Maize Kernel Components and their Roles in Maize Weevil Resistance:
My Summer in México

Kevin Joseph McNee

My name is Kevin Joseph McNee, and I attended the fall 2002 Youth Institute, representing Xavier High School of Cedar Rapids, Iowa. The program interested me because I have always felt a nagging curiosity about the problem of the inequity of the world's food situation. The paper on the topic of water shortages also piqued my scientific interest and I greatly enjoyed researching it and writing out my ideas regarding it. I felt very privileged to have distinguished figures from all over the world listen to my paper and give me feedback. I figured that if it was that great on just a three-day excursion, a whole summer working with the World Food Prize would be an absolutely fantastic undertaking.

The Youth Institute chose me to go to the International Center for the Improvement of Wheat and Maize, also known as CIMMYT, its acronym in Spanish. CIMMYT is located in El Batán, México, just twenty minutes from the tremendous mega-metropolis of México City. The Center was established in 1977 as a joint venture by the government of México and CGIAR, the Consultative Group for International Agricultural Research. As a member of the umbrella group of CGIAR, it shares the goal of all CGIAR's member research centers to work on poverty reduction, thereby increasing the quality of life for the world's poor. CIMMYT's mission statement strives to emphasize the fact that it does not endeavor to simply increase the world's supply of food, ending hunger that way. It recognizes and wants others to recognize that to truly, effectively, end world hunger and the problems that accompany it, we must improve the efficiency of distributing our food supplies already extant, end wasteful farming practices and those that harm our environment, and improve the quality of life for the world's poor. Not only do CIMMYT and its staff believe this, they are wholly committed to the fact that it is doable and especially that it necessarily must be done. I am happy to say my experiences at CIMMYT has strengthened my own beliefs and redoubled my enthusiasm for this cause.

When I arrived at CIMMYT on June 3rd, I had almost no idea what the experience would be like, much less what research I would be doing. I had talked to the intern who was stationed there from last year, Emily Nieman, and she had been very helpful in calming my anxieties about the whole living and work situation there. Still, it was completely new to me and not a little bit frightening. I arrived late at night, during an intense downpour, which I would learn was typical of that part of México at that time of year. The flight had gone well, and I was excited to see the place I would be spending the next two months at. After a nice little chat with the driver who took me to CIMMYT from the airport, he dropped me off at the CIMMYT dormitories. Needless to say, I didn't get the chance to see much of anything that night, but I didn't mind so much. There was a warm, dry bed there, and I was exhausted from the flight and the preparations I had made for the trip. I flopped on the bed with my shoes and jacket still on, and was soon sleeping soundly.

The next couple of weeks I got to know everyone in the Maize Germplasm Bank, where I helped them to pull seed out of cold storage for distribution to various research centers around the world. I met and talked with Dr. Taba, who explained to me the way things worked at the Germplasm Bank. My main task at the Germplasm Bank was packaging seeds for distribution. I enjoyed it because I got to know several of my fellow bean-counters well, and it was exciting for me to be immersed in the Spanish language. They were interested in learning about Iowa and the part of the United States where I come from as much I was interested in learning about their culture. Despite being transferred soon to the Entomology Unit, I made fast friends with my fellow staff in the Maize Germplasm Bank.

About two weeks into my internship, I was still waiting for a project of some type to pop up, which I was required to carry out as per the requisites of my internship. One day I was counting seeds when Dr. David Bergvinson from the Maize Entomology Unit showed up and offered me the chance to work with his staff. There was a higher chance of me being able to find a project there. That day I walked from the Germplasm bank to the Entomology building not knowing what to expect, but I am very pleased to say that the group of people I found turned out to be just what I needed. There was Silverio García-Lara, a Ph.D. student who would be my supervisor and soon enough my close friend. He and I worked very closely over the remaining six or so weeks I had at CIMMYT. Silverio was so kind as to invite me to his birthday party, which was an extraordinary event. His family accepted me as their own. I really enjoyed that because I could understand what they were saying and could reply in kind. I took the opportunity to teach them a card game that was taught to me, in turn, by a group of visiting Afghani scientists that lived in the CIMMYT dormitories.

Besides Silverio, there were five others that I worked in close association with at Entomology with, all Mexican nationals. The favorite part of my day was getting to work in the morning to eat breakfast with them, as they ate breakfast early before most of the international staff came in. That's one thing I miss about México: the comradeship and intimacy that people live and work with each other. Silverio and the others helped me so much that I don't think anything I've done can really be called solely my work, and I couldn't have achieved nearly as much without them.

The work carried out in the Entomology Unit focused on the relationship between agricultural pests and their targets. This area is still being explored; little is known about the exact methods of how certain pests select what crops to attack, which species of insects like which species of grain, and other certain peculiarities of the problem. Study in this area is important because much of smaller farmers' harvests are lost in the field to insects, but the most damage is done to grain in storage. The goal of CIMMYT's Entomology Unit is to help farmers deal with pest-related problems, hopefully preventing the farmers from turning to pesticides and other short-term and potentially hazardous solutions.

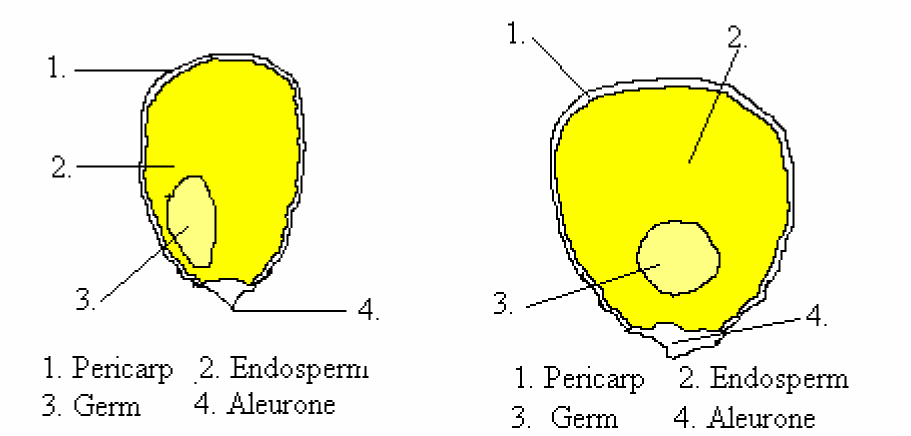
The Entomology Unit work at CIMMYT focused on two major pests: the common maize weevil (*Sitophilus zeamais*) and the larger grain borer (*Prostephanus truncatus*). These two insects are classified as granary pests. They inflict heavy damage on maize because not only do they feed on it in the field, they also lay their eggs in the seeds. After hatching out of their eggs inside the seeds, the weevils will spend their larval and pupal stages hollowing out the maize kernels. When they reach maturity, they break out of the seeds. The reason weevils are so harmful to stores of grain is that one or two ears of infested maize can hatch dozens of weevils that will continue the cycle, infesting more ears. Also, the weevils produce maize powder and fragments when they bore through the maize seeds, creating fodder for secondary pests to establish themselves in the store on.

I myself decided to focus my research on the maize weevil's detailed behavior regarding maize kernels. Towards this end, Dr. Bergvinson and Silverio directed me to conduct a project in which I delved into the world of the relationship between maize and the common maize weevil. I also designed an olfactometer to more closely examine the factors influencing maize weevils' choice when picking maize to infest.

Before I began my investigation, I talked with Silverio and Dr. Bergvinson and we established the general form my experiment would take. It was decided I would use two populations of maize specifically bred for their vulnerability and resistance, respectively, to weevils. By quantifying, in a way, the differences between these, it was thought we would see the characteristics that make a strain more vulnerable to storage and field pests. When we knew exactly what made the one more susceptible to the other, we postulated we would be able to encourage farmers to grow certain varieties which did better against storage pests more certainly. What we found was even more encouraging than that.

It was decided it would be best for me to use CML244 x 346, a variety known to be susceptible to the larger grain borer, and Population 84 c3 Mix., a variety known for its resistance to the larger grain borer because at the time, the supplies of these varieties were most abundant in the Entomology laboratory, and their qualities would make clear contrasts to each other.

We determined that one of the grounds that we would compare the varieties to each other would be through kernel hardness. We went so far as to compare not only the whole kernel hardness of the varieties to each other, but also the discrete components of the kernels. The components we compared were the pericarp, the whole kernel, and the endosperm. The pericarp is the thin layer of harder tissue surrounding the endosperm, protecting it from damage.



Figures 1 and 2 components of maize kernels, head-on and side view. Note the position of the germ.

Silverio and Dr. Bergvinson asked me to especially focus on the pericarp. They had conducted research on the pericarp and its chemical composition. Their study of the pericarp had led to more questions regarding its role in weevil resistance and we thought this investigation would answer them.

One thing we wanted to figure out was how the pericarp related to the whole kernel and to the endosperm in terms of hardness. This was because it had been noted that the pericarp in the more susceptible varieties was somewhat thicker. However, the knowledge in this field was limited because not much research had yet been conducted.

Abstract

Hardness is one quality of maize seeds, which may affect their resistance to maize weevils, specifically the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Hardness varies from seed to seed, from plant to plant, and from variety to variety. The harder a seed is, the more resistant it is to storage pests, such as the larger grain borer. Using a displacement force resistance testing station, we measured the hardness of hundreds of seeds. The seeds we used were of two varieties: CML244 x 346, a variety known to be susceptible to the larger grain borer, and Pop. 84 c3 Mix, a variety known for its resistance to the larger grain borer. We also used a micrometer to measure thickness of the pericarp and of the seed itself. We found a relationship between hardness and thickness, both in the pericarp and the whole kernel. A relationship between the hardness and thickness of seed and seed pericarp and the subject's saturation with moisture was observed. Lastly we observed a correlation between the seed variety and the pericarp's and kernel's thickness and force.

Introduction

Maize is one of the world's most important food staples, and its importance is projected to rise in the coming years. It is a source of great nutritive value and is irreplaceable in the diets of billions of people around the world. It is important to protect this valuable food source. It is vulnerable because of the fact that it is so widely cultivated and eaten; a plague of some sort that develops in one part of the world could feasibly spread to everywhere it is grown, stored, prepared, etc. One example of this would be the problem of maize weevils, which were first noted in grain stores in Central America. This granary pest spread through the economically important trade of maize. No longer isolated to Central America, the maize weevil became a huge problem in the United States in the middle of the 20th century, and later decimated Africa's grain stores beginning twenty years ago. Weevils bore into maize kernels in the field or in storage, laying eggs. The young grow to maturity inside the kernels, eating them from the inside out. This causes loss in the maize's nutritive value and weight. Also, a contaminated supply of seed will most likely contaminate other stores within a certain range. This is especially severe on sustenance farmers, who may depend on one year's store of grain to provide germinating seeds for next year. While boring through the seed, the insects create powder and kernel fragments that encourage other, secondary storage pests to take root. By learning more about how certain maize seeds resist contamination through seed hardness, we will be able to reduce the amount of grain lost to storage pests and related problems.

Materials and Methods

Kernels. Kernels were taken from a seed bank in the CIMMYT Entomology Unit Laboratory where they were stored at 65% relative humidity. These kernels had been grown in the El Batán research station in Mexico. Fifty kernels were used in each treatment. No kernel was used more than once.

Obtaining pericarp. The aleurone was removed from kernels using a stainless-steel razor blade. This allowed water to enter the kernel more readily. The kernels were then fully submersed in water for fifteen minutes. After this time they were removed from the water and the softened pericarp was cut using razor blades. The pericarp was placed in a large book overnight to flatten and dry. It was necessary to flatten the pericarp to obtain as accurate a reading of their thickness as possible. Drying them overnight helped to make sure that we achieved accurate readings of their hardness, as moisture can greatly affect this.

Obtaining endosperm. The same procedure was followed as was used for obtaining pericarp, except that the entire kernel was stripped of all pericarp to achieve the naked endosperm. These were left to dry overnight as well.

Measuring hardness. Using a displacement force-testing machine, the kernels were probed and their hardness was measured and recorded by a computer. The pericarp we placed between two small sheets of metal with holes punched into them, centered between the holes, so that the probe could descend straight through and test the pericarp. The pieces of metal were held together with two clips at each end. To achieve accurate results, we needed to mill a probe tip to the proper diameter of .80 millimeters. All samples were placed embryo side down when probed unless we were getting the readings from the embryo side, in which case they were placed embryo side up.

Measuring thickness. Thickness of kernels and pericarp were measured using a micrometer.

Statistical analysis. The data was analyzed using Statistix 7.0.

Results

The relationship between force and thickness was observed. Force generally increases with an increase in thickness.

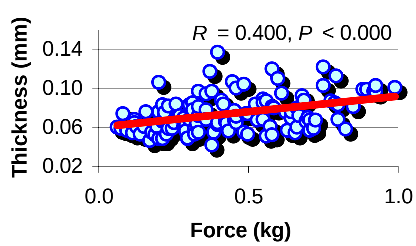


Fig. 3 positive correlation between thickness and force

There is a difference in hardness between the two genotypes. The following is a graph made using readings from the kernels on the side opposite the germ. The pericarp readings were multiplied by a factor of ten so that one could more clearly see the difference between them on the graph.

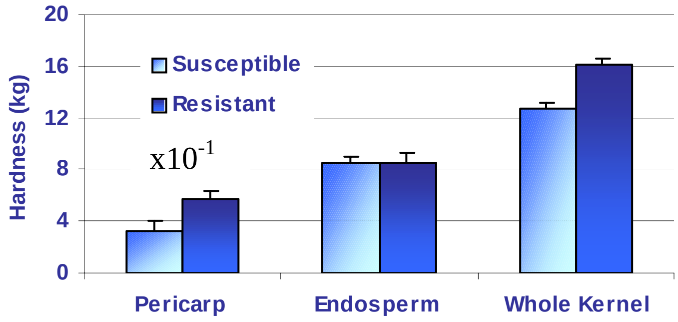


Fig. 4 probe readings when entered through the side opposite the germ

The difference is more greatly pronounced in the tests we ran with the Displacement Force Testing probe entering on the germ side:

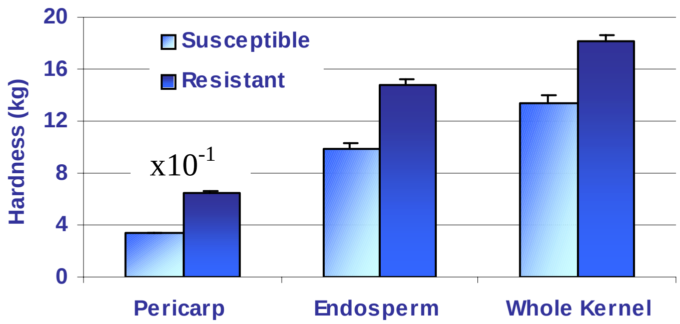


Fig. 5 probe readings when entered through side closest to the germ

The following graph shows the differences between the two genotypes in thickness.

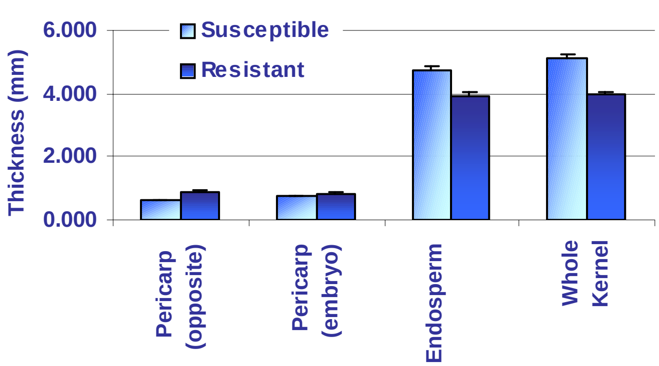


Fig. 6 genotypic difference in thickness

The following two graphs illustrate readings we obtained during moisture saturation experiments. When you compare these with the previous graphs, you can see that moisture saturation does indeed affect the attributes of the pericarp that deal with kernels' resistance to weevil infestation.

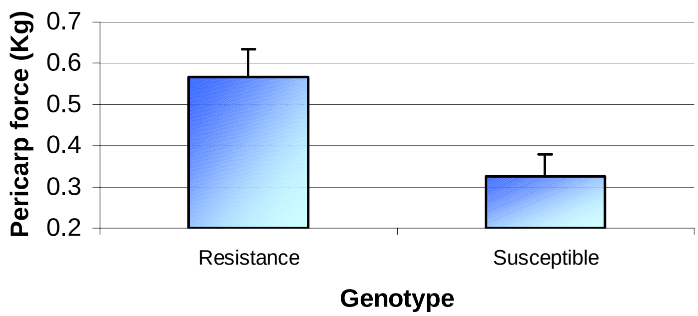


Fig. 7 pericarp force comparison between genotypes

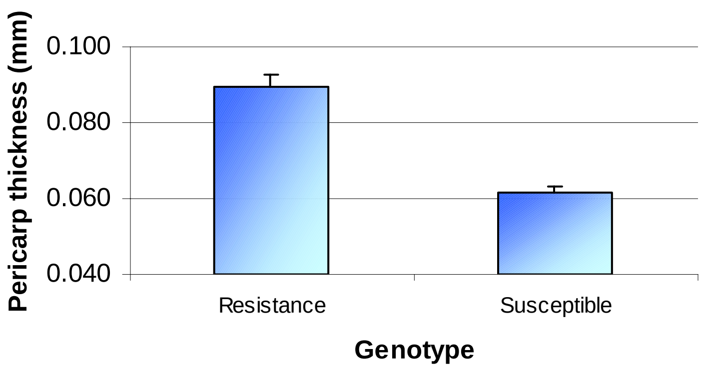


Fig. 8 pericarp thickness comparison between genotypes

Discussion

Studying the relationship between pericarp thickness and force, we observed a positive correlation. That is, an increase in thickness of the pericarp generally signifies an increase in that pericarp's force. This is true of both the resistant genotype we studied and the susceptible, and on both sides. However, the resistant genotype is able to have more force at a lower thickness. This is important. The main hurdle that exists in getting grain that has been genetically modified (GM) for increased force is keeping the pericarp thin. A thick pericarp discourages sustenance farmers from planting the GM grain because it is harder to grind into flour and is harder on the animals it is fed to. The large grain producers prefer grain with thin pericarp as well because it is easier to process through the mills and other machinery. With this research we have been able to prove that grain can be more resistant through harder pericarp without necessarily thicker pericarp. This can be achieved through selective breeding or genetic modification.

We also established a connection between kernel moisture content and kernel force. As could be seen in the graph, the force of a kernel was diminished when it was submersed in water. We submersed three sets of forty kernels (of both susceptible and resistant genotypes) in water after removing the aleurone of each. One set was submersed for fifteen minutes, the next, thirty, and the last, sixty minutes. The force of the kernels of both genotypes in the first set had significantly lowered force levels. The second and third sets were negligibly less hard than the first. This establishes that the moisture saturation point is somewhere around fifteen minutes. The most important thing that we found is that moisture decreases the force of both resistant and susceptible genotypes. This means that even if we do invest in developing and distributing the resistant genotypes, it is still important to keep in mind storage conditions. If conditions in a grain store are damp, this makes it easier for weevils to infest the grain, and other storage pests will follow.

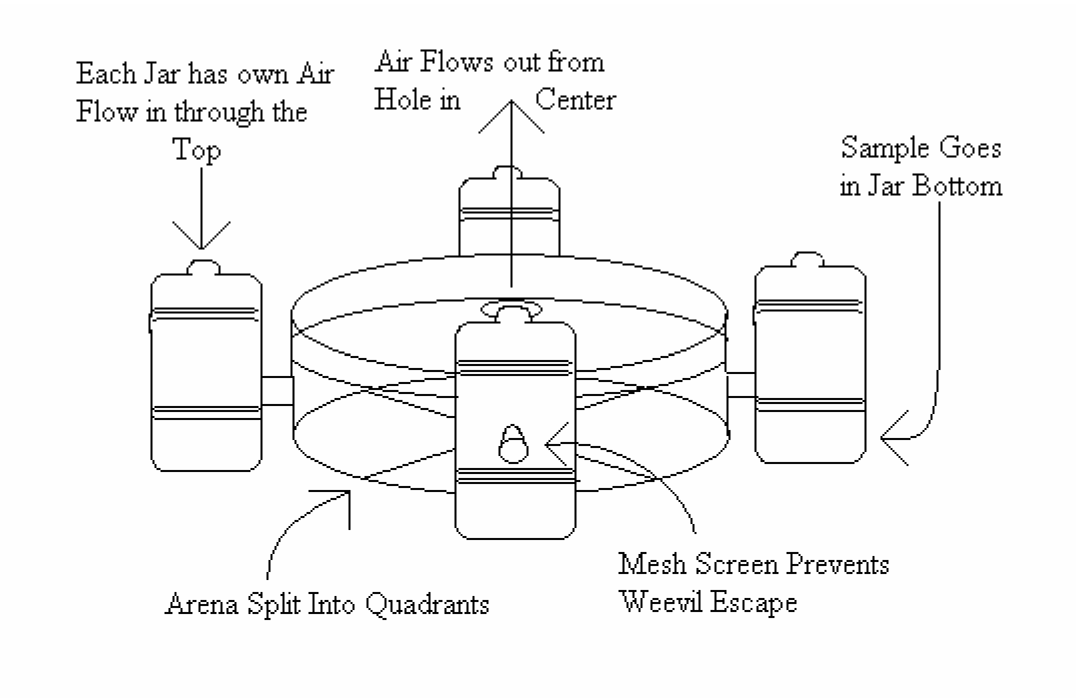
Comparing sides of the pericarp, we found the embryo side to be less hard than the opposite. This is a negligible amount, however. In other studies, anti-feedants such as phenolic acids were found in high amounts in the pericarp on both sides. Also, structural supports, for example, extensins, were found highly prevalent in both sides of the pericarp. The majority of weevils and other grain borers prefer to bore through the pericarp on the side opposite the embryo. There was a hypothesis that the pericarp was thicker on the side of the embryo. However, the two sides seem to be almost like peas in a pod in terms of thickness. More study is needed to determine what causes this phenomenon. In my opinion, the weevils simply find it favorable to drill through that side because the germ may be harder than the endosperm, or less nourishing, or it contains more anti-feedants. The endosperm being more preferred by the weevils makes sense because it is used as food by the germ, and so is highly nutritive and most likely has fewer anti-feedants.

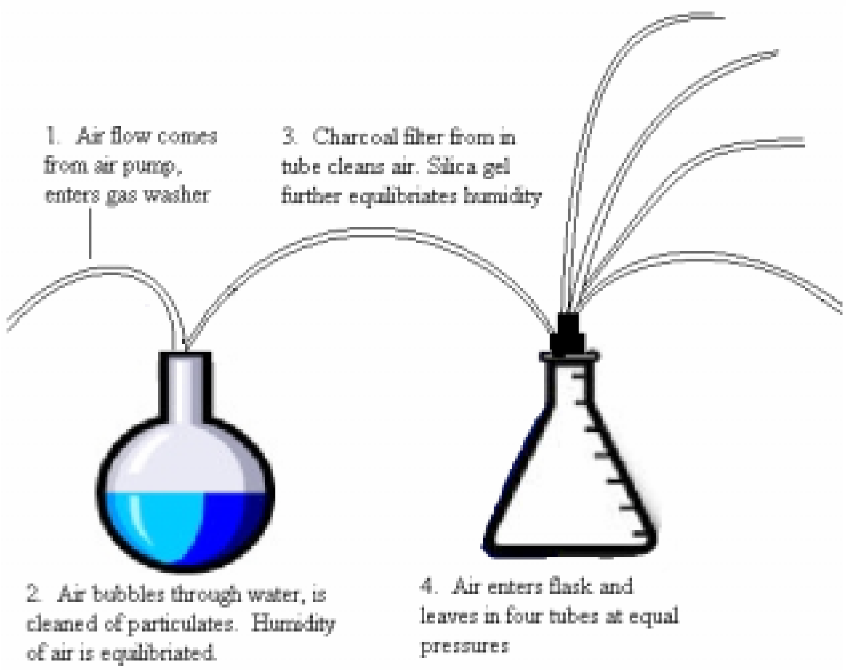
When we tested the separate components of the kernel, we found that the pericarp contributes much more to total kernel force than previously thought. This is especially true in the resistant genotype we studied. Although both genotypes had about the same pericarp thickness on average, the resistant variety had more force. This may seem to contradict the earlier finding we have, that force increases with thickness. However, there is another factor: the extensins I mentioned earlier and other structural proteins. These do not add much to pericarp thickness, but they add much to the strength. Taking a look at the graphs, you can see that the pericarp contributes a disproportionately high amount of force to the kernel for its percent composition in the kernel. If it was possible, we would have been interested in comparing the two genotypes in terms of their composition. This finding shows that it would be worthwhile to attempt to increase the resistance of maize to storage pests by working with the pericarp force, either through selective breeding or genetic modification. This can be done, as we have shown, without increasing pericarp thickness, which is the main hurdle. If we can discourage the weevils from boring through the pericarp, it will cut down very much on all the storage pests, because they are generally dependent on the weevils to create powder and pieces of broken seeds to feed on.

A blueprint for the olfactometer I designed appears on the next page. The idea behind the olfactometer was to find more about exactly how the weevil chooses the kernel or cob of corn it wants to infest. The idea was that we would place a sample of different types of corn, for instance, weevil-resistant and weevil-susceptible varieties of maize, into two of the jars opposite each other. The other two jars would be left empty. Air flowing from the filtering and diverting apparatus would enter and flow through the jars at an even rate. This would expose the weevils to the air. We made sure the air entered all four sections of the center chamber at an equal and even rate, that it was free of particulates in the air, and that it was at a low relative humidity. Other studies have shown that weevils have an adverse reaction to high levels of relative humidity.

After construction, the olfactometer was tested with weevil aggregate hormone to see if it was successful. Silverio and I both agreed that, since the weevils spent the most time in the sectors that were closest to the sample jars that had the hormone in them, the olfactometer was practical. We would place weevils in the center of the chamber at the beginning of five minutes. After that time, we would record how long the weevils spent in each sector. In between test runs, we would soak the device overnight in a solution of bleach and water to get rid of all trace chemicals that might affect results. We designed it so it can be fully disassembled, and so easily cleaned. I didn't have enough time to actually run any tests with this part of my project. However, it is ready for next summer's Youth Institute intern if he or she chooses to work with it.

Fig. 9 partially disassembled olfactometer and I in the lab


Each Jar has own Air Flow in through the Top
Air Flows out from Hole in Center
Sample Goes in Jar Bottom
Arena Split Into Quadrants
Mesh Screen Prevents Weevil Escape


1. Air flow comes from air pump, enters gas washer
3. Charcoal filter from in tube cleans air. Silica gel further equilibriates humidity
2. Air bubbles through water, is cleaned of particulates. Humidity of air is equilibriated.
4. Air enters flask and leaves in four tubes at equal pressures

My summer in El Batán, México, was my most well spent summer ever. I made many friends in CIMMYT and in Texcoco, the small town nearby where I shopped for groceries. In the following pages I would like to show some pictures detailing my life from June 3rd, 2003, to August 1st, 2003, and hopefully delineating some changes I have gone through as a result of my internship.

One thing that really changed my perspective on things was the presence of visiting scientists from such diverse countries such as Bangladesh, Afghanistan, China, Sweden, Switzerland, and the United Kingdom. They gave me a refreshing outlook on life and everything from work to play. I realized through my interactions with them, especially the Afghanis, that I had grown complacent with what I have and what I can do. This exposure to other cultures has made me less disillusioned with the power of one to make a difference. Visiting scientists, through playing cards with me, cooking ethnic specialty dinner with me, or inviting me to a piñata party, have had a great positive influence on my mindset and my belief in what I can do. Above and to the left, I am standing in front of CIMMYT's guard booth with some of the visiting scientists from Afghanistan, Iowa State University, and Kenya.

I thought it would be a good idea to ask around and see if I could get experience in other departments. Here is a picture of Jens and José-Luís, from the Computing Services Department. I helped them take soil temperature and moisture readings in the fields sometimes. Jens and I had a discussion about affirmative action that convinced me that, although it is necessary, there really was nothing else that can be done to even things out. He also tried to teach me to speak Spanish faster by refusing to speak to me in English, or even slowly in Spanish. José-Luís was the handy-man of the Computing Services Department, and we would discuss popular cartoons like *Pinky and the Brain*, a cartoon about a pair of mice who plan to take over the world. He called me 'Cerebro,' or 'Brain,' in the Spanish. The story behind that is that I had gotten people to play me in the board game 'Risk,' a game where you take over the world, and had earned a reputation for being ruthless in my tactics. Needless to say, he was too scared to challenge me.

Silverio and I worked very closely over the course of the two months. We got to know each other very well, so well that he invited me to his 32nd birthday party. I invited him to play 'Risk' with me as well; however, he claimed to be too busy. This picture is of him and me working at the DFR station. I learned much of the Mexican culture from him. He liked to discuss with me matters of family, because he had recently become a proud father. One day we were discussing politics and matters of society when he asked me, "What do you think the purpose of the family is?" I answered that families are meant to provide a caring environment for children. He replied that his idea of a family was one in which the family members all strived to care for each other, and help each other to thrive. The way he thought about families was pretty radical to me because I had never really realized that parents depend on children for support just as much as children depend on parents.

Through discussions with people from different backgrounds, I gathered a broad idea of how different people do different things around the world. I was playing soccer one day at CIMMYT, and my friend Erich, who grew up there, said something along the lines of the reason he was skilled at soccer is because he lived at CIMMYT and played people from all over the world with different styles, strengths, and weaknesses. I think that is a good analogy for what happened to me: I met and talked to people all over the world, and now I have a more eclectic world view. I am no longer the sheltered, isolated Iowa boy I was last spring.

Speaking of Iowa, many people would ask me about it. "Where are you from?" they would ask. Most of the time I liked to ask them why they thought I wasn't from México, was it my accent that gave me away? When I said 'Iowa,' the most common reply would be, "How far is that from New York?" Everything to them is located relative to New York, California, or Chicago. I enjoyed telling them about Iowa, frightening them with stories of snow that sometimes reached waist level or temperatures below freezing. Mostly, they were surprised that an Iowan knew Spanish as I did, and they wanted me to teach them English or about the United States. Many Mexican nationals I met, especially

taxi drivers or students would tell me their goal of someday becoming a citizen of the United States.

I met some very pleasant people from Afghanistan. They appear in the picture above and are, from left to right, Paimon, Amin, Mil, and Zora. It surprised me how much we had in common. Left, Mil and I mess around in a dormitory. They had witnessed the daily presence of American troops in Afghanistan, and asked me if I had completed a tour of duty there. Even though I told them I hadn't, they liked to call me their 'Young American Soldier,' especially when I wore my bandanna. They were visiting scientists working for the United Nations, and they were working on establishing maize as a viable crop in Afghanistan. This is a challenge because Afghanistan is a mountainous and barren country. They were playful, especially Mil. They left halfway through my internship and I miss them greatly. They made me appreciate what I am, an

Iowan and a citizen of the United States. They also gave me an appreciation for other cultures and ideologies.

In conclusion, I would just like to thank everyone at the World Food Prize for all their efforts that made this possible. You made it possible for me to meet so many diverse and interesting people. I was able to see the splendors of México City, watch kung-fu movies with a renowned scientist, challenge a Nobel Peace Prize Laureate to a game of 'Risk,' and gave me so many innumerable opportunities. I will never forget watching the All-Star Game with a Yugoslavian, playing video games with an Indian, being captain of the Biotech team in the CIMMYT volleyball championship, discussing the Troubles of Northern Ireland with a subject of the United Kingdom, or seeing the splendors of México: La Plaza de Las Tres Culturas, El Zócalo, the pyramids at Tenóchtitlan, and the fountains and buildings of México City.

I look forward to continuing to stay in contact with all the friends I made. I hope that someday I will be given the opportunity to return to CIMMYT. I wish everyone would get to experience the same wonderful things I have. I only wish that I have taken full advantage of this opportunity: enriching other people's lives as well as my own, sharing the perspective of an Iowan with citizens of the world, and helping to fulfill the World Food Prize's goal of increasing awareness of world hunger issues home & abroad.

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