



AEIC Fall Meeting 2013 Minutes

October 23-24, 2013
Sacramento, CA
Hosted by Monsanto Co.

P.L. Hunst, Secretary

AEIC BUSINESS MEETING

Spring 2013 Secretary's Minutes: Motion was made, seconded and voted approved to accept the Spring Meeting minutes as posted on the AEIC website.

Treasurer Report (Y. Dudin):

	Planned	Actual
Beginning balance (as of 1/1/2013) (includes CD which was \$25125.31 as of 1/30/13)	\$32439	\$32439
Dues	7500	8200
Interest on accounts	125	2
Advance payment for 2013 Fall Meeting (Monsanto)		10000
BALANCE	7625	18202
Expenditures		
Scientific paper	5000	
Wire transfer fees		
DE franchise tax	25	25
ANSI/ISO	2900	
Board meeting		
Spring Meeting 2013	4500	2735
Website	350	240
Bank service charge		
Fall Meeting Expenses	4500	9789
Graphic Design Material Creation		
Reprints (Brochure)	800	
Subscriptions	100	
Miscellaneous	100	
TOTAL EXPENSES	18275	12789 (YTD)
BALANCE (Checking + CD)	21789	37852.30 (estimate as of 10/21/2013)



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

Membership Update (Y. Dudin):

Member	Total	Dues Not Paid	Outstanding Dues
Large Companies	14	7	\$3500
Small Companies	14	8	\$2000
Associate Members	2	1	\$100
Individual Members	3	1	\$50
TOTALS	33	17	\$5650

AEIC Brochure (Y. Dudin):

No changes to the brochure have been sent to Dean Layton. It would be good to update and reprint the brochure for the upcoming Spring Meeting 2014. Yelena, Ray, Gina and Denise volunteered to work with Dean to update the brochure.

AEIC Website (P. Hunst):

The webmaster just recently returned from maternity leave. Sections of the website need to be updated, particularly "Past Accomplishments". Yelena, Matt, Frank, Don, Penny and Dean (in absentia) agreed to work on making recommendations to the webmaster.

AEIC Goals and Activities (D. Thiede):

The paper on sub-sampling was discussed as to whether this should be dropped as the lead author (B. Kaufman) has moved on. It was decided to shelve the decision to the 2014 Spring Meeting.

Another potential activity for AEIC is the initiative on composition methods (see below). Matt B. presented an overview.

Another area for AEIC to consider is new genomics methods acceptance, i.e., new generation sequencing (NGS) methods for copy number and in lieu of Southern data. It was mentioned that agencies are starting to accept NGS. It was suggested that the topic be focused on in an upcoming meeting with the goal to publish a paper. It was also suggested to have a session on digital PCR standards (maybe 1 hour). Ray S. (Bayer) and Pearce (Eurofins GeneScan) volunteered to look into this. Frank S. (Eurofins GeneScan) also volunteered to put together an educational piece to distribute to the membership.

Lectin Method Update and Composition Methods Initiative (M. Breeze, Monsanto):

The lectin single lab validation manuscript is progressing towards a goal of submission to JAOCS in January of 2014. In addition to the method and validation data, quantitative soybean data from a population containing commercial varieties from 1972 to 2008 will be included to demonstrate the utility of the test method. The global collaborative method study will be conducted in as many locations/labs as possible, currently 17 labs in five countries are included in our draft list. In addition, all agricultural biotechnology companies are in the process of identifying soybean varieties and data generation options to provide comprehensive data on lectin levels via ELLA for publication and ILSI-CCDB submission by April 2014.



A discussion was initiated concerning AEIC and participating in an initiative around crop composition methods. Crop composition analysis methods are not optimized for purpose and regulatory requirements in this area are inconsistent and becoming increasingly burdensome. Current methods are also not applied consistently between labs. The mission of the initiative would be to develop and validate robust, fit for purpose methods for composition analyses that are scientifically valid and can be applied with no restriction as to freedom to operate (FTO). The vision is continuous improvement of crop composition analytical approaches as part of a focused open consortium.

The “ask” of AEIC is to support the formation of working groups to address method questions and the development of a methods working group structure. A structure would be needed to allow direct funding of method development and validation efforts which would be shared by interested companies. AEIC members were asked to consult within their organizations and determine whether they would be willing to support such an initiative, i.e., having AEIC develop and maintain working group structure and whether companies would monetarily support (or not) specific working groups. Matt will make the presentation available to AEIC members for their internal discussions. Matt, Gina and David L. agreed to start organizing volunteers for initiative, however, the AEIC Board will also need to discuss and make a recommendation to the membership for AEIC’s interaction in the initiative.

Spring Meeting 2014 (D. Thiede):

The Spring Meeting will be held in San Antonio, TX on May 6-7, in conjunction with the annual AOCS meeting. The meeting will be held at the Convention Center which is located on the Riverwalk in downtown San Antonio. A block of rooms at La Quinta Hotel (several blocks from Convention Center) at a rate of \$129/night (breakfast/internet included) is being held by AOCS for AEIC. AOCS will send out a notice in early January 2014 for housing accommodations and AEIC members will need to book by late February. After late February, the block of rooms will be opened to AOCS members. AEIC members are invited to the AOCS mixer the evening of May 5 and the AEIC group dinner will be in conjunction with the AOCS Biotech Division the evening of May 6 (\$45/person).

A few suggested topics for the meeting were discussed. These included:

- Discussion of methods work for harmonization
- Discussion of vitamin methods (relevant measurements, current methods, etc.)
- Impact of new generation sequencing digital PCR on analytical approaches
- Lectin method wrap-up/validation process
- Measurement of allergens (methods)
- New developments in equipment technology (LC-MS/GC-MS, etc)

Nominations for Vice President (D. Thiede):

Nominations were opened for Vice President of AEIC. This is a three year commitment—one year as VP, one year as President and one year as Past President. The AEIC Secretary will send an e-mail to the membership for nominations. The election will be held via e-mail ballots during November. The Secretary will send/receive the ballots. David Levin (Covance) and Guomin Shan (Dow) were nominated at the meeting. David accepted the nomination. The Secretary will contact Guomin for his acceptance.

ISTA Proficiency Program (R. Shillito, Bayer CropScience):



The GMO Committee within ISTA has a Proficiency Test Program Working Group. Ray S. (Bayer) is on the working group and is in charge of resource procurement. The Proficiency Program tested soy in 2013 and cotton and alfalfa will be tested in 2014. Whole seed is used for the testing and each laboratory decides how they will test the samples. Two SOPs have been developed which describe all the steps required to execute a single round of testing. The challenges for the program are procuring GM and non-GM seed and funding to cover costs. Also a laboratory is needed to put together the samples to be sent out. An audit checklist for GMO testing labs has also been developed. ISTA has also written a GMO rules chapter.

ISO/TC 34/SC 16 (G. Clapper, AOCS):

This is the molecular biomarkers group which has several documents in development which includes a definitions. The U.S. is hosting the 5th Plenary Session in 2014 in Durham, NC. The group is currently looking for members for the technical advisory group (TAG) in the U.S. The group is working on moving away from GMOs to just detection methods. There is currently a working group on qualitative methods and this group needs input from kit manufacturers. Volunteers should contact Gina Clapper at AOCS.

Business Meeting was adjourned.

INVITED TALKS

Overview of the Vegetable Seed Business (Y. Shapiro, Monsanto): In the U.S., there are 1.8 million acres of fresh vegetable cultivation and 1.2 million acres of processing vegetable cultivation. The growth in vegetable production is driven by the increasing world population, improved standards of living, innovation and value-added traits. The market may reach \$324 billion by 2015. One percent (1%) of vegetable production is done in protected culture, i.e., greenhouse or glasshouse, soil-less hydroponics, plastic tunnels. The European Union has most vegetable production in protected culture, predominantly in Spain.

Monsanto acquired Seminis in 2005, Poloni, Peotec, De Ruiters in 2007-08. In 2012, Monsanto launched their new vegetable seeds branding. Seminis produces seeds predominantly for plastic tunnel culture and De Ruiters has seeds for glasshouse production. Monsanto vegetable seeds operates in 160 countries and has 18 breeding crops with over 2000 varieties. Globally, there are 4000 employees, 55 breeding stations with an annual R&D budget of \$180 million. Net sales were \$856 million in 2013. The crops are divided into six categories: Solanaceous, cucurbits, root/bulb, large seed (sweet corn), Brassica and leafy. All are hybrids with the exception of beans and lettuce. Advanced breeding tools such as molecular markers and seed chipping are used. Throughput for development has increased 100-fold since 2008.

Monsanto has launched VT Triple Pro sweet corn (a GM product) in the U.S. in 2011 and plans to launch in Brazil in 2015. This is an insect-protected trait using Bt proteins and is targeted at the fresh market. Syngenta launched a Bt sweet corn call Viptera. Currently there are 25,000 acres of sweet corn in the U.S. but the market size is 250,000 acres. Customers demand aesthetics for sweet corn, i.e., perfect ears with no insect larvae. Also, farmers growing GM sweet corn also grow GM field corn so they are familiar with the traits. GM corn varieties have reduced insecticide applications by as much as 85% as well as decreased fuel consumption and energy use. The CO₂ emissions have also been lowered 2-3



times. There is no good non-GM source for insect resistance. Monsanto also has virus-resistant squash (also GM).

Molecular breeding has been used to allow quick addition of downy mildew resistance to cucumber types. Resistance provides the opportunity to reduce costly fungicide applications for disease control. The genes are from wild cucumber. The varieties were launched in 2012.

EasyHarvest broccoli improves harvest efficiency since its head is even with the leaf canopy. There are also fewer leaves on the stem which cuts down on the “hand-stripping”. It also has uniform maturity and consistently greener florets.

A wild donor source has been used to identify genes for Phytophthora resistance in peppers. Molecular markers are being used to assist the breeding. Monsanto is also working on combining multiple resistance sources into elite tomato germplasm for geminivirus resistance.

Output traits provide benefits to the value chain. Vegetables are a sensory crop, i.e., taste, aroma, flavor, color, texture. The nutrient density has also been raised and consumption has increased by the improvement of flavor. Convenience has been improved through increased shelf life, freshness and vegetables being fresh cut. Monsanto’s vegetable analytics lab looks at pigments, value-added components (vitamins), texture, shelf-life, processing and flavor. The lab processes over 60,000 samples.

Monsanto has developed a number of vegetable varieties via advanced breeding technologies—all non-GM. These include Beneforte broccoli, Bellaverde broccoli (sweet stem), Bellafina bell peppers (small size, sweet, crunch), EverMild onions (sweet, mild, longer shelf life), Melorange winter melons (small, deep orange color), Sweet Peak honeydew (has orange flesh), SummerSlice watermelon (loses less juice when sliced), Frescada lettuce (sweet, crunchy, like Romaine but tastier) and a slicer tomato that maintains its visual appeal longer after slicing.

Breeding Vegetables (J. Djordjevic, Nunhems): Nunhems is part of Bayer CropScience. It was established in the 1980’s and currently has 1800 employees in 43 countries, 26 breeding stations, 2 research centers and \$450 million in sales. Nunhems has 28 vegetable with 2500 varieties crops. Seed is produced in 20 countries.

Plant breeding is defined as the effective development of superior cultivars over a long time span; cross-pollinating plants with desirable qualities and traits. The goals of breeding are to produce novel varieties that meet the demands of customers. To create novel varieties, the best attributes need to be combined which entails measuring qualities/quantities of descendants and selecting the right plants with the right combination of genes. Ninety-nine (99%) percent of descendants are discarded in the process. Breeding is like a funnel going from parent varieties → F1 → F2 → F3 → testing, testing, testing → commercial varieties. New technologies (molecular breeding, double haploid plants) spin the funnel faster. Testing involves a lot of phenotype evaluations which is sometimes difficult to measure since the plants may produce fruits over a 9 month period. Breeding teams are large and consist of the breeder, molecular scientists, plant pathologists, product development specialists and sales/marketing.



The number of breeding generations varies between crops and thus, the plant breeder must look 10-15 years out. Molecular markers are very useful when putting more genes together in a plant to obtain a trait. Sequencing is a new technology that has increased breeding capabilities immensely. Sequencing has become much quicker and more affordable. For example, the human genome sequencing was done in the 1990's at a cost of \$300 million and years of work. If the work had been done in 2000, it would have cost \$200,000 and one year of work. If it had been sequenced in 2012, the cost would be \$1000 and one day of work.

The total seed market is \$35 billion and vegetable seed is \$4 billion of this market. Tomato is the biggest crop comprising 21% of the vegetable seed market with 5000 varieties. Complexity is the world in vegetables with many traits to work on. In Nunhems, the vegetable crops are divided into 4 buckets: international fruit, international outdoor, Europe and processing. Greenhouses allow production control closer to the consumer.

Production of Vegetable Seed (E. Schatz, Syngenta): Vegetable seeds are a global industry. There are different crops which use different technology, logistics, infrastructure and challenges. Some of the challenges of vegetable seed breeding include:

- Quality demands of the consumers
- Consistency
- Innovation/new genetics/shorter life of varieties
- Era of high quality, cheap labor is gone
- Complex trade issues.

The drivers in vegetable seed are quality, quantity, timing and cost. It is an inventory business. Many of the crops are hand-pollinated and now much of this labor force is disappearing due to the movement to the middle class. Also, seed-borne pathogens are a problem as seed is moving around the globe. Seed is now mechanically harvested and irrigated. Open field hybrids require hand work and bees.

Vegetable growers also present demands such as the desire for perfect seed, more use of transplant vs direct seeding, more protected culture of vegetables and physical appearance and packaging are more important. The "customer" for a vegetable seed business is the grower, produce chain and consumer. It is a wholesale business but must think beyond the distributor.

Growers expect the seed to be available when they are. Otherwise, they will use someone else's seeds. This means strategic inventory management of key items. Growers expect year round production of a consistent product.

Innovation is very important since variety life cycles are shorter. Thus, there is less time to learn how to grow a variety. There is a higher inventory risk for obsolescence but speed to market is imperative. Customers are very reactive to market changes. In the labor area, it is much harder to find skilled laborers in the U.S. The evolution of China and India as emerging economic powers has provided more opportunity for their laborers to seek opportunities away from farms.

Rules around country of origin are a political football because they are constantly changing and cause trade issues. Countries can protect their own industries by their import and export rules. Not all ports of import apply the same regulations. Import permit processes can hold up imports by 45 days or more.



Seed-borne diseases also cause trade issues. Some may be allowed in one area but not in another. The CGMM virus has been documented for the first time in the U.S. in 2013. Other known diseases are going to new species such as bacterial fruit blotch on squash. There are new races with little resistance in current breeding lines. Also, there are new diseases such as *Pseudomonas* on squash. Many restrictions and trade issues are linked to country of origin restrictions.

International Seed Health Initiative (J. Cucuzza, Monsanto): The ISHI is a group of seed pathologists that develop and disseminate reliable and sensitive seed health detection methods. The objective is to minimize the use of different methods by countries and seed companies. Standard methods are based on comparative testing worldwide and are scientifically validated for sensitivity, selectivity, reliability and reproducibility. The methods should be practical and usable globally. New technologies may be too expensive for some countries. Methods are developed according to ISTA Method Validation for Seed Testing (2007).

ISHI began in 1993 when seed companies in the Netherlands and France entered non-compete project to monitor seed health. In 1994, U.S. companies joined and then companies from Japan and Israel. Seventy percent (70%) of the world trade for vegetable seed is now involved in the initiative. There was close collaboration with ISTA in the mid-1990's which resulted in the joint ISTA/ISHI guideline. However, ISHI is not part of ISTA. In the late 1990's, ISHI became associated with the International Seed Federation (ISF) which increased ISHI's recognition. The Secretariat and financial administration is done by ISF so this saves costs for ISHI. ISHI has an independent structure within ISF and the funding still comes from participating country/company members.

The principles of participation are:

- Non-competitive subject
- Share methods, lab techniques, data, infected seed, experience
- Cooperate in a timely sharing of new information
- All information and data are stored in ISHI Vegetable database (access limited to ISHI members)
- Share technical lessons learned from seed health related complaints
- Companies in non-member countries can participate

The policy coordination group has representatives from 5 member companies, the Technical Coordination Group (TCG) chair and ISF Secretariat. The TCG is comprised of seed companies in member country national associations (such as ASTA), official testing bodies, private labs (such as Eurofins), ISHI-Veg staff and guest speakers. There are also crop-based technical groups representing a) bean, Brassica, pea, radish; b) cucurbits; c) root/bulb; d) tomato and pepper. There are 10 core voting members/group. There are meetings every 9-10 months and the locations rotate between countries. The structure of the meetings is a plenary session, core technology meetings and a plenary follow-up session. Conference calls are held in the interim between meetings.

The ISHI has 22 established methods for 10 different vegetables. Eleven of the methods have been reviewed and accepted as ISTA rules. Eight methods have been accepted by the USDA National Seed Health System. There are 26 host-pathogen combinations that are also being assessed. ISHI also has published ISF position papers and contributes to the ISF Pest lists database with science-based



information for phytosanitary requirements. ISHI is also participating in TESTA (testing, evidence for seed transmission and analysis) which is an EU project that parallels ISHI.

More about ISHI can be found at: www.worldseed.org/isf/ishi_vegetable.html

Novel Technologies in Vegetable Crop Research and Breeding (A. Van Deynze, Seed Biotechnology

Center, UC-Davis): The value of vegetable production is increasing. Novel technologies are being used increasingly in vegetable seeds research and development. Genomics has become very cheap with the cost being less than \$0.01/mb. The sequencing of four human genomes can be accomplished in a 24 hour period. UCD collaborates with the Beijing Genome Institute which has a facility at UCD. UCD works on sequencing and re-sequencing crop genomes to look for diversity. High-density genetic maps are created to link and track traits in breeding. This work has led to the finding that pepper shape and hotness have no relationship. Also, *Phytophthora* resistance is complex in pepper so sequencing assists in determining what genes and combinations of genes are needed. Molecular markers are used to help locate the genes.

Another technology is high throughput SNP genotyping which is an evolving field thus instruments are usually only good for about 3 years. Marker-assisted selection helps put into the field what plants will have the trait. The cost of sampling a genome with sequencing is less than a field plot:

- \$33 for 48-plex
- \$19 for 96-plex
- \$9 for 384-plex

Genome selection saves time but not necessarily money. It makes parent selection in one year vs three years. Markers allow access to novel germplasms.

Reverse breeding is based on engineered meiosis. This allows control of which chromosomes recombine. Haploid plants are produced centromere-mediated genome elimination. The centromere protein (CENH3) is needed. There is no tissue culture.

Data from genomics is created faster than it can be analyzed. iPlant Collaborative (National Science Foundation) develops tools for the plant sciences and works with the largest computer (located in Texas). Scientists can analyze data via a cloud. High throughput phenotyping is also very important to breeding. Mechanized solutions are being developed.

In the future, there will be quality control for all seed with trait verification and tracking. Recombination will be controlled and large populations will be handled. Genomics assisted breeding will help in understanding pathways, genes, alleles that contribute to phenotypes.

Fresh Produce Testing (T. Suslow, UC-Davis): Wholesomeness combines quality, availability and safety. Produce makes up a large percentage of illness from food. Lettuce/Romaine and fresh tomatoes make up the largest proportion of outbreaks of illness. Sprouts are in a different category.

Commodity-specific GAP and food safety audit checklists have been developed, usually defensively when an outbreak occurs. Guidance documents are comprehensive from the farm to the point of sale (published in 2013). California defensively developed mandatory standards for cantaloupe in response to the Colorado case. In many outbreak and recall investigations, there was a moment in time for



someone to recognize a microbial hazard that would have averted the situation. The Produce Safety Alliance has modules and learning objectives which are being developed by Cornell University. Micro-greens and sprouted seeds are niche-sourced. Seeds come from all over globally and there are not standards for production and cleaning. Most producers are not ready to take on challenges for systems from FDA. Most producers want exemption from FDA produce law. FDA and USDA are looking hard at commodity-based risk attribution. For instance, in agricultural water quality standards, the presence of indicators does not reliably reflect recent episodic fecal contamination. Pathogen presence in water does not accurately reflect risk of persistent crop contamination. Irrigation sources may be seasonally at risk of EHEC/STEC contamination.

Application of New Generation Sequencing Technologies for Developing Diagnostic Tools for Seed-borne Pathogens (P. Sudarshana, Monsanto): The goal of seed production is to ensure pathogen-free seed materials. Traditional methods involve plating seed wash on selective media. This process is tedious, time consuming and requires a lengthy lead time. For example, testing for tomato canker can take 28 days and bacterial fruit blotch can take 24 days. PCR methods offer quicker testing time but have false positives.

Primer design for molecular tools is difficult due to the high level of genetic diversity among pathogen isolates. New Generation Sequencing (NGS) helps to identify conserved sequences in pathogens of high economic significance (bacterial fruit blotch, tomato canker, bacterial spot, black rot, bacterial blight).

Tomato canker (*Clavibacter michiganense* subsp. *michiganense*; Cmm) spreads mainly via contaminated tools and personnel. Yield loss may reach 70-80% and the pathogen can survive in or on seeds for a long periods. Latent infections are also common. Seed health testing is required for international trade and movement. Currently, the testing consists of plating the seed wash on media and observing colony morphology to identify Cmm. Related non-pathogenic subspecies may cause confusion when trying to diagnose the presence of the pathogen. Molecular work has been undertaken to find regions of the Cmm genome that are common across geographically distinct Cmm isolates and that are not present in Cmm look-alike strains.

A defined panel of 11 Cmm isolates, 4 related species and saprophytic bacteria were assembled. The genomes were sequenced using Illumina technology and then the genomes were assembled. There were 2808 common regions identified. Cross-reactive regions were eliminated so that 21 contigs were identified. PCR methods were developed using the best 4 primers. These were screened against Cmm and saprophytic bacteria. The PCR method has been validated internally and has gone to ISHI members. Validation will be carried out on a larger panel for seed health industry. Assays are also being developed for *Xanthomonas campestris* subsp. *campestris*.

NGS is a cost effective assay development technology. Cost is reduced through the increased specificity that is identified. It will help increase seed quality for growers.

Let's Get Wild About Sunflower: Native Trait Integration in the Center of Origin (G. Cole, DuPont Pioneer): Sunflower has a wealth of natural diversity and North America is the center of origin. There is similarity between tribes in the family. San Joaquin Valley has lots of wild sunflower, thus, there is no seed production since it would require 1.5 miles of isolation. The Sacramento Valley is okay for seed production since much less wild sunflower.



Sunflower has 52 species and this number keeps growing. There are annual and perennial species with different ploidy levels. Sunflower is an obligate outcrosser. The seed shatters from the head. The seed is generally small with a low oil content. It grows in disturbed soils and has weedy tendencies so it is broadly adapted. Early Native Americans domesticated the sunflower. They developed the single headed plants with seed held in the head without shattering. The plants also tended to have larger seed and a higher oil content. In the 16th century, sunflower was brought to Europe and its popularization in paintings led to it be growing throughout the continent. Russia developed sunflower into an oilseed crop in the 19th century. This was driven by a scientist named Vavilov who was an early innovator driving center of origin concepts and utilized global genetics. In the 20th and 21st centuries, a germplasm repository was established in the U.S. Multiple disease and pest resistance, cytoplasmic male sterility, self-compatibility and herbicide tolerance derived from natural selection in wild species has been identified. There are also novel alleles for yield which are captured through heterosis.

Sunflower is fairly recent to California. *Helianthus californicus* grows in the muck of the bay but also in very dry areas. *H. inexpectatus* is a tetraploid which may be a cross between *H. californicus* and an extinct species. *H. winterii* grows on steep rocky outcroppings and flowers every month.

Creating Genetically Modified Potatoes Using the Innate™ Platform (C. Rachael, Simplot Plant Sciences): The Simplot biotech program was started in 2001 and is part of J.R. Simplot which is a privately held company. The mission of Simplot Plant Sciences is to create Innate potatoes with only potato elements—“all potato in potato”. The Innate technology uses no foreign genes, no antibiotic resistance markers, no backbone sequences but is not fully cisgenic. Acceptance of GM potatoes in the U.S. has progressed since NatureMark potatoes.

Potato is a tetraploid crop which is clonally propagated. Recurrent selection occurs over 10-13 years. There are 4 varieties that dominate the North American French fry industry:

- ✓ Russet Burbank (1914)
- ✓ Ranger Russet (1991)
- ✓ Umatilla Russet (1998)
- ✓ Shepody (1980)

Ranger russet has broad adaptation, high yield, lower disease susceptibility and a better grade-out than Burbank. It is also readily transformed. It is susceptible to blackspot bruise and cold induced sweetening. During cold storage, an enzyme triggers the breakdown of starch to sucrose and then fructose/glucose. When these potatoes are deep fried, they turn dark. Blackspot is caused by polyphenol oxidase and cold sweetening by invertase.

Simplot has used marker-free transformation via an *Agrobacterium* vector. The vector backbone contains the cytokinin synthesis gene. Those cells receiving just the backbone are identified by the strange phenotype of the plant that develops. Both Burbank and Ranger have been transformed as well as the potato chip varieties, Atlantic and Snowden.

Innate 1.0 potatoes are low bruise, low CIS, low asparagine. The polyphenol oxidase is not completely reduced so there is still some bruising but the browning is very low. Low asparagine is important because under heating (i.e., frying), asparagine combines with sugars to make acrylamide. Innate 2.0



potatoes have low bruising, lower CIS, low asparagine and late blight resistance. Future potatoes will have resistance to potato virus Y and additional late blight resistance genes.

Innate 1.0 potatoes have recently completed the USDA public comment period and had few public comments as compared to the apple, alfalfa and salmon files. These potatoes may be launched next year pending regulatory approvals. Simplot expects USDA and FDA approvals in 2014 and approvals are pending in Canada and Japan. Submissions will be made for Innate 2.0 potatoes in 2015.

Laboratory Techniques for Vegetable Seed Health (D. Maddox, Endless Sky Partners): Endless Sky Partners was established in 2009 and does seed quality management systems, seed health programs, accreditation systems and small business marketing and evaluation for the seed industry.

The most important words in healthy seed production are prevention, prevention, prevention. Market requires seed health programs, growers demand programs, regulatory requirements to move seed, seed pests affect seed quality and there are lawyers lurking behind every tractor. The goals of seed health programs are to a) minimize losses from seed-borne diseases, b) prevent pathogen introduction, c) avoid costly seed treatments and mitigations and d) keep customers happy. For clean seed, the seed should have a good genetic background, a seed stock program should be in place, production should occur under controlled conditions, harvest and treatment and testing. Production should be done under conditions that are unfavorable to pathogens—dry, arid, little rainfall. Fields and plants should be isolated in time and space by growing in the off season, keeping away from similar crops, adequate isolation between fields, seed beds, varieties.

Good cultural practices and good sanitation should be followed as well as adequate seed cleaning. Seed treatments should also be used. These include field disinfectants (acid extractions, fermentation, bleach) and warehouse treatments (hot water, bleach, acid treatments, fungicides, seed enhancements). These all may affect seed testing results.

Plant pathogens in seed include bacteria, fungi, viruses and nematodes. Seed-borne pathogens are those that may be seed transmitted. These pathogens are also referred to as “regulatory pathogens” as phytosanitary certificates are needed for seed export to prevent introduction into other countries and/or regions. These pathogens affect the entire seed industry and most tests are designed to detect them at threshold levels. Seed-transmitted pathogens are referred to as “quality pathogens”. Tests for these are designed to confidently detect the targeted pathogen.

The challenge with any seed testing is the sampling and the diagnostic method. Sampling should yield a representative sample which is randomly drawn, of a meaningful size and condition appropriate for the method. The challenge is that the disease/pathogen is seldom evenly distributed in the field and the pathogen is rarely randomly distributed in the seed. Also, seedlots tend not be thoroughly mixed. AOSA and ISTA sampling methods are generally utilized.

The threshold level is the level of the pathogen that causes disease. Tolerance is the level of contamination that can be tolerated without causing economic disease. There are very few thresholds or tolerances known. Tests are generally conducted at 3-5X the threshold for confidence. Samples with debris may give false positives and those with pesticides may give false negatives. In PCR and ELISA, false positives occur if there are inactive or dead organisms that react with components of the tests.



Test methods should have a) specificity, b) sensitivity, c) reliability, and d) be cost effective, simple and quick. Methods are one of the tools to measure the quality of seed production. If there is a positive test, the field is considered infected and the seed program may need to be re-assessed. Testing does not tell if the infected seeds will result in seed-borne disease in crops produced from the seed. But testing is useful to help determine mitigation and what treatments to use to control the disease. Also, a negative result does not mean the field is free of the pathogen.

Standardization and validation are very important in testing now and for the future. The food industry has zero tolerance for food-borne pathogens so millions are spent on the development of test methods. PCR is widely used in the food industry because there are actively growing pathogens and the populations tend to be high and detectable. For seed-transmitted pathogens, most methods are developed with a few thousand dollars or methods are adapted from other industries. There is a reluctance to use PCR tests in the seed industry due to cost of development, difficulty in identifying primers of the pathogen, labs not equipped. Also, Koch's postulates are ingrained in plant pathologists and thus, pathogenicity tests are favored.

Diagnosis of Plant Pathogens in Vegetable Production (F. Filho, Agdia): Agdia was founded in 1981 and easy, quick, reliable, economical, practical tests are its foundation. Agdia has more than 210 tests available for pathogens, GM traits and plant hormones.

Plant diseases are economically important and may also cause adverse effects on humans (i.e., mycotoxins). In Sri Lanka, the coffee rust outbreak caused the population to move drinking coffee to drinking tea. Late blight of potatoes in 1845 in Ireland caused over 1 million people to starve to death. And chestnut blight in the U.S. devastated the chestnut tree population. Important diseases today include citrus greening (Florida), citrus canker (Florida, Louisiana), sudden oak death (northwest U.S.) and plum pox virus in stone fruits (northeast U.S.). Modern agriculture practices favor diseases because of host plant density, host plant genetic uniformity and production areas foster host plants meeting pathogens. Fungi predominantly cause most of the plant diseases. Plant diseases move around via vectors (insect, fungi, nematode, human), infected plant parts, seed, air, soil, rain.

Pathogens affect the quality and yield of crops. Tomato spotted wilt virus causes mottled fruit which is unacceptable by consumers. Papaya ringspot virus reduces the quality and size of papayas and zucchini mosaic virus affects photosynthesis.

Seed-borne pathogens affect seed germination, seed commerce, seedling diseases and require testing which adds cost. Methods used for testing include microscopy, bioassay, serology, electrophoresis, dsRNA, nucleic acid hybridization, PCR, isothermal amplification, arrays, immunosensors, flow cytometry, new generation sequencing. Agdia uses microscopy, bioassay, serology, nucleic acid hybridization, PCR and isothermal amplification most frequently. There are no perfect methods and the best methods depend on the customers' needs. ELISA is most often used because it is flexible, economical and high throughput. The immunostrip test is a point of site test which is quick, sensitive and requires no skill or special equipment. PCR is used for pathogens where there is no good serological test available and it is also used for confirmation. Isothermal amplification can be used in the lab or field and requires just crude sample extraction and no thermocycling. Results are obtained within 30 min by use of a portable fluorescence reader or by immunostrip.



Novel Diagnostics and Disease Resistance Systems in Food Crops (P. Feldstein, UC-Davis): The RISE program (Research Investments in the Sciences and Engineering) is a program at UCD designed to launch new research activities. There are currently 13 projects funded which were chosen based on their potential for societal impact. One of these projects is RAPID-NEED (RNA-based amplification-free pathogen identification using nano-enabled electronic detection). This is a process for rapid, efficient, low cost detection. Six UCD faculty are involved in the project. Traditional methods provide a skewed view of microbial world. The first step in controlling plant diseases is rapid and accurate identification. Approximately 10% of the global food production is lost to plant diseases. There are clear links between civil disruptions and food supply in countries. Fresh produce consumption is increasing and it is a significant vehicle for food-borne pathogens. Enteric pathogens can persist in low quantities on plants and multiply post-harvest.

The RAPID-NEED targets pathogen RNA for detection as all microbes express information via RNA. The method steps are: Pathogens → release nucleic acid → electrochemical (hybridization electrode) → molecular conductance. The electrochemical step allows fast screening for multiple pathogens. The molecular conductance provides selectivity within a pathogen group. Small RNAs have been developed *in vitro* as well as protocols to efficiently prepare them.

The electrochemical step uses nano-porous gold sensor elements to enhance and optimize the detection sensitivity. The pore size can be varied to select RNAs and to increase surface area. The redox reaction produces current under applied voltage. Hybridized DNA increases the measured current. For molecular conductance, the gold electrode is lowered and if the molecule is present, it attaches and stretches. This results in a “stretch” profile being observed. The bp changes are detected via changes in resistance. A perfect match has lower resistance. DNA-RNA hybrids are 10X more conductive than dsDNA resulting more sensitivity.

Another project is utilizing surface plasmon resonance which detects hybridization of DNA at the surface. Binding kinetics can be detected which allows investigation of temperature effects on hybridization.

Structural biochemistry of plant pathogens is also being investigated. Plant recognition of an invading pathogen is an important event. The food supply depends on it. Plants do have molecules that function as immune receptors called PAMPS (pathogen-associated pattern). Chitin of fungi and EF-TU of bacteria are two examples. There is a cascade of reactions in the plant following the recognition event. The goal is to reveal the receptor-effector interactions at atomic resolution in order to modulate or extend plant defenses. An example is tobacco mosaic virus (TMV). Plant resistance is manifested as localized lesions and susceptibility as an overall mosaic. The N immune receptor can be used a model system to study innate immune signaling. The NRIP1 protein is found in the chloroplast and is released during infection. This activates the N protein. The N protein goes to the nucleus and transcription is activated. Avr4 is a small secreted protein from *Cladosporium fulvum* which has a chitin binding domain and is recognized by Cf4 resistance protein in tomato. The Cf4 gene can be transferred into banana from tomato.

Research and Collaboration Opportunities at the Seed Biotechnology Center and Seed Central (K. Bradford, UC-Davis): California presents unique opportunity for seeds. It has a range of latitude and altitude, coastal and inland valleys, a Mediterranean rainfall pattern, chilling for vernalization and climate diversity.



The Seed Biotechnology Center (SBC) was founded in 1999 to mobilize research, educational and outreach resources of UCD to facilitate discover. Seeds are the delivery system of traits with many tools to assist in the transfer of traits—genetic modification, marker-assisted breeding, male sterility, double haploids, seed enhancements, etc. SBC provides continuing education for the seed industry. The course Seed Business 101 is taught by former seed company CEOs. Two other courses are Seed Biology, Production, Quality and Marker-Assisted Breeding.

The SBC Plant Breeding Academy was established to train more plant breeders. It is a 2 year program and re-trains people in other fields of agriculture to do plant breeding. SBC also does public service work such as engaging in policy issues. SBC also participates in a collaboration for plant pathogen strain identification to help standardize naming and create a repository for different hosts and pathogen collections (USDA will maintain/distribute but will located at SBC).

Seed Central is an initiative of SBC and SeedQuest. The premise is to energize a seed industry cluster around UCD much like the technology cluster around Silicon Valley. Seed Central will foster communication, collaboration, commercialization of technology and attract companies. Currently, monthly networking sessions are held at UCD with about 120 people attending. There are also quarterly research and technology presentations. Seed Central also fosters and facilitates collaborations and technology transfers via 3 tiers—educational networking, research consortia and sponsor research. The goal is to attract more innovative companies and talented individuals and more plant science students. Seed Central is currently trying to build a plant and seed collaborative research lab at UCD. This facility would for industry to work with faculty and students. The building funding would come from industry.

Update on Agricultural Biotechnology (M. Newell-McGloughlin, UC-Davis): Biotechnology is needed to help feed the global population by producing crops that use less water, pesticides, fertilizer. The world will need 70% more food in the future. Without yield increases, land use will double by 2050. Biotechnology has already save 108 million hectares from being converted to agricultural production.

The next wave in biotechnology is genome editing. What is changed can be detected but there is no trace of the change agent. How will regulatory authorities deal with these products?

Biotechnology has been more rapidly developed by less developed countries. Ninety percent (90%) of farmers in less developed countries are growing GM crops vs. the number of farmers in developed countries. There are 1 billion pounds less of pesticides used since biotech crops have been introduced. Biotech crops have also contributed to 93% less soil erosion and 90% reduction in mycotoxins. Climate change is moving crops development to drought tolerant varieties such as the drought tolerant maize. There are also many output traited crops being developed such as potatoes (Simplot), apples (Okanagan) and oils (Monsanto soybean).

The challenges for biotechnology include technical challenges, intellectual property, liability (coexistence), asynchrony in regulatory approvals and acceptance (countering fear and misinformation).

New Member: 20/20 Seed Labs (K. Zaychuk, 20/20 Seed Labs): 20/20 Seed Labs opened a new facility in April. The company was established in 1989 and is independently owned and operated. 20/20 Seed Labs provides services to farmers, seed growers, seed production and crop protection companies. It is



accredited through Canada CFIA, ISTA and Canadian Seed Institute. 20/20 Seed Labs is also ISO 9001 and AQIS (Australian Quarantine and Inspection Service) compliant and authorized.

Seed quality is based on germination, purity and vigor. Canola is the biggest part of the business for 20/20 Seed Labs followed by wheat and barley. For seed health, the lab uses molecular analysis for pathogens, triffid flax and varietal identification. The lab also does research and training and crop inspections.

The company has locations in Edmonton, Lethbridge and Winnipeg and contra-season lab in Chile. In Kuwait, the company collaborates with the Ministry of Agriculture on a seed system and lab working to reclaim the desert for production.

20/20 Seed Labs has a collaboration with ScanBi Diagnostics of Sweden which is focused on molecular diagnostics. They work with companies throughout the seed chain. The Lynx diagnostics partnership was established in 2013. In this partnership, proficiency testing is being worked on as well as low level presence and GM detection in canola and detection of triffid flax and plant pathogens.