Soil Borne Pathogens of Wheat

Molly McKneight

Borlaug-Ruan Intern 2010

International Maize and Wheat Improvement Center (CIMMYT)

Ankara, Turkey

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Personal Background

A little over a year ago, I never would have dreamed that one college visit would have such a great impact on my life. My mom had arranged an appointment during the spring of 2009 with Donna Keener, an advisor in the school of Food Science at Purdue University. At that time I was thinking about pursuing a nursing degree, so I did not expect much out of my appointment. However, after meeting with Donna, learning about the program, getting a tour of the building, and being introduced to some faculty, I was blown away. Going into the appointment I had only a small idea of what Food Science actually was, but I came out quite sure that it was what I wanted to study.

I came from a suburb on the outskirts of Indianapolis, Indiana, and my exposure to agriculture was minimal. Although the national FFA headquarters were less than thirty minutes away from my house, there was not actually a chapter I could join nearby. In my high school of over 4,000 students, only a handful thought to pursue a career in agriculture. So while attending the World Food Prize Youth Institute in October of 2009, I was exposed to a world that was completely foreign to me. It was through the World Food Prize that I learned about global food insecurity and I became determined to be part of the solution. I began to realize that agriculture was more than just the corn field down the street; it was about feeding the world. My new knowledge pushed me more toward a career in agriculture.

At the Youth Institute I was able to have conversations with several of the 2009 Borlaug-Ruan interns. Even though I was just getting my feet wet in the world of agriculture, I began to develop a strong interest in the program. I was in awe as I talked to each student and learned about their research and cultural experiences. Although they traveled to different countries, all of the interns came away from their trips with a new outlook on global food insecurity. I vowed from then on that I would do everything in my power to obtain an experience of my own. I wanted to live up to the small button I proudly wore at the WFP Youth Institute that bore the words “Hunger Fighter”.

I sent in my application in hopes of earning an internship, but also knowing that there were many other exceptional students vying for a spot. I was elated when I learned I was granted an interview, becoming one step closer. A few weeks after the interview I saw an email from Lisa Fleming in my inbox and my shaking hand clicked to open it. Upon learning that I was going to be a Borlaug-Ruan intern during the summer of 2010, I could not believe that my dream was finally becoming reality. Truthfully I do not think that reality set in until I boarded the plane to Ankara, Turkey on June 4th, 2010, just four days after graduating from high school.

Working with CIMMYT-Turkey

My internship placement was with a regional office of CIMMYT (International Maize and Wheat Improvement Center) in Ankara, Turkey. When I first learned of my placement I was ecstatic that I was going to be involved with the very organization that Dr. Norman Borlaug had been heavily invested in. Over the past year I had learned and read so much about CIMMYT, and I was now being granted the opportunity to work for it.

CIMMYT has its roots in Mexico, where it originated as a pilot program by the Rockefeller Foundation and the Government of Mexico in 1943 ("About Us"). After the organization's full establishment in 1963, Dr. Norman E. Borlaug became head of the Wheat Program. Under his leadership, a team of international researchers created a network to assess experimental varieties of wheat and maize. Over the coming years, the researchers were able to create varieties of wheat that were resistant to stem rust, had better reactions to fertilizers, and performed well at varying latitudes. It was because of the program that Mexico became an independent wheat producer by the end of the 1950s. Success in Mexico spurred researchers to duplicate the innovations in other countries across the globe, expanding CIMMYT to the size it is today.

Dr. Norman Borlaug's work is generally associated with the Green Revolution in Pakistan and India. Not as many people are aware that there was another "Green Revolution" in Turkey in the 1960s. A national winter wheat program was formed in by the Turkish Ministry of Agriculture in 1967. Partnering with the government was staff from Oregon State University and CIMMYT. In the years to come, seeds for spring wheat varieties were sent from Mexico to Turkey. The results were extremely positive, with the new varieties growing to be at least twice as tall as the local crops. The winter wheat area also experienced improvements due to new farming methods and cultivars.

Currently, the CIMMYT office in Turkey focuses on wheat research. There are both breeding and Soil Borne Pathogens programs that collaborate to develop wheat that is resistant to disease and produces a high yield.

Historically, wheat has played a major role in the lives of humanity. It has been grown for over 8,000 years and remains the most crucial food grain source for humans. Recently the United Nations predicted that the global population will increase rapidly in the years to come, with the number of world inhabitants potentially reaching 10 billion by 2100. If these predictions prove to be true, it will likely be difficult to keep up with the world demand for wheat. The depletion of global grain reserves and unstable wheat prices threaten the supply of wheat, especially in developing areas. To avoid a future crisis, research must be done to improve the yield of wheat around the world.

Upon my arrival in Turkey, I was quickly made a part of the existing Soil Borne Pathogen of Wheat Program research team. Since it was a regional office of CIMMYT, the number of staff was relatively little and everyone worked together to complete tasks. The SBP group was small, so I was able to become very familiar with them and their work. Despite their size, they worked incredibly hard and I was constantly amazed at what they were able to accomplish over the course of a day. I was grateful that they placed their full trust in me and allowed me to work alongside them as one of their team members. Although the SBP program researches the Pratylenchus species of nematodes as well as fungi, my work was mostly concerned with Cereal Cyst Nematodes (CCN).

While in Turkey, I worked with a team of SBP researchers. The director of the Soil Borne Pathogen of Wheat Program in Turkey was Dr. Julie Nicol, a wheat nematologist. I also cooperated with Dr. Amer Dababat, a post-doctoral fellow at CIMMYT, and Dr. Gul Erginbas, who specialized in fungi. My host sister, Didem Saglam, was a nematology PhD student at Ankara University as well as an employee of CIMMYT.

All studies completed at the research center are done on a long term basis and conclusions about wheat lines and cultivars are only drawn after years of close study. Therefore, it was not possible for me to work on my own project like most of the other interns. The following report reflects the issues of CCN, how the research is conducted, and the contributions I made.

Background of Cereal Cyst Nematode (CCN)

Economic importance

In recent decades, cereal cyst nematodes have been identified as a major cause of yield loss in wheat (Riley *et al*). CCN is a global problem, and its effect is worsened by drought stress and rain-fed conditions. Although the most common species that causes damage to cereals globally is *Heterodera avenae*, in Turkey the most economically important species is *Hetereodera Filipjevi*. During my research*, H. Filipjevi* was the species that I worked with. *H. filipjevi* can be found across northern Europe and Central and West Asia, as well as the Middle East and India. In some field trials in the Central Anatolian Plateau (CAP) of Turkey, CCN caused yield losses up to 40%. Other countries have observed similar occurrences. One of the biggest challenges nematologists face is lack of financial and governmental support. For many years foliar wheat diseases have been a focus of research. On the other hand, unseen pathogens such as CCN are often overlooked and therefore research in the area is minimal. Another problem is that nematode damage is non-specific and can easily be confused with other factors such as drought and nutrient deficiency (Ferraz and Brown).

Introduction to Plant Parasitic Nematodes

In general, nematodes are microscopic, colorless animals similar to worms (Kleynhans). They can be found in nearly all environments conducive to life including soil, fresh and salt water. Since average size of nematodes is around 1mm and the animals' bodies are translucent, they cannot be viewed easily by the naked eye.

Symptoms

As stated previously, damage caused by *Heterodera* nematodes is not easily identified by its physical symptoms alone. Above ground, damage is show by light green patches and wheat plants that contain less tillers (Figure 1). Below ground, white females are sometimes visible on the roots depending on which part of the growing season they are in. However, to truly diagnose CCN, the wheat plants must be further examined under the microscope.

Fig. 1: Cereal Cyst Nematode symptoms in wheat fields. Tolerant (left) and intolerant (right) cultivars growing in fields in Xuchang, Henan, China. (Photo by Ian Riley, Nicol *et al*)

Life cycle

Heterodera species have only one life cycle each year (Ferraz and Brown). They are also described as sedentary nematodes, meaning they remain in the same general location throughout their life cycle. *H. Filipjevi* and other cereal cyst nematodes all follow a basic life cycle. First, juveniles enter the roots of the wheat plants and puncture the cells using their stylets. When a nematode infects a cell, the cell becomes enlarged and the nematode absorbs most of the energy normally used for plant development. These sedentary endoparisitic nematodes damage the wheat as a result and cause the symptoms described above. Males then fertilize the females. Those females later mature into swollen bodies that contain masses of eggs. These adult females later die and become hard brown cysts to protect the eggs inside. This process is depicted below. Hatching of H. Filipjevi eggs generally occurs twice each year in Turkey: once in November and then again in March or April.

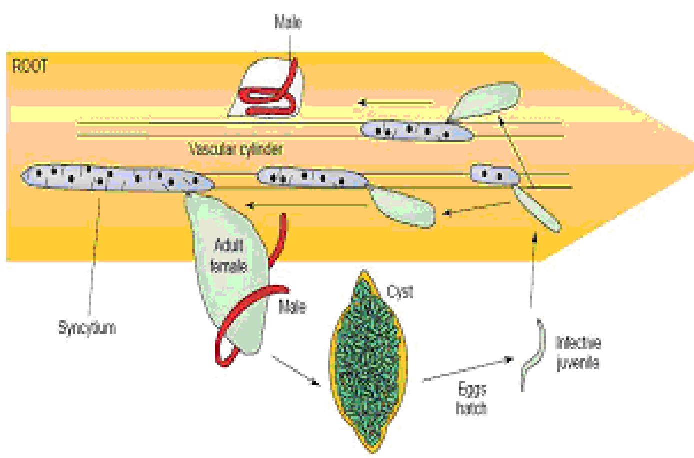


Fig. 2: Life cycle of *Heterodera* species

Control

Several practices are used to maintain a population of nematodes that is below the economic threshold. The cultural practice of crop rotation can be utilized, alternating wheat crops with non-host plants or resistant cultivars (Riley *et al*). Certain fertilizers and nematicides may also reduce nematode populations. However, these treatments can be rather expensive, especially for smallholders who lack financial resources. In addition, there are many environmental costs that are associated with their use. At this point in time, the development of resistant and tolerant wheat cultivars is most preferable. Using plant host resistance to keep nematode populations below the damage level threshold provides the greatest long term solution to the problem. Damage level threshold is defined as "the minimum population density at which a particular nematode specides is considered to cause economically important damage to a particular host crop" (Ferraz and Brown). The goal is developing a line of wheat that has enough resistance to keep the nematode population below the economic threshold level. In the Soil Borne Pathogens program in Turkey, an emphasis is placed on this method of control because it effectively reduces and inhibits nematode population growth and is cost effective.

International Collaboration

Sources of resistance to CCN have been developed in many places around the world, but their effectiveness varies by region. Through the combination of the CIMMYT-Turkey collaboration and international partners, fifty-seven wheat lines have been identified as having resistance to one or more pathogens (Riley *et al*). Resistant sources can be readily installed through the communication of many global centers, as well as the use of effective germplasm exchange. To date, more than 1000 wheat genotypes have been screened as a part of the International CIMMYT-Turkey partnership. So far twenty-seven sources have indicated a high level of genetic resistance and further field research is being conducted to confirm their resistance and tolerance in natural conditions. The germplasm showing the most promise have been shared with over 15 other countries.

Use of Genetic Host Resistance to Control CCN

In vitro experiments

The initial method to test the resistance of wheat lines and cultivars to CCN is to test them in a growing chamber. The reason that this type of experiment takes place first as opposed to field testing is that all conditions are under the control of the person running the tests. All variables can be controlled with the touch of a button. Many specific strains can be tested quickly because experiments are done on a small scale, with one germinated seedling placed in each tube. During my internship I worked on several in vitro experiments by preparing tubes for inoculation and counting cysts.

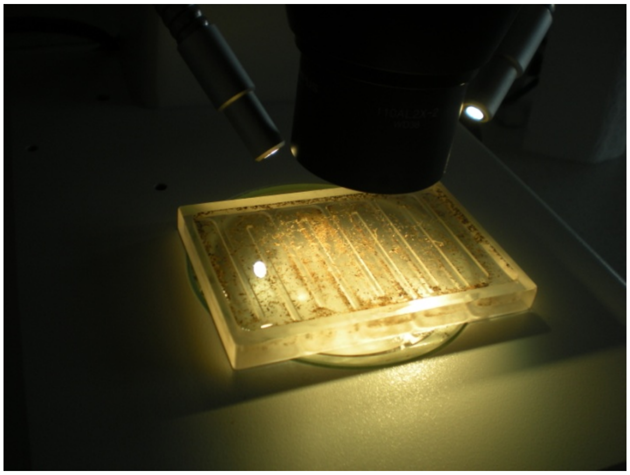
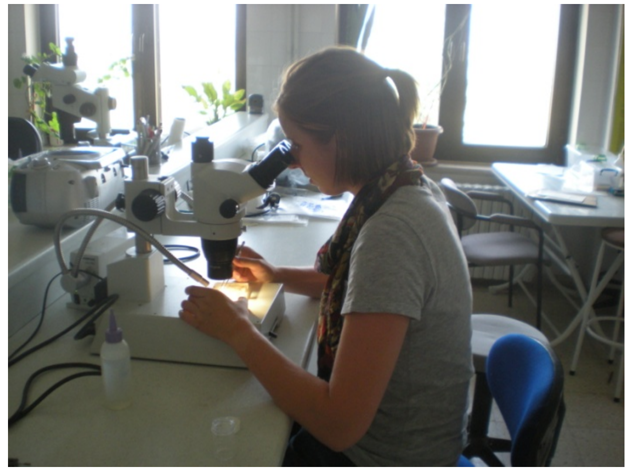
Methods:

Although each experiment has a different aim, there is a general pattern that is followed. Seven replications of each cultivar are generally tested to insure that the results are consistent. After inoculation, the tubes are kept in the growing chamber for a period of about 2 months. However, exact growth time varies by experiment. The growing time is approximately 2 months. The standard potting mixture (70:29:1 mixture by volume of sand, field soil and organic matter) is placed in each pot, and one seed is sown per tube.

To extract sedentary nematodes (cysts) from soil and plant samples, the sieving method was used. It involved mixing soil with about 5 liters of water and stirring to blend all contents of the bucket (Figures 3a,b). After allowing heavy particles to settle for 30 seconds, the top two-thirds of the mixture were poured through two sieves (Figure 3c). For plant samples, the roots were washed thoroughly to obtain any cysts that might be attached to them. This process was repeated two more times to ensure that all nematodes were retrieved. The larger sieve (850µm) was used to catch large debris and the smaller sieve (250µm) collected the nematodes along with smaller debris. Nematodes caught on the smaller sieve were washed into small tubes, labeled with the sample's identification information (Figure 3d).

Figure 3: Sieving method
(Photos by M. McKneight)

Once the cysts and soil particles were collected in the tubes, each sample was viewed under a dissection microscope (Figures 4 & 5). The number of cysts and white females were counted to determine the resistance of the wheat cultivar. After all samples were counted, a decision was made as to how many cysts qualify a sample as being susceptible or resistant. The lines or cultivars that showed the most promise of high resistance were recommended for use in field trials. To insure that results were correct, several check lines (lines that have already been determined to be resistant or susceptible) were also tested. The two susceptible winter wheat check lines that were most often used were Bezostaya and Kutluk, while the resistant check lines were Katea and Sönmez.

Figures 4 and 5: Viewing cysts under a dissecting microscope

<u>Field Experiments</u>

Objective

The majority of the cultivars being tested in the field had already shown high resistance when screened under the in-vitro conditions described above and were therefore promoted to field tests. The purpose of field trials is to study the yield of the wheat plants despite the presence of CCN. It is used for validation purposes as well because it most accurately reflects the conditions of crop plants. The resistance of each line or cultivar being tested is determined by calculating the multiplication factor of CCN:

$$Multiplication\ factor = \frac{Final\ population\ density\ (Pf)}{Initial\ population\ density\ (Pi)}$$

Initial populations (number of cysts in a plot at the beginning of a growing season) are usually taken around November, a few weeks after the seeds are sown. Final populations are recorded after harvest, which generally occurs in early August. Typically if the multiplication factor is around one, the cultivar or line is considered to be resistant to the nematode growth. If it is above one then it is regarded as susceptible to nematode population expansion.

Methods

In the 2009-2010 growing season, four different experiments were set up at three sites in Turkey: Yozgat, Eskişehir, and Haymana. 50 lines from the International Winter Wheat Improvement Program (IWWIP), 24 germplasm from Turkey's national program, 25 elite germplasm from the national Soil Borne Pathogen program, and four from the Field Resistance Cereal Cyst Nematode (FRCCN) project were tested in the four experiments. Each experiment name relates to the different collaborations that the CIMMYT-Turkey SBP office possessed.

At least three replications of each cultivar or line were used under both low and high nematode populations. Although there were some variations in organization, the three field sites used the same general pattern. Each field was divided into parts: high population of nematodes and low population. This was done to observe the response of the wheat plants to the attack of nematodes. At the Yozgat and Eskisehir sites, half of the plots were treated with chemicals to simulate a low nematode population. In Haymana, a low nematode population was created by rotating with safflower, a non-host crop. High population of nematodes was created by rotating with Bezostaya, a susceptible wheat cultivar.

As mentioned before, the resistance of wheat lines and cultivars were determined by calculating their multiplication factors. Initial and final populations of CCN were found by obtaining soil and plant samples from each plot at the beginning and end of the growing season. Each sample was washed thoroughly in a uniform manner in the same fashion as in vitro experiments, described and pictured above. After collecting samples in tubes, the cysts in each one were counted under the dissecting microscope and recorded. This process was often quite time consuming, as there were thousands of samples to be washed and counted.

Although resistance of a wheat line or cultivar is extremely important, it must also display tolerance to qualify as being elite. To determine tolerance, physical measurements were needed, which required several visits to the fields in Eskişehir and Haymana. The variables that were measured included plot size/length, plant height, spike count, and grain weight. The first three variables were measured approximately two weeks before harvest to record each plot's overall growth. Although all plots were planted uniformly, the resulting yields varied. These differences could be attributed to problems in planting, insect damage, or the overall tolerance of the specific cultivar.

Figure 6: Collecting field measurements

After all data is collected, it is sorted by cultivar or line name. The replications are compared to insure that the results are indeed valid so that conclusions can be made regarding their effectiveness. Once effective lines and cultivars are identified, their information is returned to wheat breeders. The breeders either continue to cross the lines or directly recommend them to farmers to grow in order to improve overall yields.

Drawbacks

There is a wide array of variation within the field. There might be an uneven distribution of nematodes, different soil types, and other diseases present. Also, field screening can only be done once each year. As a result, a combination of both in-vitro and field screening must be used.

Applying the Research

One of my favorite conversations that I had over the summer took place during the Soil Borne Pathogens Master Course. One of the experts that came to Turkey was Francis Ogbonnaya, a molecular geneticist at the International Centre for Agricultural Research in Dryland Agriculture (ICARDA) in Syria. He gave a lecture that did not directly apply to my area of research, but I truly enjoyed being able to gain a broader view of the issue at large. Dr. Ogbonnaya emphasized that research must not be contained in a lab or only published in a report. Research is only beneficial if it is applied to the world for the purpose it is intended for. In the case of wheat research, newfound information and technologies must be passed on to farmers rather than simply being documented. However, this is not always as easy as it sounds. It is not possible to simply tell farmers what changes they should make because they want to feel as though they are part of the solution. If new methods are to be spread, they must be initiated with the smallholders themselves. Once one individual adopts the techniques as his own and has success, his neighbors or friends will be more apt to follow. People must see the benefit with their own eyes before they make the decision to adopt the method. They do not want to feel as though they are inferior to the researchers. Therefore researchers and farmers must work together in order to make significant progress towards solving food insecurity. This conversation inspired much thought for me. I realized that the work being done by CIMMYT did not end with the research; that was only half the battle.

4th International SBP Master Course: June 20th – July 2nd, 2010

During the third and fourth weeks of my internship, CIMMYT-Turkey hosted the 4th International Master Course on Soil Borne Pathogens of Wheat in Eskisehir, Turkey. The twenty-five participants consisted of cereal pathologists from the countries of Iran, Iraq, Syria, Australia, Afghanistan, USA, Tunisia, Morocco, and Algeria. This course had occurred three times before, aiming to provide training to individuals working in plant pathology where SBPs are considered to be a problem. My supervisor, Julie Nicol, was the coordinator of this particular year's event. As a result, our research team took a two week break from lab and field work to run the course.

Figure 7: Group of SBP Master Course staff and participants (Photo by Ian Riley)

Before the course, I assisted in assembling about 40 bags for participants and staff that contained books and materials to be used during the course. My job during the course itself was to assist my supervisors logistically in any way possible. Usually this involved setting everything up before lectures and practical sessions in the lab. I also tried my best to make sure that all participants and teaching staff members were well taken care of, whether it was serving refreshments or helping them with their laundry. Although these actions were simple, I was happy to act as an ambassador to so many people around the world. I had only been in Turkey for two weeks before the course occurred, yet I was included in the host team. I was especially proud of the fact that the nametag that was provided to me even had "Turkey/USA" listed as my home country. I had attended conferences like the World Food Prize Youth Institute before but I had never been on the administrative side. By helping with the background work for an event such as this, I gained a greater appreciation for the larger conferences I had attended in the past.

Figure 8: Dr. Julie Nicol with two SBP course participants

Figure 9: Me and several course participants during a tour of Eskişehir, Turkey

Although I was not able to help with research for two weeks, I feel that the experience was well worth the time. I was able to sit in on most of the lectures and labs during the course, gaining valuable information about nematodes, root diseases, plant genetics, conservation agriculture, and more. My knowledge of agriculture expanded beyond the realm of nematodes, and I was able to see the bigger picture of the threats to wheat production. Learning how to interact with many various cultures at once is something that I am sure will become increasingly common in the years to come, as the world becomes more and more connected.

Cultural Observations

Travelling to a Foreign Land

When I first learned that my internship would take place in Turkey, I was ecstatic. The thought of living in a completely foreign location by myself exhilarated me. The fact that I would be living in the Middle East for two months never worried me. However, I cannot say the same about my parents, family, or friends. When I would mention where I would be working for the summer, people were excited but also apprehensive. As the summer progressed I realized that sometimes we allow stereotypes to replace the truth for our views of the world. Learning to form my own views rather than relying on ones of others was a critical lesson of the summer.

When Boundaries Disappear

Living in the Middle East for a summer allowed me to broaden my cultural horizons and my view of the world. At the SBP Master Course for example, I was able to meet people from Afghanistan, Iraq, Iran, Morocco, and a handful of other countries. Those were the countries that I had heard about in the news all the time. I had an image in my mind about them and what their citizens would be like. But once I actually met people who lived in those countries and had conversations with them, those stereotypes were quickly erased. I was able to meet people from all over the region, which exposed me to cultures I never thought I would come in contact with.

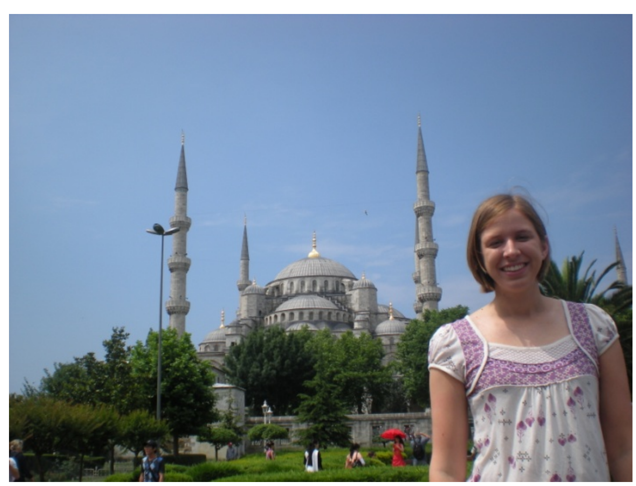
Although some of the friends I made during those two weeks came from countries that do not have the best relations with the United States of America, that never affected how we treated one another. I began to realize that in science and with a common goal to help humanity, boundaries disappear. It would be nice if the world was more concerned with bettering life for all humans than who is right with morals and ethics. With collaboration comes success in every area- not just agriculture. The course, with over 10 countries in attendance, consisted of scientists around the world joining together to share their expertise on wheat pathogens in hopes of improving programs in their respective countries. All they saw was the end goal: increasing the yield of wheat around the world. That common goal was enough; to them it was stronger the animosity between their countries. The collaborations I witnessed gave me hope that we can truly solve global food insecurity in the years to come.

A New Family

One of the cultural aspects of my internship that I enjoyed the most was living with a host family. My own family had hosted students from Japan several times, so I knew that bonds are quickly formed in those situations. It was a great experience to be on the opposite side of the host family situation this time. Arriving in a country in which I did not know anyone was a bit frightening at first, but when I met the Sağlams, my Turkish family for the summer, my fears quickly subsided. As soon as I arrived in Turkey I was taken under wonderful care and was shown every aspect Turkish life. I was not just a guest in their home; I was part of the family. I will remain grateful for that experience for the rest of my life. For me, a trip to the grocery store was just as valuable as a side tourist trip. I did not want to leave the country without doing everything I could to experience the full culture. Because only one member of my host family spoke fluent English, I tried my best to learn their language. In my spare time I had impromptu Turkish lessons with my host sister Didem, who patiently responded each time I asked her "Bu ne?" (What's that?). By the end of my stay I was able to shop on my own, order off of a menu, and have small conversations with my family. After a long week of work it was nice to have another fun way to spend my time. I enjoyed the language aspect so much that I began Turkish language classes when I arrived at college in the fall in hopes of being able to communicate with my host family better.

Extending the Experience

For me, my Borlaug-Ruan internship experience did not terminate when I left Turkey on August 2, 2010. Instead, I feel that it served as just the beginning of the next stage of my life. I realized that agriculture is so much more than what most people believe it to be. There are endless opportunities in the field to make a lasting impact on the world, which further convinces me that I made the right choice in choosing my area of study in college. I hope that my eight weeks in Turkey was only a small taste of my involvement with international agriculture. Currently I am studying Food Science at Purdue University and although I am not settled on an exact career path, I know I want to use my education to fight global insecurity.



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