AEIC Fall Meeting 2014 Minutes

October 15-16, 2014

New Orleans, LA

Hosted by Eurofins GeneScan

P.L. Hunst, AEIC Secretary

The AEIC was welcomed to New Orleans by Frank Spiegelhalter, Eurofins GeneScan.

AEIC BUSINESS MEETING

Secretary's Minutes of Spring Meeting: Motion was made, seconded and voted positive to accept the minutes as published.

Treasurer's Report (D. Layton): Motion was made, seconded and voted positive to accept the treasurer's report.

	Proposed Budget ($)	Actual ($)	Notes
Starting Balance	**39006**	**39006**	
Dues	8000	9600	
Interest	190	2	
Other		1076	CLI payment for Fall Meeting
TOTAL	**8190**	**10678**	
Expenditures			
Scientific paper	5000	280	
DE Franchise tax report	25	30	
ANSI/ISO	2900	2900	
Board Meeting	900	212	
Spr Meeting	2500	675	
Website	3500	3291	
Bank Service		4	
Fall Meeting	2500		
Graphic design			
Reprints	800	375	
Subscriptions	100		
Miscellaneous	100		
TOTAL Expenses	**18325**	**7767**	
Projected Balance	**28871**	**41916.15**	**Current balance**

Membership Update (D. Layton): It was announced that the new website will soon have the functionality to accept credit cards for dues payment.

	Expected Amount ($)	UnPaid Amount ($)	# Unpaid
Large Companies	8500	2000	4
Small Companies	3500	1000	4
Individual	400	100	1
Associate	100	0	0

Website Update (D. Theide): The new, redesigned website was put into operation for the Fall Meeting registration. There were some glitches which were fixed and more functionalities will be added (credit card payment for dues and for meeting dinners). Denise will discuss with the website company about adding a Sharepoint site functionality for exchanging documents that are in progress. Denise gave a brief online "tour" of the website. The "Login" which appears at the top of the website is for access to member information. To register for meetings, click on "Meetings" and then "Register for meeting". Once the credit card functionality is ready, paying dues will be available under "Membership". The AEIC updated brochure PDF will be posted soon. AEIC members are encouraged to visit the website and provide feedback to the AEIC Board. A committee will be appointed to work on updating the website content in the near future.

Upcoming Meetings (G. Shan): The current schedule of host companies for future meetings is:

Spring 2015	Illumina in San Diego, CA
Fall 2015	Agdia in Elkhart, IN
Spring 2016	EPL BioAnalytical Services in Illinois

Proposed topics for the 2015 Spring Meeting were discussed and are listed below:

- Unintentional presence of unidentified events
- Composition Working Group>Update on questions being worked on such as methods for endogenous allergens
- Explore the technology companies in the San Diego area that may be working on new technologies applicable to agriculture
- Update from AquaBounty on the GM salmon
- New breeding technologies update

Composition Working Group (M. Petty, Monsanto): The Composition Working Group met the afternoon of Oct 14 in New Orleans. The group is focused on looking at all methods used for regulatory analyses. There are several active projects. The lectin project to replace the hemagglutination test with an immunoassay method, ELLA, has developed an article for the AOCS journal. The ILSI crop composition database has also been updated to accommodate data generated by the new method. Thirteen labs have also participated in a study in which they ran the method on provided samples. This data will be used to drive for AOCS official method status for ELLA. Other projects include harmonizing methods for

endogenous allergens, fat soluble and water soluble vitamin methods and a new method for trypsin inhibitor. The group is also moving forward with a method for analyzing cyclopropenoic and straight chain fatty acids that will combine two methods into one. The method has been tested by several laboratories and is moving toward a collaborative study for AOCS official method status in 2015 along with two publications.

Scientific Paper on Detection (D. Layton, EnviroLogix): The paper has been drafted and the editor has made comments which need to be addressed. Dean will contact C. Alarcon (DuPont Pioneer) as to how to finish the paper. The paper was not intended to be submitted to a peer-review journal as it is more appropriate for a trade journal for educational purposes.

Multplex Method Validation (G. Shan, Dow AgroSciences): A proposal for a working group to put together consensus on validation criteria for multiplex detection methods has been drafted. A draft paper on validation criterial for mass spectrophotometry for proteins has also been drafted. These have been forwarded to the AEIC Board for consideration.

Nominations for AEIC Vice President: Y. Dudin asked the membership to nominate individuals for the office of Vice President. This office is a 3 year commitment—one year as Vice President, one year as President and one year as Past President. David Levin (Covance) was nominated and accepted the nomination. Ray Shillito (Bayer) was also nominated but has had to decline the nomination due to previous commitments. Nominations can be submitted to the AEIC Secretary (Penny Hunst) via e-mail through Oct 28. Voting will commence on Nov 3.

UPDATES

Biosafety Protocol (R. Shillito, Bayer CropScience): The Meeting of the Parties (MOP) to the Protocol was held in Korea in October. The BSP was established to protect biological diversity amongst participating countries, thus it affects trade and regulatory approvals. The major exporting countries USA., Canada, Argentina and Australia are not parties to the BSP,. Brazil is the only major exporting country which is a party to the BSP. At the MOP, there were 44 industry participants, representing 16 countries. Among the key issues was the transboundary movement of living modified organisms (LMOs). There was an initiative proposed to publicly post information about events in all field trials of LMOs, however, this was not adopted. In addition, grain shipments will continue to be able to state "may contain" in regards to LMO content. More of the countries are adopting GM technology. South Africa and Ecuador have requested to have workshops in their countries on sampling and detection, and Mexico on Sampling.

Update from the American Association of Cereal Chemists Int'l (AACC) (R. Shillito, Bayer CropScience): The biomarkers committee is now under the AACC as well as the ILSI workshop program. A meeting was held to discuss new methods for components of whole grain and grain/seed purity testing for wheat. Workshops were also held on sampling in Brazil and Peru for seed inspectors. G. Shan is also writing a book for AACC on detection methods.

ISO/TC 34/SC 16 Update (G. Clapper, AOCS): Subcommittee 16 (SC 16) is the molecular biomarker analysis subcommittee. R. Shillito is the U.S. TAG chair and M. Sussman (USDA AMS) is the chair of the international committee. Working Group 4 (WG 4) deals with antibody or chromatography methods for

the identification and detection of plant pathogens. There is currently a document for ready for review from WG 4 and there is a need for volunteers to participate and review documents. This is not for GMO detection. If anyone is interested, please contact Gina Clapper, AOCS.

INVITED PAPERS

International Seed Trade Association (ISTA) Proficiency Testing (C. Dollard, CFIA on behalf of ISTA): ISTA is a non-profit association under the governance of member countries. ISTA's goal is the standardization of seed quality for international seed trade. The annual meeting was held in June. There are 72 voting delegates. The Executive Committee consists of 11 members and the Secretariat has 11 professional employees. Beni Kaufman is the Secretariat Head. There are 18 technical committees who deal with such topics as proficiency testing, GMOs, seed purity, rules, etc. The International Standardization of Methdologies has been publishing methods since 1933. In 2013, GMO testing was added. General aspects of testing are included but no protocols are provided but rather, a performance-based approach is followed. The International Standard of Laboratory Performance deals with proficiency testing. It is open to any lab. The official language is English. All documents are posted at www.seedtest.org. The International Standard of Reporting Results issues the ISTA certificates which assure that the sampling/testing was done in accordance with ISTA rules. For the accreditation program, labs must be members, use established ISTA protocols, be audited and participate in the proficiency testing. There are 120 labs globally in the accreditation program and each must perform well to maintain the use of the certificates. There are three parts to the program: a) basic tests, b) seed health tests, c) GMO tests. The test fee is covered by the yearly membership fee. Non-members are welcome to participate but a service fee is charged. There are several rounds/year with the process being a) lab testing of blind samples, b) submission of results, c) review of results and statistical evaluation of lab proficiency. The submitted results influence the accreditation status. Labs are rated as A, B, C or BMP (below minimum performance). The overall lab rating is given after 6 rounds have been completed and only the mandatory test results are included. GMO proficiency testing is performance-based, i.e., no prescribed method thus bioassay, protein-based or DNA-based tests may be used. Each round consists of one species with 1-3 events. The results can be qualitative or quantitative. Samples are spiked between 0 to 4% and there are 10-12 samples of 1800-3000 seeds each. ISTA has a database to track all results. Qualitative results are reported as yes or no and the labs are given a % misclassified. Quantitative results are reported as % seed, mass of GM seeds or % DNA. Labs are rated as A, B, C, BMP. There are two SOPs—one for administering the program and one for sample preparation. ISTA has recently started to pay a third party to prepare the samples for the program. The majority of lab participants are from the EU (65%) with 18% from North America. There is an ISTA expert team for statistical analysis/interpretation of results to ensure the secrecy of the program and to finalize details/logistics for a round. There have been 20 rounds to date.

Design and analysis of proficiency testing plans (T. Perez, Monsanto): For reference materials, GM seed is required to have a purity of >99.25% purity with 95% confidence. Conventional seed is required to demonstrate purity of <0.01% with 95% confidence. The cost of proficiency testing of reference material is too high so instead of a binomial distribution, Bayesian statistical methods are used. For Bayesian, must have prior information and then match with current information. Seed provider normally has performed some purity tests (posterior probability). The flat prior information assumes 1% adventitious presence. This is then compared with current information. When rating proficiency testing, true value is known or can be estimated. If there are too many too far from the truth, the rating

is BMP. Z-scores (standard scores) are also used. The Z score is the number of standard deviations a result is from the mean. For a BMP rating, more than ½ half of the sample results are outside the standard deviation. For a C rating, the probability to observe a more extreme value of sum of the absolute spiking levels Z-scores when assuming the lab provides accurate results is <0.01% of the time. For the B rating, more than 1/6 of the sample Z-scores are outside. For the intra-lab standard deviation, Cochran's test at the 95% level is used to see if the lab with the highest variation has an outlying spread. Over time, the number of A and B ratings dropped over time due to the labs being more precise. The intra-lab standard deviation became too small which caused the Z-scores to become too large. The intra-lab standard deviation is now 25% of the true GM content. In summary, the reference purity requirements are check using Bayesian methods. The labs' ability to detect present/absence is rated on the % misclassified samples and the labs ability to quantify is rated based on Z-scores.

GIPSA proficiency testing (B. Beecher, USDA GIPSA): GIPSA is part of the Federal Grain Inspection Service (FGIS) whose mission is to facilitate U.S. grain marketing. The BASB is the biotechnology group that does commodities testing and has the reference lab. GIPSA does not provide official testing for GM products. The grain market cannot visually distinguish GM from non-GM, thus, the need for testing. Testing is done in the case of a regulatory breach (inadvertent release) and for the market since there are still asynchronous approvals of GM products globally. Industry benefits from the increased confidence that there are reliable tests/proficiencies in place. In the GIPSA proficiency program, no methods or reference materials are specified or provided as cooperators are able to use their own systems. The first sample round was done in 2002 and the most recent round was sent out in April, 2014. Participant labs may remain anonymous. Qualitative and quantitative protein-based and DNA-based testing may be used. The samples are continually modified to include the most relevant GM events. Overall, 159 organizations participate—32 from the U.S. and 123 labs outside the U.S. (46% from Europe). Samples are prepared and disseminated twice per year. The sample packets contain six corn and four soybean flours which have been fortified with 0 to several GM events. In 2014, 77 labs participated of which 69 returned results: 27 returned qualitative results, 5 returned quantitative results, 31 returned both qualitative/quantitative results and 6 proved protein-based results using LFS or ELISA. Overall, the performance of all labs was generally good. Results are available on USDA GIPSA website (http://www.gipsa.usda.gov/fgis/biotech/quarterly_reports/april_2014_final_report.pdf). GIPSA would appreciate feedback on the proficiency program.

SPARCL technology (D. Astry, Lumigen/Beckman): SPARCL (spatial proximity analyte reagent capture luminescence) is a homogeneous chemiluminescence immunoassay technology developed by Lumigen. Lumigen was bought by Beckman Coulter in 2006 and is located in Detroit, MI. Lumigen makes chemiluminescent substrates for western blots, ELISA and clinical applications.

SPARCL (pronounced "sparkle") utilizes a light emitting compound, acridan, which is triggered to release a photon following oxidation catalyzed by a closely bound horseradish peroxidase enzyme (HRP).

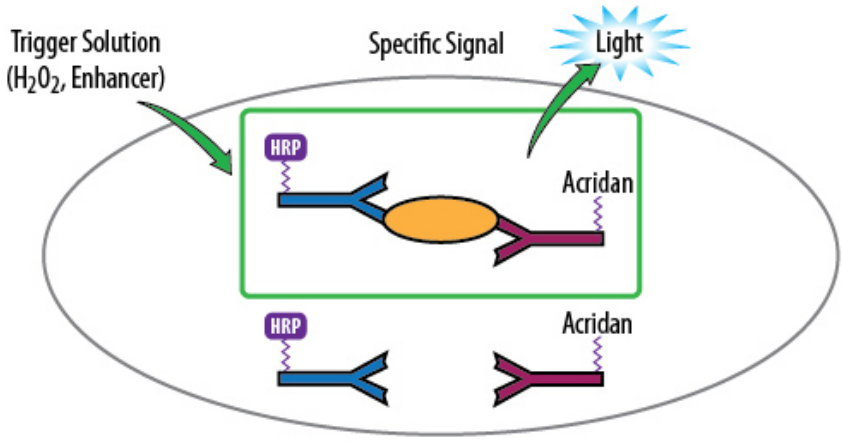

Figure 2. *A Simplified Mechanistic Scheme of SPARCL Technology.*

From http://www.lumigen.com/products/elisa/lumigen-sparcl

The technology can be used in all immunoassay formats and facilitates rapid immunoassay development. The technology is based on solution phase kinetics thus there is no solid support needed. The light is emitted when hydrogen peroxide is added to the system. If an "antibody sandwich" is not present, no light is emitted as the acridan and HRP are not in close enough proximity. The SPARCL kit consists of the trigger solution, background reducing agent (free radical scavenger) and the acridan reagent. One antibody is labeled with the acridan and another antibody is labeled with HRP. These are placed in a microtiter plate well along with the sample and incubated up to 30 min (dependent on the antibodies used). The background reagent is then added which limits the proximity peroxidation of HRP. No special microtiter plates are needed as there is no binding to the wells for the assay to function. Monsanto has adapted their trait immunoassays to SPARCL with assays being completed in 45 min. They have found the assays to be cost effective, reduce water and energy consumption by eliminating the use of incubators and plate washers. Different protein assays can be run simultaneously so it increases the testing capacity. In addition to the SPARCL kits, an instrument is needed to measure the light emission. The approximate cost of these instruments is $20,000, however, Lumigen does not sell these instruments.

New member: seqID and Eureka Genomics (H. Koshinsky, Eureka Genomics): Eureka Genomics has a partnership with seqID for DNA-based genotyping (Next-Gen Genotyping). DNA-based genotyping is used for detecting SNP variance via triplet detection using two LHSeqences and one RHSequence. Taq DNA ligase is used to ligate DNA between LHS and RHS strands. Each reaction well contains a unique identification index, many copies are made along with the bits that let them go on a flowcell. Reaction wells are pooled and sequence data is generated. A computer is used to pull it all apart and the statistical clustering is applied and probabilities to called genotype. The error rate of the machine is 1%. There are controls within the assay to determine failed samples. NGG has been used mainly for animals but there is not a kit developed for barley. In summary, NGG is cost competitive, generates more data, is a flexible multiplex technique and allows the pooling of thousands of samples in one run. NGG has high reliability, accuracy (99.5% concurrence in cattle) and is user friendly for library preparations. In the plant world, potential applications would be event verification, zygosity, adventitious presence detection, etc.

PANEL DISCUSSION

Technical challenges in GMO testing (R. Shillito, Bayer): GM products move throughout a value chain as commodities and eventually into food feed and fiber. Row crops are harvested in large volumes and grain handling systems are on a large scale. Testing has to be consistent throughout the value chain. Export markets are very large and complex. In 2013, the U.S. exported $141 billion in agricultural exports and it also imported $103 billion in value. The major country importers of US grain exports are the EU, China, Mexico, Japan, Taiwan, Korea and Southeast Asia. Grains are generally shipped via panamax vessels. One typical panamax vessel may be approximately 38 barges = 22000 semi-trailer trucks = 2 million bushels = 45000 metric tons = 330 trillion soybeans. What are we trying to find when we test? Protein-based, DNA-based and herbicide bioassays all used to answer the question. In order to determine a probability of detection, you need to know what question you are asking. There is a need for harmonization in testing methods, and this has been achieved in many cases.

Seed semi-quantitative methods (R. Johnson, BASF): In testing, there is no such thing as zero. Every test is an estimate. Statistical inferences on a seed/grain lot are based on estimates. Results are only as good as the sample that is submitted and testing is more variable at lower levels of adventitious presence. Protein testing indicates what is in the sample at that point in time. DNA testing indicates what the plant is capable of. In PCR testing, data is taken at the plateau stage thus it is more difficult to implement hard cut-offs. It requires at least one negative pool. If the data spread is not distinct, positive and negative pools start to merge. Intermediate signals make it difficult to identifiy target from non-whole seed sources. Non-biologically equivalent sources may be present such as dust or partial kernels that can skew results. Intermediate signal are more evident in assays with less specificity such as a promoter assay. This not an issue with the science of testing but rather with the business of testing. It is dependent on where the customer wants to put the risk, i.e., prevent the use of non-conforming product or prevent the disposal of conforming product.

Methods for detecting and measuring biotech traits utilized in the grain distribution system (C. Seward, EnviroLogix): Contracts for grain are writeen with specifications of the end user in mind. The price, volume, delivery point/period are all determined in the contract. A premium is associated with a specialty product and is noted in the contract as well as specific varieties. Growers plant, grow and harvest within the guidelines of the contract. The grain is harvested and segregated on the farm and/or delivered to an elevator in the contracted timeframe. Upon delivery the grain is subject to analysis to determine if load is within the specifications of the contract. The load is sub-sampled and subjected to analysis with a LFD (which may be qualitative, quantitative, single event or multi-event). If the load passes, it is segregated. If the load fails, it may be unloaded but it is channeled as a generic commodity and no premium is paid. Failed loads are generally not added to the volume determined in the contract. Contracting elevator must originate more specialty grain to fill the end user contracts. LFD testing is performed to ensure quality prior to shipping the product. A retain sample is kept for further analyses. Converging factors (increase in GM crop acres, increase in low level presence, increase in number of stacked trait products which produce higher PCR values on release testing) all make it increasingly difficult to fulfill specialty grain contracts for export. As the number of stacked trait products increase, more grain loads will be rejected. Screening levels will have to be lowered to avoid rejection. Finding non-GM grain below these lower screening levels will become increasingly difficult.

Grain qPCR methods (F. Spiegelhalter, Eurofins): When sampling whole grain, sub-samples for a very large barge can lead to great differences in results. For non-GM corn, cannot test for each event separately. Instead a promoter assay is used which covers many events. PCR give an approximation and not an exact number for the amount of GM present.

$$\frac{\text{(biotech corn)} \times 100}{\text{(corn DNA)}} = \%\ \text{GM DNA}$$

In this equation, biotech corn contributes to both the numerator and denominator.

$$\frac{\text{(biotech soy)} \times 100}{\text{(corn DNA)}} = \%\ \text{GM DNA}$$

In this equation, soy only contributes to the numerator which ups the % GM corn.

For stacked products, the analytical test is skewed due to homogenization, thus, individual kernels need to be tested.

Challenges facing the grain industry in managing biotech grain (J. Stitzlein, Consolidated Grain & Barge): Grain companies do not want to test but they must do it and they are not well prepared to deal with the complexities of testing. The current challenges for the grain industry include a) the launch of GM products without appropriate import approvals, b) keeping stuff separated, c) expectation for zero thresholds or close to zero for trade, d) cross-pollination risks exist and e) stacked products and other complications in reporting test results. The U.S. Biotech Crop Alliance is a broad-based industry effort to improve communications and risk assessments needed for commercialization of new traits. Jim's lessons: a) zero threshold is unreasonable, b) unreasonable to assume that major markets won't change over time, and c) stewardship is a worthy goal but will be nearly impossible. The case of MIR162 corn is an example. MIR162 corn was launched without EU and China import approvals. China started buying corn from international sources in 2011. In July, CGB and Bunge announced they would not accept MIR162 grain. China did not reject shipments until Nov 2013 which also affected DDG shipments. Duracade (event 5307) has also been launched. CGB checks and re-checks throughout the pipeline to confirm non-GM bulk shipments within tolerance. Many import countries have documentation requirements to show that identity preservations has been followed, i.e., starts with the seed that was planted. Japan tests ships when they arrive even though testing was done prior to the ship leaving its destination. CGB achieved ISO9001:2008 certification for identity preservation department. CGB chose ISO because system was customer-focused and allows employees to have a deeper understanding of the customer's objectives. CGB is understanding that zero is not achievable. Commercial expectations and outcomes are much lower than the 3 or 5% threshold in Japan and Korea labeling laws. Protein tests are a useful screen to catch loads with high adventitious presence. The U.S. domestic interest in non-GM is being pushed by activist groups pushing labeling of GM products. Industry will need to respond or be transparent. There is a federal proposal to do voluntary labeling. Mandatory labeling will not provide the consumer with meaningful or useful information. Giving choice does not change people's minds.

Hybrid rice production (J. Plaza, RiceTech): Rice the biggest cash crop globally and is second only to wheat in production. Approximately 85% of the rice produced globally goes directly to food. China is the leader in rice yields and India is the leader in number of acres. U.S. brings 2% production but all of it

goes to exports. In the southern U.S., rice is produced on 2.2 million acres and in California on 0.5 million acres. The recent drought has impacted rice production in Texas and California.
Rice is a self-pollinated plant which is planted dry and then irrigated. Continuous irrigation helps the rice to out-compete weeds. Rice is seeded from airplanes in California onto flooded fields. In the early 1970s in China, certain plants were found not to be fertile (male sterile). These male plants were crossed with females and then with a fertile male to create hybrid rice. Hybrid rice produces more grain per panicle, more panicles, bigger root masses and as much as 20% higher yields. RiceTec has taken the lead in hybrid rice production. RiceTec is located in Houston with facilities in North America and South America. A winter nursery is in Puerto Rico. RiceTec just recently started operations in Hyderabad and Haryana, India. RiceTec has 54% of long grain market in the U.S. There is no GM rice in the U.S. Herbicide tolerant rice is provided via a mutant, Clearfield rice. Rice product development is a long process which starts with mining the germplasm, recombining and then to the hybrid platform. Rice breeders look for morphology, grain types, outcrossing, heterosis response. Selected plants are crossed. The process can take 6-8 years for release a new product. U.S. production is all mechanized whereas in Asia, much of the work is done by hand. Split planting is usually done, i.e., the male is planted first followed by the female a few days later if female flowering occurs >7 days after male flowering. If female flowering occurs <7 days from the males, manipulation of nitrogen levels and watering is done to control the flowering to synchronize. Pollen flow is assisted by flying of helicopters over the production fields. At harvest, the female is harvested for the seed and the male seed goes to sale. Hybrid seed has a higher value. Hybrids tend to be pubescent (fibers contain silica) which is hard on the equipment and itchy to humans. Current work on hybrids to select those that are not pubescent. There are many players in the rice supply chain: seed suppliers, chemical suppliers, growers, buyers, intermediaries, exporters, etc. The rice crop is consolidated for export which requires uniformity in quality. Market is generally moving to identity preservation instead of co-mingling for export. The to do list for traits includes higher yields, increased resistance to cold, drought, heat, submergence and diseases as well as being more sustainable (less water, less emissions, less chemicals).

Breeding strategies in rice (S. Linscombe, Louisiana State University): In the U.S., rice was introduced into South Carolina around 1685 and into Louisiana by Bienville in 1718. The modern rice industry was in the coastal prairies of southwestern Louisiana around 1880 and spread rapidly due to the completion of the railroad, migration of northern and Midwestern farmers, cheap land and mechanization. Farmers quickly developed irrigation systems. In 1884, Brien used a wheat binder for rice which quickly lead to an explosion of binders being sold. The 1890s marked the beginning of the development of canal irrigation. By 1900, 25 canal irrigation plants were in Louisiana. In 1909, a rice experiment station was established near Crowley, LA. In 1949, LSU purchased land northeast of Crowley which is the present day location of the experiment station.

Louisiana primarily grows long grain rice whereas California produces medium/short grain rice. Crosses are made initially and then F1 populations are harvested. The F3 populations are grown in progeny rows (one panicle plants one progency row). The station can plant 25000 progeny rows per day. Yield testing is done on 10-12,000 plants per year. Havesting is all mechanized. Anther culture is employed to speed selection. Microsatellite markers are being used to select blast resistant lines in segregating populations. The winter nursery in Puerto Rico allows the speeding up of the breeding process (3 generations/year) which results in releasing varieties in 4 years versus the normal 7 years. Red rice is a weed that is the same genus/species of normal rice so it is difficult to control. Clearfield rice, which is herbicide tolerant mutant, assists in controlling red rice and is now on 62% of rice acres. BASF is

releasing Provisia system which provides an alternative trait mode of action to complement Clearfield. Lines may be released in 2-3 years. Average rice yield in 2013 was 7300 lbs/acre. A rice hybrid program has been initiated to increase yields. The experiment station also has cooperative programs globally with Brazil (Rio Grande de Sul) and China (high elevation rice that is cold tolerant).

Diagnostic methods for trait detection for Clearfield rice (S. Whitt, BASF): Weeds reduce crop yield and quality and are the greatest threat to productivity. Herbicide tolerant (HT) crops thrive in the presence of herbicides and provide increased grower flexibility. Approximately 100 resistant weed biotypes have been added in the last 4 years. HT crops have seen rapid adoption over the last 18 years in the U.S. Clearfield HT (tolerance to imidazolinone) rice has about 70% of the rice market. The Clearfield system is in nine different plant species (corn, canola, wheat, rice, sunflower, lentils, etc.). BASF also has a GM HT platform which includes Cultivance (imidazolinone tolerance) which will launch soon in Brazil. Research efforts are ongoing to identify new mode of actions for herbicide tolerance.

Imidazolinone inhibits AHAS (acetohydroxyacid synthase) in plants and blocks the biosynthesis of branched chain amino acids (valine, leucine, isoleucine). A single amino acid substitution confers tolerance to imidazolinone herbicides. HT diagnostics include phenotypic assays, protein-based assays (enzyme assays) and DNA-based assays (PCR). PCR assays are available for all Clearfield crops. Allele-specific PCR is used for zygosity testing which is accomplished by scoring unknown samples relative to examples from known reference samples. It is a low-cost method that yield qualitative results. RT qPCR SNP genotyping is the gold standard for determining copy number. It is a high throughput method and cost effective. Reference samples are critical and it requires modified primers. Non-specific amplification also occurs due to probe cross-talk which causes drift in pools on graph. The KASP assay is endpoint SNP genotyping. Kits are available for purchase by licensed seed partners. Clustering allows calls to be made but need good sized clusters to make confident calls.

Export grain handling and sampling (J. Hobbs, Russell Marine Group): The Russell Marine Group (RMG) is a privately owned surveying company owned by Pat and Tom Russell. There are 70 employees with the main office located in New Orleans. RMG handles 80% of the bulk grain (and by-products) not handled by USDA FGIS.

The successful execution of a contract for the seller might be the logistics of moving the product from the origin to the destination in the most efficient and cost effective manner. For the buyer, it might be the timely delivery of the product. There is much behind-the-scenes logistics work of the shipper such as documentation, testing (GMO, pesticies, pathogens, etc.). The U.S. river system is the key to the grain market. Two dozen barge lines operate 1200 covered hopper barges on the rivers which provides a continuous flow of products within the U.S. New Orleans supports 50% of the exports due to the presence of the Mississippi River. One barge = 1500 metric tons = 15 jumbo hopper cars = 58 large semi-trailers. One panamax vessel = 37 barges. Farmer silos and country elevators are connected via barges to New Orleans. Barges connect river terminals to 10 export elevators, three of which are floating elevators on the lower Mississippi River. The Ohio River directly impacts the lower Mississippi River since if the Ohio is too high, barges may break away and it is too low, barges cannot travel. Trucks and railroads are the typical means of transport of grain to river terminals. FGIS samples and grades before dumping. By-products (such as DDGs) are sampled by RMG. The FGIS origin grade is final. Origin analysis of non-regulated by-products by independent labs is also considered final. The origina grade follows the barge to point of export. Barges are accumulated in fleets. A visual inspection is conducted

of the commodity prior to sampling. Surveyors walk through cargo looking for animals, other contaminants, odors. A 12 ft probe is used to sample each barge door and take the temperature. Sampling is geared to protect buyers. Heavy grains are sampled by FGIS (corn, soybean). By-products, such as DDGs, are sampled by independent inspection services. FGIS rule mandates that a sampler must be located at the highest elevation point before a commodity begins its descent into a ship. Analyses include proximates, GM testing for all commercial GM events, mycotoxins, heavy metals, chemical residues and FGIS grading standards. Floating elevators deliver product one way direct from the barge to the scale to the ocean vessel. Land-based elevators go from barge to scale to the ocean vessel or shipping bin. This allows a pass or fail before the cargo enters the ship. Unloading of barges is accomplished via a marine leg which is lowered into the barge. The marine leg can unload a barge in one hour. Some loads will not completely fill a ship section and then a separation may be done to fully utilize the space. First a bulldozer levels the initial cargo. The initial cargo is then covered by a tarp followed by plywood. Separation generally requires about 6 hours of work at a cost of $20,000.