**Final Project QMEE**

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**Introduction**

The crop establishment is key factor for soybean growers at the beginning of the season where most of the time, seedlings are subject of the attack of soilborne pathogens, resulting in damping off and poor plant establishment. Soybean seedling diseases impact the plant root system at the beginning of the season, however the root infections can occur at later stages, impacting crop physiology and yields. In 2005, seedling diseases caused losses around 829 tons in the US (Wrather & Koenning, 2006). Different factors could influence the outcome of the interaction of soilborne pathogens and soybean plants, such as seed genetic background, the climate, edaphic factors and their interaction with the soil inhabitants (Broders et al., 2009). These soilborne diseases have been attributed to several pathogens most of them fungi and oomycetes (fungi-like organisms). However, the key species playing a role in disease are not fully known (Broders et al., 2007).

In fact, the oomycetes *Pythium* and *Phytophthora* are two of the main causal agents of soybean root diseases, for instance losses have increased by four-fold in the last ten years (Koenning & Wrather, 2010). The increased incidence is related to some of cultural practices now being used by growers, like no-till and early planting. These practices and the environment interact strongly with the microbial communities present in the soil, causing shifts in the different species that exist in this ecosystem (Arcate et al., 2006). The knowledge of the diversity of species causing soybean seedling diseases in the US Midwest is limited. At present, we carried out a classic culture-based approach to start understanding the diversity of oomycetes associated to soybean and corn seedling diseases in the state. We have identified multiple species affecting either or both crops, all of them *Pythium* species that accounted for 33 different species (22 of them were isolated from soybean and 24 isolated from corn).

The understanding of these soil communities and the pathogens there present has traditionally been done using culture based methods. The caveat for the media-based isolation is the introduction significant biases due to differential growth of organisms such as influenced by temperature, medium preference, and antibiotic/fungicide sensitivity. Even “semi-selective” medium designed specifically to increase the recovery rate of a target organism or group of organisms introduce biases such as selection of isolates within the target species or group reducing representation of isolates within the target group that are slower growing and more sensitive to antibiotics.

Nowadays, culture independent microbial community analysis provides a significant advantage over culture based methods as no selection pressure is placed on the group of organisms and a far more complete snap shot of organisms present can be achieved, at least at the level for which primers are designed. The availability of different technologies have reduce the cost of the deep sampling of a community (Desai et al., 2012), providing even more information in reduced time and less cost, like MiSeq that could generate a 1.5 Gb of data for 8 different samples in just one run for about thousand dollars; while the identification of 100 isolates just considering sequencing (ignoring isolation materials) could be around 800 dollars.

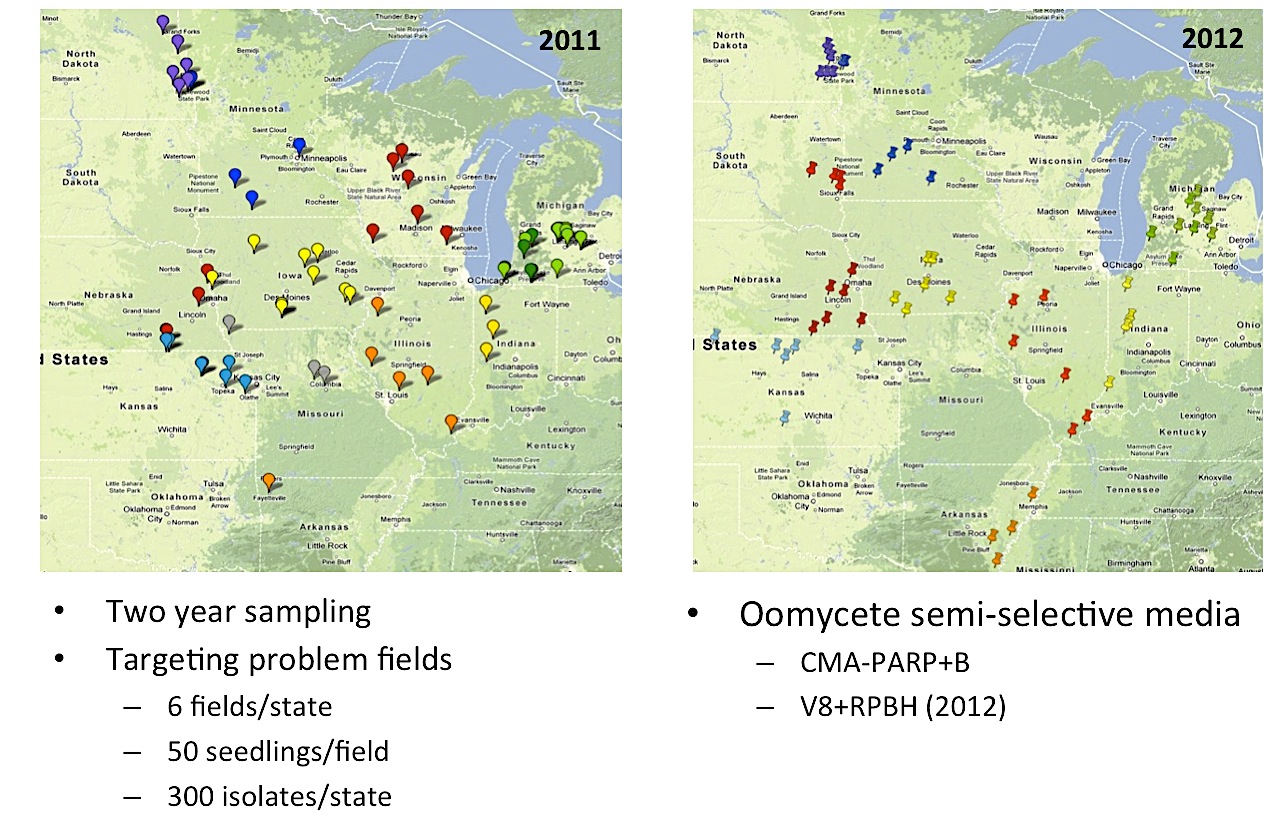
The use of metagenomics to study the diversity of fungal and oomycete pathogens associated to crops like soybean and corn will provide a better understanding of the complexes of organisms that might be associated to disease under specific conditions, such as crop rotation or plant genetic background. The reduce cost of sequencing facilitates the evaluation of the microbiota at different time points, which can be important to detect population shifts. These shifts could be result of plant age and might show pathogens present at different plant stages.

The understanding of microbial communities associated will be significant since corn and soybean are major crops with economical importance not only as staple food, but also as biofuel sources to reduce the impact of potential pathogens, as we understand their distribution, frequency and abundance.

My research is focused on the causal agents of root rot and damping off on soybean seedlings, so far is known that *Phytophthora sojae* is the major pathogen causing root rot on soybean, recent survey have found more oomycete species associated to seedling diseases, these are mostly *Pythium* spp., however the full extent of *Pythium* spp. causing this disease is not well known. Few states have extensively sample to address the diversity of oomycete root rot pathogens on soybean. The question of my research focuses on what are the oomycete species associated with soybean seedling diseases. Our hypothesis is that seedling diseases are cause by multiple oomycete species, which some of this species could be acting as pathogen complexes to cause disease across the Midwest. As other fungal systems, we also hypothesis that there are biotic and abiotic factors driving the presence and abundance of these oomycetes on the different fields, abiotic factors such as soil physical properties, temperature, precipitation and other environmental factors. Last, some of these species are going to vary in the different levels of virulence, we hypothesize that those abundant species will be primarily pathogenic species.

The dataset is from a study focus on the diversity of oomycetes associated with soybean seedling diseases in the U.S., therefore 10 to 12 states were sampled in two years (2011 and 2012), the goal was to target fields with a history of seedling diseases, with stand establishment issues and damping-off. For these reason 6 fields per state were sampled and within each field a total of 50 symptomatic seedlings were collected in W-shaped transect. Thus, we sampled single fields (unit of replication) within states in the Midwest. The goal of this study is to determine the extent of oomycetes species present in those fields and address the role of those species as plant pathogens, also determine what factors are driving the diversity, presence and abundance of oomycetes in these fields. We can say that most of the data collected is observational since samples were collected from non-experimental sites, and the oomycetes were isolated using a semi-selective media.

The scheme below represents the locations sampled and what we aimed for, however since we are limited by the presence of disease and the success on isolation the design is not quite traditional, and replication is difficult to carry on. The sampling is hierarchical sampling plants within fields within state. There is pseudoreplication within state since not the same fields were sampled across the years, but one can hypothesize that fields within the state are replicates. However, we acknowledge that these boundaries could be artificial in our study.



The purpose of the study is descriptive, looking for patterns related to the abundance and presence of some of this species, so some metadata was collected like planting data, GIS coordinates (to include more information based on GIs databases) and soil analyses. The main questions are: What are the oomycete species associated with soybean seedling diseases? Are pathogen complexes responsible for these diseases (co-existing species)? Are oomycetes species distribution driven by environmental-geographical factors?

We also subsampled the isolates collected, and we determined the pathogenicity for some isolates of the different species found during the survey, in order to resolve the question: are all the species found pathogenic? We setup a controlled experiment for this purpose, but for the class I am going to focus on the diversity data.

**Methods**

Statistical methods will help me to understand if there is a pattern across the different states sampled (10 states total) to determine what are (if there is any) difference of community structure across the soybean fields on different states, or if there is different factors that affect the community structure of oomycetes promoting a conducive environment for pathogenic species. Therefore methods on multivariate statistics, correlation of diversity with different factors (e.g. temperature, soil moisture, soil type) will provide some basis on the distribution of these species. If those factors are determined, we can also use to better approach to model the presence of this oomycetes (e.g. general linear model using a reduction of dimensions on my diversity data to compare that to different environmental data) and the risks implied with their presence/abundance also considering their environment.

The null hypothesis for my research question should be that the diversity of oomycetes associated to seedling diseases is not different from has been found at smaller scales, being *P. sojae* the most aggressive and prevalent species. In addition a lack of distinction across the fields sampled. If there are not differences, it would not be possible to determine if there are factors associated with distribution of the species, therefore none of them will be correlated to their presence on the different fields.

**Results**

**Appendix**

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