and waiting for an environmental freeze before being able to harvest. Organic cotton growers must plant organically produced seed. If organic seed is not available, growers can plant non-GM seed. Breeding methods for organic seed included traditional methods, conjugation, fermentation, hybridization, molecular/genomic tools. No GM methods are allowed as well as no cell fusion, microencapsulation, rDNA, genome editing. Native traits are compatible with organic. Farmers have few options for planting seed which usually include farmer caught seed. There is also limited processing capacity for organic seed.

The National Organic Program (NOP) exemption is required for use of HCl gas. The green marketing option is not as restrictive as organic certification. Bayer's e3 sustainable cotton program is an example.

For organic breeding, the Organic Research Environment Initiative has provided funding. The challenge in organic breeding is adventitious presence of GM events. This requires testing logistics in breeding nurseries which leads to added expense due to the use of LFS devices and other tests. Farmers cannot control pollinators. Fiber production isolation is not planting seed isolation. Growers often ask if it is cheating if GMO detected in their product. The answer is "no".

Methods for oil analysis in cotton—P. Horn, East Carolina University: Seed oils are complex or simple. To analyze oils, approaches require resolution, speed, sensitivity, qualitative vs quantitative, targeted end user, ease of use and costs.

Time domain NMR can analyze lipids and proteins since each give different signals. Seed remains intact and it takes 45 sec for analysis. A project was carried out to survey genetic diversity of cotton measuring oil content. High-oleic *Gossypium barbadense* varieties were identified. The cons of the method include the necessity to heat sample to 40°C, relies on chemometric setup and the current protein signal is weak. Applications for the technology include breeding and stress treatments.

Diverse lipids and their functions can be analyzed via supercritical fluid chromatography (SFC). SFC supplies detailed composition but the methodology is still being understood. The pros for SFC include good separation, identification and quantification for a complex mixture.

Oil composition may be visualized by on-stage sampling, microphase extraction and nanospray mass spectrometry (MS). This allows the investigation as to whether all organelles are the same. The pros of MS include spatial resolution and complements other techniques. The cons are that not all compounds can be analyzed, sample preservation and the cost/time of method.

The important point about oil analysis is that the question to be answered needs to be known in order to apply appropriate methodology.

Hemp as an alternative row crop—K. Edmiston, North Carolina State University: Hemp is a fiber crop which can be mixed with cotton. High quality varieties are now coming from China. Hemp is planted at 180,000 plants/acre. Canada grows hemp primarily for seed and as a possible rotation crop. Hemp is a very messy crop to harvest. Farmers in North Carolina want to grow hemp for CBD (cannabidiol) which is popular to treat many human conditions. Hemp would be an alternative crop to tobacco. Since hemp is dioecious, female plants are needed. Mother plants are produced in greenhouses. Hemp varieties are not pure and produce a lot of variability in the field. Root-bound clones are starting to cause problems in the field since they do not make adventitious roots like tobacco. Hemp is planted with 4-5 feet between plants or with the use of plastic between plants to keep weeds out. Hemp is a short day plant so it cannot be planted too late. Hemp does not like "wet feet" so cannot be planted in wet areas or areas prone to excessive moisture (flood areas). Hemp does have pests such as bollworms (really love it), however, many pesticides are not approved for use on hemp. Hemp does need water and fertilizers. Hemp is harvested by hand and tobacco barns

are now being used to dry it. Trichomes contain the CBD and generally turn from clear to cloudy when time to harvest. FDA is considering regulating CBD in food and beverages. NCSU has an internet portal for more information:

<https://industrialhemp.ces.ncsu.edu/>.

Gene editing in cotton for oil improvement—J. Shockey, USDA ARS: Oils are all about the fatty acids. Fatty acids determine whether the oil is liquid or solid. The more double bonds in fatty acids means the oil will be liquid at room temperature. The goal at USDA ARS is to improve the diversity of fatty acid composition in cottonseed oil. This can be done by traditional breeding, MAS/GWAS breeding, reverse genetics, transgenesis and targeted genome editing (CRISPR Cas9). CRISPR has worked well in medical applications and in agriculture. USDA has started work using Arabidopsis as a quick screen tool to screen mutations. With genome editing the two biggest concerns have been off-target recognition and sluggish, inefficient guide RNAs (gRNA) that fail to act. Many groups continue to develop algorithms for the prediction of the best gRNA sequences which are needed for the most effective specific targeting and recruitment of Cas9 which having minimum off-target activity. The gRNA is still the weak link in genome editing. Promoters and terminators are important in transgenesis. gRNA choice and design is the most important mystery. There have been several publications for using transgenesis and CRISPR Cas9. A transient leaf system has been developed to optimize CRISPR Cas 9 genome editing and allows testing of a suite of gRNAs. Genome editing will be a powerful toolkit for metabolic engineering.

Regulatory status of gene edited products—P. Hunst, BASF: Gene editing is another tool in the breeding toolbox. Gene editing methods introduce small DNA changes at a desired position in existing gene sequences which is accomplished by site-directed nucleases (SDN). Gene editing is also referred to as “new breeding technologies”, “plant breeding innovations” and or “targeted genome optimizations”. Gene editing is a continuum from targeted deletion mutation to targeted gene editing to targeted gene insertion (cisgenesis) to targeted gene insertion (foreign DNA). Conventional breeding (spontaneous mutations) and mutagenesis (radiation/chemical) are considered “natural” and not regulated. Transgenesis (foreign gene insertions) are regulated under GMO regulations globally. For targeted genome editing, regulation is unclear as resulting products may have genetic changes that are comparable to conventionally-bred plants. Some countries have made decisions on some of these products. Argentina, Chile, Brazil, US (USDA) have indicated that targeted deletions, targeted edits, targeted allele replacement and null segregants will not be regulated whereas targeted insertions will most likely be regulated. In the EU, the European Court of Justice ruled that organisms obtained by mutagenesis are GMOs and subject to obligations of GMO DIR 2001/18. However, the ruling exempts organisms obtained by mutagenesis techniques (radiation/chemical) which have conventionally been used and a long safety record. Other countries such as Australia and Japan are considering possible exclusion for certain gene edited products (case-by-case). In summary, countries are struggling to accommodate gene editing under current regulations and thus, inconsistencies between countries for these products are developing.

Gene editing detection methods—J. Chen, Syngenta: Gene editing technologies include Zinc fingers, meganucleases, TALENs, CRISPR and base editing. CRISPR Cas9 is a site-directed nuclease (SDN) and uses gRNA. Cas9 causes double-stranded breaks in DNA. There are 3 classes of SDN referred to as SDN-1, SDN-2 and SDN-3. SDN-1 causes break in DNA without addition of foreign DNA. This leads to a deletion or mutation. SDN-2 produces a double-stranded break with a small nucleotide template being supplied that is complementary to area of break. The template contains one or several small sequence changes which are copied into plant's genome. SDN-3 also induces double-stranded break in DNA but is accompanied by a template containing a gene or other sequence of genetic material. The gene template is incorporated into plant's genome.

Conventional plant breeding utilizes random mutagenesis whereas genome editing delivers targeted mutagenesis. rDNA methods result in a random insertion of foreign DNA material.

Genome editing process is basically as follows:

gRNA/construct → transformation/regeneration → plant analysis → crossing/selection.

When no foreign DNA is introduced, a line is called NEDEL (no exogenous DNA edited line) and each generation is designated as "E" for edited. Thus, E0 is the first generation. Edits are screened and verified by sequencing. T-DNA coding editing machinery in E0 plant are segregated out producing null segregants with no detectable foreign DNA.

Edited plants can be tested by target capture NGS and multiple PCR assays on Fluidigm platform. There is potential for off-target edits since plant populations are constantly accumulating genetic changes. Thousands of crop varieties have been produced via natural mutations and no regulatory analysis has been required. The CRISPR systems are increasingly specific and off-targets are very low.

Handling of gene edited plants in the field is dependent on government regulations and the global regulatory environment, the biology of the crop and the presence of foreign DNA. It is feasible to develop PCR assays to distinguish gene edited DNA alterations from the original sequence in the value chain. The development of sensitive or practically feasible PCR assay depends on the specific change, size and genomic location. PCR assays can be developed for large deletions. SDN-1 and SDN-2 are indistinguishable from those similar changes that occur in nature. Thus, specificity of PCR assays will be an issue as well as method validation and compliance. In the grain channel or for value chain testing, PCR methods probably cannot detect a single edited seed in bulk or heterogeneous samples.

In summary, appropriate processes are required to manage gene edited materials to maintain product integrity. Most gene edited products will be indistinguishable from naturally-occurring or conventionally-bred mutations.

Breakout Session: Testing for gene edited products (B. Schoel, FoodChain ID):

“Can we test for the presence of gene edited products in bulk seed/grain or in foods?”

Changes of Gene Edited (GE) organisms are known and monitored at the design and production of GEs.

(for the purpose of this report the term GE is used to denote a product of the gene-editing process, where insertion, replacement or deletion – not to be confused with other uses of the term GE)

If significant foreign DNA is inserted by GE methods such as CRISPR/Cas9 the GE is probably regulated as a GMO and the GE is able to be detected with conventional PCR methods.

If no foreign DNA is inserted and only deletions occurred the GE is not regulated in certain jurisdictions (except EU and NZ to date) and it is virtually impossible to detect if nothing is known about the changes.

For small inserts, the question was raised at what bp length a PCR based test is possible. For example could an 8 bp insert or even a 4 bp insert be detected?

The point was made that the GE may be IP protected with patents. For IP protection the GE maker would be expected to be able to detect and discriminate a GE from wild type varieties during seed production in order to keep the GE separate from WT varieties. However, in the field due to the large number of varieties within a species and the lack of a “consensus” sequence it remains difficult to discriminate unambiguously a SNP GE from WT varieties. Therefore, even deep sequencing will not be successful for GE detection. Also, some SNPs have been demonstrated to be detected at a 1 in 100 or 1% level but not less than that, and a general rule is not known for other SNPs. This makes it virtually impossible to detect GE with SNP or minor changes in bulk samples.

If the GE product is patented a test could theoretically be developed from public information. If only the trait is known, depending on the nature of the GE, it may or may not be possible to develop a test. An example where it is not possible to develop an assay is in the case of a GE with yield increase where a multi-copy gene is used to increase its level.

From the perspective of testing laboratories, assays for GE are highly desirable for purity and adventitious presence testing. Assay development requires (i) a testing method (i.e. target sequences) and (ii) reference material.

In summary, the following points, questions, and suggestions were raised:

- 1) GE with transgenic inserts should be detectable with conventional PCR methods.
- 2) Testing laboratories desire a testing method and reference material to be available
- 3) It was suggested that AEIC publish technical guidance on GE detection methods.

- 4) To what degree is it possible to discriminate GE from WT varieties if the change in the GE is a minor change, for example what is a minimum insert length such as 8 or 4 bp to discriminate GE from WT? Is there interest in defining a lower limit that can be easily detected?
- 5) How to protect IP preserved GEs in the field?
- 6) How to deal with GE detection in bulk samples?

Breakout Session: Next product opportunities for cotton (M. Dowd, USDA ARS): Some next product opportunities suggested were:

- For the non-woven sector for fiber, need a disposable wipe that degrades when flushed;
- More sheen and drape properties for the fashion sector;
- Fire retardancy for children's clothing and carpeting
- Disease resistance in cotton
- More medicinal uses such as bandages with healing properties and anti-microbial

Bioengineered food disclosure standard review—T. Findley, USDA AMS: The law amended the Agriculture Marketing Act (1946) and was signed on July 29, 2016. It directs the Secretary of Agriculture to establish the National Bioengineered Food Disclosure Standard for disclosing bioengineered food (BE) and food that may be bioengineered. Food manufacturers, importers, retailers are all regulated, however, restaurants and very small food manufacturers (<\$2.5 million in receipts). A bioengineered food is determined from the nutritional label. If the first ingredient is subject to regulations under FMIA, PPIA or EPIA (pork, beef, sheep, goat, catfish, chicken, turkey, domestic birds and egg products), then the food is not subject to the BE standard. For example, if labels are as follows:

- Label has pork, salt, water, food starch > first ingredient is subject to FMIA, PPIA or EPIA, therefore food is not subject to BE Standard
- Label has vegetable broth, potatoes, chicken, peas, carrots > first and second ingredients are not subject to FMIA, PPIA or EPIA, thus food is subject to BE Standard
- Label has vegetable broth, turkey, egg noodles, water, corn > first ingredient is not subject to FMIA, PPIA or EPIA but second ingredient is, thus food not subject to BE Standard
- Label has freeze-dried egg, freeze-dried egg yolk, nofat dry milk > first ingredient is subject to FMIA, PPIA or EPIA, thus food is not subject to BE Standard

BE food is defined as food that contains genetic material that has been modified thru *in vitro* rDNA methods and for which the modification could not be obtained via conventional breeding or found in nature. Current BE foods include alfalfa, apple (Artic), canola, corn, cotton, eggplant (BARI variety), papaya, pineapple (pink flesh), salmon (Aqua Bounty), soybean, squash and sugarbeet. The key is detection of the

modification. Modified genetic material is not detectable if a) records verify food is made from a non-BE food; b) can verify food has been refined using a process validated to render GM undetectable; c) testing records for specific food confirm no detection; and d) incidental additives are not BE food. Exemptions to the BE Standard include a) 5% threshold as long as inadvertent or unavoidable; b) animals fed BE feed; and c) food certified under the organic standard.

Disclosure will be made on food container as an information panel adjacent to the manufacturer label, on the principal display panel or may be made on any other panel likely to be seen by the consumer. There may also be an electronic or digital sign on food or the consumer can be notified by text message. The text for disclosure will say “bioengineered food” or “contains bioengineered food”. Electronic or digital may say “scan here for more information” and must go directly to product information page and must include BE disclosure. For entities that are exempt, they may do voluntary disclosure.

Records need to be kept that verify food is not BE or no longer contain a detectable amount of BE. Entities must keep sufficient records which are customary and reasonable. Regulated entities may determine which records to maintain, format and store them at any business location (bills of lading, inventory records, supply chain records, etc.). Records must be kept for a minimum of 2 years but may have to be retained longer for verifying a process.

Enforcement will be done based on consumer complaints and begins January 1, 2022. USDA AMS will determine if an investigation is needed based on complaints. Enforcement penalty is the posting of entity name on website indicating that they are out of compliance. More information may be obtained at <https://www.ams.usda.gov/rules-regulations/be> or emailing at befooddisclosure@ams.usda.gov.

Seed health testing—J. Mizicko, Eurofins BioDiagnostics: Seed health testing is done as an import/export requirement with the market requiring it and the growers demanding it. Companies want to know the quality of seed. Target organisms cause serious losses in the field. And there are lawyers lurking around tractor. Seed health testing is costly, takes time, slows down sales, uses valuable seed and the results may not be appreciated. It affects risk management decisions and affects relationships with third party producers. The pathogens tested for include bacteria, fungi, viruses, viroids and nematodes. Many of these are not associated with the seed. There are seed-borne and seed-transmitted pathogens. Seed-borne pathogens are on the seed but do not affect the following generation. Seed-transmitted pathogens are on the seed and will affect the next generation. They use the seed as their pathway and are the primary focus of testing. For a good test, a good representative sample of a lot is required. The sample size must be adequate. The protocol should be validated and the lab should have right maintained equipment. No test is 100% accurate since not every seed can be tested. An increased sample size increases the confidence of the test. However, infected seed is not uniformly distributed and thus, different samples may give different results. Test results reflect the sample and not the lot. Thus a negative test may not be negative. The types of tests include grow out, semi-selective media, blotter assays, ELISA, PCR and pathogenicity tests. Direct methods actually isolate the pathogens

whereas indirect tests (ELISA, PCR) do not. There is growing acceptance of indirect tests.

Seed health is not a competitive arena since what affects one, affects all. The International Seed Health Initiative (ISHI) was established in 1993 and is composed of seed health professionals from multiple countries and companies. ISHI is organized by countries and crop, with working groups for topics. ISHI meets every 9 months. The National Seed Health System was established in 2000 and aligns with ISHI protocols. It requires validated tests and the results can be used for phytosanitary certificates. It is administered by USDA and Iowa State University. Both groups focus on emerging diseases and reflect the global nature of the seed industry. There is an increased focus on seed quality and changes in phytosanitary requirements.

Testing is changing by becoming faster with more molecular methods which are more sensitive, more selective and multiplexed. Future issues in testing include:

- Will pathogenicity matter;
- Will a test be too sensitive, i.e., go below economic threshold;
- Deep sequencing is a Pandora's box;
- Trading seed pathologists for molecular biologists;
- Know the organism but not the disease
- Harmonization of protocols is important;
- Will methodologies be affordable.

Canola club root testing—T. Blois, 20/20 Seed Labs: Canola in Canada is a hybrid from the Brassica napus family. It is a broad family of which all are hosts to club root. Canola adds \$27 billion to the Canadian economy, 250,000 jobs, \$11 billion in wages and includes 43,000 farmers. Canola is primarily grown in the prairie provinces in western Canada. Club root is caused by *Plasmodiophora brassicae* which causes galls on the roots which interfere with water uptake. A 50% infection will result in a 25% yield loss. Resting spores can survive for 20 years in soil. Spores germinate and produce zoospores which move to the plant roots, invade the cells and produce galls. The organism moves from field to field by soil movement on machinery or flooded fields.

Club root was reported in Alberta in the 1970s and was detected in canola in 2003. It was declared a pest in 2007. There have been cases of population shift to overcome variety resistance. Club root is less common in Saskatchewan and little is found in Manitoba. Club root is not evenly distributed in fields as it is the equipment bringing the soil in. It is also present in low-lying spots, water runs and garden sites. Sampling is done by collecting the top 10cm at 5 points in a "W" pattern. Infected plants are concentrated sources. Testing soil nearby is not effective. 20/20 Seed Labs uses a proprietary method. The primers for the method have been published. One half cup of soil is tested and it is not a quantitative method. A semi-quantitative method has been done which classify results as detected or trace or not detected. The method uses a 5g sample. The test satisfies market demand for quantitative data but there have been challenges with consistency between labs. If a grower does a 2 year break in his fields from canola, this will give a 90% load reduction of club root.

Broad spectrum control of pathogenic and mycotoxigenic fungi in transgenic cotton expressing synthetic peptide—K. Rajasekaran, USDA ARS:

Aflatoxin fungi are destructive to tree nuts, cottonseed, corn and peanut. In corn, losses have been \$200-250 million and in cotton \$5-18 million. *Aspergillus flavus* produces a carcinogen which causes illness from ingesting food containing it and can cause liver cancer. Feed contamination is toxic to livestock. It is a perennial problem in warm climates.

There are several approaches to controlling the organism. The first is to prevent the fungus from reaching the crop. This can be done by biological control methods and farming practices. A biocompetitive fungus has been used in Arizona and Mississippi. It does not remove the fungus completely and how it works is not known. The second approach is to prevent the fungus from invading the crop. This is done by enhancing host resistance, i.e., looking for varieties that are resistant. Most upland cotton is susceptible. Antifungal products do not work effectively. However, unusual novel genes have been found that cause disintegration of the fungus but they are not hemolytic. They have broad spectrum control of fungal diseases. The *A. flavus* invades the seed at the broad end of the seed and causes it to be mushy in 3 days. For the black root fungus, transgenic has been made which expresses the peptide. Plants expressing the peptide had healthy roots. Peptides have been found to also work in transgenic corn lines. The third approach is to prevent the fungus from producing the toxin. Knowledge of the toxin pathway is being used to develop a RNAi solution.

Screening of GMOs in cotton and textiles—L. Burzio, Bayer: An international workshop agreement (IWA32) was initiated by the Organic Cotton Industry in 2018. IWAs are developed outside the normal ISO committee system. The IWA32 was sponsored by the Netherlands. The intent was to create a standard protocol to screen cotton and textiles for potential presence of known GMOs. The intent was to create clarity in the sector. It was necessary for the protocol to be clear and most importantly must outline process to obtain good quality DNA. Sample collection and the identification and quantitation of GM events were out of scope of the IWA32. The protocol is moving to a final draft and the lab work to support was done by RIKILT (Netherlands). Organic cannot be qualified on a molecular basis. The nucleus is destroyed during fiber formation and ginning removes the seed from the fiber.

Eighty-eight experts from 22 countries were brought together to deliberate the IWA. The first draft had 47 pages of comments with the importance of obtaining quality DNA at the forefront. The second draft was improved but was still not a method. It had more details but was still lacking specifics.

A WD3 meeting was held in New Delhi, IN in January 2019 with 30 participants. RIKILT presented some results at the meeting. Even though the protocol lacked validation parameters and the scope still did not include sampling, the protocol was approved by popular vote. The RIKILT data was limited but did talk about how to obtain DNA from all 4 species of cotton. The Qiagen stool kit was settled on as the preferred DNA isolation method. RIKILT tested leaf, seed and fiber samples. Since no raw data was shared, the validity and accuracy of the conclusions were questionable.

The final document has not yet been posted. It is not really a protocol but does provide recommended methods for detection of 6 elements found in GM cotton (but does not account for possible contamination). The document will put the responsibility



on the grower. IWA32 will be implemented but it is questionable whether it will resolve the issue.

Handheld scanners for moisture testing—D. Sharon, Consumer Physics: Consumer Physics was founded in 2011 and has invested over \$45 million. Efficiency is the name of the game. Digital analysis using the scientific method to capture the power of data. Moore's law of spectroscopy is similar to the computing revolution. It is democratization of near infrared (NIR) which expands the market exponentially by using tens of millions of sensors. Minaturization is possible due to smartphones and optics.

Consumer Physics' product is called SCiO, a handheld sensor that scans the molecular footprint of physical matter and instantly provides useful information about its chemical makeup. The first pocket spectrophotometer has been developed which is simple and intuitive and costs \$500 (compared to thousands for other spectrometers). It is run through a mobile app with the models residing in a cloud which can integrate with digital ag systems. The company has been partnering with companies such as Cargill and third party labs such as Eurofins. With Cargill, the company collaborated to introduce Reveal to US dairy producers. Reveal is a real-time forage analysis service that puts the power of a Cargill forage lab analysis in the palm of a hand. With Eurofins Agro UK, the pocket sized analyzer was used for forage analysis on-farm. The company has recently worked with Olam Cocoa by equipping their field and purchase managers at cocoa collection points with SCiO devices to analyze the full moisture range in the cocal beans non-invasively. Consumer Physics have also done some commodities analysis with the Soybean Board for protein and have also some done some work with corn for moisture analysis. The system can also be used for checking raw materials quality for crop protection materials.

For the future:

- Additional uses, more developers, embedded sensors;
- New hardware sensors for more accuracy;
- Third part spectrometers on the platform;
- Enhanced digital experiences;
- Improved connectivity and location;
- March to a billion sensors.

Meeting Attendees:

	Name	Organization
1	Ament, Chris	Eurofins FI
2	Balvin, Kevin	SGS North America
3	Bednarcik, Mark	Syngenta
4	Blois, Trevor	20/20 Seed Labs
5	Brix, Kalyn	SoDak Labs Inc
6	Burzio, Luis	Bayer Crop Science
7	Cheever, Matt	BASF
8	Chen, Jingwen (Spkr)	Syngenta
9	Cummings, Simone	Syngenta
10	Dever, Jane	Texas A&M AgriLife
11	Doornink, Anna	Eurofins BDI
12	Dowd, Michael	USDA Southern Regional Research Center
13	Edmison, Dustin	SGS North America
14	Edmisten, Keith	North Carolina State University
15	Fang, David	USDA ARS
16	Findley, Trevor	USDA AMS
17	Fisher, Ashley	Simplot
18	Gadola, Mary	Neogen
19	Ghavami, Farhad	Eurofins BDI
20	Haudenschild, James	Merieux NutriSciences
21	Horn, Patrick	East Carolina University
22	Houchins, Donna	Romer Labs
23	Hunst, Penny	BASF
24	Johnson, Brenda	Eurofins BDI
25	Kouba, Kristen	Corteva Ag
26	Liu, Zi Lucy	Bayer Crop Science
27	Matthews, Beth	Syngenta
28	Maxwell, Carl	Corteva Ag
29	Messmer, Valerie	Syngenta
30	Miller, Anthony	AOCS
31	Mizicko, John	Eurofins BDI
32	Muschinske, Luke	Eurofins Microbiology Labs
33	Onisk, Ann-Christine	Romer Labs
34	Persons, Keith	Eurofins NAC
35	Rajasekaran, Kanniah	USDA SRRC

36	Ross, Margit	Corteva Ag
37	Schoel, Bernd	FoodChainID
38	Secrist, Heather	FoodChainID
39	Shillito, Ray	BASF
40	Shippar, Jeffrey	Eurofins FI
41	Shockey, Jay	USDA
42	Singsit, Chong	OMIC USA
43	Smith, E Pearce	Eurofins GeneScan
44	Spiegelhalter, Frank	Eurofins GeneScan
45	Wedegaertner, Tom	Cotton Inc
46	Wiegel, Scott	AgriPlex Genomics
47	Woo, Richard	Bayer Crop Science
48	Wu, Xin	AOCS

AEIC Composition and Protein Working Group Meeting Minutes

1:00pm – 5:45pm, April 9, 2019

Attendees:

Corteva: Kristen Kouba, Carl Maxwell, Norma Houston (call in)

Syngenta: Simone Cummings, Valerie Messmer, Beth Mathews and Mark Bednarcik

Eurofins: Luke Muschinske, Keith Persons, David Levin, Chris Ament, Brenda Johnson and Farhad Ghavami

Romer: Ann-Christine Onisk, Donna Houchins

AOCS: Xin Wu

Bayer: Zi Lucy Liu, Call in - Tao Geng, Yanfei Wang, Yongcheng Wang, Rong Wang, Andy Deffenbaugh and Mohamad Bedair

BASF: Ray Shillito, Matt Cheever

I. Composition Working Group (CWG)

Discussion led by Luke from Eurofins:

- Began with Statement for Anti-Trust Compliance & Mission
- **Project List:**
 - Crude protein method comparison: Dumas vs. Kjeldahl
 - Crude fat in Canola

1) Next Step – Flesh out testing scope

Volunteers: David Levin, Anthony Miller, Keith Persons, Brian Schuld, Nancy Gillikin, Carl Maxwell, Mohamad Bedair, Chris Ament

2) **Multiplexing of vitamins:** A, E, D, K

Action item: EPL to share draft method to the group

3) **Action item:** Call for co-leads of CWG to replace Luke

II. Protein Working Group (PWG)

Discussion led by Matt from BASF:

- Began with Anti-trust compliance statement, agenda, PWG mission statement and PWG workstreams and leads.
- **General PWG topics (Matt):**
 - Selection of a new PWG co-chair: Survey Monkey. Kristen Kouba from Corteva was elected.
 - What to do about the topic on intractable protein analysis:
 - a) Paper published on *Regulatory Toxicology and Pharmacology*, Vol. 69, Pages 154-170, 2014.
 - b) **Action items:** Lucy to reserve time on Fall AEIC conference; Kristen to follow up with speaker(s) from Corteva. Matt to follow up on progress/updates.
 - c) Fall AEIC possibilities: Introduction to the topic and action items/interesting topics.
 - Update protein detection slides content on the AEIC website:

- a) **Action items:** PWG to lead and update the contents on the AEIC website: Valerie and Beth from Syngenta volunteered to help.
 - b) PWG SharePoint (Bayer) to keep all documents: contact Bob Eilers or Lucy Liu for teamsite access.
 - c) What parameters/methods should we be considering for standardization now to avoid having a future mandate which we do not support? AEIC serves as a technical platform/channel for the industry standardization:
 - i. End points of protein characterization: shall AEIC start to work on standardization?
 - ii. AEIC Coordination with CLI:
 - ✓ Is CLI willing to share the efforts on a paper focusing on protein characterization? **Action item:** Matt to follow up with CLI.
 - ✓ CLI's focus is on position/policy. Need scientific /technical review for publication that have bigger impact. AEIC plus labs may work together from scientific perspective.
- **1) Working Stream I: Allergen - presented by Tao from Bayer and Norma from Corteva:**
 - Mission
 - Team members
 - **Projects and activities**
 - Standardize methods for 10 soy endogenous allergens
 - a) Multiplexing by MS
 - b) Database harmony
 - Publish paper of MS to evaluate the *in vitro* digestion
 - a) Technical paper on 9 aa detection by MS; draft outline
 - b) Include a response to EFSA
 - **2) Working Stream II: Mass Spec - presented by Mark from Syngenta:**
 - Mission statement
 - Team members; 3 meetings since last AEIC
 - Working on a scientific review paper on the value of ELISA and MS to quantify proteins in biological matrices. Publication will provide options based on situation; reiterate both options are scientifically sound and viable. Timeline: draft; Q4 of 2019
 - **Action items:** Validation and MS working stream to coordinate publications.
 - >15 publications on MS for protein quantification
 - **Next steps:**
 - Members to review 2-3 papers and provide high level summary
 - Discuss details on who/what/how for publication abstract etc.

- **3) Working Stream III: Validation – presented by Kristen from Corteva and Simone from Syngenta:**
 - Goals: produce scientific literature
 - Technologies: Multiplexing platforms – MS, Luminex, MSD
 - 9 meetings since Spring AEIC on technologies, validation parameters, literature references etc. Encourage people to add literature to the SharePoint
 - Timeline: detailed survey by summer 2019 with preliminary parameter set and ask for feedback. Outcome: get agreement. First draft by winter 2020.
 - List of validation parameters: open discussion for anything missed
 - **Action item:** Ray to send the document/database on definitions of each parameter
 - Validation parameters: first principal approach - what we will do if no background information; what we will consider if start from fresh (Ray).
 - Publication strategy: it begins with publication (Ray).
 - ESFA note on extraction efficiency: EE Correction Factor, antibody, proteinase inhibitors, temperatures for incubation to be also included; **Action:** add Robustness to the validation parameter list.
 - **Action items:** Bayer is drafting a White Paper addressing EFSA's note on NEPs that will need to get industry review/collect feedback/alignment. AEIC should have a separate paper than CLI (Matt). AEIC focus should be on technical while there could be a separate effort from policy/position perspective by CLI.

III. PWG Breakout Sessions

- 1) Mass Spec for protein quantification
 - Current publications – allergen related
 - Current publications – transgenic or non-Allergen protein related
 - Ray suggested to review with the same criteria/categories. Such as:
 - Methods
 - Targets
 - LOD/LOQ/Robustness
 - GM crops
 - Authors/companies
 - Start with draft outline
 - **Action items:**
 - Chris from Eurofins to generate a spread sheet with the criteria, guidelines for publication review etc.
 - Allergen Working Group has one-page summary. Mark to work with Tao/Norma
- 2) Validation parameters
 - General:
 - Single vs. Multiplexing: accuracy (80-120%) as an example: may not be achievable for multiplexing platform

- Publish validation parameters for single plex; guidelines/criteria for multiplexing
- Uncertainty/variability of the method: AACC official methods
- Statistical approach may be different for multiplexing
- Need to establish analytical/scientific standards
- Both ELISA and MS are valid methods; may measure different things. So the validation parameters may be different
- Specifics:
 - Curve Fit: R square efficient? 4- or 5- parameter curve fit? Limit at the bottom and top quantitative limit: 25%? Other areas within the quantitative range: 20%?
 - Accuracy: 70-130%?
 - **Action items:**
 - Ray to send out documents on POD
 - Industry to share what we do: may use published documents on validation parameters and share what we agree upon with?
 - Ray suggested to have a spreadsheet listing 4 columns: questions; answers; positive; negative impacts/comments