AEIC Spring Meeting 2012 Minutes

April 18-19

Durham, North Carolina

Prepared by: P.L. Hunst, AEIC Secretary

The AEIC 2012 Spring Meeting was hosted by Bayer CropScience at the Hotel Indigo in Durham, NC. The group was welcomed by Judy Speas, Bayer CropScience, who gave a bit of background on the company. Research Triangle Park is the headquarters for Bayer CropScience in the U.S. The groups located at the RTP headquarters include BioScience (biotechnology), Crop Protection and Environmental Science. A new 60,000 sq.ft. greenhouse will be opened at the RTP site in May. Along with the RTP site, the BioScience research and Regulatory Science groups are located at the Innovation Center site in Morrisville, NC (close to the RDU airport). Bayer has a global network of research facilities which include the U.S. (RTP, NC; Davis, CA; Lubbock, TX), Canada (Saskatoon), Europe (Ghent, Belgium; Haelen, Netherlands; Lyon, France), India (Bangalore, Hyderabad) and Asia (Singapore). Bayer CropScience has products in crop protection (insecticides, fungicides, herbicides), traits and seeds/germplasm.

Ray Shillito gave a brief background on RTP. RTP was created in 1959 out of 7000 acres of pine forest with 630 acres available for development. It is managed by a private non-profit organization called the Research Triangle Park Foundation. RTP is home to BASF, Syngenta and Bayer CropScience (three of the big 6 companies).

AEIC BUSINESS MEETING

Fall Meeting 2011 Secretary's Minutes: A motion was made, seconded and voted positive to approve the minutes.

Treasurer Report: The treasurer's report was given by Dean Layton. The budget/spend for 2011 was as follows:

	$ Planned	$ Actual
Beginning balance	23563	23563
2011 Membership Dues	8000	6920
Total revenue	8000	6920
Expenditures		
Scientific Paper	8000	1000
DE Franchise Tax Report	25	25
ANSI/ISO	2900	2900
AEIC Board Meeting	100	107
AEIC Spring Meeting	2500	222
AEIC Fall Meeting	2500	610

Reprints (Brochure)	300	
Subscriptions	100	
Miscellaneous	100	
TOTAL Expenditures	17025	5135
Projected Balance	14538	25375
Certificate of Deposit	11548	11581
CD Interest	120	
TOTAL	26206	36956

The 2012 budget was presented as follows:

	$ Planned	$ Actual
Beginning Balance	36956	36956
Membership Dues	7200	3100
Checking interest	25	23
TOTAL Revenue	7225	3123
Expenditures		
Scientific Paper	7000	2000
DE Franchise Tax Report	25	25
ANSI/ISO	2900	
AEIC Board Meeting	125	136
2012 Spring Meeting	1500	
Website	350	
2012 Fall Meeting	2500	
Bank Service Charge		10
Reprints (Brochure)	800	
Subscriptions	100	
Miscellaneous	100	
TOTAL Expenditures	15400	2171
Balance	28781	37908

There was discussion on the fact that the budget is not spent year to year. It was suggested that AEIC could put more money toward meeting expenses. It was also brought up that if AEIC does a workshop, a healthy fund is needed to support that activity. Also, there may be other analytical issues for AEIC to work on that will require funding.

A motion was made, seconded and voted positive to accept the treasurer report, with the proviso to discuss an increase for the ANSI/ISO fee.

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

Large Companies	14
Small Companies	12
Associate Members	2
Individual Members	2
TOTAL	30

For 2011 dues, four companies have not paid. Six member companies have already paid their 2012 dues. It was noted that J. Brady has decided to no longer be a member.

Members are encouraged to recruit new member companies who may be interested in AEIC and its initiatives. D. Layton is still working to make contact with Cargill and see if they again want to participate.

AEIC Brochure (D. Layton): The AEIC brochure will be posted on the website for member companies to download and use. The brochure is due for updating of the material and members are asked to download a copy, make suggested edits and send to D. Layton.

AEIC Website (P. Hunst): Member suggested updates were made by the webmaster earlier this year. More suggested updates were called for from the members. Suggested edits, comments, etc. should be sent to P. Hunst.

2012 Fall Meeting (Y. Dudin): USDA GIPSA has support from their management to host the meeting at their newly renovated site in Kansas City. R. Jenkins will coordinate the local arrangements. Suggested dates were Oct. 10-11 or Oct. 17-18. R. Jenkins will determine which dates can be accommodated and will get back to the AEIC Board.

A list of suggested topics was offered by the members as follows:

- Update on mycotoxin analytical methods
- Predictive breeding technologies
- Quantitative testing for the presence of 35S, nos, etc.
- Non-traditional technologies replacing mutagenesis, i.e., also known as "stealth" GMOs
- Further discussion/action on new methods for lectin analysis—possible working session
- High throughput analysis

Protein reference material

- Plant DNA reference material for DNA quantitation (update from NIST)
- Food safety (general topic heading)
- Sample preparation—is there a standard?
- New member talk by Douglas Scientific

AEIC Publication Updates: AEIC members are currently drafting three publications.

General process for biotech product development and the testing involved (L. Privalle)—

The paper is currently in its final draft version. The plan is to submit the paper to the Journal of Food and Agricultural Chemistry within the next couple of months.

Protein vs DNA methods for biotech traits (C. Alarcon)—A rough draft has been put together, however, some sections still need to be added. Once all the sections have been drafted, C. Alarcon will compile everything into one document for internal review.

Quantification by sub-sampling and the use of SeedCalc (B. Kaufman)—B. Kaufman has put together a rough draft and sent to C. Alarcon for review.

Other Updates:

Codex/ISO Sampling (R. Shillito)—There is currently nothing happening in the group at the moment.

New Working Group (G. Clapper)—A working group has been approved by ISO TC34 to put together an over-arching statistics document for use on all grains and other commodities. The U.S. will host the working group. Larry Friese or Paul Wehling have been suggested as a leader of the group. The group may meet in Beijing in August (2nd week) when the Chinese cereals/oil association is meeting.

ISO/TC 34/SC 16 (G. Clapper)—The USDA is ending their contract to fund the SC. G. Clapper requested AEIC members to work with their government liaison people to convince the USDA to support the SC from central funds. The WG needs $50 – 75,000 per year to administer the SC. If the U.S. cannot secure this funding, the international community will be invited to become the host.

Other business—It was briefly discussed that AEIC may be asked to increase its funding amount to ANSI/ISO WG 16.

INVITED TALKS

AEIC: The First 20 Years (P. Hunst, Bayer CropScience/AEIC Secretary): AEIC began as an organization focused on environmental immunochemistry and has move to an organization focused on bioanlytical tests for agricultural biotechnology. In 1992, a discussion was held at the EPA Immunochemistry Summit (Las Vegas, NV) around the use of immunoassays for regulatory testing of new chemical products for EPA registration. This discussion brought forward several issues:

- Need for better communication among kit developers, users and regulators
- Need for development of standards/regulatory guidelines for acceptance of immunoassay data
- Need for educational programs for regulators and new users of immunoassay technology
- Need for policies to safeguard the quality of commercial immunoassay kits, as well as the application for which they were being developed that utilized the kits.

EPA suggested that industry take on these issues. In late 1992, the first organizational meeting of such an industry group was held at Dow Chemical in Midland, MI which would become the Analytical Environmental Immunochemical Consortium (AEIC). The first official AEIC meetings began in 1993 at American Cyanamid (in NJ) and were used to develop the by-laws of the organization. The first AEIC Board was elected and dues were collected to fund activities. From 1993-1998, AEIC focused on immunoassays for environmental applications by publishing immunoassay and kit insert guidelines, hosting workshops for EPA OPP, EPA Office of Solid Waste and EPA Office of Water. The workshops focused on educational efforts to familiarize government scientists/regulators with immunoassay technology. AEIC coordinated the workshops and hosted them in Washington, DC to be in proximity to the agency.

In 1999, agricultural biotechnology was developing quickly in the U.S. and some of the AEIC founding member companies were biotechnology trait providers and other member companies supported the trait providers by providing immunoassay technology for the expressed proteins. This lead AEIC to incorporate a biotech initiative into the group. The points of the initiative were:

- Develop performance-based method validation guidelines for proteins in GM plants, food commodities and derived products
- Serve as an educational resource to regulatory bodies and end-users (food processors, food companies, seed companies, etc)
- Engage in an industry-wide effort of cooperation to obtain consensus on the proper use of immunoassay methods
- Remain abreast of industry developments of other methods/technologies for quantitative/qualitative detection of GM crops.

In 2000, AEIC sponsored a joint workshop with USDA GIPSA called "Grains Biotechnology Methods Validation". The objective of the workshop was to discuss the validation and application of detection methods for GM grains.

In 2005, AEIC was renamed to incorporate the new focus of the organization. "AEIC" now stands for "Analytical Excellence through Industry Collaboration". AEIC has focused more on biotechnology detection methods. Member companies now include analytical labs involved in both protein-based and DNA-based testing, equipment manufacturers for both protein- and DNA-based testing, food/mycotoxin testing companies and GM trait provider companies. Education is still a pillar of AEIC's mission as well as bringing "one voice" to regulatory authorities for bioanalytical methodologies.

The AEIC Board has had members from 16 companies since 1992. Meetings have been held in 16 different U.S. states—East Coast, Midwest and West Coast.

Analytical Challenges in the Ag Biotech Industry

Challenges in the determination of lectins (J. Sabbatini, Covance): Lectins are proteins that bind to sugars. Lectins have high affinity for specific sugars and may bind with free

sugars, polysaccharides, glycoproteins and glycolipids. Lectins are also found in lower animals such as snails. In early work, it was recognized that castor beans caused agglutination of red blood cells. Ricin from castor beans was identified in 1888. In plants, lectins are a defense mechanism. Fungal infections and wounding will increase the content of lectins in a plant. Lectins are also involved in the recognition of nitrogen fixing bacteria to bind with plant roots. There are also involved in storage of proteins and mitogenic activity. Many plant lectins are toxic but not all lectins are toxic. The most studied lectins are from legumes. Stems and leaves may have different lectins than are found in seeds and the concentration may vary with the age and developmental stage of the plant.

The Liener agglutination method was published in 1955 and is still used today. The method uses trypsinized rabbit blood cells. Lectin extractions are serially diluted and then the blood suspension is added. The mixture must sit undisturbed for 2.5 hours. At the end of the incubation period, a photometer reading is performed and plotted on a blood curve. From the curve, the dilution equivalent to settling of 50% of the cells is determined. This is standardized on a known control of soybean lectin. The hemagglutination unit (H.U.) is defined as the level of test solution which causes 50% of the standard cell suspension to sediment in 2.5 hours under the conditions described by Liener. Lectin levels in soybean can vary between 37 to 323 H.U./mg protein.

The method has a few issues such as obsolete equipment, a vague description of the spectrophotometer adapter and variability due to blood preparations. Some recent modifications have been made such as the concentration of blood cells, dilutions used, etc. The results after these modifications have been somewhat lower but are the same "ballpark" as the OECD reference data. General problems with the method include low sensitivity, subject to interferences, poor correlation with other methods and other agglutination assays under different conditions.

There have been some results published utilizing ELISA (Rizzi et al., 2003. Food Res Intl 36(8): 818). Bridging studies between agglutination and ELISA may not give much information, however. Both methods may be needed for certain samples.

Suggested steps to improve the agglutination method include:

- Consideration of new approaches such as using a Colter counter;
- Publication of updated single lab validation data;
- Performing a collaborative study with several labs;
- Standardization of results again a mutually agreed upon control substance;
- Publication of reference data using a new method;
- Officialize method via AOCS, etc.

Absolute quantitation of seed allergens using tandem mass spectrometry (S. Stevenson, PepPro): PepPro is a small company located in Columbia, MO.

PepPro's technique is a bottom up quantitative proteomic technique which analyzes protein fragments from proteolysis. Peptide fragments are analyzed during tandem mass

spectrometry (MS) and the liquid chromatography (LC) retention time, peptide mass and fragment masses provide specificity.

Tandem MS uses chromatography-dependent quantitation or chromatography-independent quantitation (spectral counting). For chromatography-dependent quantitation, the whole peptide signal is measured as the peak elutes (via multiple reaction monitoring (MRM)). The peptide fragment is then measured via peak integration. Chromatography-independent quantitation is done by counting the number of times a protein is identified by database searching of MS spectra.

For MRM, peptides are first selected by mass and the signal intensities are measured. The samples are doped with internal standards to provide absolute quantitation information.

AQUA peptides are synthetic peptides with a single stable isotope-labeled amino acid. They are incorporated into peptides to become known standards. When assaying seed allergens, the labeled standard is added. The MS distinguishes the labeled from unlabeled and a ratio provides the quantitation. Back calculations are then performed to provide protein abundance. Bottom up quantitative proteomic strategies measure peptide abundances. Tandem MS has multiple quantitative output.

A study has been done to monitor levels of allergens in crops, specifically GM crops. The objective was to determine the natural variation in allergen expression in conventional soybean germplasm. The study has been conducted in collaboration with ILSI PATC and DuPont/Pioneer. The ILSI samples were supplied by Bayer CropScience and were derived from 9 locations and 3 genotypes—all from Iowa. The DuPont samples came from 6 locations and 4 genotypes from North American sites. The allergens of interest were glycinins, trypsin inhibitor and conglycinin.

For the ILSI PATC, 3 biological replications (5 seeds/replication) were analyzed. The protein was isolated from each replicate and quantified by MRM. For the glycinins, the results showed that these differ among genotypes G1 and G2. In genotypes G3 and G4, these differed among environments. The trypsin inhibitor family members were affected by different variables. Gly m Bd 28k and 30k were affected by both genotype and environment. Beta-conglycinin was affected by genotype.

For the DuPont/Pioneer study, genotypes G1, G2, G3 and G4 differed among environments. Trypsin inhibitor and beta-conglycinin were also affected by environment. In summary for the two experiments, for ILSI, genetics played a larger role but in the DuPont/Pioneer experiment, environment played a larger role. The allergens were affected individually by genetics and environment. AQUA-MRM was found to be accurate, precise and quantitative and provide a high throughput, multiplex method to quantify hundreds of proteins.

Metabolic profiling platform technology to study plant dessication tolerance (A. Evans, Metabolon):

Metabolon is in the business of metabolomics. Metabolytics engages in biomarker discovery, biomarker monitoring and bioprocess monitoring. The diagnositics area is engaged in patient monitoring and pipeline molecules.

Metabolomics involves dealing with only the biochemical, i.e., in extractions, discard the DNA, RNA and proteins and analyze the small molecules left. HPLC is utilized to detect as many molecules on the biochemical chart as possible. The types of HPLC are MS/MS with or without EIS and GC-MS with EI. The real challenge to the analysis is the data processing, quantitation and identification.

In data processing, one ion feature does not equal one molecule. There can be a number of ion features per molecule and is dependent on concentration and chemical behavior of the molecule. More ion features per molecule may result in an increased false discovery rate (FDR). One ion feature is sent to statistics and an in-house authentication standard library (based on retention time, MS mass profile, fragmentation spectra) for identification. This process occurs in less than one minute. Peaks with no library signature are flagged and then entered into the library as "unknown molecules". The ion tracker system can separate co-eluting compounds by cross-sample correlation analysis. The software was developed to look at all ion features of a sample and tease apart which ions belong to which molecule.

Standards are used for quality control. There are recovery standards, internal standards and derivatization standards (for GC/MS). Approximately 30% of the samples are dedicated to quality control. Matrix standards are run after every 6 injections as are process blanks.

Data interpretation is the hardest part of the analysis. It can take weeks to make sense of all the data/information that is gathered.

Metabolon has been working on plant dessication tolerance—comparing Sporobolus stapfianus (resurrection plant) with S. pyrimidalis (which cannot "resurrect"). The question posed was whether there is any difference between the two species at full hydration. The answer appears to be yes—S. stapfianus is primed for dessication during periods of hydration. Next, the metabolic responses to early water loss and regulation during during the late dessication stages were investigated. It was found that S. stapfianus has higher levels of amino acids and lower level of energy metabolites (lower metabolism) so that there is less disruption by stress conditions than for S. pyrimidalis. There are also higher levels of osmolytes and nitrogen storage compounds in S. stapfianus. S. stapfianus grows slower and appears to save energy in anticipation of stress conditions. During early dissication, there is a significant shift toward protective molecules (amino acids, osmolytes, tocopherol). During late dehydration, 50% of the metabolites show a significant change in concentration. Amino acid accumulation is fueled by protein breakdown. There is also an increase in glutathione production and sugar storage.

In summary, S. stapfianus has the ability to respond and regulate metabolically per stress condition. In late stage dehydration, the focus is on detoxification and additional osmotic regulation. The regulation of nitrogen metabolism plays a key role in the dehydration process. The next step in the project will be to determine what genes may be involved.

Applications for Genome Sequencing (J. Clarke, Syngenta): The cost of genomic sequencing is dropping rapidly which is due to the next generation sequencing methods introduced in 2008 and breaking away from Moore's law (data storage computation). There are assemblers and aligners (algorithms) used. Assembly of a sequence is *de novo* whereas as alignment uses a reference sequence. There are numerous algorithms published every day. Ultimately, it is not about the sequencing but about what the data allows you to do such as gene cloning, structural variation, trait association, etc.

Marker discovery is an area that sequencing is used. For soybean, markers and candidate genes have been identified for tolerance to soil applied herbicides. In bulked segregant analysis, susceptible and tolerant lines are crossed and then a segregating population map can be made. It could take years without sequencing information. For rice, 150 rice recombinant inbred lines were re-sequenced. The authors of the study claimed a genotyping accuracy of 99.94%. They identified recombination breakpoints with 40 kb resolution. In watermelon, parental lines were sequenced for SNP discovery. Approximately 200,000 high confidence SNPs were found. These were used for reference for genotyping by sequencing. Sequencing is an open system since it allows for discovery and is cost effective. Not everything needs to be sequenced to get the necessary information. Information may be pooled and it is still possible to achieve the experimental objective.

An area that sequencing has been used in is resolving population structure which occurs during breeding. For corn, association panels were used from the Plant Variety Protection library. Five hundred (500) CIMMYT elite hybrids and 3500 Syngenta lines were used. Each SNP was associated to each trait. The allowed a successful identification of new leads for future Agrisure Artesian traits. The sequences are being used for SNP chip development.

Application of Probability of Detection (POD) as a Model for Characterization and Validation of Qualitative Methods (R. Shillito, Bayer CropScience): Qualitative detection methods are "yes/no" or "binary" analytical methods. These methods include lateral flow devices (LFDs), qualitative ELISAs, allergen tests, PCR, herbicide tolerance tests, etc. The question is how to validate these binary methods.

A validation process tests the method and proficiency tests if the lab is able to do the test and achieve the correct results. ISO 5725 is the international standard for validating a method. It defines accuracy (how close to the real value), precision (how often the same result is obtained). For binary tests, accuracy does not really apply since there are only two states—yes or no. Currently there is no standard for validating a binary method. The ISO/TC 34/WG 16 has been proposed to develop a standard for binary methods. AOAC, IUPAC, MoniQA are all studying the same problem, i.e., how to develop a standard.

The Probability of Detection (POD) plots data on a number of positive and negative results against analyte concentration. This can be used to determine confidence limits of a result along the concentration axis. Two methods can also be compared. It has been proposed for the validation of a binary method, the method should be tested in-house using multiple levels of the target analyte. The method should then be tested in multiple laboratories using multiple levels of the analyte. A minimum of 5 levels should be run and should also include a matrix blank. There should be a high concentration sample and a sample to validate the response at or near the lower limit. The number of replicates per level should be determined by the study manager. The number of replicates at the limits may be higher than at the other levels. There should be at least 12 samples at each level but preferably, 20 samples at each level would be desirable. At least eight labs should be used. These criteria are still under discussion. False positives/negatives are difficult to generate good data for and are not used in this approach, but classical LOD_{95} can be derived from the data.

In conclusion, there is agreement on using the POD model for binary tests. There are two published statistical approaches on how to analyze the statistics provided by POD curves. A binary method can be validated and the statistics carried out using one or both approaches depending on which information is needed to make a decision about method performance. For the proposed ISO standard, the outline includes: an outline of general principles, application to binary methods, will include PCR methods, LFDs, etc. and will also be pertinent to microbial tests. The standard was proposed in 2008 and there was a vote to proceed after review by TC 34 (Nov 2011). Comments were received and resolved. The standard was discussed with AOAC, IUPAC, MoniQA in March, 2012. The bottom line is that the ISO/TC 34/WG 16 is currently developing it the standard in cooperation with other interested parties.

Protein Production in Plants

Synthetic Biology Approaches to Optimized Gene Expression (J. Salmeron, Intrexon):
Intrexon's approach is to have a set of platforms to do a better job of genetically engineering organisms and ultimately, make the timelines of agricultural biotechnology development closer to that of electronic manufacturing timelines (i.e., the iPAD development timeline). Intrexon has >350 employees and has raised over $300 million to date. They have facilities in San Francisco, San Diego, Blacksburg (VA), Germantown (MD), Research Triangle Park (NC) and Charleston (SC). The facilities are engaged in industrial products, protein production, cell engineering, UltraVector design, molecular engineering, animal sciences, human therapeutics, agbiotech and antibody discovery. The agbiotech site (in RTP) was founded in 2011 and now occupies the former site of Athenix.

UltraVector is a rapid, scalable customization platform for rationally-designed better DNA. It allows the ability to mass produce thousands of rationally-designed, custom DNA programs which power faster product development cycles across many product opportunities/markets for less cost per R&D cycle. It also includes testing, reporting,

learning and designing and enables scalability/reuse of modular genetic parts. Components can be changed quickly in gene cassettes. Gene promoters can be fractionated to provide more granularity. *Agaricus bisporus* (mushroom) is used as the expression host since it has no human pathogens (viruses/prions) and has a long history of safe use as a food. The genome has also been sequenced and it possesses microbe-like and crop plant-like features. Another advantage is that growth occurs all indoors so there is containment. Currently, the system is being used to produce typrsinogen.

Another system is the RheoSwitch Gene Induction System. In this system, expression can be induced without a metabolic drain or toxicity during early plant growth and development.

Large Scale Manufacturing of a Vaccine Based on a Virus-Like Particle Produced in Tobacco (T. Talarico, Medicago): Medicago is based in Quebec City, Canada and has recently built the site in Research Triangle Park, NC. Medicago received a $21 million commitment for production of pandemic influenza vaccine from the U.S. Dept. of Defense. The conditions for obtaining this funding included building a pharmaceutical production facility in the U.S., hire the staff, transfer the production process from Canada to North Carolina, scale up 30-fold, perform engineering runs and make 10 million doses (for testing on ferrets)—all to be completed by the end of 2011. Most of these tasks were completed in 2011. The production campaign is to be completed by April 24, 2012.

The manufacturing process is transforming tobacco (*Nicotiana benthamiana*) with *Agrobacterium*-H1N1 flu virus. After an incubation period for virus particle production within the plants, the plant tissue is extracted and the virus particles are purified. The virus particles have the hemagglutinin protein on their surfaces but there is no DNA within the particles. It is surmised that the whole virus particle will give a better immune response than just injecting the hemagglutinin protein only.

Currently, Medicago has some automation integrated in the process and has plans to fully automate in the near future. Tobacco seeds are sown at two densities and then manually thinned. At 35-39 days after seeding, the plants are infiltrated with the *Agrobacterium*-H1N1. The infiltration process takes about 7-8 hours/lot of *Agrobacterium* and is semi-automated. The *Agrobacterium* solution is infiltrated to leaf biomass of plants at a given growth state. The plants are then incubated in a controlled environment to drive protein expression. To release the virus-like particles (VLP), the plant tissue is diced and then an enzyme solution is added to dissolve the cell walls. All the waste tissue is autoclaved. The biomass is liquefied and the VLP are extracted via the use of columns.

The vaccine has to meet the requirements of an injectable. It must go through release testing, characterization assays and other quality attributes are examined such as immunogenicity, stability, lack of allergic reaction, etc.

Regulatory agencies have limited experience with a vaccine produced in this manner. Thus, the pathway is not clear. The vaccine is expected to be reviewed like any other biological. Some guidelines are published by the FDA (2002).

Focus on Wheat

Innovation in the Wheat Industry (J. Demarchi, National Assoc. of Wheat Growers):
Wheat provides 20% of the world calories. By 2050, the global food production must double to feed the expected 9 billion population. The task for wheat associations is to make wheat an attractive crop to growers. Wheat is currently grown in 42 U.S. states with a harvest of 50 million acres/year.
There are 6 different classes of wheat. Hard red winter wheat is the predominant class grown in the Midwestern plains of the U.S. It is primarily used as flour in breads. The soft red winter wheat is grown in the eastern U.S. and is used in cakes, cookies, pies. Hard red spring wheat is used in bagels and soft white winter wheat is used in Asian noodles and breads. Durum wheat is primarily used in pasta and hard white wheat is used in whole grain white bread.

The challenges for wheat growers are grain quality, pests (wheat stem sawfly, Hessian fly, nematodes, aphids), diseases (rusts, head blight, root diseases, foliar diseases, powdery mildew, *Septoria*, crown rot), stress (post-harvest sprout tolerance, dought/heat, cold, spring freeze damage) and weeds.

Approximately 70% of the wheat varieties come from the public sector. USDA is currently conducting the core of wheat research in the U.S. The U.S. consumes about 134 lbs/person/year. However, 50% of the wheat grown is exported. It is important to maintain good export relationships in markets such as Asia, Mexico, Colombia, Nigeria, Egypt and the European Union. A typical wheat yield is 45 bushels/acre which is much less than corn (160 bu/ac). Some authorities believe that a yield plateau has been reached in wheat due to stresses and insect pests. A grower realizes $159/ac net return on wheat whereas corn give $400/ac. In the U.S. wheat acreage is actually decreasing due to corn and soybeans moving onto those acres. There are approximately 29,000 U.S. farmers that grow >500 acreas of wheat annually.

The National Association of Wheat Growers (NAWG) is a federation of state wheat associations. It is an advocacy association supporting wheat research and biotechnology. For wheat, a goal is to achieve 20% yield increase by 2018 which would mean >50 bu/ac. Biotechnology is viewed as one avenue to achieve this. NAWG published the paper "Paving the Way for New Technology: The Case for Biotech Wheat" in September, 2009. NAWG has joined with Canada and Australia to promote the use of biotech in wheat.

The principles for commercialization for companies includes several areas. First, is technology provider dialog with U.S. Wheat and NAWG and to obtain major market approvals (countries with over 5% of export market). Technology providers also need to have education/outreach programs as well as stewardship processes for the biotech. The value capture model is a certified seed system since there is a high level of saving seed among growers but it is recognized that companies will need to make money to bring the technology to market. The traits of the most interest to wheat growers are drought

tolerance, improved yield, disease tolerance, nitrogen use efficiency, nutritional improvement and protein quality/quantity.

Companies have already made investments in wheat research/products. Some of these companies are Syngenta, Monsanto, Bayer CropScience, Limagrain, Dow AgroSciences, Arcadia Biosciences and KWS Cereals. Arcadia Biosciences has been investigating gluten-free wheat. There are also many collaborations with academia (U. of Nebraska, S. Dakota State U., Texas A&M, Idaho State, Kansas State and Virginia Tech). Timing is everything for industry. It is particularly important to get the messages out about new traits utilizing social media.

Wheat related organizations include the following:

- NAWG (www.wheatworld.org/research)
- U.S. Wheat Association
- National Wheat Improvement Committee
- Wheat Foods Council
- Grain Foods Foundation
- International Grains Program
- Northern Crops Institute
- Wheat Marketing Center
- American Institute of Baking
- AACC
- Wheat Quality Council (wheat variety introduction)
- International Wheat Genome Sequencing Consortium
- North American Millers Association (NAMA)
- American Bakers Association

Innovation is needed for wheat and NAWG and others are working toward commercialization of biotech products in wheat. The public and private organizations have responded to calls from wheat growers for more investment in research and traits.

Growing Wheat in the Southeastern U.S.: Issues and Challenges (R. Weisz, NCSU): Soft red winter wheat is predominantly grown in North Carolina. Fifty (50) percent of the wheat grown goes to animal feed. Wheat is planted in the fall and requires a winter cold period. Generally, planting occurs 7 days from first frost (usually last 2 weeks of October). Wheat planting is always in competition with soybean harvesting.

Wheat needs pre-plant fertility such as phosphorus, potassium, sulfur, lime and a small amount of nitrogen. Leachable soils are a real problem for managing fertility.

Tillers are important on wheat plants. They are semi-independent plants which produce a single head of grain. Lots of tillers are needed for yield in North Carolina. Nitrogen is needed in February to stimulate tillering. In the EU, tillers are not wanted in wheat.

The main weed problems in wheat are chickweed, henbit and wild garlic. Weeds compete with tillering, thus impacting yield. Wild garlic is a problem for smell and taste

since it will taint feed and flour. Ryegrass is resistant to all herbicides including Roundup.

In the winter, tillering will stop. During vernalization, the plant is switching from vegetative to reproductive growth. There is no photoperiod sensitive wheat grown in the South. In early March, vernalization is done and the plants are highly sensitive to freezing. During this time, the stems elongate and the grain head forms. In April, the grain head swells inside the stem (swellings called "boots"). The boots split in May and the plants die in June. The grain is considered dry when it reaches 15% moisture and then it is harvested.

The Hessian fly produces maggots that move into the leaf and kill the tillers. The maggots then pupate and generally most die. There are some resistant wheat varieties available. Aphids are usually kept in check by frost and other insect predators. The aphids themselves do little damage but they are vectors of the Yellow Dwarf Virus (YDV) which stunts and yellows the plants. The cereal leaf beetle is the major insect pest in the Southeast. There are no natural insect predators and the larvae can defoliate the crop within days. Currently, there are no resistant varieties available.

Powdery mildew is the most common wheat disease in North Carolina. There are resistant varieties, however, the resistance is broken within 1-5 years. Left unchecked, powdery mildew would eliminate wheat production in the Southeast. There are university programs to find new sources of resistance such as from wild grasses. Head scab (caused by *Fusarium*) infects kernels and causes them to shrivel and turn white or pink. The *Fusarium* produces the mycotoxin DON and thus, growers cannot sell the grain. The fungus can only infect wheat during flowering. There is no simple fix for the disease, i.e., no resistant varieties, no late planting (organism likes warm weather). The grower needs to monitor weather around flowering time and apply fungicide sprays when the weather is warm and humid. However, fungicide spraying is only about 50% effective.

Dissecting the Major Disease Resistance Pathways of Hexaploid Wheat by Virus-Induced Gene Silencing (S. Scofield, USDA ARS):

Isolation of wheat genes with particular functions is difficult due to the hexaploid genome. The genome also prevents conventional mutational analyses. The wheat genome is 16,000 Mbp and is difficult to transform. Thus, many modern approaches to gene isolation cannot be utilized.

One method to look at wheat genes is a system that will generate gene knockouts, especially a system that creates knockouts without having to transform the wheat. One such system being investigated is virus-induced gene silencing (VIGS). VIGS is a form of RNA-mediated gene silencing. Replication of RNA viruses causes a large amount of dsRNA to accumulate. The silencing mechanism targets the sequence represented in the dsRNA for homology-dependent degradation. If the plant virus carries sequence homologous to plant genes, the transcripts of the plant genes will also degrade. The

barley stripe mosaic virus (BSMV) contains 3 RNAs and was originally cloned by Andy Jackson. Plant sequences can be inserted in the γ RNA downstream of the γb gene. The α and β RNAs are not changed. The constructed γ plasmid is linearized, *in vitro* transcribed and then mixed with the α and β RNAs. The RNA mixture is then used to inoculate plants. The VIGS phenotype can then be observed.

To test the system, phytoene desaturase sequence was used. Phytoene desaturase is in the carotenoid pathway and when the gene is silenced, bleaching is observed on the plants. For a positive control, the cloned R gene was used. When the R gene is silenced, a resistant plant then becomes susceptible to leaf rust. Susceptible and resistant rust varieties were used in the experiment. Ten (10) plants were inoculated for each treatment and all were infected with the viral constructs. The plants were then sprayed with *Puccinia* (rust) and scored 8 days later.

The results showed that photobleaching was present in the resistant plants thus the phytoene desaturase was silenced. When the R gene was silenced, resistant plants exhibited susceptibility.

Another project was to look at the Hm 1 genes in nonhost resistance of grasses to *Cochliobolus carbonum.* This is a devastating pathogen that infect all parts of susceptible maize plants. The fungus makes a toxin which causes the disease. The gene is limited to grasses. Silencing of the Hm 1 gene revealed an ancient, conserved mode of fungal resistance. The gene has remained durable through 40 million years.

Another area for VIGS was to look for the functional identification of wheat genes to *Fusarium* head blight. QTLs are known but no gene sequences have been shown to be required in resistance to the fungus. The VIGS was adapted to obtain silencing in the grain heads. SAM synthase affects resistance since if it is silenced, the fungus will infect a resistant grain head. Ethylene is needed for senescence to occur. 1-MCP plants have delayed senescence and *Fusarium* moves out of the infected floret. Resistance to fungus is improved with an ethylene spray (Type 2 resistance). The spray also improves Type 1 resistance (spreading of the fungus).

In summary, BSMV-VIGS is capable of silencing genes in a wide range of cereal tissues. The variation in the extent of silencing from plant to plant remains the greatest challenge of the system. VIGS is an important new way to confirm gene function in cereals.

New Member Presentation

<u>*Covance (D. Levin):*</u> Covance has 11,000 employees with offices/facilities in 60 countries. It generates $2.1 billion in revenue.

Covance supplies services in the following areas:

- Toxicology
- Dose preparation/analysis
- Antibody products

- Biopharma development
- Biorepository
- Biotech services
- Environmental sciences
- Genomics
- Nutritional chemistry/food safety

Biotech services are located in Harrogate, UK and Greenfield, IN. Covance has invested >$30 million in state-of-the-art facilities.

Nutritional chemistry and food safety is the oldest business area of Covance. It was developed from WARF (Wisconsin Alumni Research Foundation) where vitamin D and warfarin were discovered. In 1997, Covance was publicly traded. It has 24/7 analysis capacity with over 350 personnel. Facilities are located in Madison (WI), Greenfield (IN), Battle Creek (MI) and Singapore. Methods are fully validated and Covance conducts over 1 million tests/year on 250,000 samples.

Covance's capabilities include consulting (training, investigations, auditing), analytical (nutrition labeling, vitamins, minerals, etc), microbiology (PCR, BAX, allergens), and residue (pesticide analysis, melamine, acrylamide, allergens, mycotoxins). Data is handled by an integrated automated data management systems. Covance also has crisis response, i.e., flexible scheduling. Twenty (20) percent of tests performed are done on a priority basis. SampleKinect is an intuitive tool which allows data to be viewed in real-time. It allows the customers to self-administer their account and gives spectrum of view, export and print options for each report or summary.

A Covance signature account entails the assigning of a program manager who becomes the key contact person with the customer. All activities for a signature account are handled by a dedicated team.