Molecular Identification of *Diaporthe/Phomopsis* Complex Isolates from Soybean

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Beginning

Growing up in rural Indiana, I had agriculture all around me from a young age. Looking at my surroundings, agriculture may have seemed like an obvious career path. However, a career in agriculture was not even on my radar. There were times I wanted to be a doctor, an engineer, an astronaut, and even a football player, but a career in agriculture was never of interest. This remained the case until my freshman year of high school when I met Kraig Bowers, a teacher with a passion for agriculture that changed everything.

In his time at Fairfield, Mr. Bowers had gained a reputation as the kind of teacher you want to have. No matter who I talked to, everyone liked him. I entered high school without a definitive plan for my career, and he was the kind of teacher that I had to try. From my first class, he exceeded every expectation that I had. He introduced me to ideas that I had never thought of before, and every class left me wanting to learn more. I joined FFA, and as I dug deeper into soils judging that fall, I started to feel passionate about agriculture. Soon enough, I became an "ag kid" and was involved in every FFA Career Development Event our chapter offered.

The next year, Mr. Bowers found a new project for my plant and soil science class where each student wrote a paper about food insecurity based on the outline from the World Food Prize Youth Institute. I chose to research Haiti, and as I began my paper, I was amazed at the challenges they face on a regular basis. I learned about so many factors affecting food security, and I wanted to find a solution. I began to feel personally invested in their struggle. From that point forward, I knew I wanted to fight hunger. As we progressed, he approached a few of us about submitting them to the Indiana Youth Institute at Purdue in the spring. When he told us about all this institute offered, he also told us about the Global Youth Institute and the Borlaug-Ruan International Internship. Right away, this became my goal. I was going to qualify for the Global Youth Institute, and I was going to become a Borlaug-Ruan Intern.

Four students including myself represented Fairfield at the Indiana Youth Institute, and when the long-awaited email came, I didn't make it. This was a very humbling experience, and I realized that I still had a lot to learn. I pushed myself to learn more to achieve my goal. The next year, I qualified for the 2016 Global Youth Institute, and I was eager to go to Iowa for what I anticipated would be a once in a lifetime experience.

My time in Iowa was simply incredible. The first day, I saw 200 other students in the same green polo as I was, all ready to expand their knowledge, and all with the same passion for solving food insecurity as I had. I attended educational sessions with some of the brightest minds and most influential figures in the world. The Global Youth Institute further reinforced my inclinations that fighting food insecurity was for me.

After a few months, I received the email I had been anxiously waiting on. I was thrilled to be named a Borlaug-Ruan Intern. As excited as I was, I had no idea what was about to come.

Off to Brazil

When my mom dropped me off at the airport in Chicago, I was understandably nervous about what I was about to do. For the first time in my life, I was on my own, without my parents, a teacher, or even my brother to help me out. As I tried to remember all the advice I had gotten before my trip, I felt somewhat overwhelmed. Fortunately, everyone around me was very friendly and helpful, and with some help, every connection was easier than the last.

After many hours of travel, I finally landed at the airport in Londrina. I was anxious to get my bags and meet my host family, who was scheduled to pick me up right after I landed. I walked out of baggage claim and saw Marcelo and his son Allan standing and waiting for me with a sign bearing my name. I went over to them and said hello, but to my surprise, they did not speak English as well as I expected. I walked with them to the car, and Marcelo talked to me in broken English, assisted by Allan, Google Translate in hand, and explained to me a little bit about the city of Londrina. I did my best to keep a positive attitude, but I was questioning how this would be knowing that my host family didn't speak much English, and I definitely didn't speak much Portuguese.

Aside from the language difference, I was very surprised to see how similar Londrina was to a western city. There were tall, white buildings on either side of a nicely paved highway, very similar to the United States. As we entered my home, I was optimistic that Brazil might not be as different as I anticipated.

My first meal in Brazil consisted of salad, chicken, and rice with feijoada, a Brazilian bean sauce typically put over rice. As I ate, we discussed my travel and first impressions of Brazil. I also learned some new words in Portuguese, which would become a theme at the dinner table in the upcoming weeks.

I had heard about the warmth and kindness of people in Latin America, but I did not expect it to the same level that I got it. Everyone I met was eager to get to know me regardless of how much English they spoke, and even those that spoke none showed their kindness through warm facial expressions and body language.

EMBRAPA Soja and Londrina

The Brazilian Agricultural Research Corporation (EMBRAPA) was created in the spring of 1973 with the goal of "[enabling] research, development, and innovation solutions for the sustainability of agriculture for the benefit of Brazilian society" (Mission), and to that point, the growth in agriculture has helped to facilitate growth in the Brazilian economy. When Embrapa was founded, Brazil was a net importer of food products, and has now grown to the second

largest exporter in the world, behind only the United States. This growth can be largely attributed to the work of 46 Embrapa research institutions across Brazil, each focusing in specialized areas, like Embrapa Soja in Londrina, Paraná.

Paraná's rich soil and more moderate temperatures are particularly conducive to agriculture, which is why it is one of the leading producers of soybeans in the country and the site of Embrapa's soybean division. For many years, Londrina was one of the leading coffee producers in the world, but a series of devastating frosts coupled with an increasingly profitable soybean market led many farmers to turn to soybeans in the late 1950s, with the biggest increase in soybean production beginning in the 1990s. Advancements in soybean technology by Embrapa Soja, especially the introduction of a tropical variety that could be grown in central Brazil, has allowed it to become the second leading producer of soybeans in the world as of 2017 (Dall 'Agnol).

This Embrapa division is broken up into several different departments that each play different roles in supporting soybean production in Brazil, from business-oriented functions to research based departments. With my interest in plant biotechnology, I was assigned to the Plant Biotechnology lab working on a project with doctoral student Everton Ferreira under the direction of Dr. Francismar Correia Marcelino-Guimarães. Given that this project would not last for the entire duration of my internship, I also had the opportunity to assist other researchers to gain a broader research experience and get a better picture of the research that makes Brazil a global leader in soybean production.

Research

In my primary project, I investigated fungal samples obtained from around Brazil to determine if these samples were merely another fungus within the *Diaporthe*/*Phomopsis* complex, or if they were in fact new races of *D. aspalathi* that had broken resistance.

To do this, we extracted the genomic DNA from each isolate, amplified the DNA, then used enzymes to digest it and make fragments of different lengths that are specific to each species. Lastly, we identified these samples with gel electrophoresis, comparing them to previously identified isolates from each variation within the *Diaporthe*/*Phomopsis* complex to determine which one these isolates belonged to.

In addition to my primary assignment, I also had the chance to briefly assist on other projects in the Plant Biotechnology lab. I collected foliar discs from soybean leaves, cut and tagged soybean plants for later testing, and helped to evaluate leaves for symptoms of rust. Additionally, I was able to assist other graduate students with RNA extraction. This project in the lab gave me my most independence, where I was able to use many of the things I had already learned in my main project to my advantage. While unrelated to my project, this experience broadened my horizons

and allowed me to experience other aspects of agricultural research that I may not have otherwise.

During my main project in the lab, I closely observed procedures and practiced with graduate students, and as I gained experience I was able to perform some protocols on my own. This process gave me a great opportunity to learn through observation and controlled practice before practicing on my own, so I was constantly learning and improved at many of these processes very quickly. Additionally, the lab protocols we followed were in Portuguese, which gave me a chance to improve my reading comprehension in a different language as well. While after only two months, I still have plenty of room to improve, I gained invaluable experience that have certainly helped me advance toward the ultimate goal of proficiency in these skills.

Abstract

Among the major fungal diseases that affect soybeans is the *Diaporthe*/*Phomopsis* complex, which is associated with seed decay, pod and stem blight, and stem canker of soybean. The causal organisms are *Phomopsis longicolla* and three species in the *Diaporthe* genus: *D.caulivora*, *D. aspalathi* and *D. phaseolorum var. sojae*. Stem canker disease is caused by the pathogen *Diaporthe aspalathi* and is responsible for significant losses in the yield of soybean cultivated in infested areas in the world, including Brazil. Currently this disease is controlled by using resistant soybean cultivars, however it has a high capacity for damage if there is a break in genetic resistance.

Therefore, it is the important to monitor the possible emergence of new races of pathogen, which can break down the resistance of genes present in the current soybean cultivars. The aim of this study is to determine if the fungi isolates belong to species from the *Diaporthe*/*Phomopsis* complex. The fungi isolates obtained from the fields will be identified using the PCR-RFLP technique. The molecular identification of these isolates is essential to provide a current overview of the genetic diversity of these species in the soybean fields, and will also assist breeders in faster and more accurate cultivar selection.

Introduction

The *Diaporthe*/*Phomopsis* complex is an important group of pathogens in the soybean. This complex is composed of three species of *Diaporthe*: *Diaporthe phaseolorum* var. sojae (pod and stem blight), *Diaporthe aspalathi* and *Diaporthe caulivora* (stem canker) and *Phomopsis longicolla*, which causes seed decay. The most devastating disease within this complex is stem canker. There are two distinct varieties of stem canker: northern stem canker and southern stem canker. The latter is caused by the fungus *Diaporthe aspalathi*, and can cause devastating yield losses due to the severity and dispersion capacity of the disease.

The first symptoms of *D. aspalathi* become apparent in the reproductive stage, where reddish-brown lesions form near the leaf node. As the disease progresses, these lesions expand to form cankers, and may later turn darker in color as cells die (Stem). This necrosis blocks the flow of water through the xylem, leading to cell death in the upper portion of the plant. This causes a reduction in seed size and quality, leading to a reduction in yield. This disease is especially problematic because symptoms do not appear until late in the growing season, so farmers may not recognize their crop has been infected until it is too late to apply fungicide (Jeschke). By this time, farmers have already committed an abundance of time and resources to a crop that will not produce, leading to significant economic losses for the grower.

The identification of *Diaporthe*/*Phomopsis* isolates from soybean has been based on morphological characteristics including color and colony appearance, size and shape of stromata, the ratio and size of α and β conidia and other characteristics. The identification of these organisms has been difficult because the morphological characteristics are often too variable to establish the appropriate taxonomic rank. Due to this, molecular techniques have been used in order to correctly identify the species and study the genetic diversity of the species from this complex.

Furthermore, the results obtained from this study and the results of pathogenic variability studies in the future are of great relevance, considering that most breeders do not have exact knowledge of which stem canker soybean resistance genes are being introduced into the released soybean cultivars. It should be noted that obtaining SNPs (single-nucleotide polymorphisms) for each of the resistant genes will accelerate the process of cultivar selection to this disease, since this selection is currently only performed by phenotyping (and not genotyping). This requires much more time, greenhouse space, and a large specialized team.

Materials and Methods

Fungal Isolates and DNA Extraction.

56 fungal isolates were previously obtained from plants in Brazilian fields showing signs of infection. DNA was extracted from the mycelium samples by freezing samples, then crushed into a fine powder using a mortar, pestle, and liquid nitrogen. This was used to increase the contact surface area of the cells to assist in cell lysis. The samples were incubated for 60 minutes at 65°C, then centrifuged at 6000 rpm for 10 minutes. The liquid phase was transferred to a new tube where an equal volume of chloroform was added, and the two phases were mixed with soft inversions, then centrifuged at 6000 rpm for 15 minutes. Afterward, the upper phase was transferred to another tube, and isopropanol was added (two-thirds the volume of liquid phase). Then the samples were left at -20°C for 2 hours, and later centrifuged at 13,300 rpm (maximum speed) for 10 minutes. Afterward, the upper phase was discarded and 500 µl of ethanol were

added. Then, each pellet was resuspended in the ethanol and the samples were centrifuged again at 13,300 rpm for 5 minutes. Next, the liquid phase was discarded and the pellet was allowed to dry for 40 minutes at room temperature. Then, the pellet was dissolved in 150 µl of TE buffer (10mM Tris-Hcl, 1mM EDTA pH 8.0) and 2 µl of 40 µg/mL *RNAse* A and incubated at 37°C for 45 minutes. Then, DNA integrity was confirmed with gel electrophoresis, using 1% agarose gel with 1X SB buffer and viewed with ethidium bromide under ultraviolet light. In each well was 5 µl of DNA sample and 3 µl of bromophenol blue, and the samples were run for 30 minutes at 100 V. Lastly, DNA concentrations were calculated with a NanoDrop Spectrophotometer in order to dilute the DNA to the correct concentration in the next step.

PCR-RFLP.

A 50 µl PCR mixture was prepared using: 5.0 µl of PCR 10X buffer (100mM Tris-HCl, 500mM KCl), 2.5 µl $MgCl_2$ (50mM), 2.0 µl dNTPs (2.5 mM), 1.0 µl of each the ITS4 and ITS5 primers (10µM of each primer), 3.0 µl of DNA sample (10 ng/uL), 0.3 µl of Taq DNA polymerase, and 35.2 µl of ultra pure water. The reaction was incubated in the thermocycler for: 5 minutes at 95°C initially. Then, 1 minute at 94°C, 1 minute at 56°C and 2 minute cycles at 72°C during 35 cyles; followed by a final cycle at 72°C for 7 minutes. This process amplified the ITS1, 5.8S rDNA, and ITS2 regions, the region used to determine the species of each isolate. The amplified fragments were separated in a 1% agarose gel with 1X SB buffer and visualized with ethidium bromide under ultraviolet light.

The PCR products obtained were then digested with *Alu* I and *Rsa* I restriction enzymes. The same protocol was used for both enzymes. The reaction included 2.0 µl of 10X reaction buffer, 0.3 µl of 10U/µl *Alu* I enzyme, 7.7 µl of ultrapure water, and 10.0 µl PCR product in each tube. This 20 µl mixture was kept for 3 hours at 37 °C.

Lastly, gel electrophoresis was performed and the resulting bands (DNA fragments) were compared to a DNA ladder and six control samples to determine the species of each sample.

Results

The following are the results from the digestion with the two restriction enzymes. After digestion by the *Alu* I, most of the isolates were found to be *D. phaselorum*, *D. phaselorum* var. *sojae* or *P. longicolla*, since the *Alu* I enzyme is able to separate only *D. caulivora* from *D. aspalathi.* Samples 1803 (Figure 1) and 1868 (Figure 3) were not identified as any species of the complex because they showed different DNA fragment patterns than expected. In figure 2, however, samples 1890, 1891 and 1892 were identified as *D. aspalathi*.

No isolates were characterized as *D. caulivora*. Despite this, the sample 1186 previously characterized as *D. caulivora* showed a different band pattern than expected for this species, possibly this isolate was identified erroneously. Isolate 513 was previously identified as *D. caulivora* by both PCR-RFLP and by the sequencing of the ITS1, 5,8S rDNA and ITS2 region (BRUMER, 2016).

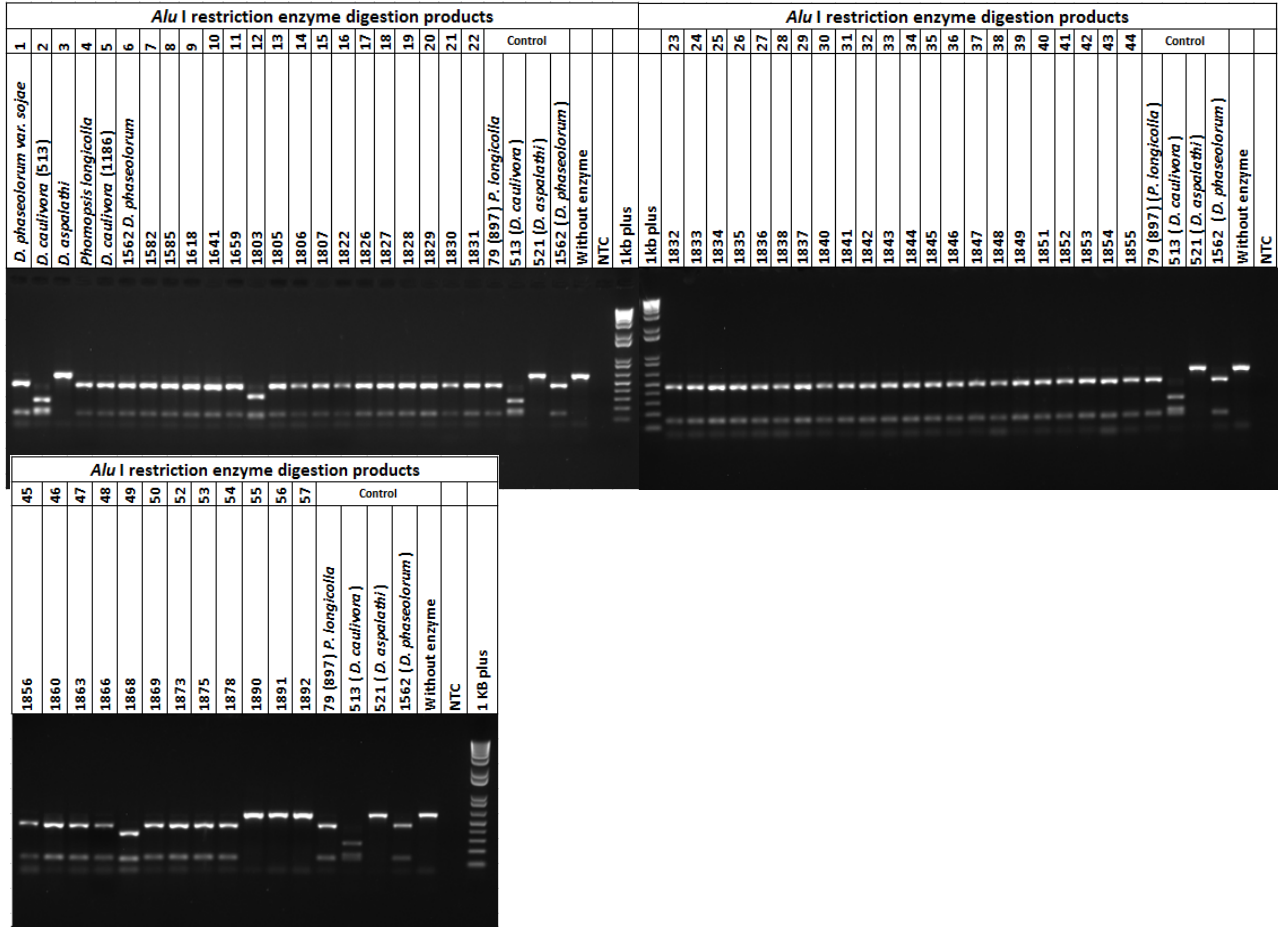

Figure 1. Restriction patterns of amplified DNA fragments of the ITS region with *Alu* I enzyme

Figure 2. Restriction patterns of amplified DNA fragments of the ITS region with *Alu* I enzyme

In order to separate *P. longicolla* from the *D. phaseolorum* var *sojae* and *D. phaseolorum,* the digestion with *Rsa* I enzyme was performed. As shown in Figure 3 and Figure 4, most of the

remaining samples were identified as *P. longicolla*. It should be noted that the *Rsa* I cannot differentiate between *D. phaseolorum* var *sojae* and *D. phaseolorum.*

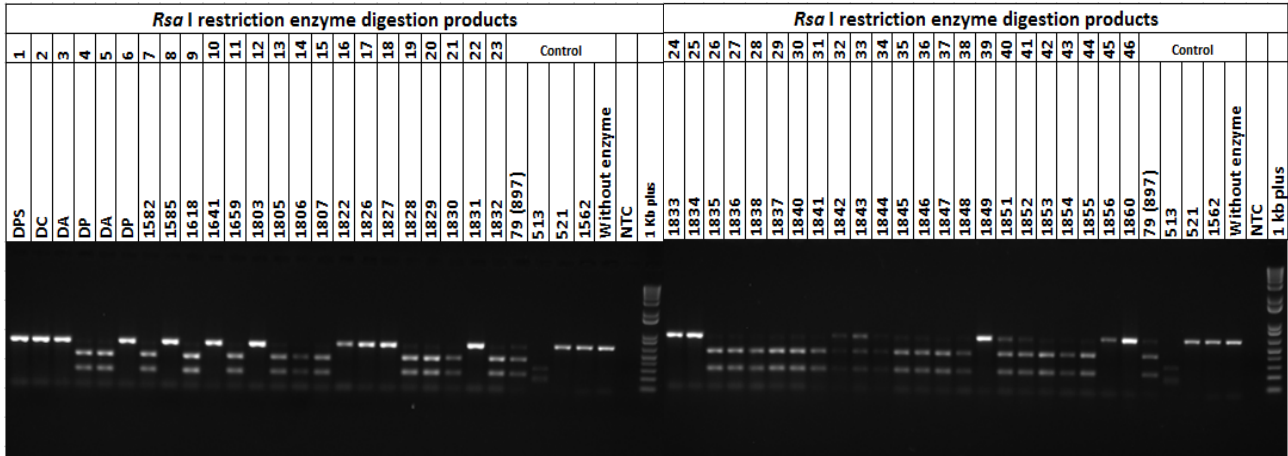

Figure 3. Restriction patterns of amplified DNA fragments of the ITS region with *Rsa* I enzyme

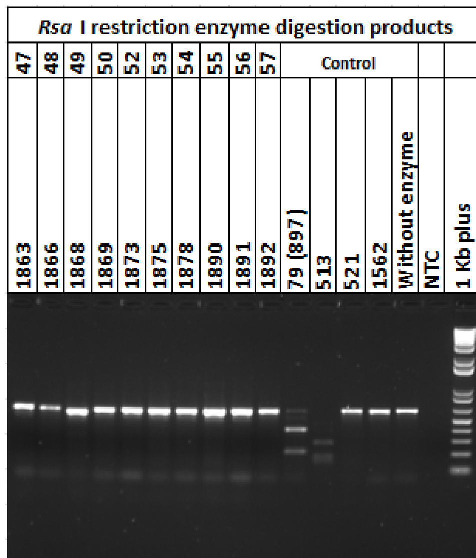

Figure 4. Restriction patterns of amplified DNA fragments of the ITS region with *Rsa* I enzyme

Table 1. List of isolates belonging to the *Diaporthe/Phomopsis* complex used in this study, with its respective collection date, location and type of sample from which the pathogen was isolated.

SAMPLE ID	PCR-RFLP IDENTIFICATION	DATE COLLECTED (DD/MM/YYYY)	STATE	SAMPLE LOCATION
CMES 508	*D. phaseolorum var.sojae*	25/05/2007	MA	SEED
CMES 513	*D. caulivora*	29/07/2007	RS	STEM
CMES 521	*D. aspalathi*	01/08/2003	PR	STEM
CMES 897	*Phomopsis longicolla*	-	RR	STEM
CMES 1186	*D. caulivora*	-	-	-
CMES 1562	*D. phaseolorum*	04/11/2014	PR	SEED
CMES 1582	*Phomopsis longicolla*	08/09/2015	PR	ROOT
CMES 1618	*Phomopsis longicolla*	16/02/2016	PR	POD
CMES 1641	*D. phaseolorum/ D. phaseolorum* var.*sojae*	-	RS	STEM
CMES 1659	*Phomopsis longicolla*	01/03/2016	MT	ROOT
CMES 1803	*D. phaseolorum/ D. phaseolorum* var.*sojae*	24/01/2017	SP	STEM
CMES 1805	*Phomopsis longicolla*	30/01/2017	-	STEM
CMES 1806	*Phomopsis longicolla*	30/01/2017	-	STEM
CMES 1807	*Phomopsis longicolla*	30/01/2017	-	STEM
CMES 1822	*D. phaseolorum/ D. phaseolorum* var.*sojae*	22/02/2017	PR	STEM
CMES 1826	*D. phaseolorum/ D. phaseolorum* var.*sojae*	22/02/2017	PR	STEM
CMES 1827	*D. phaseolorum/ D. phaseolorum* var.*sojae*	02/03/2017	MT	STEM
CMES 1828	*Phomopsis longicolla*	09/03/2017	PR	STEM
CMES 1829	*Phomopsis longicolla*	09/03/2017	PR	STEM
CMES 1830	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1831	*D. phaseolorum/ D. phaseolorum* var.*sojae*	16/02/2017	MT	STEM
CMES 1832	*Phomopsis longicolla*	17/02/2017	MT	STEM
CMES 1833	*D. phaseolorum/ D. phaseolorum* var.*sojae*	16/02/2017	MT	STEM
CMES 1834	*D. phaseolorum/ D. phaseolorum* var.*sojae*	17/02/2017	MT	STEM
CMES 1835	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1836	*Phomopsis longicolla*	18/02/2017	MT	STEM
CMES 1837	*Phomopsis longicolla*	18/02/2017	MT	STEM
CMES 1838	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1839	*Phomopsis longicolla*	17/02/2017	MT	STEM
CMES 1840	*Phomopsis longicolla*	17/02/2017	MT	STEM
CMES 1841	*Phomopsis longicolla*	17/02/2017	MT	STEM
CMES 1842	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1843	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1844	*Phomopsis longicolla*	16/02/2017	MT	STEM

CMES 1845	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1846	*Phomopsis longicolla*	08/02/2017	MT	STEM
CMES 1847	*Phomopsis longicolla*	08/02/2017	MT	STEM
CMES 1848	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1849	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1850	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1851	*Phomopsis longicolla*	16/02/2017	MT	STEM
CMES 1852	*Phomopsis longicolla*	18/02/2017	MT	STEM
CMES 1853	*Phomopsis longicolla*	07/02/2017	MT	STEM
CMES 1854	*Phomopsis longicolla*	25/01/2017	MT	STEM
CMES 1855	*D. phaseolorum/ D. phaseolorum* var.*sojae*	25/01/2017	MT	STEM
CMES 1856	*D. phaseolorum/ D. phaseolorum* var.*sojae*	08/02/2017	MT	STEM
CMES 1860	*D. phaseolorum/ D. phaseolorum* var.*sojae*	22/02/2017	PR	POD
CMES 1863	*D. phaseolorum/ D. phaseolorum* var.*sojae*	10/02/2017	PR	ROOT
CMES 1866	*D. phaseolorum/ D. phaseolorum* var.*sojae*	16/03/2017	PA	STEM
CMES 1868	*D. phaseolorum/ D. phaseolorum* var.*sojae*	16/03/2017	PA	STEM
CMES 1869	*D. phaseolorum/ D. phaseolorum* var.*sojae*	16/03/2017	PA	STEM
CMES 1870	Could not amplify	27/03/2017	RS	STEM
CMES 1873	*D. phaseolorum/ D. phaseolorum* var.*sojae*	-	TO	STEM
CMES 1875	*D. phaseolorum/ D. phaseolorum* var.*sojae*	-	TO	STEM
CMES 1878	*D. phaseolorum/ D. phaseolorum* var.*sojae*	-	TO	STEM
CMES 1883	*D. phaseolorum/ D. phaseolorum* var.*sojae*	-	TO	STEM
CMES 1885	*D. phaseolorum/ D. phaseolorum* var.*sojae*	10/04/2017	PR	STEM
CMES 1890	*D. aspalathi*	-	GO	STEM
CMES 1891	*D. aspalathi*	-	PR	STEM
CMES 1892	*D. aspalathi*	-	GO	STEM

*CMES: Collection of Microorganisms of Soybean – Embrapa.

Discussion

Our results show that three samples from two different states are *D. aspalathi*, as seen in Table 1. This suggests that there may have been a break in resistance in the soybean cultivars from these fields, but more experiments need to be conducted to confirm this. It was also observed that no isolates belonged to the *D. caulivora* species. This result was expected, considering that this species is adapted to the climatic conditions of the United States, temperatures colder than those of Brazilian soils. Until the moment, the abundant occurrence of this species has not yet been reported in other countries.

The occurrence of other species from complex was expected, but these species are responsible for other diseases, such as seed decay and pod and stem blight. Despite this, of the 56 samples, only three were found to be *D. aspalathi*, showing that resistance in the soybean cultivars is still strong in Brazil as a whole.

Future stages of this study will include pathogen diversity characterization of the *D. aspalathi* identified in PCR-RFLP, as well the sequencing of the ITS1, 5,8S rDNA and ITS2 region to confirm the species obtained. The pathogen diversity characterization will be performed with the soybean genotypes, each one with a different resistance gene, in order to observe if the 1890, 1891 and 1892 could be able to break down the resistance genes.

The results obtained from the pathogen diversity characterization with the new isolated is very important to the developing of SNPs markers. In the future, all these results led to the development of specific SNPs for each resistance gene, as well as helping in the search the new sources of resistance. With this, accelerating the process of selection assisted by markers, as well as the introgression of different genes in the cultivars, resulting in the end, in the release of new cultivars more resistant, in case the resistance break occurs.

Impact on Food Security

While it is currently well controlled around Brazil, a widespread break in resistance could be potentially catastrophic to the soybean industry. By monitoring genetic diversity of these pathogens, breeders can develop new resistant cultivars before the disease can expand. The scope of this research does not only apply to Brazil, but it can have an impact on the rest of the world as well. In order to appreciate the significance of this research, it is necessary to consider the importance of the soybean to the global food supply, Brazil's role in soybean production, and present and future demand for soybean products.

The leading use for soybeans worldwide is in animal production. Due to its high crude protein content, soybean meal is a main ingredient in many feedstuffs. Soybean can also be used for direct human consumption, particularly in Eastern Asia in the form of popular foods like tofu, and is also a popular plant-based protein source in Western countries, although this represents a significantly smaller share of the market. Soybeans also have a wide variety of industrial applications, and are also a popular source of biodiesel as many countries search for alternatives to fossil fuels.

Soybean products are of the utmost importance in the global economy, as soybean products are the most traded agricultural commodity in the world, accounting for 10% of global agricultural trade. Brazil combines with the United States to account for 80% of the total soybean exports

worldwide, and is anticipated to increase exports by 35% in the next decade, increasing its already world-pacing volume to over 76 million tons by 2026 (Lee, Tran, Hansen, & Ash, 2016). As previously referenced, the *Diaporthe*/*Phomopsis* complex could have a potentially devastating impact on the Brazilian soybean industry. Given that their large share of the global market continues to grow, it is imperative that diseases like this are kept under control to mitigate detrimental effects that would be felt worldwide.

While the soybean is already among the most important commodities worldwide, its importance figures to only increase in the future with demand for animal protein expected to increase. This is caused by the change in consumer preferences driven by a growing middle class in many developing countries. Identifying pathogens like those of the *Diaporthe*/*Phomopsis* complex is the first step in developing plants with greater resistance, and in turn reducing chemical application to satisfy this demand in a more efficient and sustainable manner.

Personal Growth

In the Lab

Before my internship, I had relatively limited lab experience. Some basic experience in high school provided a framework of skills to build off of, but there were still many things that I did not know, which left ample room for improvement. I learned how to work in a lab – from learning how to use tools to carrying myself in a responsible and professional way. I also learned how to perform basic lab procedures that I will use in the future.

I also expanded my knowledge about fungi by reading literature on the topic. In high school I developed a strong base of knowledge about plants, but I had limited knowledge of fungi. To do this, I had to learn how to read scientific papers. Even after I am finished working with fungi, I will use the ability to learn through scientific literature in the future.

Working in the lab also gave me the opportunity to become more patient. I have the tendency to work very fast to accomplish as much as I can in a short period of time, but I realized in science that does not always work. In one particular instance, I was adding ethanol to our samples during DNA extraction, and as my mentor was moving faster than me, I tried to keep up. As I tried to do everything just a little faster, I lost track of what sample I was on, and I accidentally added ethanol to the same sample twice. Although we were still able to use the sample, this served as a great lesson for me about patience in the lab.

Outside the Lab

There were many different aspects of this internship that combined for a completely transformative experience. One change was my growth in responsibility and organization. Suddenly I was in a foreign country all by myself, and I had no other option but to be organized. As soon as my mom dropped me off at the airport I realized this, and became obsessed with

planning and details. I moved through the airport with a purpose, got to my terminal as soon as I could, and waited, continually checking the monitor for gate changes. On my final flight, I received a paper from Brazilian immigration that I had to give to them to leave the country in 2 months. When I realized that I had to write things down, keep things tidy, and keep track of my belongings, I buckled down and rose to the occasion.

Communication was another area of growth for me. When I arrived, I spoke almost no Portuguese, and as I arrived expecting near-fluent English from my host family, I was surprised to find out that was not the case. Looking back, that was the best thing that could have happened. For the first few weeks, I had to learn to rely on context from the words that I did know, and I also had to pay more attention to nonverbal communication than I ever had before. This let me become more in tune with others' emotions, while also being forced to recognize the emotions I was conveying through my expressions and tone.

I also became more open to new adventures. One thing that took a lot of courage was ordering for myself at a restaurant. I knew enough words to get by, but to actually pronounce them correctly and execute was a completely different story. I tried to order by myself at a Burger King my second week, and I said the words as confidently as I could, but the cashier still did not understand. Although I laughed it off on the outside, as I stood at the front of a long line of Brazilians pointing out what I wanted on a placemat, I was mortified. Fortunately, I had people around me that spoke English and were able to help me with everything I needed. As I began to reflect on my experience, I realized that is not always the case. When people immigrate to the United States and do not speak English well, it is easy to criticize them for not learning to speak it before they came. While I do believe it is wise to do so, I realized that some people immigrate do not have the luxury of time on their side. I became a lot more sympathetic for those who are in similar situations.

Views on Food Security

When I got accepted to this internship, a part of me expected to come right in and change the world. I had no doubts, I was the next Norm. I had always been talented, I am passionate about solving food insecurity, so why not? When I first arrived at Embrapa, I was in love with the lab. I love science, and when I get to do something that serves a purpose in the world, it feels all the more rewarding.

When I began reading reports from other interns, I was surprised to find myself becoming somewhat disappointed, and this was a feeling I wrestled with for quite some time. I read all of these amazing stories about former interns that saw poverty first hand, meeting people that were hungry, and making an impact on their lives. I wondered, "*Am I doing enough? Is what I'm doing really going to help? How am I going to be the next Norm if I'm in a lab all day?"*

Among the sources for my answer was a quote from none other than Norman Borlaug. He said, "I am but one member of a vast team made up of many organizations, officials, thousands of scientists, and millions of farmers - mostly small and humble - who for many years have been fighting a quiet, oftentimes losing war on the food production front."

Food insecurity isn't going to be solved by just one person. It is a massive problem that will require many people dedicating themselves to solving it. People looking to do this job for individual recognition will not succeed, and more importantly, without selflessness and cooperation, the world will never be food secure. In order to truly succeed, we have to check our egos at the door and be willing to accept the fact that we are not going to solve this problem alone.

I believe that is exactly what the World Food Prize youth programs are doing. We're not looking for THE next Norm, we're looking for lots of them. Farmers, humanitarians, scientists, government leaders, healthcare professionals, educators, and even more job titles than I could possibly name each have a role to play. It's our job as individuals to find our role and perform it to the best of our ability. And if we work to do our best while working together, someday we may realize the dream of a food secure world.

Different ≠ less

I also realized the importance of communication in solving food insecurity. When I arrived, I spoke very little Portuguese, and tried to rely on three years of high school Spanish to get by. As I tried to learn more, I was very reliant on body language, tone, and context. While many people I met spoke at least some English, there were some situations where I had to listen in Portuguese. Naturally, I perceived those who spoke more English as more intelligent than those who did not. I also believe the opposite was true, where on some level I was perceived by some as less intelligent because I did not speak Portuguese.

When I was immersed in Brazilian culture, it became more to me than the surface level comparisons to the United States. Brazilians are warm, kind-hearted people, and even if we don't speak the same language, that's okay. I also believe there is a difference between patriotism and feeling that your country is better than others. You can be proud of your country, for all the work they have done, and you can be proud of the people that fought so hard to defend it. But at the same time, your country is not better than other countries just because it is. Different does not equal less.

Situations like this made me realize an important fact about solving food insecurity. If we try to approach international problems from our own perspectives without putting ourselves in their shoes, then we lose our credibility and our solution cannot work. We can't approach this from the perspective of a developed nation solving problems for an undeveloped nation, that

perspective inherently implies a feeling of superiority. However, if we go in with the intention of working together with locals, we can be much more effective.

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Photos

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