AEIC Spring Meeting 2014
May 6-7, 2014
San Antonio, TX

P.L. Hunst, Secretary

On behalf of AOCS, Gina Clapper welcomed to AEIC to San Antonio and to the AOCS Annual Meeting.

AEIC BUSINESS MEETING

Prior to the start of the business meeting, the AEIC membership held a moment of silence for Brian Skoczenski who passed away in March, 2014. Brian was a long standing member of AEIC and one of its founding members.

Secretary's Minutes of Fall Meeting 2013: A motion was made, seconded and voted positive to accept the minutes as distributed to the membership.

Treasurer's Report:

2014 Budget

	Planned	**Actual**
Beginning Balance	$39006	$39006
Dues	8000	3600
Interest	190	1
TOTAL REVENUE	8190	4677
EXPENDITURES		
Paper	5000	280
DE Franchise	25	30
ANSI/ISO		
Board Mtg	900	212
Spring Meeting	2500	
Website	3500	
Bank Service Charge		
Fall Meeting	2500	
Graphic Design		
Reprints	800	375
Subscriptions	100	
Miscellaneous	100	
TOTAL EXPENDITURES	15245	898
PROJECTED BALANCE (Checking + CD)	31771	42784.41

A discussion was held on the ANSI/ISO monetary support. No invoice was received in 2013 so no support was sent. Gina gave an update on ANSI/ISO activities for those members who were not aware

of its functioning. The membership then voted to give monetary support to ANSI/ISO and to do this indefinitely. The Treasurer will add $2900 for this to the projected budget.

It was also noted that the AEIC website will be updated as a new company will take over its maintenance.

A motion was made, seconded and voted positive to accept the 2014 budget.

Membership (D. Layton):

Members	**Number**	**Projected Dues**	**Dues Unpaid**
Large Companies	17	$ 8500	$ 7000
Small Companies	14	$ 3500	$ 3000
Associates	2	$ 100	$ 100
Individual	4	$ 400	$ 150
TOTAL	37	$12500	$10250

New members: Charm Sciences, SeqID, Critical Path Services, AP Biocode, Vaske Enterprises, Strategic Regulatory Solutions, Primera, Eureka Genomics

AEIC Brochure (D. Layton): The new version of the printed brochure was distributed. Thanks to everyone who contributed comments and edits. If anyone needs copies for distribution at meetings, e-mail or call D. Layton.

AEIC Website (D. Theide, D. Layton): The AEIC webmaster has changed jobs so a new webmaster (or company) was sought. A bid was received from Pula Tech, a US-based companies whose programmers are located in India. The proposal covers all the work to upgrade the website (ability to make online payments, Doodle for arranging meetings, dropbox function for documents to be worked on, etc.). The bid is for $3500 to do the initial work. The bulk of the cost is for moving the website to its own host site. Beyond this initial work, the cost will be $30/hr to do any further updates or post information.

A motion was made, seconded and voted positive to accept the bid from Pula Tech.

AEIC Goals and Activities (G. Shan):

The lectin method paper is at the AOCS Journal and contains the data generated to date. It was suggested that AEIC should have the rights to PDF once it is published in the journal so that it could be posted on the AEIC website.

An update on the protein vs DNA methods paper was given by D. Layton. A draft is now ready for review by the authors and the intent is to have it published in a trade journal.

A motion was made, seconded and voted positive to stop the work on the sub-sampling paper which had originally been proposed and worked on by B. Kaufman.

Larry M. (Critical Path Services) briefed the group on a publication that is being drafted. Currently, there is no regulatory guidance on detection method and their validations. The intent of the publication is to

present guiding principles and convince the regulatory authorities to adopt. A survey has been developed on what methods are currently being used (ELISA, LC-MS/MS, etc) and was presented at 2013 ACS Agriculture Section Session. A draft publication has incorporated the data to date from the survey but there is no intended journal for publication yet. It is meant to be a consensus document from industry, however, concern was raised that there are only 8 participating companies which is not really "industry consensus". It was also noted that regulatory authorities do not specify detection methods and keep their guidelines vague as they do not want to exclude registrant based on the inability to purchase equipment needed for specific analyses. If the paper is intended to be a work product of AEIC, it was suggested that the scope of the paper needs to be formulated and presented to the membership. An AEIC working group was suggested and volunteers are sought. Currently, the volunteers are G. Yeaman (Monsanto), L. Mallis (Critical Path), M. Cheever (Bayer), C. Maxwell (DuPont Pioneer), M. Yarnall (Syngenta), David Levin (Covance) and Fred Claussen (EPL). If any other members are interested, please let one of the AEIC Board members know.

G. Yeaman (Monsanto) also proposed a paper for methods validation since there is much needed consistency in validation due to cross-licensing of technologies between companies. It would also be useful to have some form of proficiency testing for multiplexing technologies. The intent would be to have a publication on this.

AEIC Fall Meeting 2014: Eurofins GeneScan has volunteered to host the meeting in New Orleans, LA (NOLA). October 15-16 were proposed as the dates, however, there is a conflict with the CLI Detection Methods Team meeting. It was suggested that the CLI group move their meeting back to Oct 14-15 and have it in NOLA rather than St. Louis. R. Guo will check with the CLI group and get back to the Board.

Suggested topics for the meeting include proficiency testing (approaches, results from ISTA and GIPSA, etc); QC process for test methods; genome editing and GMO testing (possibly visiting an elevator). Since NOLA does not have a port and only loading facilities, this may be a bit time consuming since these facilities are located a distance away from NOLA. The Board will discuss this more in the planning process.

2015 Meetings: Agdia or Illumina have tentatively offered to host the Spring 2015 meeting. OMIC USA has offered to host the 2015 Fall meeting.

Composition Methods Working Group (C. Dharmasri): A need for harmonized methods for crop compositional methods has been recognized and organized. The reasons for forming the group include a) analyses to support GM crops have not been optimized, b) regulatory requirements are inconsistent and growing burdensome, c) current composition methods are not applied consistently between labs, and d) there is a lack of an efficient mechanism to drive change. The scope of the working group is: To support the development of the AEIC meeting program as well as offer separate, specific sessions focused on work products (papers). Funding for the working group will come from the working group members and not from AEIC. The first meeting was being held after the conclusion of the AEIC meeting to start discussions on fatty acids methods.

ISO/SC 34 Update (G. Clapper): The next meeting is scheduled for Sept 2-4 in Durham, NC. The last meeting was held in London, UK and 14 different countries participated. It is hoped that South American countries may participate this time. R. Shillito is still the head of the US TAG.

ILSI Update (P. Smith): The sampling/detection has dissolved but has moved to AACC. The workshops are still ongoing with one being held in Brazil the week of May 12. The sampling statistical paper will be coming out soon.

The business meeting was then adjourned.

INVITED TALKS

Cotton will increase its contribution to global food security (T. Wedgaetner, Cotton Inc.): Cotton is an oilseed crop and produces 1.5 times more seed than fiber. Cotton is a perennial that is grown as an annual and its oil profile is closer to that of tree nut. One bale of cotton produces 500 lbs of lint; 500 lbs lint times 1.5 = 750 lbs seed; 750 lbs seed times 16% = 120 lbs oil. Contrary to public misperception, cottonseed oil is not full pesticides. Flavored cottonseed oils have been introduced into the retail market to foster that cottonseed oil is a good frying oil. The cotton kernel, once the external seed coat is removed, resembles a pinenut and has a nutty flavor. Cotton linters are used in filters and papers. Cotton by-products can be found in a number of products since they are thickeners (in shampoos), flow agents, stabilizers (beer), used in TV screens, cooking oil for potato chips, etc. The brand of CRISCO shortening was made of crystallized cottonseed oil (which is the derivation of its name). It is now made with soybean oil. Humans consume 3 pints of oil/year/person.

Plant chemical defense mechanisms include terpenoids which are anti-feedants and gossypol is one of the best known terpenoids from cotton. It has been known for about 100 years. It is polyphenoloic dialdehyde made of two isomers (+ is preferred isomer). Gossypol is a cumulative toxin and binds with divalent cations (especially iron) and lysine. It is toxic to mono-gastric animals. Calves cannot tolerate gossypol until their rumen is fully functioning. Gossypol-containing glands are visible on leaves and in the seed. In the 1950s, a mutant cotton was discovered that had no glands and thus, produced no gossypol. Without gossypol production, the glandless cotton was susceptible to insect pests so the cotton was commercially nonviable and had poor fiber quality.

Cotton Inc. and Texas A&M have now produced ultra-low gossypol cotton (ULGC) via genetic engineering. There are no glands in the seed but the leaves do have glands. In ULGC, the enzyme delta cadinene synthase, necessary in the gossypol pathway, has been blocked via RNAi gene silencing in the seed using a seed-specific alpha globulin promoter (AGP). This cotton is going into regulatory trials in 2014 and is a quality output trait. Since protein for food and feed is becoming more of a demand, cottonseed protein without gossypol would help alleviate some of this demand, particularly for feed. Also, the oil from ULGC looks like refined cottonseed oil so it requires only gentle refining rather than caustic refining. Cottonseeds contain oil since it is necessary for germination.

Aquaculture has 1.1 feed conversion (1 lb of feed will give 1 lb of fish). For aquaculture to be sustainable, the supply of protein feed needs to increase and substitute is needed for fish meal. ULGC cottonseed protein has been used in trials on shrimp with no negative effects. Trials are currently ongoing with striped bass, pompano, flounder and black sea bass. Shrimp and fish still need some marine lipids in their diet, along with cottonseed protein, to thrive. Aquaculture will play a significant role in food availability as the world population increases to 9 billion.

Cotton breeding activities (W. Smith, Texas A&M): Cotton yield has increased dramatically over time from 176 lb/ac in 1910 to 807 lb/ac in 2013. In the 1980s yield went down due to hot, dry years but in the 1990s, yield increased due to the introduction of genetically-modified cotton varieties with insect

resistance and herbicide tolerance. Also, the boll weevil was eradicated in the US (there are still a few parts of southern Texas where boll weevil still exists). The realized gain in cotton yield has gone from 3.8%/year (1940-1960) to 1.2%/year (1990-2009). To keep up with the population growth, a realized yield gain of 1.3%/year is needed. How to bend this downward trend? Genomics will help with this. Qualitative advances with insect resistance and herbicide tolerance but there still exists the possibility of weed and pest resistance developing. GM cotton may be a blip and not the future for cotton. Multiple traits that are quantitative (yield, fiber length) are needed. There are many genomics-based tools such as genotyping by sequencing, whole genome genotyping, genomic selection and genic networks which are and will be used to help bend the curve. Also, research is moving back to phenomics—high throughput phenotyping, photosynthetic capability and plant phenology. The new mantra is to map the genetic basis of a plant's desirable traits and use those data to breed new custom-designed varieties by traditional breeding techniques (a combination of low and high technologies).

Public cotton breeders are contributing in many areas of research. Mike Gore (currently at Cornell) is working on high throughput phenotyping. North Carolina State University has programs fro germplasm enhancement and map-based cloning of leaf shape. The USDA ARS is looking at fiber quality with Pee Dee cotton breeding material as the base material. University of Georgia has programs in the molecular genetics of fiber quality and germplasm/variety development. Mississippi State University is using mutation induced variation and also working on reniform nematode resistance. New Mexico State University directs the acala cotton program. Texas A&M (TAMU) has research programs in Verticillium wilt resistance, fiber quality elongation, organic cotton production, mutation breeding, fiber quality, mapping of populations, cytogenetics, whole genome characterization for fiber quality, RNAi for glandless seed. In TAMU's cotton improvement lab, unique fiber quality is being investigated. An extra long upland staple was found in TAM 94L-25 progeny. The fiber length is better than pima cotton and DPL491 upland cotton varieties. Long fiber length has no commercial value unless the yield increases. Genic networks have been used to look at QTLs which affect fiber length. These networks have shown that there the interaction of genes for longer length is different, i.e., fewer genes are turned on.

The challenges for upland cotton include finding the appropriate genetic variability that will allow breeders to regain the genetic gain. Whole genome analysis will assist in this by helping to understand the complexity of the genomes.

TAMU has launched a distance breeding educational program which offers MS and PhD degrees. This is a new paradigm in plant breeding education. The educational requirements are the same as for on-campus students but the delivery of the information is electronically. TAMU is the only university to off degrees in Plant Breeding.

Development and commercialization of GLA safflower oil (F. Flider, Arcadia Biosciences): GLA is gamma linolenic acid (18:3). It is desaturated with the enzyme delta desaturase which is the same enzyme used to produce stearidonic acid in soybean. This enzyme is a limiting factor in human metabolism. GLA is a general dietary supplement and also has medical food applications. It has a complementary function to fish oils, has been found to reduce weight gain following major weight loss, reduces PMS symptoms, topical and oral application for healthy skin, is used for acute respiratory distress syndrome and for healthy joints and to ease joint stiffness.

The primary source of GLA has been borage oil and evening primrose oil which are primarily produced in China. The shortage of supply, unpredictable availability, unpredictable price swings and erratic GLA levels are issue for dietary supplement manufacturers. Safflower has ideal agronomic characteristics, is

high in linoleic acid, has a long history of contract production, is a self-pollinating crop (low outcrossing), high seed oil content and no known allergenicity. All of these make safflower ideal for GLA production.

Arcadia Biosciences introduced delta desaturase gene via *Agrobacterium*-transformation methodology. Two genes were tested, each driven by oleosin promoter (seed-specific). Fatty acid analysis was used for event screening. Chosen events exceeded the >20% threshold increase for GLA. The GM safflower is an identity-preserved crop and did not need USDA deregulation or FDA consultation. Arcadia used the FDA NDI (new dietary ingredient) process for approval. The same data that is generated for the safety of a new crop is also submitted for a NDI. The GM safflower is grown under USDA notification (up to 5000 acres) and requires crop containment. It is also the first crop approved under the USDA BQMS system. The NDI process was completed in 2009 and took 5.5 years from transformation to the NDI approval.

There are 3 types of safflower oil—oleic, linoleic and GLA. To prevent co-mingling of GLA with others, identity preservation was used. Arcadia has established GLA safflower as a rotation crop in Idaho, being rotated on premium irrigated acres with potatoes, wheat and sugar beets. Commodity safflower is not on irrigated acres. All GLA safflower is grown in Idaho and Arcadia personnel are on-site.

<u>*Oilseed processing (C. Dayton, Bunge):*</u> No cotton acres are specifically planted for cottonseed oil production. The same is true for soybean. Cotton is grown for fiber and soy is grown for protein source.

Impurities in oils are naturally occurring compounds that are generated in storage and processing. Free fatty acids produce oil rancidity, phospholipids will turn oil black and are natural emulsifiers, chlorophyll makes the oil green, tocopherols are left alone since they are anti-oxidants. Rapeseed, sunflower and cottonseed have waxes which need to be removed to avoid separation (such as in mayonnaise). Gossypol in cotton will turn crude oil black.

Phospholipids play a large role in fatty acid synthesis. There are various types of phospholipids which differ in their rate of hydration. PE and PA are relatively non-hydratable phospholipids. Phospholipids are emulsified and then removed via industrial centrifuges in matter of milli-seconds. Cottonseed oil is a fully hydrogenated oil (20%) whereas soybean oil has 12% hydrogenation.

The Miscella refining method for cottonseed is mostly used in Brazil and is done in a mixture of hexane and oil. The process is generally:
Hexane extraction → acid pretreatment → neutralization → centrifugation (separates soapstock) → solvent removal →bleaching (absorbents) →deodorization.

Chemical refining follows a similar process: acid pretreatment → neutralization → centrifugation→silica trtment or wash water
--silica trtment→filtration→bleaching→deodorization
--wash water→centrifugation→bleaching→deodorization

Enzymes are being deployed more for gum and phospholipid removal. Physical refining uses adsorption to remove residual metals, soaps, phospholipids and chlorophylls.

Deodorization is the last major step to remove impurities and contaminates that affect taste, odor and stability. High temperatures are employed to remove impurities, however, high temperatures and longer times create more trans-fats (polyunsaturated). Trans-fats formation is controlled by time and the use of packed columns (requires 5 min).

Seclect topics on analysis of cottonseed and cottonseed products (K. Parsons, Eurofins): Gossypol is a bioactive compound found in cottonseed. Processing of cottonseed allows gossypol to bind to proteins thus making it inactive. The AOCS method Ba7-58 detects free gossypol. The method calls for extraction with 70% acetone. Gossypol then reacts with aniline in a boiling water bath to form dianilingossypol. The results are read with a spectrophotometer at 440nm.

HPLC or UPLC methods use gossypol extracted with acetone. HPLC or UPLC allow higher sample throughput and select only for gossypol. AOCS methods Ba8-78 and Ba8-99 can be used. Total gossypol is extraction using a complexing reagent in a hot water bath. In Ba8-78, the total gossypol is reacted with aniline and read at 440nm with a spectrophotometer. In Ba8-99, the total gossypol extracts are diluted, filtered and then analyzed with HPLC.

Fatty acid analysis is more complicated than gossypol. Extraction is performed via acid hydrolysis. Fatty acids are methylated via saponification with methanolic sodium hydroxide but this is problematic since the cyclopropene ring is broken. Use of sodium methoxide does not break the cyclopropene ring, however, free fatty acids are not derivatized. Direct methylation may be done with alkali hydrolysis or acid alkali hydrolysis. Fatty acids are quantified via % fatty acid which measures the % area of the peak. This is a less precise measurement. Use of % weight (% full weight) is also used with an internal standard. The halphen test is a qualitative test used for the present of cyclopropene fatty acids. This test is used for the detection of cottonseed oil in other oils. Cyclopropene fatty acids may also be detected by HPLC. These fatty acids lack a chromophore so must be derivatized for spectrophotometric detection.

Cotton genome and molecular markers (C. Channabasavaradhya, Dow AgroSciences): Over 90% of the cotton grown globally is genetically modified. Cotton has a complex genome. Both upland cotton (higher yield, 90% of world production) and pima cotton (high fiber quality, 8% of world production) are allotetraploids (AADD). Sixty percent (60%) of tetraploid cotton is unchanged from its diploid progenitors. SSRs (simple sequence repeats) are still major marker resources available in cotton. The challenges of cotton include: tetraploid genome, lack of a reference genome for tetraploids, narrow germplasm base, very low polymorphism within species and a need for enhanced collaboration within the research community.

Cotton genotyping is moving toward the use of single nucleotide polymorphisms (SNPs). A cotton chip has been developed with 70,000 SNPs (Texas A&M with Dow AgroSciences). This allows a turbo-charged breeding process. Markers are being used to improve cotton traits. Resistance to root knot nematode is being mapped to two genomic regions on chromosomes 11 and 14. Similarly, resistance to the reniform nematode has been mapped to chromosomes 21 and 18. SNPs are being used to introgress this resistance into cotton varieties. Markers are also used for zygosity testing, adventitious presence testing, trait and genetic purity testing. All the testing is carried out using TaqMan and KASPr-based PCR. The thermal cycler revolutionized molecular testing and has gone from compact heat block thermocyclers to integrated water bath thermocyclers to new generation sequencing using computer flash drive type chips (point of care testing). DNA detection has gone from gel-based to fluorescence signal detection to electrochemical signal-based detection. Genotyping has progressed from PCR to genotyping by sequencing.

New Member Presentation: Critical Path Services (L. Mallis): Critical Path Services (CPS) is a contract research organization (CRO) which was founded in 2001 by Julie Eble. CPS is located in Garnet Valley, PA. In March, 2013, CPS opened an office in Research Triangle Park in North Carolina. In June, 2013, CPS was acquired by Knoell Consulting Company which is a global consultancy firm and is not limited to agriculture. Services offered include report writing, study monitoring, quality assurance, literature research, full regulatory dossier preparation and submission, professional project management and electronic publishing. CPS will do contacting to suit the needs of the customer. CPS staff can be embedded within the customer company or remain off-site. CPS will do QA audits internally and externally and they are fully GLP compliant.

Multiplexing technology for cotton traits expression (G. Yeaman, Monsanto): New GM products coming through industry pipelines are stacked trait products produced via traditional breeding or molecular methods. To determine expression of proteins from these products, Monsanto has a priority to have a multiplex method for a number of analytes.

The Luminex technology uses capture antibodies immobilized on micro-beads. The xMAP technology combines polystyrene beads with magnetic particles. A traditional sandwich ELISA on beads with fluorescence detector antibodies would require 10 separate plates for 10 different analytes. Using the Luminex technology requires only one plate and 800 results/plate. The dynamic range is 1 – 10,000 pg/ml.

The basic components of the technology are biological reagents, microspheres, fluidics and optics, high speed digital processing. Five hundred (500) distinctly colored microsphere sets are used. Red dye and infrared dye distinguish one bead set from another. Each bead has a pre-determined location on the plate. The fluidics is similar to fluidics of flow cytometry. To read, 50ul of beads are injected into a cuvette. The beads are interrogated by two lasers and a laser for phycoerythin. The light is collected by a photo-multiplier. The red laser is used for bead classification and the green laser for the assay result. It takes 20 min to read a 96 well plate, however, 9600 results/hr can be achieved.

The Luminex technology is a mature technology in mammalian biology. Its adoption in plants has been slower. US FDA has approved 84 510K applications which represents 259 analytes.

The challenges and solutions are:

Challenge	**Solution**
Universal extraction buffer	Choose most common ELISA buffer; assess extraction efficiency
Universal assay buffer	PBS/BSA
Wide range of expression levels	Assay has a large dynamic range (>4.5 logs)
Multiple plant tissues to assay	Optimize for each tissue type
Large selection of candidate antibodies is desirable	Expensive to produce a large range of candidate antibodies; pairs of antibodies used in ELISA work well

Monsanto has compared 9 proteins with diverse physiochemical properties. The proteins ranged from 25 to 132 kDa. The tissue:buffer ration was 1:100 with TBA buffer. The plate dilution was 1:20. Standard curve ranges were extended for higher expression plant tissues and the same antibodies as

used in conventional ELISA were used where possible. The 9 protein were quantified in all 4 plant tissues in a single extract. The expression results were consistent with results obtained from conventional ELISA. Thus, the Luminex technology shows high potential for use on stacked trait products. Monsanto is currently in the process of the validation of Luminex technology and will then reach out to regulatory authorities to introduce the technology for plants.

Multianalyte lateral flow immunoassays for cotton (S. Kovacs, EnviroLogix):
The first multianalyte lateral flow device (LFD) system was the Cry1Ab + Cry9C LFD in 2001. This LFD demonstated the feasibility of such a device. The next LFD was for Cry1Ac + Cry2Ab for cotton seed in 2002. Cotton multianalyte QuickStix kits have been offered over the years. No other crop has taken to multianalyte LFDs like cotton. These LFDs are simple, cost effective and reproducible. LFDs are well matched to seed purity testing needs. LFDs use crude extracts of tissues and seeds and can be mass produced at a low cost. They use "laddered detection" in that multiple bands will appear—one band for each analyte.

Multianalyte LFDs provide flexibility and can be made for specific stacked trait products. They are capable of detecting numerous trait combinations. Due to their size, they can comfortably accommodate testing for 1 to 6 traits in 10 min or less. Leaf or seed testing is done with a common buffer and the LFD can be retained for the records. Also, samples may be retested if there is poor extraction which allows more confidence with results. The workflow benefits include high throughput, little set up time, minimum sample preparation and may start/stop the test as needed. LFDs also have a small space footprint and require no sophisticated lab equipment.

Antibodies are the key to multianalyte LFDs. Antibodies have a huge repertoire of recognition with each capable of binding a distinct target. They are capable of identifying and strongly binding to a specific target present in a complex mixture. Also, antibodies can be produced at relatively low cost. For multianalyte testing, antibodies must not interact with each other.

The LFD detection system is gold conjugate. Gold forms colloidal nanoparticles which are large enough to visually detect. Gold is low cost at the LFD scale.

Has the limit been pushed for LFDs? There is always some application due to the ease of use. LFDs have been found to have 18 month refrigerated storage stability. Also, an endogenous extraction control is not out of the question. Currently, LFDs have only a functional control.

Cotton supply chain (M. Christian, Bayer CropScience): Bayer CropScience (BCS) has cotton facilities in the U.S., South America, EU and India. BCS cotton brands are FiberMax and Stoneville with trait packages that include GlyTol, GlyTol LibertyLink, GlyTol TwinLink, GlyTol LibertyLink Bollgard II, LibertyLink Bollgard II and Bollgard II Roundup Ready Flex.

Seed inventories are always a challenge for the supply chain and require lots of planning. Varieties (8-10) are tested in the field and possibly 3-4 of these will be advanced for commercialization. Traits are already introgressed into the varieties being field tested. One bag of cottonseed is enough to plant 6 acres whereas a bag of corn seed plants 2.5 acres and a bag of soybean seed plant 1 acre. A focus variety is used to expand to new farms (growers) and with existing growers. A neutral variety is sold to existing growers in combination with focus varieties. A transition variety is in phaseout.

There are approximately 11 million acres in the U.S. for cotton production. Seven million acres are in the western cotton belt and 4 million acres are in the eastern cotton belt. The cotton acres in the eastern part of the cotton belt are more volatile in number due to planting of corn and soy.
BCS' mission is to safely produce adequate quantities of high quality seed to meet sales and marketing demands by combining unique engineering controls and standards. BCS has seven groups in the cotton supply chain: quality assurance, counter season, parent seed, field production, planning/scheduling, processing and logistics. Pre-basic seed production involves the handing off of seed from breeding to go to seed increase which is usually done in Arizona where there is ample space for isolated fields to produce certified commercial seed. Harvested fuzzy seeds are stored in a receiving warehouse. The lint is removed from the fuzzy seed by acid treatment and cleaned by air-screens and gravity table. Seed treatments can then be applied and the cottonseed is packaged for distribution.

Counter-season production of seed occurs outside the U.S. which save a year on production. Arizona is preferred for parent seed production due to the dependable climate, isolation of fields, timely harvest, adequate yield and consistency. BCS contracts for needed acres for field production. Arizona is again used even though competition for acres is competitive, however, there are many experienced growers. Some field production is also done in west Texas and in Mississippi/Arkansas.

The relative timeline for seed production is as follows:

January – March	placement of acres
February – May	planting
February – September	field inspections
September- November	harvest

Growers keep the lint.

For planning/processing, forecasts are usually done 3-4 times per year. The plan is made between August – April and adjustments will be made for orders per market. BCS has five processing sites across the cotton belt. A considerable amount of monitoring is carried out which includes visual inspections, trash and tare analyses. For quality assurance, 30 individual tests are done which include free fatty acid content, temperature, visual mechanical damage, moisture, cut test, number of immatures, internal moissutre, discoloration, seeds per pound and warm/cold germination. LFDs are used for adventitious presence detection. Finished seeds go through the same testing. Traits are tested for purity, adventitious presence which is usually done by PCR.

In the warehouse, the last quality check occurs. This is the last chance to take a look before releasing the seed to the customers. Safety training occurs at all levels of the seed production process. BCS has zero safety incidents in 2013.

Digital PCR: Rain Dance Technologies (J. Therrien): Rain Dance Technologies was founded in 2004 by Harvard scientists. It was founded on the digital droplet technology which allows picoliter scale. The droplets have a fluorocarbon oil exterior and an aqueous interior. There are 10 million droplets/50 uL. Rain Dance offered commercial products in 2007, mainly for use in human health for cancer detection. It can be used for pre-amplification for next generation sequencing. The droplets may contain DNA or RNA, proteins, antibodies, small molecules or cells. Rain Dance is projecting to release a digital ELISA in the next 1-2 years.

Digital PCR is carried out with the RainDrop instrument which has been sold to agbiotech companies. It is not a high throughput screening tool since its capacity is 8 samples/run. It is a high resolution

detection device. Target nucleic acid can be distinguished against a background of wild type DNA. The sample is segregated into single molecule droplets (1-10 million drops/sample). Each drop either contains one DNA molecule or nothing. This allows the detection of low frequency mutations. The droplets also allow for multiplexing (most data points/sample) and sample diversity (heterogeneous or limited). In humans, a fluid biopsy can be done. Cancer cells are leaky and thus, blood can be drawn. The plasma is separated by centrifugation and then interrogated for known cancer variants of DNA.

When to go to digital PCR? It is most useful when there are too few mutations and too many wild type molecules or too few copies of the gene target and too many PCR cycles.

Digital PCR is basically divide and count, i.e., a single sample volume is divided into countable volume elements (droplets). Thermocycling is carried out on droplets and then the droplets are sent through a microfluidic nozzle on a chip. A laser counts the fluorescent droplets (thousands per second) which is single molecule interrogation. The resulting data is a two color system with fluorophors (VIC and FAM). The data is presented as clusters on a plot heat map. The method has a wide dynamic range thus there is no need to find the "sweet" spot dilution of each sample. True single molecule measurements enable multiplexing (10 markers can be used simultaneously). The Canadian Grain Council has used the technology for canola and barley for a certain allele detection. No agricultural applications have been published by private companies.

Attendees:

Beecher, Brian	USDA
Bell, Tandace	USDA GIPSA
Smith, Paul	Cargill Global Foods Research
Bailey, Dena	
Breck, Parker	EnviroLogix Inc
Breeze, Matthew	Monsanto Co
Carmichael, Karen	Charm Sciences Inc
Channabasavaradhya, Chandra-Shekara	Dow AgroSciences
Chen, Jingwen	Syngenta Biotechnology Inc
Clapper, Gina	AOCS
Claussen, Fred	EPL Bio Analytical Services
Currier, Thomas	Bayer CropScience
Dharmasri, Cecil	Bayer CropScience
Dudin, Yelena	Monsanto Co
Dyer, Rex	EPL Bio Analytical Services
Fendley, Ann	Bayer CropScience
Fu, Huihua	BASF Plant Science
Ghavami, Farhad	BioDiagnostics Inc
Golbach, Jenny	EPL Bio Analytical Services
Holmes, Steve	Charm Sciences Inc
Houchins, Donna	Romer Labs
Hudson, Joe	Bayer CropScience
Hunst, Penny	Bayer CropScience
Johnson, Ryan	BASF Corp
Kimble, Mark	Romer Labs

Kovacs, Stephen	EnviroLogix Inc
Layton, Dean	EnviroLogix Inc
Levin, David	Covance Labs
Liu, Kai	Eurofins Scientific Inc
Mackie, Sandra	Bayer CropScience
Maxwell, Carl	DuPont Pioneer
Miller, Suzanne	Agri-Diagnostics
Petty, Morgan	Monsanto Co
Rambow, Dave	Agdia Inc
Rogers, Hilary	Eurofins Scientific Inc
Sabbatini, Jane	Covance Labs
Schuetz, Keith	Agdia Inc
Shan, Guomin	Dow AgroSciences
Singsit, Chong	OMIC USA
Soans, Chandrasen	Illumina
Spiegelhalter, Frank	Eurofins Genescan
Thiede, Denise	BioDiagnostics Inc
Umthun, Angela	Stine Biotechnology
Yarnall, Michele	Syngenta
Yeaman, Grant	Monsanto Co
Zhong, Cathy	DuPont Pioneer
Zhou, Ning	
Christian, Monty	Bayer CropScience
Lilly, Jason	Neogen Corp
Mallis, Larry	Critical Path Services LLC
Pick, Charles	SeqID
Sizemore, Ryan	Monsanto Co
Smith, E. Pearce	Eurofins Genescan