W2168: Environmental and Genetic Determinants of Seed Quality and Performance

Annual meeting St. Augustine Beach

Participants:

Mark Bennet, Ohio State University
Kent Bradford, University of California, Davis
Dan Cantliffe, University of Florida
Bruce Downie, University of Kentucky
Bob Geneve, University of Kentucky
Xingyou Gu, South Dakota State University
Allen Knapp, Iowa State University
Andy LaVigne, President and CEO, American Seed Trade Association
Daniel Leskovar
Miller MacDonald, Ohio State University
Mitch McGrath, Michigan State University
Hiro Nonogaki, Oregon State University
Jeff Norcini, University of Florida
Hector Perez, University of Florida
Dzingai Rukuni, University of Florida
Alan Taylor, Cornell University
Greg Welbaum, Virginia Tech University

Meeting opened by Chair: Hiro Nonogaki Oregon State University.

Introduced Andy LaVigne: President and CEO of the American Seed Trade Association.

Hiro Nonogaki asked for approval of the 2007 W-1168 Annual Meeting Minutes

Kent Bradford: moved and Miller McDonald seconded. Minutes passed unanimously.

Bradford and Welbaum will be on the committee for determining executive officers.

The site selection committee will be Alan Taylor and Bob Geneve.

Call for proposals of discussions for further meetings like the translational seed biology meeting.

Bradford: mentioned that the fall out from the translational seed biology meeting. It has been suggested that we write a meeting report. The journal Plant Science will be the venue. How will this go down? Will we be trying to get the speakers to write their presentations or will we write the sections? Kent Bradford will contact the speakers with regard to providing their talks in paper format and see what their interest level is. Any ideas, concerns should be forwarded to Kent Bradford.

Nonogaki: asked whether we should start planning another meeting like the translational seed biology meeting. Perhaps toward the end of this project.Upcoming meeting International Society for Horticulture Science co organized by ISSS.

The W-2168 group may be included in organizing this meeting or a special session. Is there anything that we could propose to the organizing committee that we could do to assist in the meeting? Any objections for our group to contact the ISHS organizers? There were none. We will think of something specific later. Any other business?

Welbaum: What is going to happen with the seed biology list server? Alan Taylor said that Ralph Obendorf is on phased retirement and will probably be deciding how he will be relinquishing control of that item.

Nonogaki: If there is no other business then we will go to the talks.

Cantliffe: gave a brief overview of the time line for the meeting.
A little bit about Florida Agriculture. There are two horticulture departments. Horticulture Science Department and Environmental Horticulture. Financial crunch has hurt them badly. Live off of sales taxes. Dan Cantliffe ended by saying that the seed biotech industry is the one that is hiring the graduate students of our programs. So, how can we build up our graduate student production.

Nonogaki: introduced Andy LaVigne: President and CEO of the American Seed Trade Association. He pointed out that seed research has been suffering from underfunding and Andy LaVigne will address this.

LaVigne: US Seed Biology Meeting Report: Vulnerabilities of global agriculture. Soaring commodity prices. Record low levels of carryover stock, etc. Quality seed was identified as a key.

Cantliffe: How many people does industry hire? For example, a large seed company is looking for 300 plant scientists in the next year. The Universities have to get the information to the companies that we are here and available to educate your next hires. We need the money to pay them through a graduate degree program. We “the universities that are present” have to get the message back to the industry that we are available and willing to educate the next generation of hires. Another aspect is where are these graduate students going to come from? It would be great to have a home grown pool to chose from.

LaVigne: You have to avoid the idea of ‘how much money can you give me?”

Cantliffe: we have to avoid the competitive idea of I’m not paying into some general research fund that will provide information that will be freely available to everyone.

LaVigne: I think they know that if they don’t support the research, there will be nothing available, programmatically, to go back to in a couple of years.

Cantliffe: I would like to see an endowment made for graduate education.

Bradford: ASRF a $100,000 endowment for graduate education. You need to look at the Dutch model where industry, government goes half and half and then competes for the funds. The Dutch seed industry is doing very well because of it. This has to be seen as a collaboration.

LaVigne: There are some ideas in the loop that have an endowment that would be for graduate research training. They are also involved in outreach to K-12 programs to interest kids early in Agriculture as a career goal. Kits made available to plant seed and grow the plant though to maturity. A form to get the industry, government and universities together to talk about direction.

Cantliffe: What happened in September was great, they came to do that and only that and they came! They were able to identify things and identified the educational component i.e. what do we need and how do we get them? This should be ongoing. They get both the research done that they need and then they get the person educated in the discipline they want.

LaVigne: the demand today has dramatically changed the career path for many graduate students. Academia is no longer as viable an option. Even if we can only fund 10, it is 10 more than we did have. World Bank, Gates foundation, etc. are being talked to get them to realize that if they are serious about solving the problems in Africa then we need to be involved in educating them.

Knapp: Pleased to hear you continue to talk about physiology and not just plant breeding and molecular genetics. Collaborations between physiologists, breeders and geneticists will become more important as we continue.

LaVigne: In the land grant universities, a lot of funding has been drained to computer science/engineering.

Welbaum: Perhaps we can send Andy the annual report?

Nonogaki: Andy, how can the W2168 assist you with taking the next step in sending this further?

LaVigne: We are taking the white paper and trying to develop this further. We will distribute this further and ask for feedback.

Taylor: You talked about moving ahead but do you have any idea of what is next?

LaVigne: We hope to have that and some additional projections in February.

Perez: Wondering how some of the more regionalized seed production industries fit into the ASTAs agenda.

LaVigne: We have members that are involved in reclamation, wildflowers, etc. also biofuels so we feel we have members in all these areas so you are represented.

Norcini: Did they have a table specifically for the reclamation/wildflower industry.

LaVigne: Not specifically but they are not out of the conversation. There were one or two people that are extremely involved.

Norcini: Native seed quality group is trying to have a survey of important points.

Nonogaki: It is important at this stage to ignore specialty groups in order to focus on education so we can get the program started. Later we can try and expand it.

Geneve: It would be good to inform the National Needs USDA program that we are an important group to fund.

Perez: The NSF also has a program that seems to work in the same way.

Nonogaki: Called us back from the break to introduce Miller and his vision for the future of seed biology.

McDonald: Presentation - Seed biology at OSU 1976-2008. Future: Issues: Complexity of science means that no one institution has the strength to do everything. Global industry: Need for trained personnel. Need for basic research but it might be necessary to give up the applied. Feels that we must develop a web site with an active webmaster. Addresses visibility. Emphasizes research activities and accomplishments. This means commitment by the group to the group. We must commit to cooperative research. Utilize one meeting for identifying cooperative research activities. Commit to a minimum 25% effort in cooperative research supported through W2168. Routinely communicate using

distance learning technology. Link all W2168 members. Consider expanding horizon internationally.

Bradford: We did the weekend meetings because you could get really cheap rates.

Bradford: Miller, the 2168 as a global organization how would that work since it is a USDA organization. Would it be a group that would be responsible for liasing with some international groups?

McDonald: Perhaps that is something to bring up with the USDA 'handlers'. We can use technology to be all inclusive internationally. You participated, what did you think?

Bradford : I felt the interaction part of things was missing.

McDonald: Perhaps you were talking over their heads and the fact that there is always a language barrier issue and they are reluctant to speak.

Bennet: This is true, the language can be trouble.

Welbaum: Sometimes, but it is the way of the future whether in the state or globally.

McDonald: In the past we gave a classroom lecture to students that was Ohio based and that was not wise given that they will be hired throughout the nation.

Break for lunch.

Reconvene: 1:05.

STATE REPORTS:

Iowa (Knapp)

Susana Goggi: continuing research on frost and corn seed germination and vigor.
How early can you detect injury?
Variance between freezing damage between a single kernel and the kernels on the ear.
Al Knapp: Investigations of QTLs for seed vigor in Maize. Substantial differences in field emergence among IBM 302 population. Using the saturated cold test to determine vigor. B73XMo17 as well as 223 out of the 302 population.

California (Bradford).

Compositae Genome project. Refunded. Thermodormancy in lettuce. L. serriola very tolerant to high temperature for the completion of germination. Jason Agiris zeroed in on a QLT that explained 63% of the variance! = *Htg6.1* The best compression was a chromosome arm. Went to candidate gene approach and decided to examine *LsNCED4*. ABA biosynthesis. There is also a *LsGA3OX1* and ethylene *LsACS1*. Expression analysis showed that these genes were expressed in a manner among the parents that could explain the phenotype. Towards the end of his thesis, Jason mapped the *LsNCED4* collocalizes with *Htg6.1*. Also, in the microarray for Lettuce devised by Van Deynze and Michelmore really dense maps and *LsNCED4* still maps right in the middle of Jason's QTL. Second project. Also lettuce.
Seed Priming can alleviate thermodormancy. Can we find QTLs associated with priming? Yes, *LsNCED4*. Oxygen sensor using this to ascertain live/dead/high quality seeds. Sealing membranes is the big bug-bear in this system. Peggy Lemaux and Bob Buchanan -

Wheat proteomics. Gluten protein fraction. Characterize amyloplast proteome. Branch point sin CHO and AA biosynthesis. Thioredoxin effects on flour properties. Seed Center: Michael Campbell Executive Director of the Seed Biotechnology Center. Microarry Marker discovery project in lettuce and pepper. Allen Van Deynze has done a lot of out reach.

Michigan (McGrath)

MAP Kinase involvement in seedling vigor and growth
Oxylate Oxidase was up-regulated in good emergers versus poor emergers in Sugar beet. This seemed to suggest that hydrogen peroxide is acting as a signaling molecule in the good emergers to induce lipid utilization for carbon and energy to support the completion of germination and emergence. How could the signal be transduced? Usually through a MAP Kinase. Work in a combinatorial manner to elicit infinite responses using a finite number of genes. 60 MAP-KKK, 10 MAP-KK, 20 MAP-Ks. Somewhat more complexity than one could wish. Tried to narrow this down using Arabidopsis information to those involved in the hydrogen peroxide response. Found homologues in Sugar Beet.

Florida (Perez, Rukuni)

Germination ecology and development physiology. Working with Native wildflowers. If growers have trouble with have a species complete germination he is called in to solve it. Starting with Jeff a wild flower meadow. What would it look like in Florida with truly native species. Also using part of the area for a production area, and using some of the seed for his program. Also with Jeff working with a restoration species. Physiological dormancy associated with the seeds.

Dormancy in prevarietals of coreopsis. *Coreopsis floridana* or *lanceolata. C. floridana* is endemic to Florida. Did a lot of seed structural work on the achene. Testa surrounded by a pericarp. Both light and SE Microscopy. Also a lot of germination experiments in light or dark and over a range of temperatures. Also examined the effect of removing the covers of the embryo on alleviating thermoinhibition. Examined endobetamannanase activity. There was a positive correlation between EBM activity and the completion of germination. EBM activity was associated positively with the completion of germination over a range of temperatures. EBM activity induced in the endosperm by the presence of the embryo. The above results were all for *C. lanceolata*. No EBM activity detected in *C. floridana*. Removal of the covers resulted in the same effect for *C. floridana* as with *C. lanceolata*. Again, removal of the endosperm resulted in faster completion of germination and definitely removed thermoinhibition. Also looked at the effect of darkness and priming on *C. floridana*.

Reconvene

Ohio (Bennett)

Seed development of lettuce about 2/3 of the way through physiological development was where true desiccation tolerance was induced. Water productivity i.e. seed mass dry matter

produced per volume of water used for plants was assessed. Powerful responses of seed quality to different ratios of Red/Far red ratios. J.C. Tang. Tandem Zinc Finger genes in seed development and environmental responses in *Arabidopsis thaliana*.
Ornamental germplasm Director: originally occupied by David Tay. There seems to be good will within the Department at the Ohio State U. to rehire Miller's position as a seed biologist position. The soybean breeder is being actively searched right now and the feeling is why not hire a person to complement this position focused on seed composition and resistance. Cluster hires. Distance learning class to go on International Seed Production. Spring 2009.

New York (Taylor)

Accelerated evaluation of perennial grass and legume feedstocks for biofuel production in New York State. Seed Science and Technology of Switchgrass as a biofuel. Cleaning seeds to enhance uniformity of glume removal essential for better coating. Coating provided by seed companies. Applied using a pan coater. Very uniform results. Ernst Seed Co. is their collaborator. 6 seed coatings and none, moist chilling or not, and tillage or no till examined for their effect on emergence and subsequent stand establishment. Definitely moist chill, definitely till and some coating combinations seem to help.

Texas (Lescovar)

Development and management practices for artichoke production in southwest Texas. Management, water relations, transplant stress. Trying to influence the number and length of root hairs by applying ethylene response and production inhibitors/stimulators. AVG = long roots from the seed (radicle) applied from 0-8 DAI while it eliminates root hair growth. Ethephon and possibly ACC appears to induce root surface area through dense and long root hairs. This treatment may assist transplants to survive transplant shock/drought/heat stress. Second project: Designing ABA methods to control growth of vegetable transplants.

Kentucky (Geneve):
The water gap in *Ipomoea lacunose* seeds.
Much discussion regarding how this mechanism of imbibition and dormancy alleviation actually works.

Sunday, January 17, 2009.

Dan gave a brief introduction to the area of St. Augustine Beach.

Continuing with the state reports.
South Dakota (Gu)

Identify seed dormancy genes from wild/weedy relatives of rice and wheat.

Preharvest sprouting is a problem in the area of around Brookings, SD. So, wheat has become a species of interest in Xingyou's program. There is considerable natural variation in seed dormancy in rice. Most weedy species have considerable dormancy while the cultivated varieties have little or none. Using a QTL approach to identify loci associated with dormancy. There were 7 QTLs established with a cross between a cultivated variety and SS18-2 from Thialand which is a weedy rice with extreme dormancy but crosses well with cultivated varieties. To date they have already cloned one gene qSD7-1, which assists in developing red color of the grain pericarp. At present they have also come close with 2 additional genes, 300 Kbp and 50 Kbp (this hunt is NSF supported). This 50 Kbp gene controls dormancy through the endosperm.
Dormancy genes from Synthetic hexaploid wheat. *Aegilopes tauschii* (2n=2x=14)
Ae. Tauchii X tetraploid wheat to the F1 then chromosome doubling to form a synthetic hexaploid wheat (SHW) Seed covering tissues also contributes to seed dormancy.

Virginia (Welbaum)

LED-illuminated one dimensional programmable thermogradient table.
Greg now makes to order LED-illuminated one dimensional programmable thermogradient tables. Polymer case does not warp or distort like wood. Well insulated lids, LEDs emit very little heat and do not affect the thermal gradient on the plate surface. The LEDs can provide sufficient light intensity to grow seedlings. Vegetable seed production class 2 credit course offered at VT or through the office of continuing education at VT. Class can be taken for one credit or two. Spring semester January through April. Web-based asynchronous course with at home project. Research Projects. Microbial profiling of seed microorganisms. Seed vigor of soybean genotypes differing in their phytate content. Propagation of filamentous algae for biofuel production.

Oregon (Nonogaki)

International Visiting Scholars -George Bassel (Canada); Cristina Martinez-Andujar (Spain); Arun Kumar (India); Wioletta Pluskota (Poland). Continuing work on miRNA aspects of control of germination in Arabidopsis. Carrot seed development slides developed for growers in Washington State. Optical section reconstruction of Arabidopsis radicles.

Break

Nonogaki: reconvened. How are we going to assist Andy in his mission?
Bradford: We have to get another meeting earlier than two years from now.
University of Illinois, Texas A&M, North Carolina State, University of Florida have all received money from a large seed company. There is no traction for Seed Biology.
Geneve: Why can we not get more recognition for seed biology?
Nonogaki: There is no organization or function promoting seed biology.
Cantliffe: But we are a small group with a small voice. Add everyone that you can get here and you might have 25 people. The seed industry thinks 'we need a

geneticist, or a breeder' they don't think 'we need a seed biologist'. The seed industry does not wish to provide money without strings attached. It is discouraging to try this with all these barriers in place.

Bradford: The Dutch have a major interest in seed biology due to the huge export portion of seeds in the Dutch economy.

Cantliffe: What about the Japanese companies?

Nonogaki: There are some that have US subsidiaries.

Perez: We are giving large seed companies a lot of power over the graduate student product.

Cantliffe and Bradford: We should leave the decision of which companies to contact to Andy.

Bradford: I heard Andy say that the ASTA have been lax in pushing the agenda.

Geneve: Floriculture extension guy has a consortium that companies put money into that help run his program as well as others.

Cantliffe: If it is a foundation then there is no problem setting up an account into which they can pay money. What we are up against is that seed companies first think about new germplasm. Improved seed health and performance might be the one to have Andy target and that we should meet over next.

Bradford: Some company has made a commitment of increasing yields by 2030 by 50% with fewer inputs and a lot of this increase will come from better seed quality/performance.

Cantliffe: Large seed companies have all focused on the big crops. Cotton, corn and soybeans. The question will be what can you do for us?

McDonald: What I heard from the meeting was what we want from you are students. We shouldn't be talking about crops, we should be talking about students trained broadly in seed biology.

Geneve.: We are lacking visability. No identity as yet, but put together a seed academy and now you've got something.

Leskovar.: We need identity,

Cantliffe: Yes, a web page and now you come up in Google.

Leskovar: We can also ask the government and the industry to get money through the W-2168. The Specialty Crop Block Core has funding from the State government in Texas.

Nonogaki: Really agree with Miller, focus on the students and the companies should line up.

Cantliffe: I think we can do this but you have to have a research focus as well. You are miniscule when you go up against plant breeding.

Nonogaki: My perception is that we do not have that power.

Cantliffe: No, but we need to talk to Andy and convince them that some funding from them leads to the ability to apply for government matching funds.

Nonogaki: Action item. Will a delegation from the group go to Washington to speak with Andy?

Nonogaki: I agree that we should form a committee of two-three people who will devise an action plan and do something aggressive to move them on our agenda.

Bradford: We need to do the plan first and then go to Washington. Both seed health and conservation and germplasm is another issue that must come from us. Approach

them with that. Dan Cantliffe is right if we don't push it this will die. We also need to be ready to jump on their revision to the white paper that will come out next month.

Nonogaki: Who will be on this committee? Kent Bradford, Greg Welbaum, Dan Cantliffe, Alan Taylor, Hiro Nonogaki. We need a web page, a name other than W-2168, and a mascot, preferably a seed.

Bradford: We need to be involved in every single program for plant breeding because we (seed biologists) are an integral part of plant breeding.

Nonogaki: Report on next administration for this group.

Taylor: Location, one possibility is East Lansing, Michigan State in June or on the West side of Washington State using people in the industry to organize it.

Nonogaki: who will be vice chair and secretary?

Welbaum: We need a vice chair and secretary. Report is that Hector Perez be the vice-chair and that Mark Bennett be the secretary.

Welbaum: Just make a vote.

Michigan is the spot. End of meeting.

Outputs:

Objective 1. Identify and characterize biophysical, biochemical, genetic, and environmental factors regulating or influencing seed development, germination, vigor and dormancy.

A series of genetic experiments reached the conclusion that the rice *qSD12* QTL controls seed dormancy through the embryo or endosperm tissues. About 20 lines were developed from selected recombinants for the about 20 centiMorgan-genomic region, which encompassed the rice *qSD1* QTL, to fine map the seed dormancy locus by progeny test (SD).

Four wheat populations of about 700 double haploid or recombinant inbred lines were evaluated for seed dormancy and resistance to pre-harvest sprouting. A substantial amount of genetic variation in seed dormancy and resistance to pre-harvest sprouting was discovered in four wheat segregating populations derived from synthetic hexaploid wheat accessions by two years of field experiments (SD).

Two QTL for germination heat sensitivity were mapped on the short arm of chromosome 3A (QGhs.osu-3A) and the long arm of chromosome 4A (QGhs.osu-4A) in Intrada x Cimarron RIL population of winter wheat. These two QTL were also mapped in Jagger x 2174 RIL population of winter wheat in similar genomic regions, but their genetic distance to common SSR markers showed not exactly the same as those found in Intrada x Cimarron population (OK).

The maize RILs (IBM 302) exhibit poor field emergence. Laboratory studies were conducted to find QTLs associated with seed vigor. A saturated cold test was performed on five replications of 223 of the 302 lines available in the IBM 302 RIL set. QTL

analyses were conducted with QTL cartographer, with marker data reported previously. QTL were considered significant at $p \leq 0.05$ as determined by running 1000 random permutations on ls-means with QTL cartographer. Based on these criteria, two putative QTLs were found on chromosome 7. A follow-up study has been conducted organized in the same fashion but with the saturated cold tests conducted at 8, 10, 12, 14, and 16 C. The initial study has identified two putative QTLs located on chromosome 7. The behavior of these and other QTLs across a range of germination temperatures is currently being analyzed. The outputs from these studies are QTL maps from germination under low temperatures. Analyses of QTLs across a range of biologically important germination temperatures will be conducted (IA).

Soybean seed with low raffinose, stachyose, and phytin are desired for feeding non-ruminant animals to improve feed efficiency, increase mineral uptake, and reduce flatulence, but may have reduced agronomic quality. Composition of soluble carbohydrates in seed parts of mature soybean seeds were determined for low raffinose and stachyose seeds (LRS), low raffinose, stachyose, and phytin seeds (LRSP1, LRSP2), and normal raffinose, stachyose, and phytin seeds (CHECK). Cotyledons and axes of seeds from the three modified lines had low raffinose, stachyose, and verbascose compared to the CHECK. Cotyledons from LRS seeds had significantly higher concentrations of galactinol and the di- and tri-α-galactoside derivatives of *myo*-inositol, D-pinitol, and D-*chiro*-inositol than cotyledons from LRSP1 and LRSP2 seeds. Seed coats of all four lines were similar in soluble carbohydrate composition indicating the modifications were expressed in embryo tissues. Research results provided information on the composition of low-raffinose, low-stachyose soybean seeds with normal phytin composition. These seeds also accumulated significantly higher concentrations of galactinol and the di- and tri-α-galactoside derivatives of *myo*-inositol, D-pinitol, and D-*chiro*-inositol (NY).

Previously a major quantitative trait loci (QTL) termed *Htg6.1* was identified that confers the ability for lettuce (*Lactuca sativa* L.) seeds to germinate at high temperatures (up to 37°C) in an accession of *L. serriola* (UC96US23) in a recombinant inbred line population derived from a cross with *L. sativa* cv. Salinas. Multiple near-isogenic lines (NILs) were developed in the Salinas background carrying an introgression containing the QTL locus. Multiple NIL families were utilized to demonstrate that this genomic region conferred increased upper temperature limits for germination. Additional fine mapping of the QTL locus using single feature polymorphism (SFP) markers developed using a lettuce microarray identified a gene encoding an enzyme in the abscisic acid (ABA) biosynthetic pathway (*LsNCED4*) that localized to the center of the QTL interval. Expression analyses were conducted on over 80 genes in the abscisic acid (ABA), gibberellin (GA), ethylene and light regulation pathways in relation to thermoinhibition in different genotypes. Seeds of genotypes exhibiting thermoinhibition had higher expression of *LsNCED4* when imbibed at high temperatures, and their ABA contents were elevated. In addition, expression of genes in the GA (*LsGA3ox1*) and ethylene (*LsACS1*) biosynthetic pathways was repressed by high temperature. Molecular markers associated with different alleles of *LsNCED4* will allow marker-assisted introgression of this gene/QTL into cultivated lettuce lines. Crosses have been made to the NILs described

above to initiate this process. Gene expression studies have clarified some of the regulatory interactions among ABA, GA and ethylene in regulating seed responses to light and temperature (CA).

Modifying the maternal plant environment has significant effects on lettuce seed quality. By controlling specific environmental conditions during seed production of the commercial lettuce cultivar 'Tango' it was possible to modify seed size, germinability, thermoinhibition, photodormancy and storability. Changes in germinability include increasing germination percentage and rate under a wider range of conditions, which may be interpreted as an increase in seed vigor. Storability is considered another component of seed vigor. In some cases (e.g. seed production at higher temperatures) both aspects of seed vigor were improved at the same time. However, alterations in the maternal light environment caused opposite responses in seed germinability and storability (they were inversely related), so in this case the effects over seed vigor were dependent on the vigor test selected. During 'Tango' seed development, a peak of ABA concentration was observed at approximately 65% of the time to physiological maturity. This ABA peak seems to be involved with the onset of desiccation tolerance and accumulation of reserves in the embryo. We hypothesized that changes in the magnitude of this ABA peak could be part of the mechanism by which higher temperatures during seed development increase germinability. Testing this hypothesis would contribute to a better understanding of how maternal environment affects seed dormancy and germinability. Restricted water availability during seed production had little effect on many aspects of lettuce seed quality, although a significant increase in seed weight and the production of fewer seeds per plant were observed. Of particular interest for seed producers is the significant gain in water productivity (seed yield per volume of water consumed) attained with restricted irrigation, especially because of the arid regions and conditions in which most lettuce seeds are produced. Producing 'Tango' lettuce seeds under environments enriched in red light had a significant effect on reducing thermoinhibition and photodormancy. This is a novel and promising approach to the production of lettuce seeds with improved germinability. However, there are some aspects that should be further investigated, such as i) undesired reductions in seed storability and possible effects on seed yield, ii) unknown effects on other lettuce cultivars of commercial importance, and iii) the feasibility of modifying maternal light environment at commercial scale. Similarly, the possibility of producing seeds with improved storability by reducing the R:FR ratio of the maternal environment may be of interest for germplasm centers and seed companies (e.g. to facilitate stock management of genotypes or species without germinability problems), and should be further studied. In these studies, one of the first questions to be addressed is if the effects of light quality on storability are observed in other lettuce genotypes and, more importantly, in other species. Additionally, finding a significant correlation between light spectrum quality of the maternal environment and storability in a wide range of species would be important for understanding the seed bank dynamics of native and weed plants. Basically, it would mean that seeds produced under a canopy shadow (i.e. lower R:FR ratio) have higher storability and are better prepared to remain viable for longer periods of time (OH).

Early events in sugar beet germination were investigated using quantitative PCR. Genes

examined included those involved in signal transduction such as MAP Kinases, hormone biosynthesis and response genes, and structural proteins. One hundred ninety two gene products were tested for expression at 0, 24, 72, and 96 hours post-imbibition. Of these, 12 showed no detectable expression, 114 were expressed constitutively, and 66 showed differential expression at one or more time points. Differentially expressed genes generally showed expected patterns of expression, however results are preliminary (MI).

Seed germination as well as longevity in dry storage are attributes affected by the testa (seed coat). The *brownseed*^{1} (*bs*^{1}) gene in tomato is unknown. Recombinants between *Solanum esculentum* and *S. pimpinellifolium* were subjected to PCR with a variety of primers generating CAPS, dCAPS, and SSLP markers to continue fine mapping *bs*^{1} (KY).

Germination of orchid seeds is more successful when seeds are harvested from immature pods. Protocols for decontaminating orchid seeds with calcium hypochlorite for propagation through tissue culture have been optimized. Orchid seeds can survive freezing in liquid nitrogen and therefore can be stored in genebanks cryogenically. In collaboration with Dr. Zhiwu Li, orchid was genetically transformed with a hairy root expression cassette and this work is continuing to genetically transform orchids for physiological studies. The orchid caraspace is a thin barrier to macromolecule diffusion and it may control orchid seed germination (VA).

To identify DNA sites to which AGL15 binds *in vivo*, a chromatin immunoprecipitation (ChIP) approach was used to immunoprecipitate AGL15 and associated DNA fragments. In a ChIP-on-chip approach, the DNA recovered from immunoprecipitation using AGL15-specific antiserum or preimmune serum was converted to probe to hybridize to the Affymetrix GeneChip® Arabidopsis Tiling 1.0R Array. This allowed nearly global mapping of *in vivo* binding sites for AGL15 that numbered ~2000. Affymetrix ATH1 arrays were used to investigate gene expression changes in response to accumulation of AGL15/18. We are currently analyzing results to identify genes that may be directly regulated by AGL15 and those which may be farther downstream in the regulatory network. *In vivo* association of AGL15 and regulation by AGL15 for select targets have been verified. A number of genes relevant for embryogenesis are direct targets of AGL15. The effect of select downstream targets on embryogenesis are being tested. The effects of gain- and loss-of-function of AGL15 on somatic embryogenesis were investigated. Ectopic expression of a *Glycine max* ortholog of *AGL15* enhanced recovery of transformants by somatic embryogenesis in soybean. Because AGL15 directly represses some genes but directly induces expression of other genes, co-factors of AGL15 were also identified. Components of histone deacetylase complexes were found to interact with AGL15 and this could explain how AGL15 represses gene expression (KY).

The roles of three unique Tandem Zinc Finger (TZF) genes in seed development and environmental responses in *Arabidopsis thaliana* were studied. Several sugar responsive TZF genes were identified using microarray analyses. Sequence analysis has revealed a family of 11 genes in the *Arabidiosis* genome that contains this unique TZF. Among them, 3 genes are specifically expressed in seeds according to results of *in silico* analyses. Based on preliminary results collected so far, they hypothesize that these 3 seed specific

TZF genes are important for seed development and environmental responses. It is hypothesized that these genes are involved in mRNA turnover because their TZF motifs are homologous to human Tristetraproline (hTTP) that binds to AU rich element (ARE) in 3'UTR of short-lived genes. It is interesting to find out the roles of these genes because the molecular mechanisms underlying ARE-mediated mRNA decay are unknown in plants (OH).

All proteins containing Asparagine (Asn) or Aspartic acid (Asp) can potentially convert at these residues to isoaspartate (isoAsp), an un-coded amino acid that introduces an extra carbon into the peptide backbone, usually with deleterious results for protein function. These damaged proteins can either be degraded and re-synthesized, an energetically costly affair, or repaired by PROTEIN ISOASPARTYL METHYLTRANSFERASE (PIMT) at the cost of a few SAM molecules. To what extent is the orthodox seed proteome damaged in this manner and what are the consequences for the seed should this damage be left unrepaired? A second PIMT was discovered in Arabidopsis, the first eukaryote to possess two genes for this enzyme. The second PIMT transcriptional control was characterized and found to be complex involving both different transcriptional initiation sites and differential 5'- and 3'-splice site selection of the first intron. The various transcripts thus derived encoded proteins capable of entry into every sub-cellular domain except the vacuole. Storage proteins were found to be major isoAsp bearing proteins in seeds of Arabidopsis. Phage-display and biopanning with recombinant r*At*PIMT1 as well as 2D-gel electrophoresis followed by on-blot-methylation, spot coring and MALDI-TOF-TOF were used to acquire and identify PIMT targets (KY).

Arabidopsis seed mutants completing germination were characterized. Yeast-two hybrid, western blot, single- and double-mutant analysis have determined that the F-BOX protein COLD TEMPERATURE GERMINATING10 (CTG10) results in the destruction of PHYTOCHROME INTERACTING FACTOR1 (PIF1) (KY).

DELLA proteins are negative regulators of GA responses including seed germination, stem elongation, and fertility. GA can stimulate GA responses by causing proteolysis of DELLA repressors by the ubiquitin-proteasome pathway. This destruction requires GA biosynthesis, three functionally redundant GA receptors *GIBBERELLIN INSENSITIVE DWARF1* (*GID1a*, *GID1b* and *GID1c*), and the *SLEEPY1* (*SLY1*) F-box subunit of an SCF E3 ubiquitin ligase. Using *sly1* mutants in which DELLA proteins remain stable after GA application, we found that GA regulates DELLA repressor activity by a mechanism distinct from protein destruction. Overexpression of *GID1* genes rescued the dwarf and infertility phenotypes of the *sly1* mutants without altering accumulation of DELLA proteins RGA and GAI. This rescue required GA biosynthesis and the presence of a functional DELLA motif in RGA and GAI. Both the DELLA motif and GA are required for the protein interaction of DELLA protein and GID1. The *sly1* mutants display a less severe dwarf phenotype than the GA biosynthesis mutant *ga1-3* or the *gid1a gid1b gid1c* triple mutant despite the fact that *sly1* mutants accumulate far higher levels of DELLA protein. Based on double mutant analysis, it appears that both intermediate phenotype and high level DELLA accumulation in *sly1* mutants require GA and a functional DELLA motif. These results suggest that GA-bound GID1 can block DELLA repressor activity by

direct protein-protein interaction with the DELLA domain, and that this interaction may lead to increased DELLA accumulation (WA).

To identify and characterize key transcription factors potentially associated with seed germination and stand establishment, miRNA regulated genes were characterized. Silent mutations were created in the miRNA complementary sites of *AUXIN RESPONSE FACTOR10* (*ARF10*) and *SQUAMOSA PROMOTER BINDING-LIKE13* (*SPL13*). Transgenic plants expressing miRNA-resistant *ARF10* or *SPL13* have been isolated and being characterized. Results suggest that miRNAs play critical roles in many different stages of plant development including seed germination and seedling growth. miR160-reistant *ARF10* (m*ARF10*) seeds and seedlings exhibited hypersensitivity to ABA, suggesting potential crosstalk between auxin and ABA. miR156/157-resistant *SPL13* (*mSPL1*3) seedlings exhibit temporary arrest during stand establishment (OR).

Objective 2. Determine and model the biotic and abiotic factors affecting seed germination, seedling emergence, and establishment of sustainable populations in natural and agro-ecological systems.

Sensitivity of mature soybean seeds to imbibitional chilling were determined for low raffinose and stachyose seeds (LRS), low raffinose, stachyose, and phytin seeds (LRSP1, LRSP2), and normal raffinose, stachyose, and phytin seeds (CHECK). LRS seeds had significantly higher concentrations of galactinol and the di- and tri-α-galactoside derivatives of *myo*-inositol, D-pinitol, and D-*chiro*-inositol than LRSP1 and LRSP2 seeds. Mature seeds of LRS and CHECK were tolerant to imbibitional chilling, but LRSP1 and LRSP2 seeds were sensitive to imbibitional chilling as noted by a large reduction in seedling growth after imbibition of 6% moisture seeds at 5°C, consistent with reduced field emergence as observed by other researchers. The higher accumulation of cyclitol α-galactosides in embryos of LRS seeds may have contributed to their tolerance to imbibitional chilling. Research results provided information on the sensitivity of low-raffinose, low-stachyose, low-phytin soybean seeds to imbibitional chilling injury (NY).

A population-based threshold model was applied to quantify the responses of barley seed germination to oxygen availability, ABA and GA. The model accounted well for germination responses to reduced oxygen partial pressures and provided quantitative measures of oxygen and hormonal sensitivity associated with dormancy states. The ability to quantify the sensitivity of seed germination to combinations of oxygen, ABA and GA revealed that oxygen availability and hormones interact to regulate germination, particularly in intact cereal grains (CA).

Pritchardia remota (Arecaceae) is an endangered palm endemic to Hawaii. Management plans call for *ex situ* conservation of this species. However, the ability to store seed using conventional methods has not been investigated. Research on palm seed conservation was performed. The accumulation of reserves until shedding in *P. remota* pericarps and seeds is suggestive of desiccation sensitivity. However, the large mass of *P. remota* pericarps and seeds obscures changes in embryo reserve accumulation. When reserve accumulation

is measured separately for seed tissues, it was apparent that embryos achieve maximum dry mass about half way through the developmental program. Water relations of *P. remota* embryos early in the developmental program are similar to patterns observed for various desiccation-sensitive and -tolerant species. Unlike desiccation sensitive species, however, embryos of *P. remota* undergo a maturation drying phase later in development. A concomitant large decrease in water potential indicates that embryos surpass the lower water potential threshold reported for many recalcitrant species, but do not approach limits for orthodox species. Instead, the water potential measurements reported here resemble those obtained for tissues considered to possess intermediate storage physiology. Germination ability of excised *P. remota* embryos is gained prior to acquisition of desiccation tolerance. Germination capacity in *P. remota* embryos also precedes the ability to tolerate 'flash' drying to relatively low water contents. *Pritchardia remota* embryos increase in desiccation tolerance and are not considered recalcitrant because the critical water content drops below -15 MPa (*i.e.* approximately -60 MPa at shedding). Furthermore, metabolic activity shuts down as development progresses. Toxic by-products that may be produced, despite remarkably low levels of respiration, could be controlled by high antioxidant activity throughout development. Nevertheless, embryos seem sensitive to stresses imposed by high levels of drying and do not exhibit extreme desiccation tolerance required for conservation in genebanks. Therefore, until the effects of enforced desiccation on potential embryo dormancy can be elucidated further the feasibility of storage in conventional genebanks remains questionable. It is concluded that conclude that *P. remota* embryos posses a storage physiology intermediate to the recalcitrant and orthodox types (FL).

Existing recovery plans for *P. remota* also call for the development of germination protocols. It is concluded that for *in situ* management, incorporation of *P. remota* fruits into the soil is recommended as a treatment to improve germination. Studies indicate that *P. remota* seeds possess non-deep, simple morpho-physiological dormancy. Therefore, e*x situ* germination of *P. remota* seeds can be promoted by removal of the pericarp and/or the operculum followed by incubation at high constant temperatures (25° to 35°C) and consistent moisture (*i.e.* warm stratification). Seeds germinated at 35°C should be removed soon after germination (FL).

Summer farewell (*Dalea pinnata*) is a legume found in upland ecosystems throughout the southeastern United States and often used in seed mixes for restoration purposes. It also represents an opportunity for the emerging native seed industry in this region. Yet, germination characteristics for summer farewell are not reported. Several key outcomes from studies described below are evident. First, restoration practitioners should expect low initial germination for summer farewell after seed shedding or sowing of non-treated seeds. Second, low germination does not necessarily equate to failure since seeds of summer farewell have the ability to form a soil seed bank. Third, the majority of the seed population possesses physical dormancy but this dormancy can be readily broken through scarification. Fourth, the seed or seedling producer may increase germination by scarifying seeds. Finally, further studies dealing with seed development, polymorphism, and the role of fire on dormancy break are required to determine how these factors influence germination and recruitment of summer farewell (FL).

Paspalum notatum (bahiagrass) competition was the main factor limiting establishment (via seeds) of Florida ecotypes of *Coreopsis lanceolata*, *C. leavenworthii*, *Gaillardia pulchella*, and *Ipomopsis rubra* under simulated roadside conditions. Bahiagrass did not limit germination and emergence of wildflowers was not limited by *P. notatum*, but *P. notatum* limited subsequent wildflower growth and flowering. Seeding rates were explicitly tested for *C. lanceolata* – the optimal seeding rate was 600 viable seeds/m^2 (7 lb PLS/A). Seeding at 100 seeds/m^2 (1.2 lb PLS/A) resulted in poor establishment but seeding at 1100 seeds/m^2 (13 lb PLS/A) only provided limited additional benefit. Short-term sustainability of wildflower plantings was not affected by mowing 2-6 times/year when timed to avoid flowering and seed set. Frequent mowing (12-24 times/year), however, that did not account for flowering and seed set reduced fitness and growth of *C. lanceolata* and *C. leavenworthii*. Based on a preliminary cost analysis, and considering safety, erosion, aesthetics, and management practices, establishing roadside populations of Florida ecotypes of native wildflowers by seed is most appropriate for sites that are mowed by small to medium-sized mowers or string trimmer. Excising the endosperm of *Coreopsis floridana* and *C. lanceolata* seeds allowed germination at supraoptimal temperatures, while in *C. floridana* it alleviated dormancy of seeds imbibed in the dark. In 4-week-old *C. floridana* seeds imbibed in the dark, GA overcame dormancy while cold stratification (5^{o}C) partially overcame dormancy; potassium nitrate was ineffective. Four-week-old *C. lanceolata* seeds required 150 days of dry after-ripening to overcome dormancy; endosperm enforced dormancy for 90 days. Dormancy was not alleviated by GA or potassium nitrate. Naked embryos of both species were nondormant. Endo-β-mannanase (EBM) activity was detected in *Coreopsis lanceolata* at 90 hours of imbibition, with an association between EBM activity and endosperm rupture. Germination and EBM activity were inhibited by ABA, tetcyclacis, and supraoptimal temperatures. No EBM activity was detected at any time during germination of *C. floridana* (FL).

Seed ecology studies were conducted on seeds collected from Hawaii and Taiwan and in collaborations with seed scientists in Australia, China, India, and Sweden. The Subfamily Convolvuloideae of Convolvulaceae has many species water-impermeable seeds (physical dormancy), but also some with nondormant seeds and few with physical as well as physiological dormancy. Seasonal cycles has been documented in terms of the ability of seeds with physical dormancy to respond to dormancy-breaking factors in the environment, determined/modeled the mechanism of opening of the water gap. The phylogenetic relationships of seed dormancy in the whole subfamily were elucidated (KY).

Eastern gamagrass (*Tripsacum dactyloides*) is a native, warm-season perennial grass that can be used for forage, biofuel and conservation plantings. The combination of dormancy and low germination limits the widespread adoption of gamagrass. Germination in untreated seeds averaged 12% across 13 commercial seed lots from six cultivars. Viability estimated by tetrazolium analysis was 56% with a range between 14 and 90%. Stratification between 2 and 8 weeks at 5 or 10^{o}C as well as H_2O_2 application enhanced germination and reduced dormancy compared to untreated seeds. However, total

germination following dormancy release was still relatively low (~45%) due to poor seed lot viability. Germination temperature had a significant impact on germination percentage in both stratified and H_2O_2 treated seeds. Alternating temperatures were generally more effective in promoting germination and minimizing dormant seed than constant temperatures (KY).

The principles of PCR and Density Gradient Gel Electrophoresis have been applied to studying microbial seed ecology. This technique along with 16S gene sequencing will allow use to genetically profile microbial populations residing on seeds (VA).

Objective 3. Develop, evaluate, and transfer technologies to assess and improve seed and seedling quality, health, performance, utilization, and preservation.

Many seed crops have been identified as suitable for biofuel production producing a need for high quality seed lots that will produce optimum plant stands with the potential for maximum biomass production. Seed dormancy, suboptimal soil temperatures, and pathogens often lower seed quality and can negatively impact stand establishment. Seed testing methods provide an overall assessment of the quality of a seed lot; however, variants in the germination test method can have a major influence over final germination rates and percentages. The germination results are greatly influenced by temperature conditions and mold growth can also confound germination test results. The objective of this preliminary work was to study the seed biology and to apply seed technology to enhance the performance and achieve expected germination of switchgrass (*Panicum virgatum*) seed lots. The initial task was to investigate discrepancies between the labeled germination percentages and the germination results at specific temperature regimes to better predict field emergence and better quantify temperature dependence. Seed coating methods were sought to apply more uniform and adherent seed treatments. Seed lots of the varieties 'Cave-in-Rock and 'Shawnee' were provided by Ernst Seed Co. (Meadville, PA). Three seed lots were assessed for the effects of 14 day, 5°C stratification, followed by a diurnally alternating 15-30°C regime on final germination percentages. A high quality lot was evaluated over a wide range of constant temperatures to investigate the effect on germination. A seed coating method was developed to apply fungicide seed treatments that would lower mold contamination during germination testing. Germination at a constant temperature revealed seed lots with high, medium and low dormancy levels. Dormancy was broken with 14-day stratification at 5°C. The optimum constant temperature was 35˚C and there was a decline in both the rate of germination and final germination as temperature decreased or increased. Zero germination was recorded after 30 days at 10˚C and at 45˚C, indicating that the maximum temperature is approximately 40˚C. A seed coating method was developed including brushing seeds to remove glumes, and then application of a binder and filler to obtain uniform application. Seed treatments Captan and Thiram were found to reduce mold growth. Knowledge of the seed biology of species used for biofuel production is needed to understand factors or conditions limiting germination. Specific technology methods can be adapted or developed to optimize seed quality and stand establishment (NY).

Because of its wide geographic distribution and high yield potential, switchgrass is considered a leading candidate as bioenergy crop. Poor stand establishment is a frequently encountered problem in growing switchgrass. This is mainly associated with high degree of dormancy of switchgrass seeds. Global gene expression analysis using rice long-oligo arrays were conducted to identify genes that showed differential expression in dry and germinating switchgrass seeds (TN).

Sweet corn *(Zea mays* L.) seed has naturally low physiological quality compared to field corn. The use of priming treatments has been recommended to decrease the time between sowing and seedling emergence. In sweet corn seeds, hydropriming and osmopriming methods increased the uniformity of seedling emergence and reduced the range of days for germination. Priming evaluation is normally conducted by germination assays; however for vigor analysis of seed lots, more precise, quick and efficient tests are desirable (OH)

Results obtained for sweet corn sh_2 'SWB 551' and 'Obsession' hybrids demonstrated that high quality sweet corn seed lots respond positively to priming; however, priming is not beneficial for seed lots with medium physiological quality. The best treatment using the drum priming system was 36 h priming and for this duration, SVIS (vigor index ratio of 70 growth + 30 uniformity) produced similar results to seedling emergence assessments documenting this as a rapid seed vigor test that can identify the efficacy of priming treatments (OH).

Proteomic analysis of the wheat grain has advanced our understanding of endosperm and amyloplast proteins. The gluten proteins, a complex collection of highly repetitive storage proteins, are of major interest since they determine the characteristics of extensibility, elasticity and gas-holding capacity that are unique to wheat flour doughs. Proteomic studies have identified and characterized gluten proteins and have been used to assign gluten genes to specific chromosomes and to distinguish different wheat varieties. Proteomic analyses of the less abundant non-gluten proteins revealed that they function in metabolism and defense as well as storage. Some of these proteins have been identified as foam-forming proteins that may play roles in stabilizing gas bubbles in dough and influencing the crumb structure of bread. Others, especially the defense proteins, are potential food allergens. Still other proteins are targeted by thioredoxin, a widely distributed disulfide protein that regulates a number of plant metabolic processes. Proteomic studies have demonstrated that, among other changes, reduction of water-insoluble storage proteins by thioredoxin alters their properties in such a way that they become soluble in aqueous solutions, thereby facilitating their mobilization during germination. Environmental conditions during grain fill alter both wheat yield and flour quality. Comparative proteomics of the gluten and non-gluten proteins has provided insight into the accumulation patterns of these proteins during grain development and the response of these proteomes to environmental conditions during grain fill. Proteomic studies of amyloplasts isolated from wheat endosperm revealed that this organelle is involved in a number of metabolic pathways in addition to starch biosynthesis. They also revealed a number of branches and control points in carbohydrate and amino acid biosynthesis. These branch points may regulate the balance between starch and protein and be important to understanding the trade off between grain yield and protein content.

Thus, proteomics has provided new information on the identification and regulation of major metabolic pathways functional in the endosperm and amyloplast, knowledge that is fundamental to the understanding of both the quality and productivity of the wheat grain (CA).

Seed proteins of sorghum are less digestible than those of other cereals and digestibility is exacerbated by wet cooking the meal or flour, which results in significant nutritional losses. To address this problem, the properties of two sorghum lines that have a common pedigree but differ in digestibility were analyzed. Consistent with results based on a ruminal fluid assay, the protein and starch of one line (KS48) was more thoroughly digested than that of the other (KS51) using *in vitro* assays based on pepsin and a-amylase. The indigestibility of KS51 relative to KS48 was shown to be due to (i) a greater abundance of disulfide-bonded proteins; (ii) presence in KS51 of non-waxy starch and the accompanying granule-bound starch synthase; and (iii) the differing nature of the protein matrix and its interaction with starch. The current findings suggest that each of these factors should be considered in efforts to enhance the nutritional value of sorghum grain (CA).

Seed priming (controlled hydration followed by drying) is used to overcome thermoinhibition in lettuce and to speed germination in a number of species. The same *L. sativa* x *L. serriola* RIL population as was used above to identify *Htg6.1* was screened for the ability of priming to increase the upper temperature limit for germination. A QTL was identified for priming response that collocated with *Htg6.1*. This locus has also been fine-mapped, and the response to priming also collocates with *LsNCED4*. Seeds of NILs carrying the UC96US23 allele at this locus have a greater response to priming than do seeds carrying the Salinas allele. Expression of *LsNCED4* is reduced in seeds imbibed at high temperatures following priming, while expression of *LsGA3ox1* and *LsACS1* is increased during and following priming. Thus, the same locus apparently is involved in determining the upper germination temperature limit for both untreated and primed seeds. Seed priming (prehydration and drying) is widely used in high-value seeds to improve seed germination uniformity and speed and to overcome dormancy, particularly in lettuce, where it increases the upper temperature limit for germination. The ability of priming to increase germination temperature was also found to be associated with *Htg6.1* and *LsNCED4*. This indicates that transfer of the trait to cultivars may have the added benefit of making them more responsive to priming treatments.
(CA).

Effects of aqueous slurry seed coatings were examined. Marketmore 26 cucumber (*Cucumis sativus L.*) seeds were treated with commercial preparations of *Trichoderma harzianum* Rifai strain KRL-AG2 G41 [*Th*], *T. virens* G-41 [*Tv*], or their combination [ThTv] at half rates each of the single application (1 mg/seed) as a control against damping-off in soilless media inoculated with *Pythium aphanidermatum* [*Pa*]. Protection against damping-off by high pressures of Pa (16% emergence in non-coated, non-primed seeds) was increased by *Th* on non-primed (76.4 % emergence) or on osmotically primed seeds, with coating either before or after priming having on effect on efficacy (average 62.6% emergence). In a second study with lower disease pressure (58% emergence from

non-coated, non-primed seeds), slurry coating of non-primed or osmotically primed seeds with *Th*, *Tv* or *ThTv* reduced damping-off and increased final emergence percentage. The combination coating eliminated damping-off only in non-primed seeds, and tended to reduce percentage damping-off in primed seeds compared to coating with *Th* or *Tv* alone. *Th*, *Tv* or *ThTv* applied to growth media at the same rate as the seed coating (1 mg/seed) were generally as effective as the seed coatings, and only the *ThTv* growth medium application eliminated damping-off. *Th*, *Tv* or ThTv remained viable on non-primed seeds for up to 4 weeks at 21 or 4C, but 21°C storage resulted in faster seed germination by week 3 and higher colony forming units per three seeds by week 4 (DE).

Field and laboratory bioassays were conducted in multiple States (Delaware, Maryland, New York, Ohio and Virginia) to assess efficacy of thiamethoxam, and clothianidin + imidacloprid seed treatments and compare with efficacy of in-furrow applications of thiamethoxam, and imidacloprid. Cucumber (*Cucumis sativus*) was tested in Virginia, Delaware, and Ohio, while pumpkin (*Cucurbita maxima*) was tested in Maryland, New York and Ohio. The target pest at all locations was the striped cucumber beetle (*Acalymma vittatum*), but pressure was low in 4 of 5 sites. In response, excised leaf bioassays were conducted by removing cotyledons and young leaves from field-grown plants, placing the excised plant part in moist media and introducing cucumber beetles collected from other fields. In general, insect mortality and defoliation were reduced by seed treatments and in-furrow treatments, and control was provided to the 2^{nd} or 4^{th} true leaf stage. Efficacy of treatments was also assessed against infestations of thrips (*Frankliniella fusca*) in Virginia and squash vine borer (*Melittia cucurbitae*) in Maryland. All neonicotinoid treatments reduced thrips in comparison with the non-treated check on cucumber up to 16 dap. Seed treatments reduced squash vine borer for about 2-4 weeks after moths were active, but efficacy declined after this period and larvae were able to infest many of the seed-treated plots (no in-furrow treatments were included at that site). Collectively, neonicotinoid seed treatments provided comparable efficacy and duration of control to neonicotinoid in-furrow treatments (NY).

A growth chamber study investigated abiotic stress tolerance of artichoke transplants. Post-transplanting heat (35/20^{o}C vs. 25/10^{o}C, day/night temperatures) or drought (30% WHC vs. 60% WHC) stress significantly reduced shoot or/and root growth. Heat and drought stress combined strongly affected shoot water status and root growth. In order to improve stand establishment of artichoke it may be necessary to condition seedlings to increase root growth and to prevent leaf dehydration. Ethylene regulators including precursors and releasing compound (DL-MET, ACC and ETH), and inhibitors (AVG and 1-MCP) were applied to seedlings to evaluate their effect on root growth and development. ACC and ETH (1-100 $\mu M \cdot L^{-1}$) enhanced root hair, root area and lateral roots (only with ETH at 30 $\mu M \cdot L^{-1}$). We will further evaluate the potential use of exogenous ethylene regulators as root enhancers. Improving root growth of seedlings appears critical to enhance post-transplant performance, especially under heat and drought conditions, which typically occur during summer and early fall in southwest Texas. The effects of film-forming antitranspirants and ABA (500-2000 $mg \cdot L^{-1}$) foliar application on physiological responses, water status and hardiness of artichoke transplants were examined under drought stress. ABA at 1000 $mg \cdot L^{-1}$ enhanced drought tolerance of

transplants which was associated with the maintenance of shoot water status via stomatal closure. Film-forming antitranspirants were not effective for stress mitigation. The effects of film-forming antitranspirants and ABA (500-2000 $mg \cdot L^{-1}$) foliar application on physiological responses, water status and hardiness of artichoke transplants were examined under drought stress. ABA at 1000 $mg \cdot L^{-1}$ enhanced drought tolerance of transplants which was associated with the maintenance of shoot water status via stomatal closure. Film-forming antitranspirants were not effective for stress mitigation (TX).

Experiments were performed to, a) optimize concentration and frequency of ABA application to control growth of mature vegetable transplants, and b) determine morphological, physiological and overall transplant 'quality' responses in the greenhouse and after field transplanting. ABA rates evaluated were 0, 250, 500, 1000 and 2000 ppm applied either 1, 2 or 3 weeks after plant maturity (approximately 5 week-old) on the following species: watermelon seedless cv. Majestic, cabbage cv. Blue Vantage, and pepper cv. Aristotle. The initial results indicate that growth responses to ABA is specie-dependant. Pepper appear less sensitive (chlorosis) to high ABA concentrations. Best results were obtained with ABA x 2 applications at 500 to 1000 ppm. In watermelons, ABA delayed flowering and improved overall quality, dry matter partitioning to roots, and overall field survival. A follow-up implementation experiment in Speedling, California, showed that transplants treated with ABA > 250 ppm had a reduced water uptake with increase in overall plant turgidity (TX).

A method to extract true seeds from beet fruits (seedballs) was enhanced. The method involves a brief soak in water that resulted in making the seedball pliable, deformation of the wetted seedball with firm pressure until the cap separated from the seedball, and teasing the true seed from the fruit. This method is being used to investigate mechanisms of seedling vigor and assessing seed quality in sugar beet during the critical imbibition, biochemical reactivation, and early growth of sugar beet seedlings (MI).

A new instrument to measure respiration (oxygen consumption) of individual seeds in a 96-well format (Q2, www.astecglobal.net) was evaluated as a seed vigor test. Seed lots representing diverse quality levels and enhancement treatments were assessed in the instrument. Indices associated with the quality of seed lots were calculated based upon the parameters of the oxygen uptake patterns of individual seeds. The relationships of individual seed respiratory patterns were evaluated with respect to other indices of seed quality. While some technical improvements are still required, the Q2 instrument provides a useful tool for evaluating seed quality (CA).

Vigorous and healthy seeds are an essential start toward successful crop production. Seed sanitation methods (disinfectants, heat treatments, biological protectants) may be more or less successful depending on factors such as seed vigor, seed maturity and initial levels of seed pathogens. Studies were conducted to evaluate (i) the effectiveness of seed disinfectants and chemical treatment in eliminating *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the causative agent of bacterial canker, on and in tomato seed, (ii) the effectiveness of seed treatment combined with biocontrol bacteria in eliminating Cmm on and in tomato seed, (iii) the influence of seed treatment on seed vigor and disease

control effect in greenhouse tomato seedlings, (iv) the influence of seed treatment on seed vigor in storage, and (v) the influence of seed treatment on seed vigor (OH).

The Seed Vigor Imaging System (SVIS) is a vigor test that evaluates seed performance by scanned images of young (three-day-old) seedlings evaluated by computer software. In SVIS, seeds are germinated at 25^{o}C, the resulting seedlings scanned and their length and uniformity analyzed using software that computes an overall vigor index. This test provides a rapid and objective measurement of seed quality, and the images and vigor indices are stored and a data base developed for future reference. The objective of this study was to analyze the effects of priming treatment on sweet corn seeds using SVIS (OH).

Seed technology and production training DVD's have been completed. Seed testing (importance of seed testing; seed quality; tetrazolium tests; genetic purity) and seed production modules (coffee/tropical forage grasses; sunflower; maize) are currently being marketed by the Society of Commercial Seed Technologists – SCST (OH).

A one-dimensional temperature programmable thermogradient table was designed with LED grow lights to utilize new technologies for seed testing. The case and lids are made extensively from polymers that reduce weight and are not susceptible to water damage like wood framed tables. The TASCO gradient tables expand capabilities for seed testing and research because temperature gradients can be programmed and seedlings can be grown using the LED grow lights. The LED lights provide wavelengths in the photosynthetic spectrum and do not burn out like conventional lights. Since water resistant polymers are extensively used in construction, the case is water resistance (VA).

A protocol to rapidly view high resolution confocal images of purpletop (*Tridens flavus*) embryos in dormant and non-dormant (scarified) caryopses was developed. Decreased days to 10%, 50% and 90% of final germination percentage and increased length of radicle and widths of main axis and radicle were concomitant with rapid subsequent germination of non-dormant carypopses, but not of dormant ones. Thus, embryo observation using confocal microscopy may be a useful technique for establishing seed vigor (DE).

Incubating seeds (florets) of *Aristida stricta* in glycerol, lactic acid, glycerol + lactic acid, or lactophenol for up to 24 hr failed to clear the embryo coverings enough to view TZ staining patterns. Use of a press test only accurately predicted that a seed was nonviable. Based on preliminary results, viability and germination of a seed lot can be accurately determined by conducting a press test, subjecting only filled seeds to a germination test (14 days @ 15/25^{o}C in dark), and then conducting a press test on nongerminated seeds (FL).

Priming *Coreopsis floridana* seeds in polyethylene glycol (PEG) +BA or SMP (emathlite clay)+BA resulted in 100% germination at 20 or 30^{o}C in the dark. Germination of *C. lanceolata* at supraoptimal temperatures was improved by priming seeds in PEG+BA (FL).