Soja: Iowa's Parallel to Brazil

Alyssa Beatty

Chariton, Iowa

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Embrapa Soja

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II. BACKGROUND INFORMATION

A. BRAZIL

The largest country in South America, Brazil, accounts for nearly half of the continent. Its numerous neighboring countries include Uruguay, Argentina, Paraguay, Bolivia, Peru, Columbia, Venezuela, Guyana, Suriname, and French Guiana. Three point three million square miles make Brazil the fifth largest country in the world (www.worldinfozone.com/country.php?country=Brazil). The country also plays home to the largest city in the southern hemisphere: São Paulo. Ten point nine million people inhabit all of the city's 588 square miles (en.wikipedia.org/wiki/S%C3%A3o_Paulo).

The ethnicity of Brazil makes this country very unique. The largest populations, outside their countries, of Italian, Japanese, and Lebanese people reside in Brazil (en.wikipedia.org/wiki/Brazil#Economy). This very diverse combination of people has greatly affected the language. Brazil is the only Portuguese speaking country in North and South America. Brazilian Portuguese is influenced by native and African dialects and is even different among the geographical regions.

The most urgent dilemma in Brazil is poverty. Currently, more than 22 million people have been classified as living in a state of *extreme poverty*. That means they lack the basic necessities of life such as food, shelter, and clothing. One contributor is the fact that roughly 10% of the population is unemployed. This is unacceptable if Brazil is to develop economically in the future.

B. EMBRAPA SOJA

Embrapa Soja is a research center sponsored by the Brazilian Research Corporation. The farm research complex is located twenty-two miles outside the city of Londrina.

According to Embrapa Soja's website, their mission is "to provide competitive technological solutions for sustainable soybean development through generation, adaptation, and transfer of knowledge and technologies, for the benefit of society." This means that by researching soybeans, Embrapa can provide practical solutions to problems experienced by farmers in Brazil. In turn, communities will advance in society from the ideas of Embrapa Soja.

Embrapa Soja has four objectives. They are:

1. To provide technical solutions for promoting the competitiveness of soybeans and sunflowers.

2. To provide technological solutions for promoting economical and environmental sustainability to soybeans and sunflowers.

3. To provide technological solutions that contributes to decrease social unbalances.

4. To provide technological solutions that contributes to improve nutrition quality for human population. This means that by developing and supporting soybeans and sunflowers, economical and environmental manageability can be achieved. Also, the exploration of technologies can help to solve problems related to the uneven distributions of currency, food, and natural resources.

The facilities of Embrapa Soja are outstanding. For purposes of research, there are 15 laboratories and 23 greenhouses. Researchers can present their work in the auditorium or any of its three adjoining meeting rooms. Embrapa employs just fewer than 300 people (296). The environment in which they work is remarkable. At several times throughout the workday, buses transport workers to and from Londrina and its suburbs. A cafeteria type restaurant serves lunch, and workers can purchase snacks at the Cantina convenience store. A series of sidewalks connects all offices, labs, and greenhouses. I believe this work atmosphere contributes in a very positive way to the productivity of Embrapa Soja.

III. INTRODUCTION

A. Me & World Food Prize Foundation

I was first introduced to the World Food Prize Foundation when my sister attended the conference during her junior year of high school; I became determined to do the same. At the end of my sophomore year of high school, my Group Dynamics teacher gave me a World Food Prize brochure. It was filled with pictures and information about truly amazing individuals. I attended the World Food Prize Conference in the fall of 2005. Writing a research paper which explored the aspects of hunger and obesity in the United States was an eye-opening experience. How could so many individuals be morbidly obese, while a child dies of hunger every few seconds? The questions in my mind helped to shape a clearer picture of the world, one which is still changing today.

Knowledge about food security and how it personally affects our lives is something every person should learn about. It totally changes the perspectives people have about the world. This is what the World Food Prize Foundation did for me. All of the presentations, by working professionals in the world, added to my new found knowledge of food security. It is the topic which our future totally depends upon.

B. Getting to Brazil

In the time leading up to my departure, I was never nervous. Two words which better described what I was feeling were astonishment and anticipation. Receiving a 2006 Borlaug-Ruan Internship position was the greatest honor so far in my life! It was exciting to think about what it would be like to live in Brazil. To travel to a foreign nation and live with people you have never met before is a daunting task. Also, I wondered what my experiences would include. I was eager to work alongside with scientists in a laboratory.

Since my plane left Des Moines International Airport at about six o'clock in the evening on Monday, June 12th, some packing and resting still took place throughout the day. My mom, dad, and I arrived at the airport very early. After checking my luggage, we ate supper at a restaurant beside the gift shop. Jokingly, my mom referred to this as my "last meal." With hugs and kisses before security, I embarked on my journey alone.

Both flights began with rocky starts. The Des Moines plane needed to be fully powered down before take-off, due to electrical difficulties. Secondly, departure from the hectic runways of O'Hare in Chicago was delayed an hour and a half. Unfortunately for the passengers and me, this time was spent buckled into our seats while the crew rifled through the luggage compartments. Ten and a half hours later, I found myself in the crowded São Paulo Airport. Customs and

checking my luggage went great. However, directly after this I endured a six hour layover. The airport was not busy at this time of day, so I say alone and watched soccer on the television.

My mãe, Eglaida, and pai, Amélio, greeted me at the Londrina Airport. The relief I felt once I plopped into the car with them was overwhelming. Once home, I showered and prepared for dinner. It was amazing to learn about all the different fruits, vegetables, and meats Brazilians consume.

As I began the journey to work the morning after I arrived in Brazil, I couldn't even begin to imagine the experiences in store for me. My host father, Dr. Amélio Dall'Agnol, and I climbed into the elevator from the family's fourteenth floor apartment. Once in his BMW, we traveled past the lake, luxurious homes, and apartment buildings. After passing the downtown area, different images came into view. People were going on about their lives, unaware of my outside eyes. As we drove farther from the city, the homes and people became poorer. All of them were made of orange red brick, crumbling in worn areas.

The shoulders of the road were covered with trash. Most of it seemed to be papers, scattered by the wind. As I would discover, even the richest of places were dotted with papers. The city parks and downtown streets were in such a condition as this. I began thinking, comparing things in Brazil to my hometown of Chariton, Iowa; a difference in priorities exists between these places.

C. First Impressions

I found that throughout my internship, different and new situations constantly arose. In a sense, every day was met with first impressions. Whether it was food, cultural customs, or work ethics, my time in Brazil was full of wonderful and interesting surprises.

In a way, Brazil can be compared to the United States. The term "melting pot" is fitting for the racial diversity which exists in both places. During my time, I observed people of African, European, and Asian descent. Their heritage is reflected in the cuisine of the country.

In the southern state of Pananà, *gaucho* barbecues are very common (en.wikipedia.org/wiki/Gaucho#Modern_influences). My host father always told me he was "a gaucho cowboy from the south". For his and my birthdays we prepared an authentic gaucho barbecue. The meat was cooked over a pit by a professional chef who specialized in barbecue. He cooked such meats as beef, pork, chicken, and sausage. In addition to gaucho barbecue, a modern day example of the influence on today's society is gaucho pants. Last summer I experienced this trend first hand in my community in Chariton, Iowa. I think it is very important to know and understand the effects other cultures have around the world.

For hundreds of years, *feijoada* has been one of the most widely known foods in Brazil. This true-Brazilian-food is made with pork and black beans. However, my favorite food was made by my Brazilian mother. *Pão de queijo* is a kind of cheese bread, made with corn flour. It is crunchy on the outside, but soft and full of cheese on the inside. The cheese blends in with the soft part of the bread to give it a truly wonderful taste. Due to its delicious taste and texture, I loved to eat it with my host family.

In my opinion, the cultural customs of Brazil compared to the United States are vastly different. When greeting a Brazilian, a kiss on the cheek is mandatory. This took some getting used to, but it makes for closer feelings in friendships. At evening, supper is not served until 7:30 or 8:00. This gives people time to rest after work, instead of having to prepare a meal directly after arriving home. In the same way, people go out later at night. The time change, plus the difference in these two activities took some getting used to. Lastly, the age at which individuals are allowed to consume alcohol and receive a driver's license is eighteen, compared to twenty-one and sixteen in the United States, respectively. It is strange to think about having to wait two more years to drive, but then being able to drink alcohol at the same time. I feel these were the most noticeable and important cultural differences I experienced in Brazil.

As I mentioned earlier, the Embrapa Soybean work environment is excellent. In every building, coffee and tea are provided all throughout the day. Whenever in between projects, my coworkers and I would enjoy short breaks. This past school year, I had a full academic schedule. During second semester, I began experiencing stress headaches. My internship was just as demanding, but I did not have anything of the sort since arriving. Americans always have a time schedule to follow and are always on the run. In Brazil, I learned to slow down; I was able to do the same amount of work but without the added worries. Americans could learn a lot from the work ethics and ways of life of other cultures.

IV. PROCEDURES AND METHODOLOGY

A. Biological Control

Biological control is the use of pathogens, parasites, or predators in nature to disrupt the ecological status of pest organisms. Besides the method of biological control being better for the environment, it is far more cost effective than commonly used insecticides. With Dr. Daniel Ricardo Sosa-Gómez as my supervisor, I worked with two species: Piezodorus guildinii and Dichelops melaeanthus. It is important to know about the species, how they affect agriculture, and what can be done to prevent these problems.

Since the 1970s, *P. guildinii* was a major problem for South American soybean farmers (www.lsuagcenter.com /en/crops_livestock/crops/soybeans/red_ shouldered_stink_bug/faq/index.htm). It is differentiated from other stink bugs by the red band on its back, directly under the head. With a life cycle of about 30 days, the *P. guildinii* causes the most damage in seeds. At the present time, there are no biological control methods available which are effective with this stink bug (www.lsuagcenter.com/en/crops_livestock/crops/soybeans/red_shouldered_stink_bug/faq/index.htm).

D. melacanthus is most attracted to soybean plants as well. Its two sharp projections on either side of the fore body are very distinctive for identification. The adults are different shades of brown with a green underside (Sosa-Gómez).

For my insect project, I used several strains of *metarhizinm* on both species of stink bugs. Metarhizinm is a fungus which acts as a parasite to most insects. The distinctive green spores reproduce asexually in about one week's time. When the insects become infected with the conidia, it begins to grow on their bodies. Then the hyphae, the threadlike vegetative part of the fungus, enter the cuticle of the insect. The cuticle is the hardened protective covering of the insect's body. Once the metarhizinm is inside, it grows and eventually kills the insect. The cadavers are covered with growing metarhizinm, which can be taken to start a subculture (Sosa-Gómez).

B. DNA Extraction

During my time at Embrapa Soja, I worked with different methods of DNA extraction; it is the collection of DNA for molecular analysis. I extracted DNA from insects, soybean seeds, and soybean leaves. Since I began working in the Laboratory of Biotechnology and Bioinformatics halfway through my internship, I

did not work on one specific project. Instead, I was given the opportunity to work with many individuals in the extraction process.

In the DNA extraction of soybean seeds, thin slices are cut off the side opposite of the embryo using a sterile knife. The slices are placed in numbered eppendorfs, and the remaining seeds in corresponding envelopes. 400 µL of the extraction buffer is added to each 1.5 mL eppendorf. The tubes are incubated in a 60° C water bath for 20 minutes. 400 µL chroloform is added and mixed by hand for five minutes. The solution is centrifuged, and the top layer is removed with a pipette. 200 µL isopropyl alcohol is mixed, and the solution is incubated at -20° C for 20 minutes. Then it is centrifuged and the supernatant is removed. The tubes are washed with 200 mL of EtOH 70%, and the pellets are allowed to air dry. 49.6 mL TE and 0.4 ml RNAse 5mg/mL are added, and the tubes are incubated at 37°C for 30 minutes. The samples are then stored at -20° C.

The leaves collected for DNA extraction are stored in a freezer at -80° C. When removing samples from the freezer, they must be placed in liquid nitrogen. A few leaves from each sample are placed in different and sterile mortal and pestle dishes. Liquid nitrogen is added and the samples are ground into a fine powder. Each ground sample is placed in a frozen 1.5mL eppendorf tube. 900mL of extraction buffer is added and mixed well using the vortex. Then the tubes are incubated at 65° C for one hour, followed by centrifuge for 10 minutes. The

supernatant is removed and 900 ml CIAA (24:1) is added. The tubes are inverted and centrifuged for 10 minutes each, respectively. The supernatant is removed and this step is performed once more. 600 mL cold isopropanol (isopropyl alcohol) is added, centrifuged for 10 minutes, and the supernatant , 400mL 1.0M N_2Cl, is dissolved by vortex, and incubated at 65° C for 10 minutes. RNAse with a final concentration of 40 mg/mC is added, and the tubes are incubated at 37°C for 30 minutes. Add 800 mL of 70% ethanol. Discard the supernatant and add 30 mL 1/10 TE Buffer. The solution should now be dissolved completely.

C. PCR & AGAROSE GELS

Polymerase chain reaction, PCR, is a practice used in molecular biology. This process is catalyzed by enzymes, which replicate DNA in large amounts without the use of a living organism. This process can turn a tiny amount of DNA and multiply exponentially. This relatively easy process is used in labs all over the world. When performing PCR, it is important to understand the primers' role and the steps involved in this process.

Primers are select, short sequences of DNA. When performing PCR, certain primers are chosen which compliment the DNA fragment that is to be amplified. Before PCR, I used the Wallace method to determining the annealing temperature for the primers. The formula is: $4(C+G) + 2(A+T)$. The letter variables are substituted from the gene sequence of the substance. The optimum annealing

temperature is from 58 to 62° C. If the annealing temperature is not in this range, the primers will not fix themselves to the DNA, resulting in no exponential amplification.

The PCR process usually consists of about 32 to 36 cycles. Within these cycles is a series of preset temperatures. The first step is called denaturation and occurs at about 95° C. At this temperature, the double stranded DNA is separated into two single strands. After it is certain that the strands are separated, the temperature is lowered to allow the complimentary primers to attach themselves. This is the annealing process. (Remember that the annealing temperature was approximated by using Wallace's formula.) Once the primers are fixed to the DNA fragments, they travel the length of the single strands and copy the DNA. At the end of every cycle, the number of DNA fragments has doubled. Due to the primers, which produce the specific sequence desired, the number of unwanted fragments is miniscule compared to the billions of copies the PCR process has just produced!

Agarose gel electrophoresis is a technique used to visualize the PCR process. Agarose gels are prepared with agarose, synergel, and ethidium bromide. The synergel is to help clarify the solution. The ethidium bromide acts as a stain in the DNA, which ultraviolet light excites. Depending upon the machine size and desired concentration, different amounts of components are added. Once the gel

has set up, it is placed into the machine, which is filled with TBE buffer solution. The samples are placed in the wells using a pipette. At the end of each row of samples, a known marker is used for comparison.

The electrophoresis machine moves the DNA from its wells along the gel. DNA molecules are negatively charged; the machine creates an electric field which pulls the DNA through the gel. Also, the ethidium bromide slows the DNA in the gel process when it inserts itself between the base pairs. A 250 mL volume gel takes about two hours to complete. Care must be taken when watching the clock, however. If kept active long enough, the DNA could run off the edge of the gel.

After the electrophoresis, the gel is placed on a Gibco BRL UV Transilluminator. The machine has a protective clear shield to cover the gel when the UV light is on. This is a necessary safety precaution when working with ultraviolet light. A Kodak EDAS 290 camera takes pictures of the ethidium bromide excited gels. After analyzing the photos, the DNA band can be cut out of the gel for purification if necessary.

V. OBSERVATIONS AND CONCLUSIONS

A. Biological Control

I found biological control to be the most interesting subject to study. Insect control is a large dilemma for farmers around the world. Insects are unique when they adapt to resist new control agents. This is why it is necessary to continue researching new agents of control. The scientists at Embrapa constantly collect new samples of insects and test different methods of control.

The method I used for control was metarhizium fungi. Once the sample had been grown on media culture, two species of stink bugs were inoculated with different strains. The insects were kept in standard conditions of 26°C and 70 to 80% humidity. After the insects died they were placed in separate Petri dishes with damp cotton balls. The green fungi began growing after a couple of days on the corpses. One way to tell if the insects died from the fungi was by examining their legs. If they were stretched out far from their bodies, as opposed to curled up like a dead fly, this was a good sign of mortality due to metarhizium. Following the growth of fungi, I prepared several subcultures of the strains which had been most effective. The purpose of this was to obtain a clean sample in order to inoculate another set of stink bugs from the original fungus. By examining the mortality tables in appendices A, B, and C, I concluded that the metarhizium was not as affective as other possible methods.

B. DNA Extraction & PCR and Agarose Gels

During the second month of my internship at Embrapa Soja, I worked in the Laboratory of Biotechnology and Bioinformatics. Throughout this time I accompanied Danielle Cristina Gregório Da Silva. Since my time in this lab was short, I was not assigned a specific project to work on. I helped Dani to perform many DNA extractions, as well as DNA cloning. I completed molecular cloning through the process of electroporation. This is the use of electricity to increase the electrical conductivity and permeability of the cell plasma membrane. Then, when the cell pores are open, we introduced a new substance into the cell. I performed approximately two to three clonings during my time. The first was, unfortunately, unsuccessful. However, the second produced useable clones. This was very exciting for both Dani and myself.

During my internship I performed many PCRs. After the process had been done to samples of DNA, agarose gels were run. Aragose gels are simply a way to visualize the DNA and its bands. By analyzing the photographs of the gels scientists can draw conclusions about the quality of the samples, and whether or not the DNA needs to be purified or extracted again.

The many connections between Brazil and the United States are interesting to discuss. The parallels of insects in certain crops, as well as plant diseases are unremarkable. Due to the crops we have in common, many of the problems are the

same. This is why it is essential to work together to solve these difficulties. Each country has made significant advances in to research in agriculture. While living and working in Brazil, I came to realize and understand the future our planet is faced with. That is why I would like to help to continue and progress agricultural research in Brazil and around to world.

Appendix A

Dichelops

Inoculated: 06-26-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox A Ma 12	0	0	0	1	0	0	6	0	1	0	0	0

This gerbox contained 8 insects.

Inoculated: 06-26-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox B Ma 283	0	0	0	2	0	0	2	0	1	0	0	0

This gerbox contained 5 insects.

Inoculated: 06-26-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox C Ma 283	0	0	0	0	0	0	3	1	0	0	0	0

This gerbox contained 5 insects.

Inoculated: 06-26-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox D Ma 468	0	0	1	1	0	0	3	1	1	0	0	0

This gerbox contained 7 insects.

Inoculated: 06-26-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox E Ma 492	1	0	1	2	0	0	1	0	0	0	0	0

This gerbox contained 5 insects.

Appendix B

Dichelops

Inoculated: 07-05-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox A Ma 12	0	0	0	0	1	4	0	1	0	0	0	0

This gerbox contained 6 insects.

Inoculated: 07-05-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox B Ma 468	0	0	0	0	4	1	1	0	0	0	0	0

This gerbox contained 6 insects.

Inoculated: 07-05-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox C Ma 492	0	0	0	0	6	0	0	0	0	0	0	0

This gerbox contained 6 insects.

Appendix C

Piezodorus

Inoculated: 07-03-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox A Ma 468	0	0	2	3	0	0	2	0	0	0	0	0

This gerbox contained 7 insects.

Inoculated: 07-03-06

	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII	Day VIII	Day IX	Day X	Day XI	Day XII
Gerbox B Ma 468	1	0	0	3	0	0	2	0	0	0	0	0

This gerbox contained 6 insects.

Appendix D

Preparing Agarose Gels

	Agarose	Synergel	Ethidium Bromide
300 mL gel tray	2.1 grams	3.45 grams	3µL
250 mL gel tray	1.75 grams	2.875 grams	2.5µL
70 mL gel tray	490 milligrams	805 milligrams	0.7µL
30 mL gel tray	210 milligrams	345 milligrams	0.5µL

Appendix E

DNA Extraction (Leaves and Seeds)

1. Put a piece of leaf or seed in the 1.5 mL eppendorf tube and smash with 400 µL buffer of extraction (CTAB 2%, Tris-HCl 1.0 M pH 8.0, EDTA 20 mM, 2.4 M NaCl, PVP 1%)
2. Incubate at 60°C for 20 minutes.
3. Add 400µL chroloform and mix for 5 minutes.
4. Centrifuge at 14,000 rpm for 5 minutes.
5. Take 200µL of liquid phase and transfer to a new tube.
6. Add 200µL of isopropanol and mix for 5 minutes.
7. Incubate at -20°C for 20 minutes.
8. Centrifuge at 14,000 rpm for 5 minutes.
9. Discard supernatant.
10. Add 200µL EtOH 70% and centrifuge at 14,000 rpm for 5 minutes.
11. Incubate at 37°C for 30 minutes.
12. Store at -20°C.

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