

Breeding for Enhanced Food Safety

Abstracts

Breeding for FHB resistance in wheat with focus on deoxynivalenol reduction

Fusarium head blight (FHB, caused by *Fusarium graminearum*) is a disease of wheat that can occur when there is plentiful moisture and humidity just prior to and during flowering. The fungus infects spikes and developing grains and causes losses in grain yield and the infected grain can have elevated levels of deoxynivalenol, a toxin produced by the fungus. Breeding for FHB resistance in U.S. wheat breeding programs was greatly accelerated beginning in the mid 1990's following epidemics of the disease in the hard red spring and soft red winter wheat growing regions. The U.S. Wheat & Barley Scab Initiative (USWBSI) was launched in 1997 to coordinate nationwide efforts "To enhance food safety and supply by reducing the impact of Fusarium Head Blight (scab) on wheat and barley."

Although breeders have focused most of their efforts on reduction of disease symptoms, reducing DON also has been an important FHB trait, and the USWBSI funds DON testing service labs to assist breeders and other FHB researchers. FHB phenotyping nurseries have become ubiquitous but are expensive and laborious, leading to intense efforts to identify DNA markers for this quantitatively inherited disease. The FHB resistance gene with the largest effect in wheat, *Fhb1*, is a target of selection in our breeding program and many others worldwide, and reduces disease symptoms by 25-30%, depending on genetic background. We also have been investigating genomic selection as a first screen to discard FHB susceptible lines. More than half of the 30+ hard red spring wheat varieties targeted for production in the Upper Midwest have moderate resistance to FHB compared to none 20 years.

Anderson, James

Bell, Rebecca	<p>FDA's Human Pathogens on Plants (HPOP) Program: Advancing produce safety and reducing risk with a one health environmental microbiology approach</p> <p>FDA CFSAN's Human Pathogens on Plants (HPOP) program focuses on the persistence and survival of enteric foodborne pathogens, specifically <i>Salmonella</i>, <i>Listeria monocytogenes</i> and shiga-toxin producing <i>E. coli</i> in the pre-harvest produce production environment. The program has two major arms. One focuses on intense environmental surveys to understand the emergence and movement of resident and introduced pathogen populations in the environment. Bacterial isolates recovered from these surveys have been sequenced and added to the GenomeTrakr database which in turn aids in outbreak investigations and traceback. The second major arm of the program examines the mechanisms that enteric human pathogens use to survive and often flourish, on fresh produce, and the critical timepoints in production when plants maybe the most vulnerable to this contamination.</p>
Brandl, Maria	<p>Fitness of Enteric Pathogens in the Pre- and Postharvest Phyllosphere</p> <p>Enteric pathogens have been defined mostly by colonization of their primary habitat, the animal intestine. As a result of produce-linked outbreaks, new paradigms have emerged about the fitness of enteric pathogens on plants. We have identified several biotic and abiotic factors that affect the fitness of <i>Salmonella</i> and <i>E. coli</i> O157:H7 in the phyllosphere. Although field studies have revealed that populations of enteric pathogens inoculated into the phyllosphere decline rapidly, their bi-phasic decay indicates that a small subpopulation of cells may persist for longer periods of time. The ability of contaminant cells to withstand the environmental pressure at the site of their arrival on the leaf may be critical in this persistence. Likewise, the heterogeneous and fluctuating physicochemical properties of plant surfaces at multiple scales may provide rare opportunities for bacterial survival and multiplication, and dictate the outcome of a plant contamination event. Additionally, leaf tissue that is compromised mechanically or by plant pathogen infection offers a nutrient-rich environment conducive to bacterial multiplication, although enteric pathogens must also contend with the stresses imposed by active plant defenses against such insults. Transcriptome profiling revealed that <i>Salmonella</i> and <i>E. coli</i> O157:H7 undergo physiological changes in response to mechanical injury or microbial infection of lettuce and cilantro. Knowledge of the behavioral and physiological responses of enteric pathogens to the opportunities that arise or the challenges they face on plants in the pre- and postharvest phases of production may inform crop breeding strategies to prevent a contamination event from leading to human disease.</p>

Clements, Donna P.	<p>Cultivating Produce Safety Knowledge: An Extension and Outreach Perspective</p> <p>The Produce Safety Alliance (PSA) is a collaboration between Cornell University, the Food and Drug Administration (FDA), and the United States Department of Agriculture (USDA) to prepare fresh produce growers to meet the regulatory requirements in the Food Safety Modernization Act (FSMA) Produce Safety Rule. The PSA has developed and launched a standardized training course and outreach program to assist the produce industry, including growers, packers, and regulatory agencies with the implementation of produce safety practices and regulatory requirements. Since the launch of PSA trainings in 2016, more than 36,800 individuals have attended the PSA Grower Training Course and over 2,600 trainers have attended the PSA Train-the-Trainer Course, domestically and internationally. This talk highlights lessons learned from launching a produce safety extension program, including challenges growers have in interpreting regulatory language and implementing risk reduction practices.</p>
Gradziel, T.M.	<p>Extraordinary problems require extraordinary solutions: exotic genes for solving almond food safety challenges from aflatoxin, allergenicity, and salmonella</p> <p>Almond is a desirable and high-quality food source but the presence of nut allergens and a vulnerability to aflatoxin and salmonella contamination are major threats to food safety. In 2018 over 2.2 billion pounds of almonds, representing over 80% of the world total, were produced in California from a relatively few varieties and so a very narrow genetic-base. To address emerging needs from climate change, including loss of pollinators and traditional agrochemicals, new germplasm has been introgressed from peach as well as wild peach and almond species. Because almond is a clonal crop, unique genetic/genomic/epigenetic interactions can be captured and multiplied through vegetative propagation for rapid commercialization. Advanced varieties and breeding selections incorporating exotic genes in a genetic background compatible with commercial production in California have demonstrated sizable decreases in levels of amandin, the primary allergen in almond, as well as improved resistance to aflatoxin and salmonella. Breeding strategies range from direct selection from enriched germplasm (allergenicity), integrated resistance to disease and insect vectors (aflatoxin), and major modification of traditional harvest strategies (salmonella).</p>

<p>Jay-Russell, Michele T.</p>	<p>The Problem of In-Field Microbial Contamination of Fresh Produce by Zoonotic Pathogens: Low-Probability High-Consequence Human Foodborne Disease Events</p> <p>Low-probability high-consequence (LPHC) events are characterized by their unpredictability and rarity, which can hamper the ability to completely understand underlying causes and manage future risks. Human illnesses linked to pre-harvest contamination of fresh produce could be considered LPHC events with the potential for extreme public health and economic impact, but relative rarity compared with the scope of production and exposure. Additionally, there are few examples of environmental assessments following fresh produce-related outbreaks that have resulted in determination of root cause(s) linked directly or indirectly to a source; this is due, in part, to the complexity of produce production, time-delays from recognition of an outbreak to traceback, and resource limitations for on-farm sampling and testing. At the pre-harvest level, possible environmental sources of fecal-borne foodborne pathogens are numerous and may include nearby domestic animal operations, human sewage/septic facilities, infected farmworkers, contaminated agriculture water, untreated manure-based soil amendments, flies or other invertebrates and wild animal intrusion/defecation in the production area. Unique challenges in mitigating these risks include the low infectious dose of important zoonotic enteric pathogens, susceptibility of vulnerable populations (e.g., young children, elderly), attachment of pathogens to plant surfaces, cross-contamination during processing, and lack of a post-harvest “kill step” for raw and minimally processed produce. Plants for human consumption grown in open fields and orchards cannot be fully protected from low levels of contamination all of the time, thus risk mitigation strategies are followed, but sometimes fail. Plant breeding in combination with pre- and post-harvest risk reduction efforts could be a promising approach to address these challenges so that growers can continue to coexist with adjacent animal production and wildlife habitat while still protecting the public health.</p>
<p>Leveau, Johan</p>	<p>Bacterial attachment to leafy greens: contributions of leaf surface topography</p> <p>The surface topography of plant leaves has a genetic basis, which means it has potential as a breeding target. This is of interest in the context of our recent findings that leaf surface topography is a significant contributor to the foliar attachment of bacteria, including enteropathogenic <i>Escherichia coli</i>. More specifically, we were able to demonstrate, using topomimetic leaves, that a higher degree of surface topography (e.g. bigger veins, deeper crevices) is positively correlated with greater retention of water and bacteria on the leaf, with higher probability for dispersal by splash, and with increased bacterial resilience to removal and sanitation. These findings offer a quantitative basis for evaluating the merits of breeding leafy greens with altered leaf surface topography for enhanced food safety.</p>

<p>Melotto, Maeli Jacob, Cristian</p>	<p>Genetic components associated with human pathogen colonization of lettuce.</p> <p>Every year, disease outbreaks related to the consumption of fresh produce cause a significant number of illness, hospitalization, and death episodes affecting both public health and agribusiness. In this study, we sought to assess and understand the effect of lettuce genetic variation on its interaction with two of the most common bacterial species identified in these outbreaks: <i>Salmonella enterica</i> serovar Typhimurium and <i>Escherichia coli</i> O157:H7. We observed significant variation in the bacterial persistence in the leaf apoplast among eleven lettuce genotypes (<i>Lactuca sativa</i> L. and <i>L. serriola</i> L.). Contrasting bacterial persistence phenotypes in two lettuce genotypes correlated with the level of plant defense responses (<i>i.e.</i>, reactive oxygen species burst and callose deposition) against these strains. Simultaneous and temporal RNA-sequencing analysis of the plant and bacterium transcriptomes revealed an active interaction between these organisms and a significant modulation of diverse metabolic pathways, including plant defense response, secondary metabolism, and plant regulatory networks. These findings highlight the existence of sufficient genetic variation within the lettuce germplasm useful for mapping genes that are responsible for bacterial persistence phenotypes. Furthermore, these results will facilitate breeding for enhanced safety of lettuce.</p>
<p>Micallef, Shirley</p>	<p>The Odd Couple: Reciprocal <i>Salmonella enterica</i>-tomato interactions</p> <p>Good Agricultural Practices and food safety regulations have minimized fresh produce crop contamination by foodborne pathogens during production and postharvest handling in the past twenty years. However, continued foodborne illness outbreaks involving fruit and vegetables, the sporadic frequency of contamination events, and the complex, multifactorial risks encountered in the field call for innovative solutions to this problem. Identifying traits in plants that can reduce their likelihood of associating with enteric pathogens is an innovative approach that requires the identification of mechanisms at play in human pathogen-plant interactions. This presentation will discuss reciprocal responses between <i>Salmonella enterica</i> and tomato, as well as take a look at how the metabolic landscape on the surface of tomato plants impact epiphytic <i>Salmonella</i> establishment.</p>

<p>Smith, Richard</p>	<p>Heavy metals in spinach.</p> <p>Cadmium (Cd) uptake in the diet is a human health concern. The Salinas Valley is the largest spinach production area in the US. However, there are natural deposits of Cd in the south of the valley. The outwash from these rocks have elevated Salinas Valley soils. The Food and Drug Administration does not regulate the amount of Cd in spinach, however, will act if high levels of Cd are detected. In addition, international trading partners such as Canada and Mexico adhere to the International Codex limit of 0.2 ppm Cd on a fresh weight basis. As a result, growers in the Salinas Valley are under pressure to keep Cadmium levels in their products as low as possible. Cadmium uptake can be reduced by a number of cultural practices: 1) staying off the highest Cd soils (>1.5 ppm), 2) applying high rates of zinc fertilizer, 3) applying lime to keep the pH above neutral, 4) use of compost and 5) use of low chloride containing irrigation water. In addition to these cultural practices a breeding for low Cd accumulating varieties is a key method of reducing Cd uptake by spinach. Efforts by researchers at UCD are underway to develop lines that take up low levels of Cd.</p>
<p>Taylor, Gail Arnold, Libby Damerum, Anabelle</p>	<p>Making lettuce clean and safe: Opportunities from the leaf microbiome</p> <p>The surface topography of plant leaves has a genetic basis, which means it has potential as a breeding target. This is of interest in the context of our recent findings that leaf surface topography is a significant contributor to the foliar attachment of bacteria, including enteropathogenic <i>Escherichia coli</i>. More specifically, we were able to demonstrate, using topomimetic leaves, that a higher degree of surface topography (e.g. bigger veins, deeper crevices) is positively correlated with greater retention of water and bacteria on the leaf, with higher probability for dispersal by splash, and with increased bacterial resilience to removal and sanitation. These findings offer a quantitative basis for evaluating the merits of breeding leafy greens with altered leaf surface topography for enhanced food safety.</p>

<p>Teplitski, Max Walls, Isabel Kaleikau, Ed Thro, Ann Marie</p>	<p>Plant Breeding for Food Safety: NIFA-catalyzed opportunities</p> <p>Over the last decade, fresh fruits, produce, nuts, and sprouts have been increasingly associated with outbreaks of foodborne illness caused by enteric pathogens, like <i>Salmonella</i> spp and Shiga-toxin producing <i>E. coli</i>. Grains, nuts and dry fruits have also been among commodities linked to illnesses caused by mycotoxins. While interactions of enteric bacteria and mycotoxin-producing fungi with plants likely involve distinct (or at least partially overlapping) pathways, recent evidence begins to suggest that the manipulation of the underlying mechanisms of these interactions may ultimately improve food safety outcomes. Over the years, USDA NIFA invested in understanding mechanisms of interactions between crops and microbes that are pathogens of humans, and plants and mycotoxin-producing fungi. These projects reveal adaptations of human pathogens to a plant-associated lifestyle, plant responses to colonization by human pathogens, genetic traits involved in limiting proliferation and mycotoxin synthesis, and production practices that impact susceptibility of crops to contamination and proliferation of pests of food safety concern. This presentation will focus on the emerging collaborative opportunities along the research-to-impact continuum in this trans-disciplinary space.</p>
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Identifying and deploying host plant resistance to *Aspergillus flavus* in maize: a multipronged approach

A. flavus is an opportunistic saprophyte and economic problem in some oil seed crops, including corn (maize). *A. flavus* produces aflatoxin, which can cause immunosuppression and cancer in humans and animals. Maize inbred lines highly resistant to *A. flavus* and aflatoxin accumulation have been created, mostly via phenotypic selection from tropical landrace materials and their derivatives. Stable QTL with moderately large effects on resistance have been identified and validated. Transferring resistance to elite maize lines, phenotypically or via MAS, has been slow and incomplete to date. Genomewide association mapping has uncovered more genes of interest, as have studies of proteins, RNA, and metabolites created in maize in response to *A. flavus* infection. However, many of the genes identified in this way only improve resistance a little, and alone, can't help much. Pathway and network analyses using large data sets resulting from these genomic, transcriptomic, proteomic and metabolomic studies often uncover more information on mechanisms of resistance than the original studies. Taken together, a review of many such studies show some common themes on how maize may resist *A. flavus* and the accumulation of aflatoxin. Resistance steps include how maize senses *A. flavus* or aflatoxin; how signaling molecules and plant hormones are created to turn on other genes in response; how maize creates defense chemicals, possibly at the expense of continued starch synthesis; and how maize responds to oxidative stress to keep *A. flavus* from creating aflatoxin. Genes involved in these steps may become targets of genetic improvement for maize resistance.

Warburton, Marilyn