Soybeans and Solutions

My Brazilian journey as a Borlaug-Ruan International Intern

Tara Mittelberg
The World Food Prize
Borlaug-Ruan International Internship
Embrapa Soja, Londrina Brazil
July-August 2013

Embrapa

THE WORLD FOOD PRIZE

Table of Contents

An Incredible Journey

At the orientation preceding the Borlaug-Ruan International Interns' departure, Ambassador Quinn advised our parents to hold a conversation with us before we left for our internship, because when we returned we would be different people. This prediction couldn't have been closer to the truth. My Brazilian experience changed me for the better in countless ways, from teaching me lab techniques to giving me the maturity and confidence necessary for a smooth transition into college. I attained countless irreplaceable friendships and expanded my views on issues of global and local scales. Most importantly, I discovered a passion for food security, giving me direction for my future. The following is an account of this transition, from naïve student of AP Biology to passionate fighter of hunger.

My Background: AP Biology, Norman Borlaug, and the Global Youth Institute

I never thought I would be interested in biology. In fact, until my sophomore year in high school, I vehemently opposed the idea of pursuing any type of scientific career. When I reluctantly signed up for AP Biology, I saw it only as a means of satisfying my school's expectation that its students take as many Advanced Placement courses as possible.

Despite my initial diversion, however, signing up for AP Biology became one of the most influential decisions of my life. One day, my teacher Mr. Horton brought up a name that sparked my interest. He explained the legacy of Norman Borlaug, the man who had genetically engineered high-yielding crop varieties and initiated the Green Revolution. Most remarkably, Dr. Borlaug's work in agronomy led him to be credited with saving over one billion lives. Before this, I had never realized the global applications of biology. To me, Dr. Borlaug's legacy represented the type of science I wanted to be involved with – a science that advanced human society from the bottom up.

Although we learned of many more scientists that year in AP Biology, none intrigued me as much as Dr. Borlaug. The thought of his work lingered in my mind until the following spring, when I received the opportunity to represent my high school at the 2012 World Food Prize Iowa Youth Institute. At this event, I was inspired by dozens of leaders, scientists, and high school students, all of whom shared a passion for solving food insecurity. By the time of the Global Youth Institute that October, I too had developed a strong interest in the plight against world hunger.

For me, there was never a question as to whether I would apply for the Borlaug-Ruan International Internship. My love for science, interest in international development, and consideration of pursuing a career in research made it seem like the perfect opportunity. As I mailed in my application in mid-December, nothing could have prepared me for what lied ahead.

Preparing for the Trip

In March 2013, when I first found out I was headed for Brazil, I was overwhelmed with excitement. Since the Global Youth Institute, the only thing on my mind was the thought of expanding my knowledge and making an impact in the field of solving food insecurity. In May, my excitement multiplied after I attended the Borlaug-Ruan International Intern orientation and learned more about my research and host family. I had known since sophomore year that this was the type of opportunity with which I wanted to fill the summers of my college years. To finally be on track to go abroad and work towards a meaningful cause was unreal.

However, as the trip neared, I began to grow anxious. What if I couldn't communicate with anyone? Would cultural differences get in the way of forming a close relationship with my host family? Was I capable of succeeding in a biotechnology lab despite the fact that I hadn't taken a biology course in over two years? How would I cope without my family for two months? All of these thoughts circulated through my mind as I hugged my mother and sister goodbye and stepped into line at security at the Des Moines airport. For the first time in my life, I was truly on my own.

First Impressions: a Warm Welcome

After 24 hours of travel, I touched down in Londrina, Brazil. The plane crew herded us toward the baggage claim area, where I collected my luggage and walked toward the double doors separating the plane passengers from the line of their waiting loved ones. I took a deep breath, crossed the threshold, and thus began my Brazilian life.

Immediately, I was enthusiastically hugged and side-kissed by a middle-aged blonde woman and a teenage boy. They introduced themselves as Soelma and Heitor: my new host mother and brother. On the way home to their apartment, I was surprised by the modern metropolitan nature of Londrina. Although there seemed to be many construction projects, the city's infrastructure and tall buildings seemed similar to those of a mid-sized Western city.

Soon we arrived at their building and took the elevator to their tenth floor apartment. I was introduced to Soelma's mom, and was surprised by another hug and side kiss. After this encounter, the grandmother muttered something in Portuguese and Soelma informed me that I needed to work on my introductions. For dinner, Soelma made spaghetti, salad, and a dessert of strawberries and sweet condensed milk. We discussed our backgrounds and various aspects of American and Brazilian culture. I was happy to discover that their entire family had spent a year living in the United States, so they were familiar with English and American customs. They were eager to host me because they said they wanted to somehow reciprocate the kindness shown to them during their time in the United States.

This welcome was only my first glimpse into the compassion and warmth of the Brazilian people. From this day forward, I would be constantly amazed by how openly I was received by strangers and friends alike. This greatly reduced the stress of adjusting to a new culture, allowing me to devote myself entirely to the true purpose of my internship: soybeans.

Embrapa Soja, Londrina, Brazil

The Brazilian Enterprise for Agricultural Research (Embrapa) is a Ministry of Agriculture-affiliated institute dedicated to increasing the availability of food in Brazil and around the world. It has 38 research institutions across Brazil, each focusing on a particular crop or agronomic sector. Embapa Soja, located in Londrina in the Southern state of Parana, is devoted to the research and cultivation of soybeans.

It was here, at Embrapa Soja's Biotechnology Vegetable Lab, that I spent my Borlaug-Ruan summer internship. Comprised mostly of students, the people in my lab focused their work on the molecular biology, genetics, and bioinformatics of plants. Although these were the areas in which my internship was focused, Embrapa Soja has many other labs for the study of bacteria, soil, seeds, parasites, and more. In total, the main campus contains 29 laboratories and 28 greenhouses, plus various administrative, educational, and support buildings.

Surrounding the campus are hundreds of acres of fields devoted to agricultural study. Although Embrapa Soja's main focus is the cultivation of soybeans, in the winter the institute uses its fields to grow wheat, maize, coffee, sugarcane, oats, palmetto, and various fruits. At first I was curious as to why they didn't utilize their fields to plant winter soybean varieties to prolong their research season – their name, "soja" means soy, after all. It wasn't until the last week of my internship that my mentor informed me that it is illegal to plant soybeans in Parana state during some winter months. The reason? Well, that's where my project comes in.

Research

Asian soybean rust caused by the fungus *Phakopsora pachyrhizi* is one of the most devastating agricultural diseases in Brazil. It is spread rapidly by wind and can wipe out an entire field in days (Goellner, *et al* 2010). Because of North America's cold winters, the disease has had only minor effects in the United States; however, it is particularly potent in Brazil's tropical and semi-tropical climate. In fact, because Brazil's mild winter isn't enough to naturally stave off rust pathogens, most soybean producing states enforce a 90-day soybean free period between June 15 and September 15 (USDA 2012).

Despite the wide range of Brazilian soybean rust breeds, currently little is known about the genetic diversity among various *P. pachyrhizi* isolates. This makes it incredibly difficult for breeders to engineer rust resistant soybean plants. Although agronomists have discovered multiple genes for rust resistance, without knowledge of *P. pachyrhizi* isolates' unique genomes, it is impossible to implement them in soybean genomes.

I spent my Borlaug-Ruan Summer Internship doing research on soybean rust isolates at Embrapa Soja under the direction of Dr. Francismar Correa Marcelino Guimaraes and Ph.D. student Luana Darben. Dr. Marcelino Guimaraes completed most of her doctorate in Brazil and also spent one year studying at Iowa State University. Her primary focus at Embrapa was on soybean diseases including nematodes and soybean rust. Under Dr. Marcelino Guimaraes' direction, Luana's main project was to molecularly characterize the ARF, ITS1, and ITS2 regions of the genomes of 32 *P. pachyrhizi* isolates from across Brazil. After obtaining the sequences, she plans to analyze them to discover the similarities and differences between isolates. With this information, soybean breeders will be able to create broadly resistant crops.

My individual project was to obtain the sequence of the ITS2 genomic region of one of Luana's isolates, L. UB112. I was assigned to this task for several reasons. For one, because I would be focusing on only one sample, I would have time to help Luana and other students of the Biotechnology Vegetable Lab with additional projects. This would give me the opportunity to learn about many different aspects of molecular biology and soybean research. Secondly, the basic processes for DNA extraction, amplification, cloning, sequencing, and analysis that I would complete are vital to research labs everywhere. By learning these techniques, I would be able to utilize my skills in hundreds of molecular biology labs around the world. Most importantly, soybean rust is one of the biggest threats to stable soybean production in tropical regions, making it an important issue in battling food insecurity.

For the first few weeks of my internship, I did not work directly with my sample, and instead helped Luana as she prepared other *P. pachyrhizi* isolates for sequencing. Although I had no previous lab experience, I quickly learned to prepare solutions for PCR, make agarose gel, apply samples for electrophoresis, purify the PCR product, and clone the DNA sequence using bacterial recombinant plasmids. I was surprised by how readily I was entrusted with lab equipment and chemicals. By my third day at Embrapa, I was pipetting solution containing the expensive Taq enzyme and applying samples to the ghostly wells of agarose gel. Previously, these had been procedures of textbooks and experienced lab technicians – distant, elusive processes that I had only dreamed of performing myself.

By the second week of my internship I discovered something disappointing – science isn't perfect. Luana and I would often spend days repeatedly preparing and performing PCR, only for our samples to fail to amplify. One day, as I was preparing

the PCR solution for the third or fourth time, I made a mistake with my pipetting. Instead of adding 2.5 µl of Taq enzyme to a universal solution, which would later be added to each of the eight samples I was working with, I began putting that amount of the concentrated enzyme in every sample. By the time I realized my mistake, I had already added it to four of the eight samples and was certain I had caused Luana's experiment to fail once again. Because there was little we could do to fix the situation, Luana said we would still try to amplify the DNA in hopes that the other four samples were unaffected. After subjecting the samples to PCR, the gel revealed that only four samples had amplified, and surprisingly, they were the four samples to which I had added too much enzyme. Unfortunately, we made a mistake in the next step and had to start over once again, but this time we used a higher concentration of Taq enzyme and began seeing better results. This experience not only taught me to be more cautious as I measured out solution, but also of the unpredictability of scientific procedures.

In addition to helping Luana, I also got the chance to work on the projects of other students of the Biotechnology Vegetable Lab. For example, I spent several afternoons planting and tending to Arabidopsis seeds in preparation for bacterial transformation with graduate student Juliana Marcolino. I learned the complexities of keeping track of an experiment involving hundreds of samples by helping Juliane Marinho with her gene expression and soybean stress project. I even expanded my knowledge of soybean diseases by shadowing Valeria Lopez Caitar as she and her lab assistants counted hundreds of nematodes infecting soybean roots. Although unrelated to my project, these experiences were incredibly valuable to my internship as a whole. They widened my views on molecular biology and scientific research, showing me the expanse of possibilities for advancing agriculture.

By the fifth week of my internship, I had gained enough lab experience to begin to prepare my own sample for sequencing. Although Luana was there if I encountered any major problems, I was largely able to carry out my procedure independently. The following is a presentation of the procedure, results, and analysis of my individual project: molecularly characterizing the ITS2 region of the ribosomal DNA of *P. pachyrhizi* isolate L. UB112.

Abstract

Although it has yet to cause severe losses in the United States, Asian soybean rust is a devastating disease in Brazil and many other tropical regions. Currently little is known of the genetic diversity of the rust-causing pathogen *Phakopsora pachyrhizi*, making it difficult for agronomists to create widely resistant soybean breeds. The purpose of this experiment is to molecularly characterize the ITS2 region of *P. pachyrhizi* isolate L. UB112. After completing the study, it was found that this nucleotide sequence is identical to that of the ITS regions of several other *P. pachyrhizi* isolates collected from regions ranging from Africa to Southeast Asia. This is an indication of the volatility and widely dispersed nature of the pathogen.

The results from this study will be compared to 31 other isolates collected from various regions in Brazil to help establish the range of diversity among *P. pachyrhizi* isolates. This will allow plant scientists to more effectively implement strategies for combatting Asian soybean rust. This could have significant impact on food security by expanding soybean yields and increasing smallholder enthusiasm for the cultivation of soybeans.

Introduction

Soybean rust caused by the fungus *Phakospora pachyrhizi* causes significant yield losses globally. Originating in Japan, P. pachyrhizi is rapidly dispersed by wind and was first reported in Brazil in the early twentieth century (Freire, *et al* 2008). Asian soybean rust derives its name from the orange rust-like lesions and uredosori that form on its host's leaves (Figure 1). It is a biotrophic fungus, meaning its microscopic haustoria penetrate the host's leaves, allowing *P. pachyrhizi* to survive off its living cells. It requires warmth and moisture, which makes soybean rust a particularly potent disease in Brazil's tropical and semi-tropical climates. It is found in all soybean producing areas of the country, and caused yield losses equal to US$2 billion in 2002-2003 alone (Goellner, *et al* 2010).

Figure 1. Soybean leaf infected by *Phakopsora pachyrhizi*

Currently, six genes for soybean rust resistance have been identified including Rpp1, Rpp1b, Rpp2, Rpp3, Rpp4, and Rpp5. Despite this knowledge, these genes are impossible to implement in soybean plants because little is known of the genetic diversity of *P. pachyrhizi* (Zhang *et al*., 2012). The purpose of this experiment is to molecularly characterize the ITS2 region of the genome of the *P. pachyrhizi* isolate L. UB112. This sample was collected in 2012 from Uberlândia, Minas Gerais state in east-central Brazil. It is part of a larger study of 32 *P. pachyrhizi* isolates currently being conducted by Dr. Francismar Correa Marcelino Guimaraes and Ph.D. student Luana Darben. Eventually the findings of this experiment will be used in diversity studies to develop broadly resistant soybean breeds.

The internal transcribed spacer (ITS) regions of fungal nuclear ribosomal DNA are particularly useful for studying genetic diversity. They are easily amplified and can be replicated with several universal primers. Additionally, the ITS regions are non-functional genes. This means that they are not affected by evolutionary pressure and thus nucleotide sequences vary greatly even among closely related species (Gardes, *et al* 1993).

Analysis of the genetic diversity of various *P. pachyrhizi* isolates is necessary for the eventual engineering of broadly resistant soybean breeds. The objective of this experiment is to further knowledge of the genetic makeup of *P. pachyrhizi*, which will eventually lead to advances in creating rust-resistant soybean breeds.

Explanation of Materials and Methods

DNA Extraction: P. pachyrhizi spores were transferred to a microtube, crushed with diatomaceous, and centrifuged. This caused cell lysis, exposing the DNA. An extraction buffer was added and the mixture was centrifuged. The buffer ridded the cells of their non-organelle components and broke open the nuclei. Next, chloroform was added (to further aid in cell lysis) and centrifuged. Ethanol and isopropanol were used to aid in DNA precipitation. Sodium acetate aided in this process by increasing the DNA's ionic strength. Lastly, we RNAse was added to degrade the RNA molecules.

PCR: The sample was diluted to 10ng/uL of DNA and then prepared it for PCR with several reagents. $MgCl_2$ was used because magnesium is a cofactor in the polymerase reaction. dNTPs provided bases so the DNA could properly replicate. The Forward and Reverse primers marked where the DNA should start replicating on the sense and anti-sense strands. The Taq enzyme is a heat-tolerant DNA polymerase used to build DNA molecules. A buffer was also added to ensure optimal chemical conditions for PCR. In the denaturation step of PCR, the reagents were heated to 94 degrees Celsius for 5 minutes. In this step, the double-stranded DNA split into two complementary strands. In the annealing step (58 degrees Celsius for 1 minute), primers attached to the DNA template. These primers provided a place for the Taq polymerase to bind in the elongation step (72 degrees Celsius for 1 minute). After binding, the enzyme added dNTPs to the two strands in the 5' to 3' direction. Denaturation, annealing and elongation were repeated for 35 cycles. Finally the samples underwent a final elongation step performed at 72 degrees Celsius for 5 minutes to ensure any remaining single-stranded DNA fully extended.

Electrophoresis: After PCR, the samples were tested for amplification using agarose gel electrophoresis (Figure 2). In this process, samples were added to the wells of a gel and subjected it to an electrical current. Because DNA has a negative charge, it was pulled toward the positive electrical current. Strands of different length traveled different distances because shorter strands can more easily pass through the gel's mesh-like molecular structure. The sample was compared to a ladder to ensure it was approximately the expected 750 base pairs in length.

PCR Purification: PCR Purification of the single amplified DNA fragment was completed using the Promega PCR Purification kit. First, the PCR product was added to a column containing a silica resin and a Membrane Binding Solution. After centrifugation, all but the DNA was forced through the resin. The remaining liquid containing proteins, nucleotides, and other non-DNA reagents was discarded. Membrane Wash Solution was then added to the column. Centrifugation forced the solution through the membrane, washing away remaining non-DNA components. Finally, water was added to the column and subjected to centrifugation. The water eluted the DNA from the membrane, forming a purified DNA solution.

Cloning: DNA was added to a solution containing a vector and NaCl, which helped carry the electric current in the electrocompetent cell. This solution was refrigerated overnight to allow the DNA to bind to the vector, forming a recombinant plasmid.

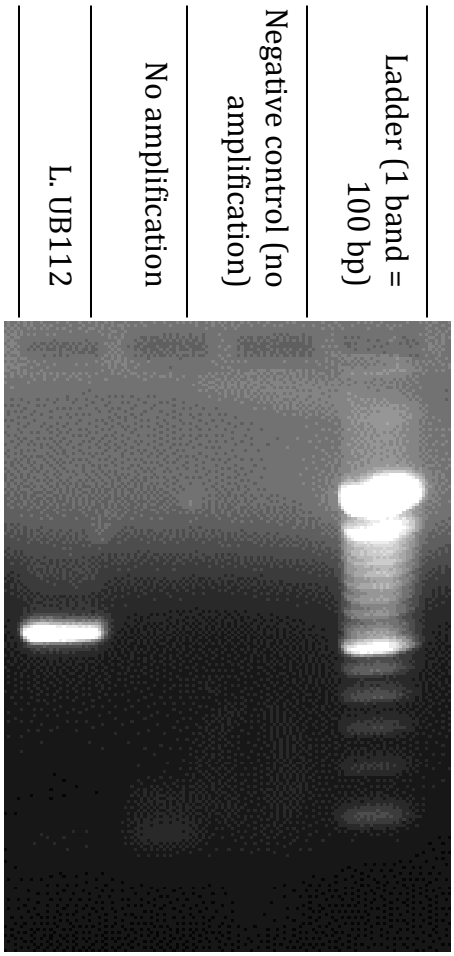


Figure 2. Electrophoresis of PCR Product.

After overnight refrigeration of the plasmid, *Escherichia coli* bacteria were subjected to transformation by electroporation. In this step, the bacteria were exposed to an electric current, which opened the pores in their cell walls. This allowed the recombinant plasmid to enter the cells and become part of the bacterial genome.

The bacteria were then cultured on a plate with an agar and ampicillin or kanamycin medium plus X-GAL/IPTG. Because the vector contained a gene for antibiotic resistance, only transformed bacteria were able to grow on the plate. Additionally, positive colonies were selected using the blue and white screen method. When the DNA insert was added to the vector, it disrupted a gene for the production of the enzyme β-galactosidase. When a bacterial colony contained a disrupted β-galactosidase gene, it could no longer break down lactose and the colony was white on the agar plate. When it contained an intact gene, the colony was blue. Thus, only white colonies were selected for cloning because their β-galactosidase genes were interrupted and therefore their recombinant plasmids contained the DNA fragment.

After selecting the positive colonies, the cloned recombinant plasmid was extracted by miniprep. With this method, a buffer and the ionic detergent sodium dodecyl

sulfate were added to lyse the cells. NaOH denatured the cells' proteins and DNA. When the solution was neutralized with potassium acetate, the small plasmid DNA was able to renature while the chromosomal DNA and protein molecules could not. The chromosomal DNA and protein molecules formed a precipitate, which was discarded after centrifugation.

The plasmids in the supernatant liquid were confirmed to contain the correct gene using the restriction enzyme *Eco*RI and then sent for sequencing using the Sanger method. The Sanger method uses different enzymes to splice DNA fragments at A's, C's, G's, and T's. Each fragment is dyed a different fluorescent color depending on the base at which it was terminated. The DNA fragments then undergo gel electrophoresis, which separates them according to length. A laser then reads the fluorescence of the bands and a computer sequences the gene based on the locations of the different colors.

Results

The nucleotide sequence of the ITS2 region of *P. pachyrhizi* isolate L. UB112 was revealed by the Sanger method to be as follows:

> 5' TCTTAAAAAAAAAATTTACATTCAATTAATGTTATAATAAATCTCAC
> ATATATTTATTTTTTTGATGAAATGATTTTATTATTACATCAAAATATA
> CTGAAACTTATATATATTTATTTATTTAAAGTGAGCTGCAAAAAAAGCA
> ACAGCAACACTCAAAATCCAACAATTTCCCTTTAAAAAAAAGAAATAAT
> GTTGAGAAGATTTCAT 3'

Using the program BLASTIN 2.2.28, this nucleotide sequence was compared to other sequences in the NCIB Blast Gene Bank database. It was found to be 100% identical to the ITS 1 or 2 regions of the genomes of 13 different *P. pachyrhizi* isolates (Morgulis, *et al*., 2008). These isolates were collected from a total of up to eight countries including Colombia, India, Nigeria, Philippines, Thailand, Vietnam, and Zimbabwe (Zhang *et al*., 2012; Frederick *et al*., 2001) (Table 1).

Table 1. *P. pachyrhizi* isolates in NCIB Blast Gene Bank database with ITS sequences 100% identical to L. UB112 ITS2.

Isolate Name	Study Associated	Isolate Source (State if known, Country)
IN73-1D	Zhang, *et al*.	Pantnagar, India
VT05-1C	Zhang, *et al*.	Vietnam
TH02-1C	Zhang, *et al*.	Thailand
CH03-1D	Zhang, *et al*.	Unknown
CO04-1B	Zhang, *et al*.	Caicedonia, Colombia
PH77-1D	Zhang, *et al*.	Philippines
PH77-1E	Zhang, *et al*.	Philippines
NG05-1A	Zhang, *et al*.	Nigeria

ZM01-1B	Zhang, *et al*.	Zimbabwe
ZM01-1D	Zhang, *et al*.	Zimbabwe
TH02-1B	Zhang, *et al*.	Thailand
TM Zimbabwe	Frederick, *et al*.	Zimbabwe
Taiwan 80-2	Frederick, *et al*.	Taiwan

Discussion and Impact on Food Security

Although the nucleotide sequence of *P. pachyrhizi* isolate L. UB112 is relatively meaningless on its own, by comparing it to other samples, it could have tremendous implications for food insecurity. L. UB112 from east-central Brazil is identical to samples from up to eight different countries from three different continents. This expanse of collection sites shows the volatility of Asian soybean rust. It confirms that Asian soybean rust is a rapidly dispersed pathogen, indicating that it is a problem worth investigating further.

Eventually this sample will be compared to 31 other isolates associated with the same study. These isolates were collected from all over Brazil, so analysis of polymorphisms between samples will offer insight into the pathogen's spread within the country. Additionally, by comparing findings from this study with those of studies from the United States, South America, Africa, and Asia, it will be possible to predict how *P. pachyrhizi* entered Brazil and Latin America. With knowledge of where the pathogen spread to and from, scientists will be able to investigate how it is dispersed. Measures can then be taken to prevent its further spread.

This study is the first step in developing soybean varieties with resistance to many types of Asian soybean rust. Currently, several soybean genes are known to aid in resistance to *P. pachyrhizi*; however, they are difficult to implement because little is known about their effectiveness due to lack of knowledge of fungal diversity. As this study continues, more *P. pachyrhizi* nucleotide sequences will be revealed and comparative analysis will be performed among the samples. With information about polymorphisms and the range of diversity among *P. pachrhizi* isolates, soybean breeders will be more knowledgeable of the scope of Asian soybean rust. Eventually, this will help them develop broadly resistant soybean breeds.

By preventing further spread of *P. pachyrhizi* and by engineering soybean breeds resistant to the pathogen, it will be possible to reduce future crop losses to Asian soybean rust. Increasing soybean yields could have significant impact on food security, for currently Asian soybean rust is a threat to global production. Not only would this help relatively developed producers such as the United States, Brazil, and Argentina provide more aid to developing nations, but it would also encourage smallholder farmers to cultivate soybeans. Because *P. pachyrhizi* thrives in tropical areas, current subsistence farmers in Africa, South America, South Asia, and Southeast Asia may be reluctant to plant soybeans in fear of losing their crops to the

pathogen. If this concern could be eliminated because of widely available broadly resistant soybean varieties, smallholder farmers may be more eager to cultivate the nutritious, protein-rich legume.

Overall Reflection: Moments of Self Discovery

In the Lab

At Embrapa I learned about valuable research techniques such PCR and gel electrophoresis, but more importantly, I learned about myself. Working in a molecular biology lab for 45 hours a week taught me patience and persistence. More often than not, our PCR or bacterial cloning would fail, meaning we would have to repeat procedures multiple times. Even when our procedure was finally effective and we were able to send in samples for sequencing, there were always more samples to prepare. In the beginning, this repetition frustrated me. Before my World Food Prize internship, I had a false impression of lab research and thought a scientist would come up with a hypothesis, prove that it's correct, write a paper, and solve world hunger in less than a year. After several weeks of working at Embrapa, however, I discovered that this isn't true – and it's a good thing it isn't. By slowly working through the issues we encountered, I gained a more thorough understanding of the procedure we were performing. After the scenario described earlier in which I accidently added too much Taq enzyme to several of our samples, I was quick to assume because only the samples with too much enzyme amplified, that we should add that amount to all future samples. Luana cautioned against this, instead suggesting that we speak with Silvanna, one of the individuals overseeing the lab. With Silvanna's help, Luana devised a new concentration of Taq enzyme to add to the samples. This number was between the original amount described in the procedure and the amount that I mistakenly added to several samples. After this adjustment, we had much more success with amplification and we didn't waste resources by using too much of the expensive Taq enzyme. Luana's reluctance to jump to conclusions showed me the value of patience in scientific research. By collaborating with other scientists and not rushing the procedure, we ended up with a much more successful experiment.

Outside the Lab

Although I learned many valuable things in the lab, a majority of my self-discovery took place outside of Embrapa. Perhaps one of the most incredible and surprising aspects of Brazil was the openness of the people. From the moment I stepped off the airplane and was kissed by the two strangers that would soon become my brother and mother, I was overwhelmed with warmth. Before going to Brazil, I never considered myself an extraverted person, but because of the Brazilian people's eagerness to speak English with me and welcome me into their culture, I never felt like I had trouble making friends.

When I first arrived in Londrina, I was surprised by how closely the city resembled many of the American cities I was used to. On my way home from the airport, we drove by a Zumba studio, a Pizza Hut, and countless middle-aged women walking small dogs. My apartment had a Wii, Apple computer, and a maid who came several times per week. My host family and everyone I met wore western-style clothes, often from brands such as Gap and Calvin Klein. When I looked beyond the material culture, however, I found that Brazilian customs were slightly different from those in the United States.

To me, the defining characteristic of Brazilian people was the inherent joy with which they interacted with people. My coworkers were always excited to see one another, and despite early mornings, would greet the entire room with a cheery "bom dia" upon arrival to work. After lunch, everyone would gather in the break room and animatedly discuss their weekend adventures or the latest soccer game. The very nature of the typical Brazilian greeting – a warm hug and side kiss – is a perfect example of this openness.

An aspect of Brazilian culture that took some getting used to was how people seemed to act less restrained than they do in the United States. During a dinner of my first week in Londrina, my host mother was describing her favorite Brazilian singer, Maria Rita. I was having trouble grasping what her music sounded like based off of a description alone, but promised my host mom I would look it up later. That night, I was blogging in my room when my host mother strode in and insisted I look up Maria Rita immediately. She found her favorite song, and soon she was passionately singing along to Rita's mellow samba. I was surprised by her directness, and even more surprised that she was so nonchalantly singing in the presence of someone whom she had met just days before. However, I quickly came to appreciate my host mother's openness and carefree nature. During my fifth week in Londrina, she, my host sister Heloisa, and I were doing dishes after a meal of traditional Brazilian *feijoada*. Maria Rita was playing in the background, and I'll never forget the sight of my host mother and Heloisa – towels and clean dishes in hand – as they samba-ed around the kitchen. By this point, I was no longer taken aback by their openness, and was instead simply overwhelmed by the joy they were emanating.

By the end of my time in Brazil, many of my old social habits had changed. I no longer cringed at hugs, and instead embraced friends and strangers with gusto. Eating breakfast and driving to work with my cheerful host father taught me to be a better morning person. And after being accepted so warmly by so many individuals, I became more confident and eagerly interacted with new people. All of these developments improved my outlook on life, made my transition to college easier, and made me an overall better person.

Looking to the Future: Striving Toward Food Security

When I returned to the United States, I was home for one week before departing for college. In this time, everything seemed foreign: my once familiar home didn't seem like my own, and interactions with good friends seemed slightly off. Most notably, I was overwhelmed with a sense of restlessness. When I finally had one week of idleness, all I wanted to meet more people, see the world, and do something meaningful.

Although I was never exposed to extreme poverty, my time in Brazil made me passionate about finding solutions for food insecurity. One of my most important inspirations came from a surprising place: the traditional Brazilian dish known as feijoada. Feijoada is a wintertime meal made with rice, beans, sausage, and several uncommon pork products such as pig ears, tendons, snouts, and hooves. Although feijoada is quite common in modern Brazil, many people pick around the latter ingredients. This confused me at first – why include something in a recipe if nobody eats it? However, when I looked at feijoada in more depth, I realized that these ingredients held a history that transcended modern tastes. And by analyzing this history, I was offered insight into food security.

Feijoada originated in the sixteenth century, when Brazil was occupied by the Portuguese. These Western colonizers used African slave labor to extract sugarcane, silver, and gold to sell in European markets. When slave owners slaughtered a pig, they took the desirable cuts of meat and gave the more unappetizing portions – the hooves, knees, snout, etc. – to the slaves. The slaves would then combine this meat with black beans to form a rich stew, which was served over rice. The result was feijoada: a warm, hearty meal and a highly satisfactory alternative to hunger.

Although making masses of feijoada isn't exactly a solution for solving modern food insecurity, the ideas behind the dish are worth examining. The slaves' creation of feijoada represents a unique solution to a lack of substantial, nutritious food. By creatively utilizing available resources, they came up with a small-scale solution that provided food security for at least one meal. To many Americans, the idea of eating pig hooves may seem unappealing, but this is an example of a solution that worked in the culture in which it was implemented. To combat world hunger, we need to think like the first consumers of feijoada. We need to be creative, flexible, and willing to utilize old resources in new ways. We need to cast aside assumptions and examine solutions that work for the society that we are trying to help. Most importantly, we need to start on a small scale. Whether it's providing microfinance loans to impoverished women or distributing rust-resistant soybean seeds to small-scale farmers, we need to find solutions that benefit individuals. Only by eliminating individual hunger can we build up to global food security.

My Borlaug-Ruan international internship instilled a sense of purpose in my personal mission to reduce food insecurity. Before Brazil, I was content to simply

learn about food security; now I want to do something about it. My experience shaped my transition into college not only by giving me confidence and independence, but by prompting me to pursue an international studies major and encouraging me to study another language. When I returned home from Brazil, I emerged from my plane a different person than when I left. Now, food security is no longer a passive interest, but a life shaping mission.

Acknowledgements

First and foremost, I would like to thank the late Dr. Norman Borlaug for establishing the World Food Prize Foundation, serving as a constant inspiration, and believing in the power of young people. I am eternally grateful to have had the opportunity to carry on your legacy as a Borlaug-Ruan International Intern. I would also like to thank the Ruan family and Ambassador Kenneth M. Quinn for your continuation of the World Food Prize Foundation and for your dedication to its incredible youth programs.

Thank you to Mrs. Lisa Fleming for your tireless support of all aspects of my internship. You were an invaluable resource for solving issues large and small and your cheery emails never failed to brighten tough days. Thank you for selecting me for the Borlaug-Ruan Internship and for helping me make my two months in Brazil the most meaningful of my life.

Greatest thanks to Dr. Francismar Marcelino Guimaraes, my invaluable Embrapa advisor, for allowing me to work on your project and for patiently answering my many questions. Thank you to Dr. Amelio Dall Agnol for organizing the logistics of my internship at Embrapa and for helping me get to work on days when Fernando was travelling.

I can't express enough appreciation for the students of the Biotechnology Vegetable Lab. Your friendship and mentorship made coming to work such a pleasure. You taught me so much about molecular biology, Portuguese, and Brazilian culture, and I hope to see everyone again soon. Special thanks to Luana Darben for allowing me to partake in your DNA sequencing project and for being such a wonderful guide and friend in the lab.

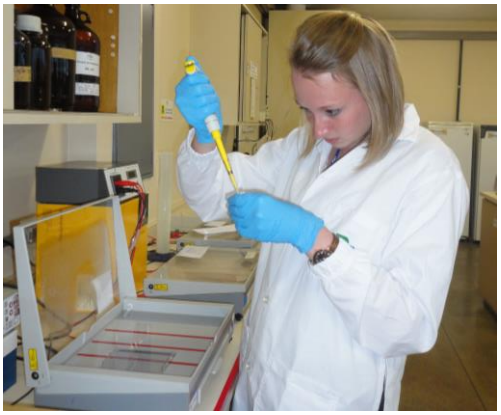
Thank you so much to Dr. Fernando, Soelma, Heitor, and Heloisa Adegas for welcoming me into your home as one of your own. Your warmth, love, and laughter made every day a little better. From Sunday lunches to travelling to Foz do Iguaçu, I will never forget the times I spent with my second family.

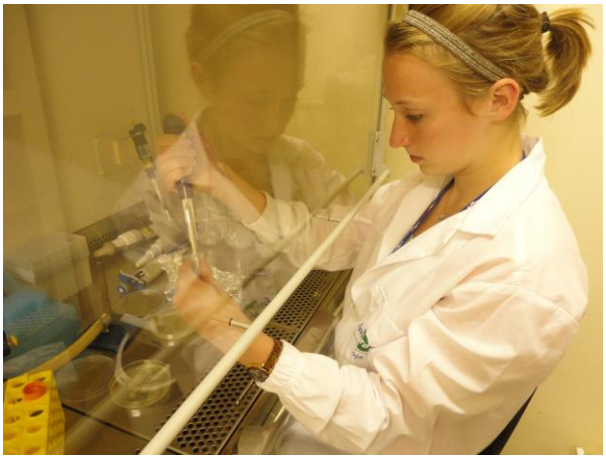
I would like to express special appreciation for my former AP Biology teacher Mr. Brad Horton. Thank you for inspiring me to pursue biological sciences and for helping me get involved with the World Food Prize youth programs. Additionally, I

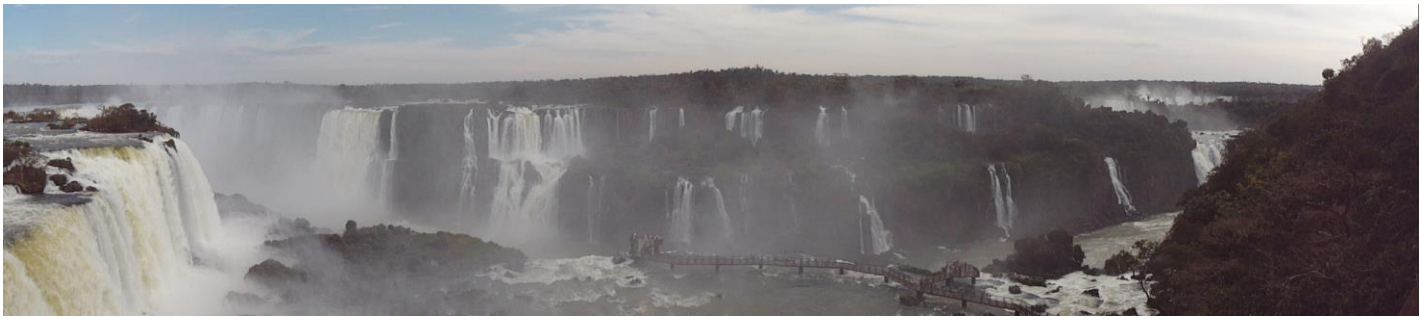
would like to thank Dr. Mary Wilcynski, former principal of John F. Kennedy High School, and all other teachers and staff members who supported me along the way. Without Kennedy's exceptional academic and extracurricular opportunities, I never could have become the person I am today.

Lastly, I would like to offer my deepest love and thanks to my friends and family. Thank you to my friends for serving as my role models, for encouraging me to pursue the Borlaug-Ruan internship, and for always being there to Skype or email during my time abroad. Thank you to my sister Renee Mittelberg for your unyielding friendship and laughter. Finally, I would like to express my upmost appreciation for my parents Neil and Cathy Mittelberg. Although it was tough to say goodbye two months earlier than many of my other college-bound peers, your support for my Borlaug-Ruan internship was unconditional. Thank you so much for always being my fiercest allies and encouragers for every step of the way.

Photos







References

Dupont Pioneer. “Increasing Food Production.” 2010

Frederick, R.D., Snyder C.L., Peterson, G.L., Bonde, M.R. (2001). “Polymerase Chain Reaction Assays for the Detection and Discrimination of the Soybean Rust pathogens *Phakopsora pachyrhizi* and *P. Meibomiae*.” *U.S. Department of Agriculture-Agriculture Research Service.*

Gardes, M., Bruns T.D. (1993). “ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts.” *Molecular Ecology.*

Goellner, K., Loehrer, M., Langenbach, C., Conrath, U., Koch, E., & Schaffrath, U. (2010). “*Phakopsora pachyrhizi*, the casual agent of Asian soybean rust.” *Molecular Plan Pathology.*

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T., Agarwala, R., Schäffer, A. (2008), "Database Indexing for Production MegaBLAST Searches", Bioinformatics 24:1757-1764.

Ospina-Patino, M., Falascina, M., Nascimento, G., & Alcantara, M. R. (2012). Analysis and forecasting of soybeans storage needs in Brazil. *International Conference of Agricultural Engineering.* Valencia.

United States Departmet of Agriculture: Foreign Agricultural Service (2012). “Commodity Intelligence Report: Record Soybean and Corn Area Expected for Brazil’s Upcoming 2012/13 Season.”