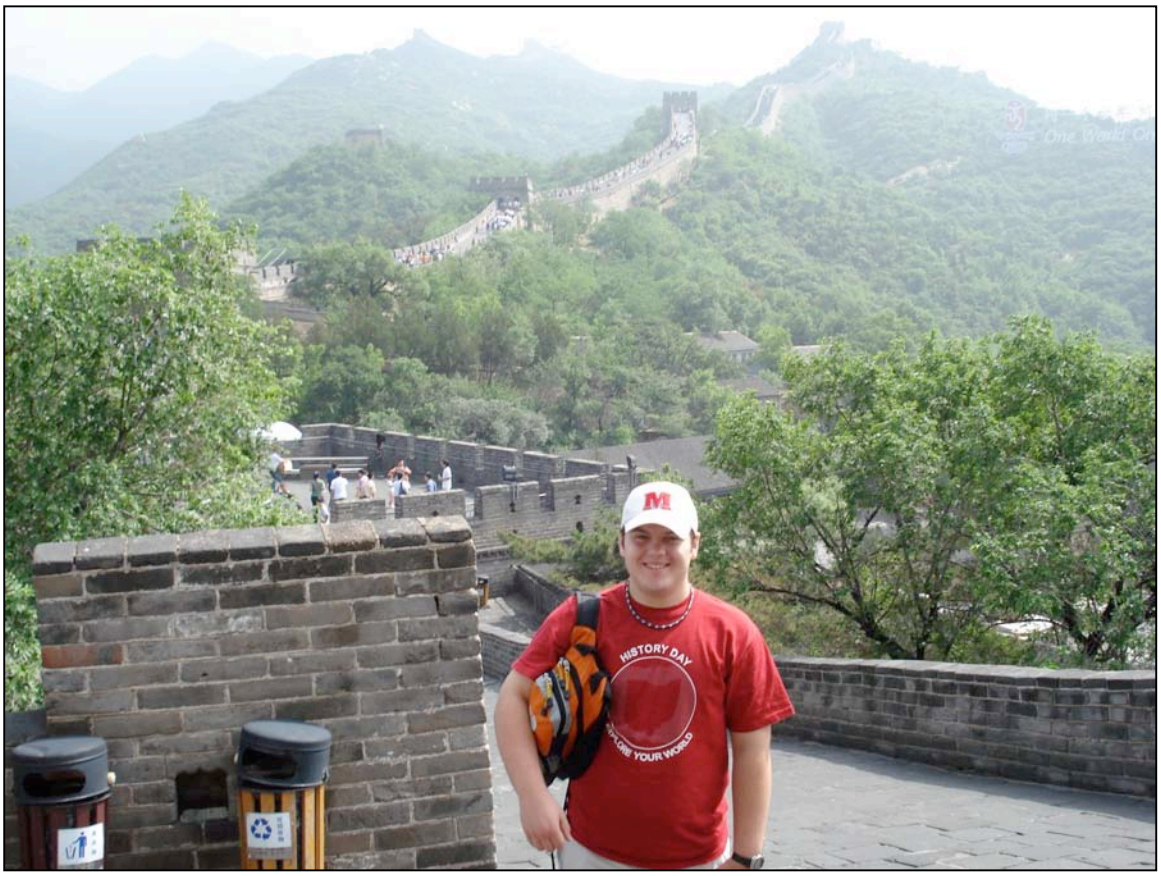
A Summer of Discovery:

My Internship at the Chinese Academy of Agricultural Sciences in Beijing



Stephen J. Frese
Melbourne, Iowa
Chinese Academy of Agricultural Sciences
Beijing, China
2006 Borlaug-Ruan International Intern

Abstract

June 16, 2006, and I was still just Stephen Frese, the history nerd, on my way home from the week-long National History Day contest at the University of Maryland in College Park. Two days later I would be the young man on my way to a place on the other side of the world where only two people would know my name, and where something other than wheat bread was the staple starch at most meals.

On June 18, I set off for Beijing, China, and the adventure of a lifetime. I spent the first couple days getting adjusted to the new time zone, and then I jumped into the lab work that would consume the bulk of the time of my internship at the Chinese Academy of Agricultural Sciences. My time was split evenly between an experiment dealing with rhamnose levels in *Arabidopsis* plants and a project in which we were trying to isolate and map the genes responsible for fiber elongation in cotton plants.

For the first four weeks of my internship I studied with Jingfeng Wang. Jingfeng was working on a project dealing with *Arabidopsis thaliana*, a little model plant that is used to observe the effects of a gene's activity over a relatively short period of time. The main goal of her project was to determine the effects of deregulating the production of the plant sugar rhamnose. Rhamnose is the component of the cell wall that causes it to retain its rigidity. We tested bacteria to find the right plasmid that would silence the gene in *Arabidopsis* responsible for regulating rhamnose. When we did find the correct plasmid, we transferred it into an Agro-bacterium solution and then to the *Arabidopsis* plants—via a glucose solution—by dipping the unopened blossoms into it and waiting until the new seeds formed. Then we planted the seeds in order to observe the final outcomes of the experiments.

Halfway through my internship I joined Gong Jian and Xiao Lin on the cotton project. We were using two kinds of Polymerase Chain Reaction (PCR) in order to find and isolate the genes that are responsible for elongating fibers in cotton plants. Once we found a gene, we had to run more PCR tests, but these were of a different sort. We used a second type of PCR to find the in-strand promoter for the gene we found. Once we located the promoter, we had to sequence it, but unfortunately I had to leave before the sequencing of the promoter took place.

Acknowledgements

I would like to thank Dr Yunliu Fan for her never-ending support, and for opening the doors of her lab to a high school student from another country. Even though her involvement in my projects was minimal, what she did for me will never be forgotten.

Another big thank you goes out to Dr. Chunyi Zhang for looking out for me in the lab. He taught me the parts of the experiments that I was unclear about, and gave me the best farewell party one could ever ask for. He was one of the most helpful members of the Chinese staff in the building and one of the most thorough teachers I had while in China.

Xiexie also goes out to Mrs. Dongfang Pan, my contact person in China before and during my stay in Beijing. She had another group of students to work with for a time but somehow still had time to make sure that I was doing well and surviving the challenges of living in another culture.

Thanks to the students in the lab, especially Jingfeng Wang, Gong Jian, Xiaolin Liu, and Jin Qin Mei. They made my life bearable by trying to understand me, my American sense of humor, and all of the other stuff about America that I shared with them.

I would sincerely like to thank the World Food Prize Foundation for providing an opportunity for students who participated in the Youth Institute to work as interns at various institutions around the world. I will always look back on the journey that I embarked upon when I set foot on the foreign soil that would be my home for two months with joy and gratitude. I would like to thank Ambassador Quinn and the rest of the World Food Prize staff for their involvement in efforts to reward those who endeavor to curb the ever-growing problem of world hunger.

Another thank you that I would like to offer goes to Dr. Norman Borlaug for his vision to send students around the world as interns in order to learn new ways to help battle food insecurity in the coming years.

Last but certainly not least, thank you to Lisa Fleming. Without her leadership, the Youth Institute would not have been as much fun or as interesting as it was. Even with all of the challenges that came because the new safety regulations, delayed flights, and canceled connections, Lisa was still making sure everything was okay for every intern. She was like a mother for all of us when we may not have been able to contact our own.

Thank You All!

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Introduction to the World Food Prize

I was introduced to the World Food Prize through my Talented and Gifted program teacher at the beginning of the 2005-2006 school year. My teacher, Mrs. Fritzell, had sent students to the Youth Institute in the past and thought that my experiences in Cambodia would make for a great paper.

After finishing my paper about poverty and malnutrition in Cambodia, I attended the Youth Institute and I can't really express how I felt when learning about the efforts that various organizations and companies were making to reduce obesity and still increase food security. Hearing those presentations after being in Cambodia, a country where the rice crop had recently failed leaving many of the country's farming families to nearly starve, made me want to be a part of the solution somehow.

In hindsight, actually taking up the challenge to participate in the WFP Youth Institute in 2005 was one of the best decisions that I've made. Not only could I apply for the internship, but I could finally tell a group of people whose life work is to help find a solution to food insecurity about the things that were bothering me about what I learned about the lives that many Cambodian subsistence farmers were forced to live.

When I applied for this internship, I thought that there would not be much of a chance for me to get one, like most participants probably thought at that stage. Then getting the letter that announced that I had made it to the interview stage of the selection process got me pretty excited. At the interview, I was probably one of the most nervous ones in the group, but getting into the hot seat and actually being able to communicate what I wanted to say in a way that was coherent made me feel better. Receiving the letter notifying me that I had been selected to serve as the intern at the Chinese Academy of Agricultural Sciences (CAAS) sent me through the roof.

My CAAS Experience

Research Oppurtunities

CAAS is a top ranked national research academy that is made up of 39 research institutes, which are located throughout China. The main campus is located in the Haidian District of Beijing. The satellite institutes specialize in areas like plant research, animal husbandry, agricultural economics, environmental studies, agricultural engineering, and new technology for use on farms. Almost two thirds of CAAS's 9000 faculty double as researchers at the many facilities of this institute. CAAS has two key state laboratories, approximately twenty key ministerial laboratories, six state crop improvement centers, and twenty-seven quality supervision and test centers. CAAS is also home to China's national Crop Gene Bank, which is one of the largest gene storage centers in the world.

The main priority for this institute is to research new areas in agriculture and animal husbandry. Recently, CAAS has reformed the logistics to accomplish the research, so as to allow for in-country leadership. The missions that CAAS wishes to perform are to be an advanced world institution that supports innovative ideas about science and technology, and also to be an advanced national incubator of new agricultural technologies that will benefit not only China, but the whole world.

CAAS Crop Science Laboratories

On the CAAS's main Campus, Dr. Yunliu Fan is one of a group of supervisors who share responsibilities for the Crop Science Laboratories. Dr. Fan is one of the most warm and welcoming women on the face of the planet,

besides my grandmother. Dr. Fan has a whole floor of laboratories under her supervision and she still has time to have coffee with an intern every morning—for almost forty-five minutes. Each of the seven labs has on average fifteen people in it. It is rather amazing that she can keep her schedule as free as she does after helping her students and conducting her own experiments.

Dr. Fan's laboratories are all studying some facet of how different plants react to certain stimuli with different genes activated or silenced. Each lab has more than one project going on at a time; sometimes groups of students would have two projects to investigate. The students take their work very seriously; every university may have the same project, so it is beneficial to get each project done as soon, and as perfect as possible, because competition is so fierce.

My Involvement:

Arabidopsis and Rhamnose Tests

When I got to CAAS, I knew that I would be working with either a cotton project or a rice project, and that was about it. My first day in the building where I would end up working was a Tuesday, and after that day I knew that everything was going to be different than what I had expected. I imagined that everyone would be in clean rooms wearing space suits, operating instruments that I would not be allowed to touch. Instead, I found a small lab with about five people in it who were very helpful in walking me

through procedures and explaining what the instruments were and why they were used.

When I realized that I would be allowed to work with college students on a project that could end up being their Masters Thesis research, it made me feel like I needed to perform perfectly. This meant that I got a little nervous when I got to work with the actual chemicals and samples that would make or break part of the experiments. I was relieved when I finished my first Electrophoresis Gel, and saw that even though I didn't find any of the correct plasmids, there were bars on the gel, which meant that I did everything right, despite my nerves.

The Plant

This is the little, little trouble-making plant. This plant is most of the way through its growing cycle, and its gene for regulating rhamnose has been silenced. That is why the main stems are not standing up straight.

That experience occurred when I was working with Wang Jingfeng, a Masters Degree candidate, on her experimentation with rhamnose levels in *Arabidopsis*. Rhamnose is one of the main components in the cell walls of most plants; it increases the strength of the cell wall, and allows the plant to stand up. Previous experiments had documented the effects of under-producing this sugar (floppy stems, soft cell walls, and plants that were easily susceptible to insect and disease damage), but they hadn't looked the other direction. It has been observed through mutation testing that without rhamnose, the main stem of the *A. thaliana* plant bends and does not stand up under the weight of the plant. One of the goals of this project is to

discover what would happen if the rhamnose-regulating gene were silenced. Our experiment investigated the effects of excess rhamnose in *Arabidopsis*. We wanted to understand those effects in order to transplant this gene into other plants.

After reviewing previous results I hypothesized that a higher than normal amount of rhamnose would cause the plant to make thicker, less permeable cell walls than its wild counterparts. I believe that an excess amount of rhamnose in the transgenic cell wall will create a plant that will be better able to survive longer in harsher conditions than the non-transgenic plant is able to withstand. If this is proven true it will mean that farmers who live in places where certain crops are unable to grow now, due to climate conditions, would be able to grow those crops and produce a food source or other crops that will generate income for their families. If this theory is proven correct, it would mean that families that were once struggling to survive might be able to support themselves and keep themselves from becoming just another poverty statistic.

Jingfeng and I studied the effects of silencing the rhamnose-regulating gene by first looking at *E. coli*—which are known to have the specific gene responsible for producing excess rhamnose, and isolating that gene, by way of Polymerase Chain Reaction (PCR), and confirming that the rhamnose gene is present by running Gel Electrophoresis. After the presence of the gene is confirmed, the bacterium is allowed to grow until the plasmid can be extracted. After the extraction, the genetic material from the bacteria is transmitted into an agro bacterium, which will then be injected into the plant tissue where the plasmid from the *E. coli* is absorbed by the plant and the plant DNA is changed so that the plant will produce an excess amount of Rhamnose.

This process was very time consuming; we had to run multiple gels in order to find just one colony that had the correct plasmid. I had the opportunity to search for a colony of *E. coli* carrying the plasmid, and there

were about five or six Petri Plates with upwards of one hundred colonies to choose from. Needless to say, it took me four tries to find one colony that had the desired plasmid. Jingfeng was extremely helpful and open with this experiment. She allowed me to conduct part of it myself. After supervising what I was doing a couple of times through the experiment, she asked if I could do it on my own and then went to work on another part of the experiment.

After isolating the plasmids that had the correct gene, we put plasmids into an Agro-bacterium. That process meant multiple steps with sophisticated computer equipment. First we took the *E. coli* cultures and measured the diffraction index—to make sure that we had enough of the bacteria in the sample. Then we inserted the sample along with the Agro-bacterium into the electronic synthesizing machine. This step caused the plasmid from the *E. coli* to bond with the plasmid from the Agro-bacterium. Once in this form the plasmid could be transmitted into the *Arabidopsis* plant. The Agro-bacterium was then put into a sugar and water solution, and then the immature flowers were dipped into this solution to transmit the plasmid. After finishing that step we had to wait for a new set of seedpods to form so we could grow the mutant plants.

As of right now, the experiments being conducted have been supporting the hypothesis, but it hasn't been long enough to see any results that will confirm or disprove the hypothesis. I would anticipate that the transgenic plant will be hardier than the non-transgenic plant, but it will take further experimentation to perfect the transgenic plants so that there are not any side effects brought about by changing the genetic makeup of the plants. I also think that this technology will someday be used to create plants that will benefit farmers in areas where drought conditions are devastating to the agriculture of the area.

My, Oh My! Look at the Cotton!

Mutant vs. Wild Type:

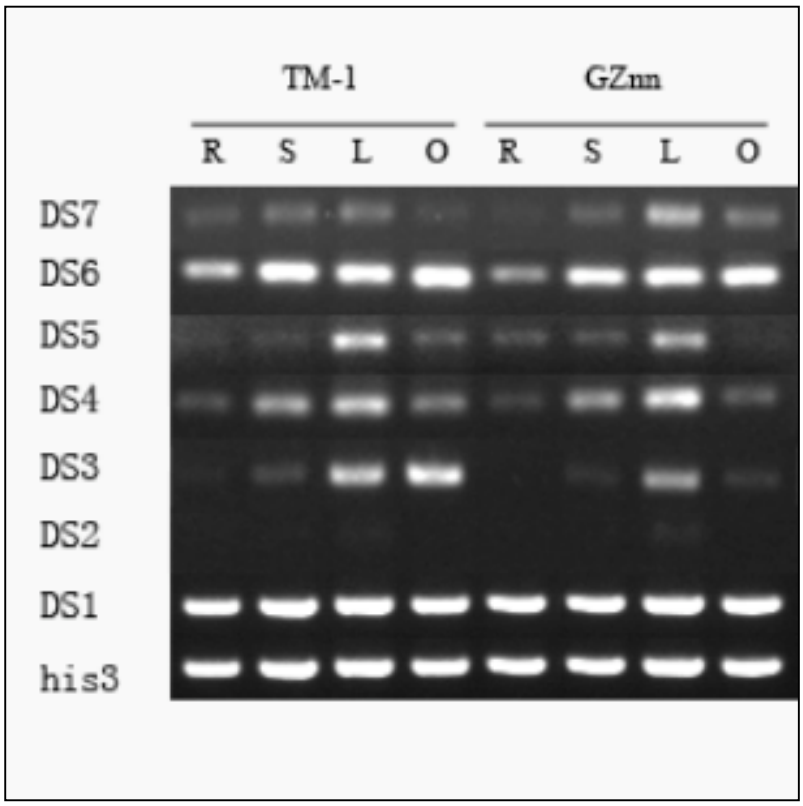
This figure shows how much of the fiber is left on the Wild Type seeds (below). The amount of cotton seen here was taken from around two seeds of each type of cotton, without having any air squished out of the harvested cotton.

The project dealing with cotton is studying the genetic structure of a mutant form of cotton plants in which the seeds do not form trichome, the main structure responsible for lint fibers being present on cottonseeds. Cotton fibers are classified into two categories: lint fibers and fuzz fibers. Lint fibers are the long fibers that are used as production materials, and fuzz fibers are those fibers that are short and remain stuck to the seeds and are unusable. Because there is no lint found on the mutant seeds, 100% of the fiber produced by the plant after fertilization has occurred is usable, rather than about 85% of the fibers produced by a wild type cotton plant. By finding the structure of this gene, it may be possible to transplant it into other textile fiber producing plants. Scientists could then study the effectiveness of this gene in increasing the amount of usable fiber in the plants.

The cotton for this project was grown in two places: the green house behind the Crop Science Building in Beijing and at the Cotton Research Institute in Henan Province. The samples that we used when I was there were taken from the plants in the green house. We took fragments from the roots, stems, leaves, premature ovules, and mature ovules. From these fragments we were able to extract enough DNA or RNA that we could isolate certain genes and study them to find the primer of those genes that are in the DNA sequence. Finding that primer would then allow other scientists to immediately find and work with the gene.

We thought that by isolating this mutant gene, and then transferring it into other textile fiber producing plants, the amount of usable fiber produced by the plant will increase. If found to be true in all textile fiber producing plants that will be tested, it could mean that farmers will be able to increase the amount of textiles produced from the same quantity of plants that they had originally cultivated. This increase in textile production will at first help the individual farmers economic status; ultimately it could stimulate the textile trade in China.

This theory is being studied by first isolating the mutant gene through a process known as DD-PCR—Differential Display Polymerase Chain Reaction—then studying the phenotypic changes that are results of that gene being silenced or over-expressed. The DD-PCR process allows the wild type DNA to be compared side by side with the mutant DNA. This makes it easy to find the differences in the genetic make-up of the two strands. After the different genes are identified, they are sent in to a corporate laboratory to be sequenced. When the sequenced DNA is returned to the CAAS lab, the gene is then put back into a wild type plant by means of an Agro-bacterium transfer—the same transfer that was done on the *Arabidopsis* project—so as to observe the effects that that gene causes until the one dealing with the trichome is found. This means that it may take a few tests on a few different genes before finding one that deals with trichome.



DD-PCR Gel Readout

This is the result of a DD-PCR Reaction. All of the spots on the gel are genes that are expressing themselves in each of the sampled tissues (R= root, S= stem, L= leaf, O= ovule). Where ever there is a difference between the two types of cotton it means that those genes will be tested further. TM-1 is the Wild type cotton plant and GZnn is the mutant.

After this gene has been found and isolated through the processes described above, it is then compared to the genetic sequence of the other textile fiber producing plants that will be tested in order to find out how close the cotton gene is to any of the genes in that plant. If it resembles the other plant's gene closely enough, the cotton gene can then be transferred to the other plant—via an Agro-bacterium transfer—to observe what effects it has on the new plant.

This project is still in the stage of isolating and sequencing the cotton genes that are different between the mutant and wild type DNA strands. However, the genes that have been sequenced thus far have shown remarkable similarities to those in other plants, so when it comes time to implant this gene into the other plants it may not be as difficult to find a compatible plant for implantation. I believe that if this project succeeds in increasing textile fiber production, it will mean that farmers raising textile fiber plants around the world will be able to support themselves and their

families when the may not have been able to in the past. This would mean that the economic status of many would increase and their lives would be transformed from simply trying to get by with a hand to mouth existence to a higher standard of living.

While I was working on this project, we were trying to compare seven genes that appeared to be associated with fiber development. That meant processing at least two or three PCR templates per day in the lab, and each of those reactions utilized a different primer. We cut the DNA from different places for better comparison against the wild type genomic information. For this part of the project, I was accepted as a fellow scientist in the lab. I did the same work as the two CAAS students with whom I was working. My only extra task was reading research papers describing previous projects that had similar goals as ours. I was scheduled to participate in the next step of the process: going to the Cotton Research Institute in Henan Province, where we would have begun to sequence the promoters in the DNA so that each gene would be easily accessible for other scientists to work with after the experiment is concluded. Unfortunately, this part of the experiment was repeatedly postponed due to cold weather in Henan Province. They ended up leaving for the Cotton Research Institute the day I left Beijing to return home.

I had to leave both projects too early to see the final results. The *Arabidopsis* project is going to be in the works for a while longer—until the gene that allows for over production of rhamnose can be successfully transferred into other plants where its effects are visible. The cotton project had successfully mapped the entirety of two of the genes by the time of my departure and we were preparing to sequence a third when I left.

Cultural Differences

Going There

Besides the food being different, cultural norms from here in the United States were not the same—at all—as what Chinese culture would dictate. One thing that I had trouble with at first was trying to use my sense of humor with my lab-mates. I had to explain a lot of the jokes that I told, mostly because the ones that I knew required a little bit of an American influence to understand. But, as my internship progressed I started to pick up a few themes of Chinese humor (not in Chinese, but rather the vocal cues that told me when to laugh), and they picked up some of the oddities that made up my sense of humor. The Chinese language presented another unique challenge: even if I knew the correct Chinese word, I might not get the tone correct which changed the word's meaning entirely.

When I got to China and had my first chance to see the sights of Beijing, I was startled by how many billboards were at street level. It wasn't

until I rode on the top of a double-decker bus that I realized that those large 2008 Olympic Advertisements actually served two purposes. In addition to announcing Beijing's selection for the summer games, they often hid either a construction site or a *hutong,* something that most Americans would mistakenly call a slum. True, many of the poorer families live in them, but those areas are rich in culture and provide an excellent opportunity to see what the "real China" is like. What I mean is that you could go into one and though at first you would attract a few wary glances, the people there would still go about their daily business. You could catch a glimpse of life in an earlier time in China through the lives of the people behind the billboards. I believe that it is one of those things that you must see for yourself in order to fully grasp the concept, and even then it is still hard to describe. But the way the Chinese government is treating them—casting them aside in favor of progress—it makes the *hutong* seem like slums. All around Beijing there is a push so become more modern and sophisticated—new styles of architecture, for example, are taking the place of traditional Chinese buildings in the skyline—as the Olympics approach. Seeing the billboards covering up what was once uniquely China in preparation for the Games made me wonder if they will be trying to hide their past from the rest of the world when 2008 rolls around. The world will be watching, and the Chinese government wants to control what we will see.

Another thing that I had a hard time with: eye contact rules. Eye contact in America is a sign that you are listening to the person who is speaking, even if that person is "more important" than you. In China, you don't make eye contact with the other person in the conversation unless you are equals. If a student is talking to his or her teacher, the teacher may look at the student, but if the student were to make eye contact with the teacher, it would be considered rude and disrespectful. Between students and teachers, that protocol only applies in the formal classroom or laboratory

situation. Beyond the classroom door—and especially during recreational times—all are equal.

For the first six weeks of my stay at CAAS, I had the unique "opportunity" to spend time with fourteen Ohio State University students. They were there on a study abroad trip, and at times it really didn't seem like school was their top priority. I spent a lot of my weekend time working as the designated room finder after some of the OSU students had consumed too much Chinese beer and other adult beverages. After having to deal with their parties on the weekends, I finally understand why Americans aren't always highly respected when visiting other countries.

Another big difference that became apparent to me was how scheduling is handled within different societies. From recreation to work, everything during my time in Beijing had a specific time to start and a time to end. The really different aspect to this system was that Chinese people in my area run at their own pace: if they say "around eleven," they may be there at ten till eleven or a quarter to twelve, depending on the importance of the event and who set the appointment. I never really got used to this. I always made sure to be at work a little earlier than the 8:30 am scheduled start of the workday. That meant that I was often the first or second one there, and would sometimes wait about hour before the rest of the people on my projects arrived in the lab.

Recreational time was something that was never shorted, however. As soon as the workday was over, young and old alike would stop what they were doing and go play football—American Soccer—basketball, table tennis, or badminton.

Especially badminton.

Behind my dormitory was the neighborhood sports complex—a couple of basketball courts, a few badminton courts, a couple of ping-pong tables, and a volleyball court that was always getting turned into a soccer field. Every morning the same elderly couple would be down playing badminton.

Seeing them out there playing was kind of comforting; it was a little bit of certainty in a world where anything could happen.

But for me basketball games and soccer games became my afternoon recreation—in fact, I wore the soles out of one pair of basketball shoes and destroyed my soccer cleats. After work I would go down to the basketball courts and play for about two hours, and during that time I would hear stories about a previous Borlaug-Ruan intern, Divindy Grant, and her ability in the lab, and her skill as a basketball player. I was surprised to hear that the Chinese students actually remembered an intern from two years ago, and at how they let me just jump in and play along with them even though they couldn't always understand what I meant when I yelled during the game.

That's it: the thing that set Chinese people apart from Americans is just that, their hospitality. They were always trying to make me feel like I was important, even when I was sitting at a table with top name scientists from around the world. No matter what, they would make a place for me, and that meant that sometimes I was on the basketball court longer than my quickly dehydrating body could handle. I never had to worry about having to ask for anything because there was always someone there with what it was that I needed, even if it meant that they had to go without. That kind of hospitality is something that I will sorely miss.

Coming Home

When I got back to the United States, I realized just how different I had become after spending sixty days in a foreign country. I am still trying to get used to some of the American things that I deal with everyday and previously took for granted. The process of returning to normal is a bit stressful at times, but I have so far been able to adjust.

I think the biggest thing for me is missing the food: the quality, the flavor, the meal structure, and the fact that Chinese food available in

restaurants here doesn't compare. One of the hardest things for me is the American custom of cleaning my plate during a meal. In China it is an insult to the cook if you clean your plate: it means he or she didn't make enough. But here—where every portion is just a little too big—everything is opposite. If you don't clean your plate you insult the cook.

Another thing that I am still having trouble with is the way meals here and there differ. In China, having two meals per day is fairly normal. Breakfast was usually the biggest meal of the day, unless you had guests for supper, and breakfast also had more of a stick-to-your-ribs quality. Then because of the heat, lunch would be small and light, or it would be completely left out of the schedule. Supper for many Chinese people would be something like lunch is here in America: light and small. Meals here are pretty much the exact opposite. Breakfast, as most of my classmates have it, is the smallest meal of the day or skipped altogether. Coming back to that when I was the hungriest in the morning meant that I have to get up earlier so I can have a little bigger breakfast. Lunch has become more of school-time thing now for me. My friends were all kind of scared when I came back and didn't eat lunch for the first week and a half of school, but because of the meal schedule that I had become used too, I was never hungry around noon time. Because most Americans are busy during the day, supper has become a family togetherness time, which also means that the meal has become larger. I would still like to just have a small light supper that goes by a little quickly. But, really, to me eating a little of a large meal doesn't phase me. I am more there to talk to my family most of the time anyway. They really like hearing the stories that I am telling them about China!!

Another thing that I noticed was that Chinese eye contact rules needed to be left in China when returning to America. It was a little difficult at first to do, but after making myself look up towards the eyes of my teachers and toward the eyes of people older than me, I have re-accustomed myself to this American show of respect.

Coming home to a place where most people don't have the same understanding of poverty and the urgent issues surrounding food security is really different. Often what is considered "poor" in The United States is a much higher standard of living than poverty in other countries. In China it is technically illegal to beg, but that doesn't stop people from doing it. There was one group of people that was always begging by the west gate of CAAS's campus, and to see the malnourished children with them made me want to give them something. However, an e-mail I received from the U.S. Embassy about an American who had been murdered because of ploys where groups, like this one, used children as a guilt trip made me think twice about giving money to them. I was torn between wanting to help and wanting to be safe. I hadn't felt that way since walking along the crowded Mekong Riverfront in Phnom Penh, Cambodia. Here at home, people in bigger cities probably have a better understanding of this, but even in my travels to New York and Washington, D.C., I have never seen a ploy like this used by the poor, or others who are exploiting the poor.

Things like that have made me really glad that I have been able to be a part of the fight against hunger through the WFP Youth Institute and Internship. It truly is a battle: people are dying everyday because of hunger and its force is stronger right now that any counteroffensive that we have come up with thus far.

Looking back on this experience, I can say truthfully that I can't think of a better profession to have; working to create a better future characterized by increased water and food security is something that will benefit all people everywhere. The "future" for many people does not extend beyond today. People who do not know where they will find their next meal have difficulty contemplating the future—they struggle merely to survive each day. Through educational programs like the World Food Prize Symposium, the WFP Youth Institute, and the Borlaug-Ruan International Intern Program,

more people are becoming aware of changes that need to take place in order to make the world a more productive, nutritious place for all of its citizens.

When it was time for me to leave Beijing, my mentors and friends at the Chinese Academy of Agricultural Sciences let me keep my key to the laboratory where I worked this summer. I placed it on the ring that holds my car keys and house keys. My CAAS lab key is a compass of sorts. It reminds me of where I spent my summer, and points toward goals that will guide my future.