

9th Annual UW- Madison Plant Sciences Symposium

† Graduate students competing in the Lightning Talk Competition from 11:20 - 11:45AM

1. Crop Rotation and Cover Cropping: Impacts on Soil Bacterial Communities

Lindsay Chamberlain†

Crop rotation, the successive cultivation of different crops on the same field, has been practiced for centuries, and it is often associated with increased crop yields. In the Midwestern United States, corn (*Zea mays*) and soybean (*Glycine max*) are two field crops that are commonly rotated. It is known that improved pest management and nutrient cycling contribute to the crop rotation effect, or yield increase associated with crop rotation, but these factors do not account for this phenomenon entirely. Cover cropping is a soil conservation practice: cover crops are grown between harvest and planting of the main crop to protect and enrich the soil. Increasing crop diversity with crop rotation and cover cropping may contribute to shifts in soil bacterial communities. Previous work in a long-term corn-soybean rotation study in Arlington, Wisconsin showed that continuous corn or continuous soybean cultivation results in distinct bulk soil bacterial communities, with the soil bacterial community from an annual rotation of the two crops similar to both continuous communities. To determine if this relationship between continuous and rotated soil bacterial communities are maintained or altered by the inclusion of cover crops, we analyzed soil bacterial communities from continuous corn, continuous soybean, and annually rotated corn-soybean crops, with two grass cover crop species within each rotational treatment. As expected, pH, soil organic matter, and certain macronutrients were essential drivers in determining bulk soil bacterial community composition. We found that continuously cropped systems had distinct bacterial communities and annually rotated communities lack distinction from each other. The incorporation of cover crops into the rotation system did not result in significant changes to the bulk soil bacterial community structure. This result was probably due to limited cover crop growth in the establishment year, and a limited amount of time for soil communities to respond to the change.

2. Transcriptome Analysis of Maternally-Transmitted Cold Tolerance in Cucumber

Madeline Olberg[†]

Cucumber is a warm-season crop that can be severely damaged by short periods of cold temperatures. Cold tolerant cucumbers would benefit growers by preventing crop loss in inclement weather as well as by allowing for earlier planting and harvest, thus avoiding heavy late-season disease pressure. Maternally-inherited cold tolerance has been reported in the heirloom cucumber cultivar, 'Chipper.' In cucumber, the chloroplast, mitochondrial, and nuclear genomes are maternally, paternally, and biparentally transmitted, respectively, indicating that this cold tolerance may be conditioned by the chloroplast genome. Phenotypic analysis of reciprocal hybrids between doubled haploids (DH) of cold-tolerant 'Chipper' and susceptible 'Straight 8' and 'Marketmore' revealed that cold recovery is maternally transmitted from 'Chipper'. Total nuclear RNA was extracted from reciprocal hybrids with identical nuclear genotypes and revealed similar expression profiles for the cold-tolerant hybrids after cold treatment. Further analysis of a non-synonymous SNP in a chloroplast ATPase subunit of 'Chipper' could further elucidate the mechanism of this cold recovery phenotype. Identification of the genetic basis of cold tolerance in 'Chipper' would provide potential targets of selection for cold tolerance in cucumber and other warm-season crops.

3. Bacteria contribute to plant secondary compound degradation in a generalist herbivore system

Charlotte Francoeur[†]

Insects and plants engage in a multitude of complex interactions. In antagonistic cases, such as herbivory, insects often specialize on a few closely related plant species to overcome physical and chemical defenses. More rarely, herbivorous insects can feed on a range of plant species. Leaf-cutter ants are generalist herbivores that forage from a variety of plant species, which the ants bring to the fungus they farm, *Leucoagaricus* sp. While we show that anti-herbivory plant compounds can harm *Leucoagaricus* sp. in vitro, it is unknown how leaf-cutter ants forage from a diversity of plants in the environment that are palatable to their fungus gardens. Here, we investigate the fungus garden bacterial community's ability to degrade plant secondary compounds. We cultured fungus garden bacteria, sequenced the genomes of 42 isolates, and found genes involved in plant secondary compound degradation, including monoterpene epsilon-lactone hydrolase. Some of these genes show in situ expression in metatranscriptomics, such as limonene-1,2-monooxygenase. A majority of the bacterial isolates grew unhindered in the presence of plant secondary compounds and, using GC-MS, isolates from the genera *Pseudomonas*, *Klebsiella*, *Enterobacter*, and *Bacillus* could degrade either alpha-pinene, beta-caryophyllene or linalool. Additionally, using a headspace sampler, sub-colonies of fungus gardens reduced alpha-pinene and linalool over a 36-hour period, while *Leucoagaricus* sp. strains alone only reduced linalool, not alpha-pinene. Our study provides evidence that *Leucoagaricus* sp. has a variable ability to tolerate and degrade plant secondary compounds, indicating that it may depend on bacteria to detoxify the diversity of plant chemistry the system encounters.

4. DcOr is Associated with Carotenoid Accumulation in Carrot

Kevin Coe[†]

Carrot (*Daucus carota*) is one of the richest sources of the vitamin A precursor Beta-carotene in the human diet. Two genes, Y and Y2 have been previously identified to be responsible for the majority of carotenoid accumulation in carrot roots. Y conditions all carotenoid accumulation in carrot roots, and one allele identified in orange and yellow carrots harbors a 212 bp insertion in the gene. Y2 is known to condition the accumulation of alpha- and beta-carotene in carrot roots. Recently, the Orange gene in carrot (DcOr) was identified in a genome-wide association study (GWAS) to also be significantly associated with carotenoid accumulation in roots. Molecular studies of Or in other plants, such as *Arabidopsis*, melon, and cauliflower have revealed mutations that result in increased sequestration of beta-carotene in tissues that are normally non-photosynthetic. Analysis of sequence polymorphisms within the CDS of DcOr found a SNP, which was nearly fixed in cultivated carrot, resulting in a substitution of a highly conserved Serine with a Leucine in OR. It is our hypothesis that during carrot domestication, a mutated DcOr allele was selected, alongside Y and Y2, for its unique ability to increase carotenoid accumulation in root tissue. Signatures of selection are being investigated using a panel of wild and domesticated resequenced plant introductions (PIs) collected from different regions of the world. Additionally, patterns of Or expression are being analyzed in a mapping population of carrots.

5. Strategies to identify and introgress production and quality traits from genetic resources into elite carrot cultivars

Keo Corak[†]

Crop breeding programs interested in using genetic resources often have difficulty identifying useful accessions from germplasm collections. We are exploring genomic prediction strategies to more effectively use diverse germplasm, using ongoing efforts to breed carrot varieties with tall canopies and mild flavor as a model vegetable crop and breeding goal. Genomic prediction leverages high-density genotype data to predict phenotypes or breeding values, potentially allowing for rapid identification of useful germplasm in a genotyped collection. Translating genomic prediction methods to diverse germplasm collections presents some challenges related to the size and design of the population used to train prediction models. In a study varying the size of a training population, we found that useful prediction accuracies were achieved only when the training population size was large enough to represent the functional genetic diversity present in the collection. Accessions with the most favorable predicted trait values using different training population designs were crossed to elite inbred lines to initiate new breeding populations. The measured trait means and variances of F2 families will be used to validate predictions and to compare the efficiency of genomic and phenotypic methods to identify valuable genetic resources.

6. Resilience and Stability of Oat Cultivars to Climate Variability

Sarah K. Bullock

Climatic extremes are becoming increasingly more frequent. This results in a greater need to identify crop varieties that are more resilient to damaging extremes and have stable yields. Oat (*Avena sativa* L.) contributes significantly to the world caloric intake, ranking 8th for world crop production for animal use and ranked 5th in terms of relative protein content. This research aimed to quantify the resilience and stability of oat cultivars to climatic stresses to reduce the risk that is associated with oat production. For this research, a stable genotype is the one that will have minimum variability in productivity and not effected by climatic changes, whereas resilience is the ability of a cropping system to withstand a crisis. Our experiment looked at both oat variety stability and resilience in historic data from Wisconsin. The two climate characteristics were precipitation and temperature. Conditions of interest were drought, water excess, heat stress and cold damage. We used a database consisting of oat grain yield from variety trials at seven locations in Wisconsin, USA, spanning 20 years from 1997-2018, and 1,035 genotypes; accounting for 168 environments and 134 site years. Crisis years for each location were identified based on minimum mean yields of each environment. The cause of the crisis year was identified using NOAA daily weather data. Resilience value was calculated for each genotype in the crisis year. The 16 crisis years identified were given one of three known causes, water deficit, water excess, and heat stress. 50% of the crisis years were found to be caused by water deficit, of those, 5 were chosen that represented the worst drought year at each location. The most stable and resilient genotypes were identified.

7. Root Nodulation Requires Multiple 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductases in *Medicago truncatula*

Zachary Keyser

Symbiotic associations between legumes and rhizobia are controlled by a dedicated signaling pathway in the roots of legume plants. Genetic and biochemical studies in the model legume *Medicago truncatula* led to the identification of receptors (MtLYK3 and MtNFP) for microbial signals, a co-receptor (MtNORK) at the plasma membrane level, as well as downstream components involved in nuclear calcium spiking, which ultimately regulates the expression of genes associated with symbiosis. We found a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (MtHMGR1) as an interactor of MtNORK. Additionally, mevalonate, the product of HMGR activity, is sufficient to trigger nuclear calcium spiking even in the absence of MtNORK. MtHMGR1 is, therefore, an excellent candidate to connect perception events at the plasma membrane level to nuclear ones such as calcium spiking and gene expression. We will present new data indicating that MtHMGR1 also interacts with the MtLYK3 and MtNFP receptors. Also, RNAi silencing of HMGR1 expression decreases calcium spiking, symbiotic gene expression, and nodulation drastically. However, when the fully sequenced genome of *M. truncatula* was released, we identified several new homologs of MtHMGR1 that were likely affected by our RNAi construct. To determine which specific HMGRs are involved in symbiosis, we used Tnt1-insertion lines, knocking-out the expression of individual HMGRs. Insertions in MtHMGR1 and MtHMGR2c decreases the ability of nodules to support nitrogen fixation but not nodule number, indicating that multiple HMGRs are involved in the nodulation process with a likelihood of some functional redundancy.

8. Agronomics of Industrial Hemp (*Cannabis sativa* L.) for Grain and Fiber Production in Wisconsin.

Haleigh Ortmeier-Clarke

In the early 1900s, Wisconsin emerged as a leading producer of industrial hemp (*Cannabis sativa* L.) as a fiber crop. The first push to regulate cannabis came in 1906, but cannabis wasn't banned until the Marijuana Tax Act was passed in 1937. Cannabis was again banned under the Controlled Substances Act in the 1970s after the Marijuana Tax Act was ruled unconstitutional. Under the Controlled Substances Act cannabis was listed as a Schedule I drug, the most restrictive category. The 2018 Farm Bill identified industrial hemp as *Cannabis sativa* L. with < 0.3% tetrahydrocannabinols (THC), separating it out from *Cannabis sativa* L. with > 0.3 % THC (otherwise known as marijuana). Farmers can again legally cultivate industrial hemp, which has led to many questions including best management practices, especially surrounding variety selection as well as weed and nutrient management. The objective of this study was to compare industrial hemp grain varieties and evaluate their response to variable seeding and nitrogen rates. The study was conducted as a factorial with 2 varieties (X-59 and CRS-1) x 3 Seeding Rates (22, 34, and 45 kg ha⁻¹) x 3 Nitrogen Rates (0, 67, and 134 kg ha⁻¹) in a Randomized Complete Block Design (RCBD) replicated four times and at two locations, Southern Wisconsin (Arlington Agricultural Research Station) and in Northwest Wisconsin (Chippewa County Farm). Plant tissue and soil samples, plant heights, canopy closure and crop density measurements were collected throughout the growing season. The results of this preliminary study will allow us to provide baseline guidance to growers producing industrial hemp for grain and fiber in the state of Wisconsin.

9. Comparing two methods for clustering genotypes in a tropical maize diversity panel

Fernando Garcia Espolador

The identification of the genetic structure among individuals is crucial to manage germplasm banks and design heterotic groups. Several approaches are addressed to identify the accurate number of clusters and their composition. However, most of them might produce different outcomes. Therefore, we aimed to compare two clustering approaches in a tropical maize diversity panel. For that, we considered a dataset with 14,560 SNP from a genotyping-by-sequencing approach filtered for only biallelic markers, minor allelic frequency > 0.05 , call rate > 0.95 , and linkage disequilibrium (r^2) < 0.99 . Then, we tested two clustering approaches: (A) software Structure, varying the number of possible groups (K) from 1 to 14, 4 replicates, 200,000 iterations with burn-in of 100,000, and a minimum proportion of 0.50 to identify an individual belonging to a group (Q); (B) K-means method, performed 30 times with K ranging from 1 to 40, and considering the Roger's distance matrix. The dendrogram was fashioned using Roger's genetic distance and clustered by UPGMA. The best K-values were identified using Evanno's delta K criteria for Structure outcomes, and the lower average Bayesian Information Content for the K-means method. According to K-means, the best fit for K was 10, while for Structure, two most likely values were assigned, 2 and 10. The Structure K=2 division underrepresented the panel diversity since there were many clear groups clustered in a major one. Hence, we consider that K=10 the most reliable partition. Regarding the results from the dendrogram, it was possible to observe a strong correspondence between the branches and the clustering from both approaches. Furthermore, computationally, the K-means method was less costly.

10. Estimation of genetic effects using distinct genomic relationship matrices in tropical maize

Júlia Silva Morosini

The incorporation of the kinship matrix in genetic-statistical analyses allows us to increase the accuracy of variance components and to improve the assessment of individuals' genetic values. Thus, we investigated how the inclusion of additive and dominance genomic relationship matrices affects diallel analysis estimates. For that, 906 single-crosses obtained from a diallel scheme of 49 inbred maize lines were genotyped in silico using 34,571 SNP and evaluated for grain yield (GY), plant height (PH), ear height (EH), and the index Harmonic Mean (HM) in four environments in the State of São Paulo, Brazil, each with two nitrogen regimes (ideal and stress). Three modeling scenarios were considered for the incidence matrix of individuals in the diallel analysis: (A) pedigree-based, (B) additive for parent lines, and (C) additive+dominance for lines and hybrids, respectively. We observed that the incorporation of genetic information in the model increased the estimates of additive variance and the narrow-sense heritability. For all traits, the total genetic variance increased from scenario A to B and decreased from B to C, which indicates that there was greater efficiency in capitalizing dominance variance by adding genomic related information between individuals. The distribution of the general (GCA) and specific (SCA) combining capabilities have little changed between models for all traits. Furthermore, the genetic estimates from the nitrogen stress regime trials were lower than the estimates from the ideal condition. Finally, the outcomes reveal that the inclusion of the genomic relationship information increases the uptake of additive genetic variance and improves genetic estimates in proportion to the level of incorporation into the model. From a maize breeding perspective, our outcomes indicate the in-depth assessment of GCA and SCA by exploring the genomic relationship between individuals has a great impact on the obtention of breeding values and heterosis.

11. Comparative Ecophysiology of Two Southern Appalachian Conifers: The Importance of Winter
Rachel Jordan

Southern Appalachian spruce-fir forests are glacial relicts, found in just seven disjunct sky islands on the region's highest peaks. Fraser fir (*Abies fraseri*), a rare endemic, dominates at elevations above 1650 m. Red spruce (*Picea rubens*) occurs at lower, warmer elevations (1380 – 1650 m) and is more widely distributed than *A. fraseri*, suggesting a potential adaptation to midwinter thaws. Previous research in northern latitude spruce-fir forests has suggested that co-occurring species may have different responses to midwinter thaws. However, very little winter research has occurred in the southern Appalachian spruce-fir forest, which has longer photoperiods, more frequent midwinter thaws, and less severe temperatures than those of the northern boreal forest in winter. Despite the potentially significant impact of winter, its importance to the ecophysiology of these trees is poorly understood, as are the potential impacts of milder winters resulting from future climate warming. It was hypothesized that red spruce would be more physiologically active than Fraser fir and have lower concentrations of photoprotective carotenoids during midwinter thaws. We compared the midday physiological behavior of both species during winter on Grandfather Mountain, NC, including current, first-year, and second-year needle pigment concentrations, photosynthesis/respiration, fluorescence, and twig water potentials.

Pigment concentrations (per gdw) were significantly higher in *A. fraseri* than in *P. rubens*, and concentrations declined with age in both species; no effect was found for canopy face (N or S). Photosynthetic rate was significantly higher in the one-year-old needles of *P. rubens* on the warmest day, but all other rates of photosynthesis did not differ by needle age. Chlorophyll fluorescence (F_v/F_m) did not differ between the two species but increased significantly with air temperature, and reduction in PSII activity increased with decreasing temperatures. Water potentials in *A. fraseri* were significantly more negative on two cold days, which may indicate a lower temperature threshold for stomatal opening. The results of this study suggest that while net photosynthesis for both species is at or near zero on cold days, the strategies by which they maintain compensation may be different and could have an influence on their relative ability to take advantage of midwinter thaws. Further exploration of differences in hydraulic conductivity, conduit diameter, and pigment cycling is necessary to conclude the underlying causes of the differential wintertime activity.

12. Strengthening a Computational Community of Practice Among Plant Scientists

Steve Goldstein & Kathryn Michel

A pervasive problem faced by bench and field plant scientists is that advances in high-throughput measurement platforms are outpacing our communities' abilities to readily analyze the datasets produced. We have formed comPS- a Computational Plant Sciences Community of Practice, to be a peer-to-peer mentoring network that works together across lab, departmental, and college boundaries.

What have we done so far?

Over the last year, we have hosted several activities to bring our community together, including:

- Creating a member directory and Slack workspace to share news, opportunities, and get help on computational needs
- Holding weekly coding coworking sessions to get together and troubleshoot programming issues
- Holding monthly meetings to discuss interesting tools and individual computational struggles and successes
- Developing and teaching a three-part workshop series on data analysis in R

But what more can be done to help those struggling with computational challenges? You tell us!

Please join us at noon on December 3rd in 1154 Discovery Building to brainstorm about these and other ideas that could help you accelerate your computational research. And come to our poster to discuss how you can help foster the growth of this community.

13. Variation for Epicuticular Waxes in Onion

Derek Hunsaker

Epicuticular waxes are present in almost all terrestrial plants and serve a variety of functions, including preventing water loss and protecting against pathogens. Onions can be classified into glossy, semi-glossy, and waxy phenotypes based on their amount and types of epicuticular waxes. Research has shown that onions with semi-glossy and glossy phenotypes show increased resistance to thrips damage. Thrips are the most damaging pest to onions. To control for thrips, onion growers must spray for them the entire growing season, which amounts to a significant monetary cost. Using gas chromatography and mass spectrometry (GCMS), it is possible to determine the wax profiles of onions. GCMS and field studies have said that the hentriacontrane-16 is the most abundant type of wax in onions, and that having lower relative amounts of hentriacontrane-16 is responsible for giving onions the semi-glossy phenotype and providing thrips resistance. The goal of my research was to assess the variation that is present in onions and find onions with unique wax profiles. This was done by running GCMS on many onions from the USDA germplasm bank to determine what variation exists in onion wax profiles. The desired wax profile is high in total wax amount but has a low relative amount of hentriacontrane-16. This gives the onion the agronomic benefits of waxes, while also providing thrips resistance. I found that there were onions with this desired profile, and further research will be done to determine the heritability of these wax profiles.

14. Mesophyll photosynthetic sensitivity to leaf water potential in Eucalyptus reflects adaptation to aridity

Amanda Salvi

The sensitivity of photosynthetic capacity to water deficits is a fundamental constraint on land-plant evolution. Declines in photosynthesis with reduced leaf water potential are often attributed to stomatal closure, but there is also a general yet overlooked tendency for leaf dehydration to reduce mesophyll photosynthetic capacity. Very little is known about how this non-stomatal limitation varies among ecologically divergent plants. We compared mesophyll photosynthetic sensitivity (MPS) among ten Eucalyptus species native to different parts of an Australian macroclimatic gradient in moisture supply, to test whether those native to drier habitats show reduced MPS. We provide the first compelling evidence that MPS decreases significantly toward drier habitats, suggesting that MPS evolves in adaptive fashion. These findings provide important insights into an unexplored dimension of plant adaptation.