Studying Whiteflies Beneath Taiwanese Skies

2008 Borlaug~Ruan Internship

AVRDC-The World Vegetable Center

Shanhua, Tainan, Taiwan

Vivian M. Bernau

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I. Acknowledgements

I would like to extend my greatest thank you to Dr. Norman Borlaug, Mr. John Ruan III, and Ambassador Kenneth M. Quinn for making the Borlaug-Ruan International Internship Program possible. Also, I would like to thank Lisa Fleming, the Youth Programs Director, for doing everything in her power to make my summer a success.

The World Food Prize Symposium has become a defining part of my life this past year. It inspired me to learn more about the importance of agriculture throughout the world and opened doors for me to begin exploring the possibilities of agricultural research. For that I will be forever grateful.

I would like to thank Dyno Keatinge, Director General of AVRDC–The World Vegetable Center, for graciously permitting me to research at the Center for eight weeks this summer. My mentors, Dr. Robert de la Peña, Head of the Biotechnology and Molecular Breeding Unit, and Dr. Kadirvel Palchamy deserve many thanks for planning my project and advising me throughout the summer.

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Thank you to all those who kept in touch and continued encouraging me throughout the summer; your support was invaluable. Lastly, I would like to thank my parents, James and Mary Bernau, for their never ending love, encouragement and support of my crazy ideas and dreams.

II. Introduction

Attending The World Food Prize Youth Institute is one of those experiences that will have an impact on your life and the way in which you view the world. However, there is no way of predicting the magnitude of this "earthquake." Last fall I arrived home stunned, and in awe of the people I had just interacted with, and the way that the whole event had invigorated me. I have always been involved in agriculture, but this was one of the experiences that helped me realize how much I love being a part of it, and how much I would love making a difference through it.

My first experiences with agriculture were on the family farm in Charles City, Iowa, where I helped my dad with the chores and showed lambs at the county fair. In 2001 we moved to Altoona, a suburb of Des Moines, but instead of leaving my connections to agriculture behind I found new ways to involve myself and expand my knowledge.

Through involvement in my high school's FFA chapter, I was able to truly explore all of what the agriculture industry has to offer. I entered my first agriscience class with no intention of ever pursuing a career in agriculture. Of course, after spending countless hours in the agriculture room (and in school vans on the way to career development events and conventions across the Midwest), I was able to see that maybe I belonged in the agriculture industry after all.

Now that we live in Altoona, my family runs a small chemical and pesticide free vegetable farm during the summer. As my mom is an elementary school teacher, she and I usually do most of the daily work. Our three main crops are asparagus, strawberries, and tomatoes; but we also grow pretty much anything else that is capable of growing in zone 5. Because of this strong background in horticulture and vegetable production, I knew that I wanted to spend my summer at the World Vegetable Center.

III. The World Vegetable Center

The World Vegetable Center (**Figure 1**) is the leading center for vegetable research and development worldwide. They are a not-for-profit institute working towards reducing malnutrition and alleviating poverty in developing countries through improved production and consumption of safe vegetables. Research at The World Vegetable Center has three broad emphases: health, wealth, and diversity. The center also has development activities to build upon this research: they provide training in vegetable production, collaborate with other researchers worldwide, and provide extensive information resources.

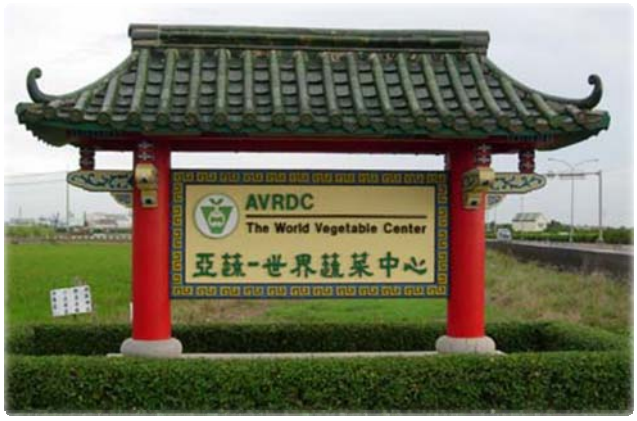

Figure 1: The sign in front of the The World Vegetable campus in Taiwan.

The World Vegetable Center receives funding from many governments including France, Germany, Korea, Switzerland, Taiwan, Thailand, United Kingdom, and the United States as well as from private institutions and foundations such as the Asian development Bank, Rockefeller Foundation, Bill and Melinda Gates Foundation, Asia Farm Africa and the Organic Center for Education and Promotion. The World Vegetable Center has a yearly budget of US $12-15 million.

Founded as the Asian Vegetable Research and Development Center (AVRDC) in 1971, The World Vegetable Center has focused on improved and safe production of high-value horticultural crops. Only through an increased consumption of vegetables can we hope to alleviate the global issue of malnutrition, an epidemic that affects developed and undeveloped nations.

IV. "A Study on White Fly Resistance"

Abstract

Tomato yellow leaf curl (TYLC) is one of the most devastating viral diseases afflicting cultivated tomatoes. Affecting tropical and subtropical areas worldwide, TYLC is a virus that scientists have been researching for nearly 40 years. The main vector of this

devastating *Begomovirus* virus is the whitefly *(Bemisia tabaci)*. In many affected areas nearly 100% of the crop is lost to TYLCV (Morales).

Natural resistance was analyzed in 11 different varieties of tomato, including accessions from *S. escluentum, S. pennellii, S. hirsutum*, and *S. pimpinellifolium.*

Introduction

Symptoms of Tomato Yellow Leaf Curl Virus (**Figure 2.**) include upward curling of leaflet margins, reduction of leaflet area, yellowing of young leaves, stunting, flower abortion, and reduced yields (Greene). This damage is in addition to that caused by the whitefly (**Figure 3.**), which includes: chlorotic spots, wilting leaves, and black mold on the leaves and stems (Morales).

Figure 2: The effects of Tomato Yellow Leaf Curl Virus (Green)

Figure 3: These whiteflies are pictured with exuviae (Johnson).

People have tried many ways of controlling TYLCV, but the best way is to manage the whiteflies. The most common form of attempted control uses frequent treatments of insecticides. This method is very rarely effective enough to reduce virus transmission and works to produce resistant strains of whitefly. Frequent pesticide applications can also have a negative environmental impact. Another method of control is to use 50+ mesh screens, but these often cause the plants to overheat and allow for little ventilation. In Central America a common procedure is to have government mandated crop-free periods in sync with the seasonal patterns of the whitefly. This technique is good in theory, but many farmers ignore it. The development of a resistant commercial crop is the best option to reduce the global devastation this virus causes (Morales).

Materials and Methods

In order to analyze and compare the natural resistance of 11 accessions and four F_1 crosses (**Table 1**) the 140 plants used were isolated in individual plastic tubes with a

mesh top (**Figures 4, 5** and **6**). 10 pairs of whiteflies were then collected and released into each tube and were left to sit for three days, giving the insects a chance to lay their eggs on the plants.

After three days the number of eggs on each plant was carefully counted and recorded. The number of first instar nymphs emerged, and the number of pupae and number of exuviae were also recorded to determine which varieties have the lowest hatching rate and which varieties the whiteflies avoid.

Table 1: Accessions, known traits of tomatoes, and number of replications used in procedure. Ty-1, Ty-2, and Ty-3 are genes known to carry resistance to TYLCV. S. escluentum, S. pennellii, S. chilense, and S. hirsutum are species that have all been shown to carry resistance to TYLCV or whitefly.

Genotypes	Remarks	Number of Replications Used
CLN1621L	*S. escluentum*; Susceptible to TYLCV	12
CLN2498E	*S. escluentum*; Carries Ty-2	12
CA4	*S. escluentum*; Carries Ty-3	12
FLA456	*S. escluentum*; Highly resistant to TYLCV	12
M-82	*S. escluentum;* Resistant to TYLCV and whitefly?	12
LA1940	*S. pennellii*; Resistant to TYLCV and whitefly?	12
LA716	*S. pennellii;* Resistant to whitefly	5
LA1579	*S. pimpinellifolium;* Susceptible to TYLCV	12
LA1970	*S. chilense;* Resistant to TYLCV	3
LA2747	*S. chilense;* Resistant to TYLCV	5
LA407	*S. hirsutum;* Resistant to TYLCV and whitefly?	3
CLN1621L x LA1940 (F1)	*S. escluentum x S. pennellii hybrid;* Susceptible to TYLCV	12
CLN2498E x LA1940 (F1)	*S. escluentum x S. pennellii hybrid;* Resistant to TYLCV	12
CLN1621L x LA407 (F1)	*S. escluentum x S. hirsutum hybrid;* Susceptible to TYLCV	12
CLN2498E x LA407 (F1)	*S. escluentum x S. hirsutum hybrid;* Susceptible to TYLCV	8

Figure 4. The 140 plants before introducing the whiteflies.

Figure 5. Introducing the whiteflies

Figure 6. Counting the number of whitefly pupae.

The DNA of the 15 accessions was also extracted, replicated, and analyzed through PCR (Polymerase Chain Amplification Reaction) using 3 different primers. The three primers (JB1, T0302, and FLUW25) were used to detect the presence of the three known TYLC resistance genes, Ty-1, Ty-2, and Ty-3, respectively.

Table 2

Protocol for DNA Isolation
1. Loosen the powder of freeze dried tissue by tapping gently. 2. Add 0.6ml fresh working solution to each tube, then stir it until no tissue powder is dry. 3. Incubate at 65˚C for .5 to 2 hours. And mix by inversion after ten minutes. 4. After allowing the tube to cool down to room temperature, fill each tube with chloroform and isoamyl alcohol (24:1) and mix vigorously. Incubate at room temperature for another five minutes. 5. Spin down for 10 minutes at 10,000 RPM. Transfer the supernatant to a new tube with label. 6. If necessary, repeat steps 4 and 5. 7. Add 2/3 volume of cold Isopropanol and invert about 10 times. Be very gentle. Let stand at -20˚C for more than 15 minutes. (The DNA will begin to become visible as a milky/clear cloud of fibers in the solution. 8. Spin down for 15 minutes at 13,000 RPM and discard the supernatant. (Directly pour out the liquid.) 9. Add 1mL 70% ethanol, and mix to wash the pellet. Let stand at room temperature for 5 min. 10. Spin down for 5 min at 6,000 RPM and discard the ethanol. 11. Add 1mL 70% ethanol again, mix and leave for 5 minutes at room temperature. ***It is possible to stop here and store the pellet in 70% v/v EtOH at -20˚C indefinitely. ***If colour is green or brown, wash in ethanol again. 12. Spin down for 5 minutes at 6,000 RPM and discard ethanol. Be careful, do not lose the pellet, 13. List all DNA samples and print the labels. 14. Resuspend the 200µL 1x TE (pH8.0) and treat with 5µL 10mg/ml of RNAse. Keep plate in the incubator at 37˚C for 2 hours. After 30 minutes check and mix gently. 15. Heat in the water-bath at 75˚C for 20 minutes to render the RNAse inactive. 16. After spinning down for 10 minutes (13,600 RPM), transfer it to a new 1.5 tube, avoid bringing any insoluble material. Store the original DNA at -20˚C with label information 17. Dilute different times (e.g. 15X and 30X) for DNA quantification in 0.8% agarose gel with λDNA and preparation working solution of DNA. 18. Store original tubes of DNA at -20˚C (label information out of box)

Results

Table 3: This table shows the average results plus standard error of my observations for each species during each stage of development. The grouping of species based on the data is included in the superscript.

Genotype	No. of eggs	No. of larvae	No. of pupae	No. of Exuviae
CLN1621L	59.25±1.07^{a}	35±0.72^{a}	26.25±0.5^{a}	35.18±0.48^{a}
CLN2498E	20.83±0.84^{bc}	17.667±0.68^{ab}	17.273±0.63^{a}	14.18±0.55^{bcd}
CA4	15.75±0.75^{bc}	6.917±0.6^{bc}	6.417±0.6^{c}	9.167±0.56^{cde}
FLA456	16.42±0.6^{bc}	11.75±0.6^{bc}	13.333±0.61^{ac}	12.67±0.62^{bcde}
M-82	18±0.73^{bc}	7.333±0.52^{bc}	8.273±0.56^{ac}	10.82±0.58^{cde}
LA1940	8.58±0.57^{c}	1.25±0.22^{bc}	0±0.0^{c}	0±0.0^{e}
LA716	3.8±0.6^{c}	0±0.0^{c}	0±0.0^{c}	0±0.0^{e}
LA1579	39.08±0.84^{ab}	12.1±0.2^{bc}	7.857±0.74^{c}	6.778±0.64^{cde}
LA1970	7.67±1^{c}	6.333±0.54^{bc}	4.333±0.69^{c}	8.667±0.35^{bcde}
LA2747	6±0.57^{c}	2.4±0.17^{bc}	6.4±0.58^{ac}	8.8±0.89^{cde}
LA407	3.67±0.77^{c}	0.5±0.63^{c}	1±0.26^{c}	1±0.26^{de}
CLN1621L X LA1940	22.67±0.68ab^{c}	8.917±0.46^{bc}	6.125±0.55^{c}	15.86±0.56^{abc}
CLN2498E X LA1940	7.7±0.485^{c}	2.083±0.23^{bc}	1.818±0.32^{c}	2.727±0.41^{de}
CLN1621L X LA407	47±1.1^{ab}	39.167±1^{a}	36.091±1.14^{a}	37.45±1^{ab}
CLN2498E X LA407	14.88±1.2^{bc}	9.625±1^{bc}	9±0.86^{ac}	7.75±0.81^{cde}

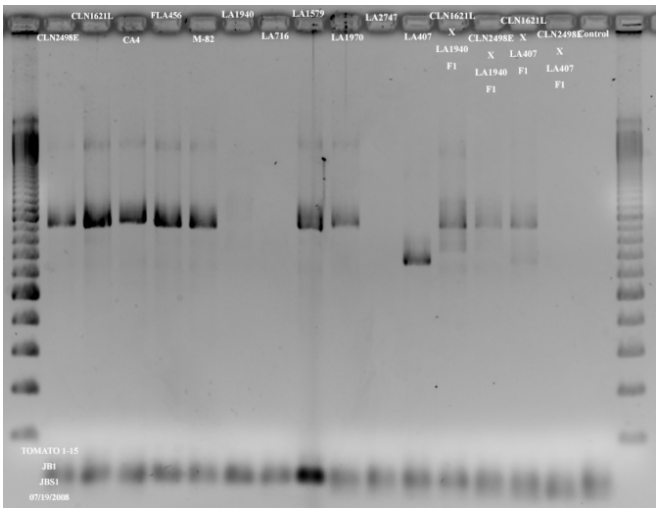

Figure 7. Primer JB1 shows the presence of a Ty-1 gene. If a Ty-1 gene is present, a band should show up at about 530bp and 450bp. None of the plants used possessed this gene.

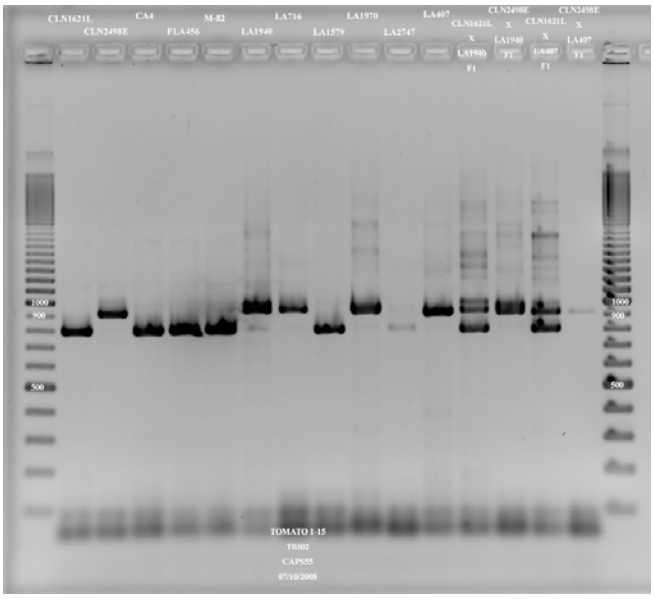

Figure 8. Primer T0302 shows the presence of a Ty-2 gene. If a Ty-2 gene is present, a band should show up at about 900bp. CLN2498E is the only tomato in this experiment that possessed this gene.

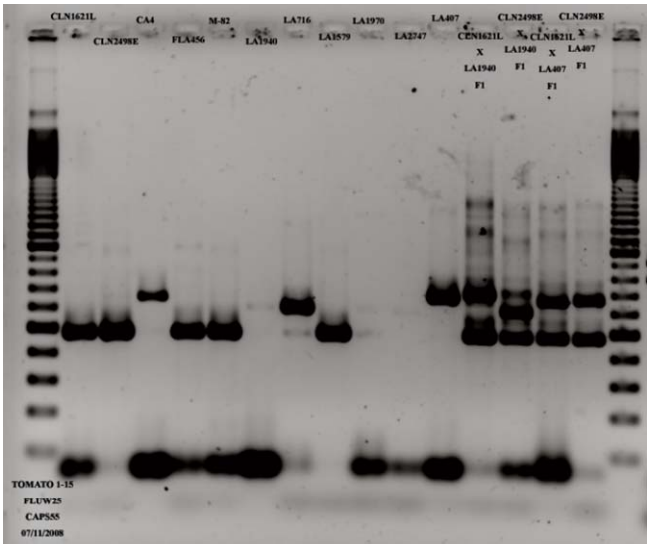

Figure 9. Primer FLUW25 shows the presence of a Ty-3 gene. If a Ty-3 gene is present, then a band should show up at about 700bp and 500bp. CA4 possesses a Ty-3 resistance gene.

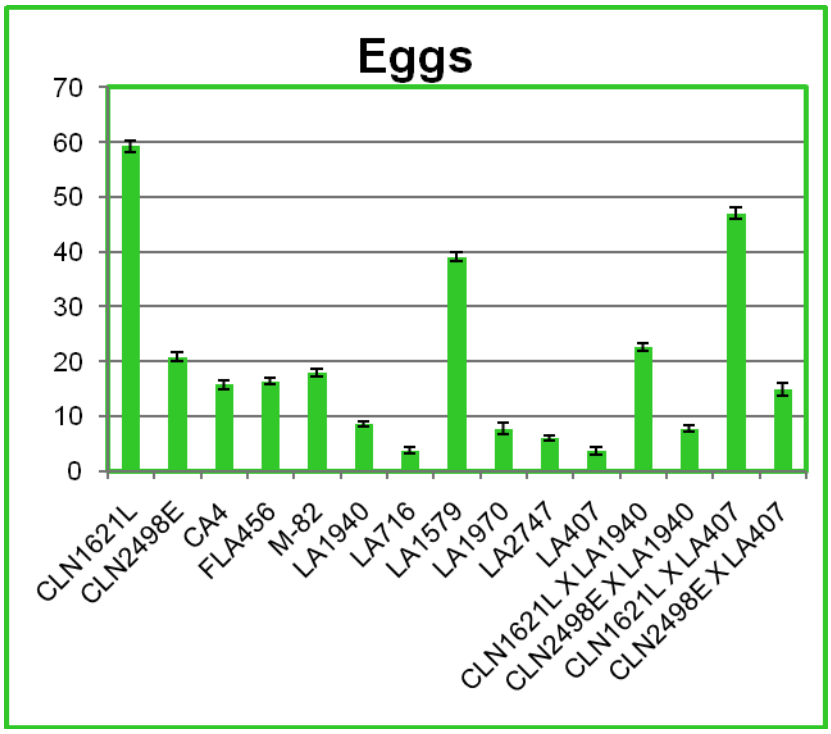

Figure 10: This graph compares the average number of eggs found on each plant after the first 3 days.

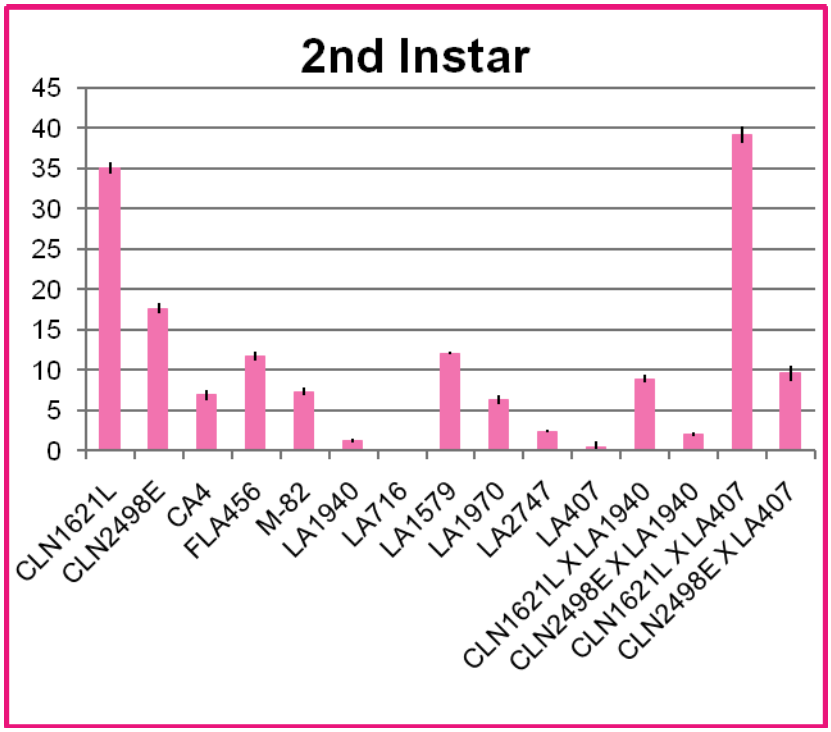

Figure 11: This graph compares the number of 2nd instar whiteflies that developed on each variety.

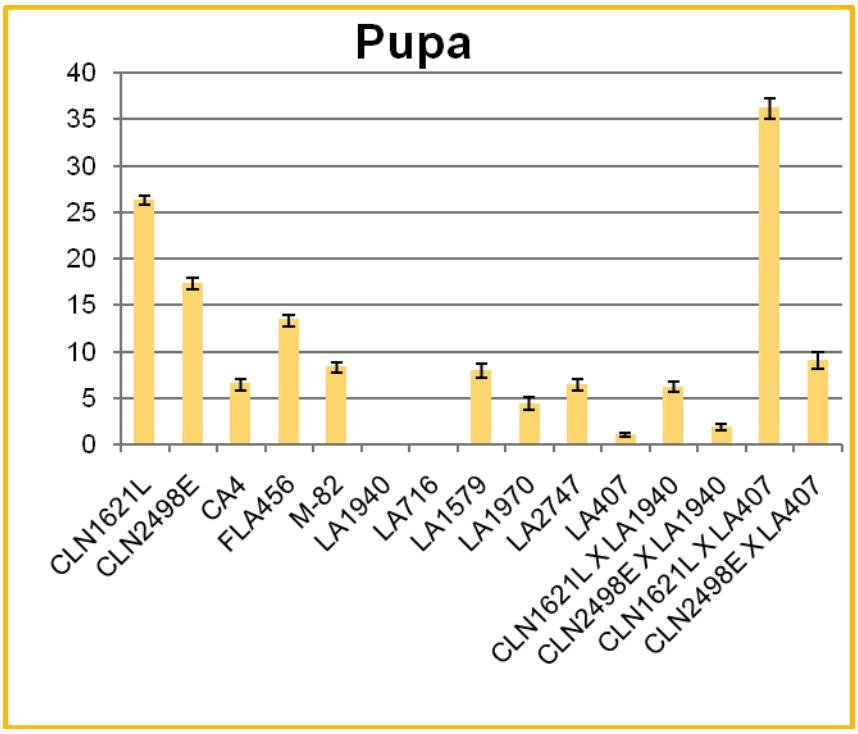

Figure 12: This graph compares the average number of whitefly pupae that developed on each variety.

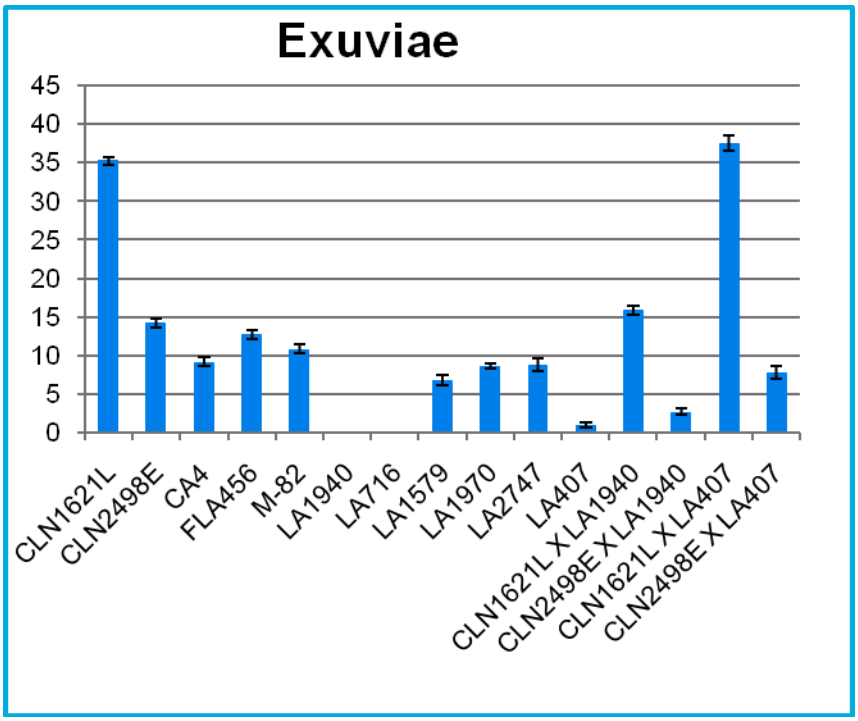

Figure 13: This graph compares the average number of whitefly exuviae found on each variety.

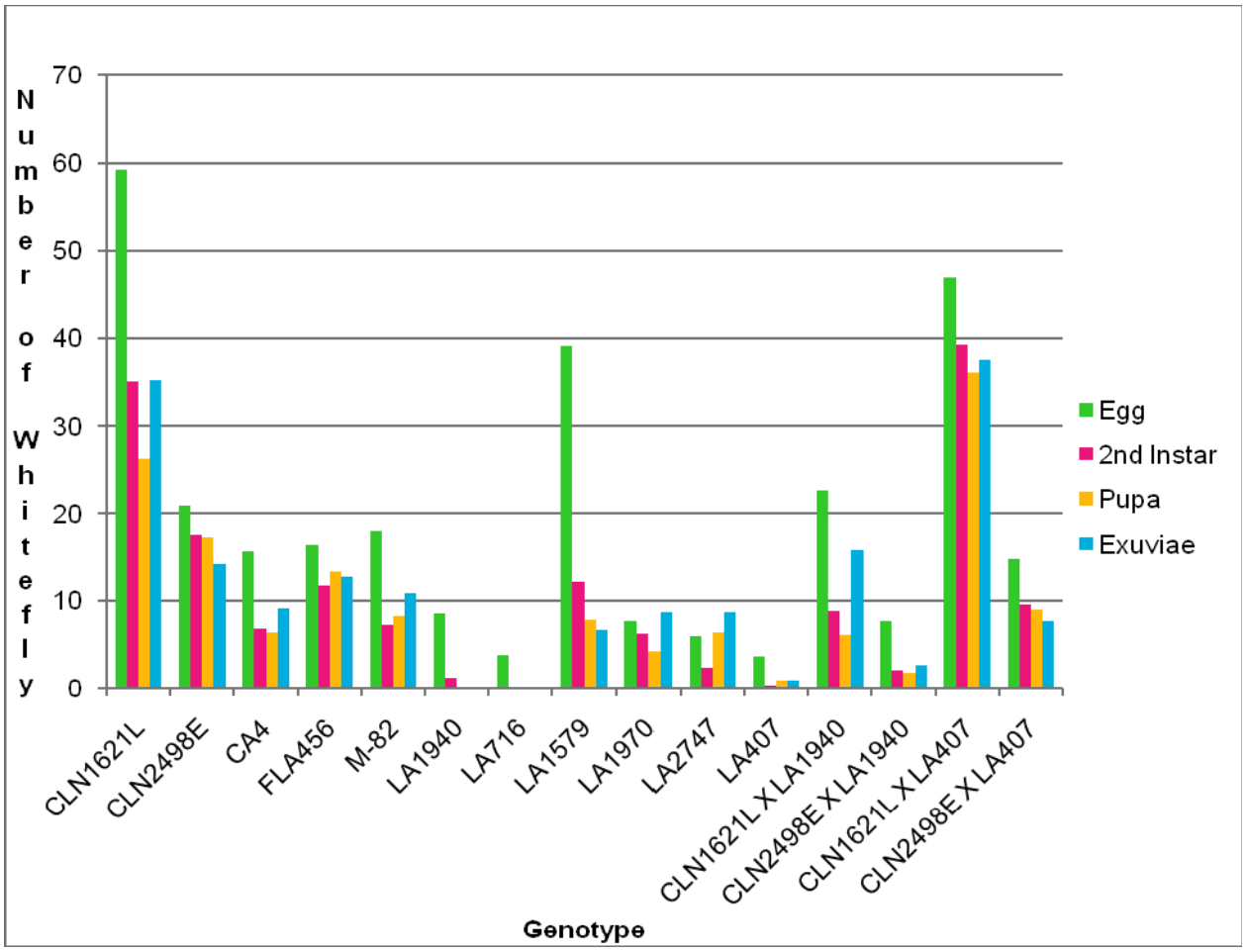

Figure 14: This graph incorporates all of the data collected, showing the average number of whiteflies during each stage on each accession. It is important to note the lack of development in LA716 and LA407.

Discussion

The goal of "A Study on Whitefly Resistance," the project that I helped with during my time at the World Vegetable Center, is to develop a variety of tomato that is resistance to whiteflies, not just Tomato Yellow Leaf Curl Virus.

Through my studies with 15 different accessions it was determined that the highly TYLCV susceptible variety, CLN1621L, was the most preferred by whiteflies. The accessions of *S. pennellii* (LA1940 and LA716) and *S. hirsutum* (LA407) were most resistant to whiteflies.

The results also support that the TYLCV resistance observed in LA1940 and LA407 may be due to vector resistance factors, as it does not carry a known Ty resistant gene.

In general, the varieties that carried TYLCV resistance were less preferred by whiteflies. It appears that virus resistance and vector resistance may go hand in hand. Host plant resistance to virus and vectors may be complementary and combining them may provide durable resistance to TYLCV problem in tomato.

V. Traveling to and Through Taiwan

I was extremely excited to go to Taiwan. The promise of new experiences, a new language, food, and culture was exhilarating. I was so excited, that I completely overlooked how stressful and emotional traveling alone was going to be.

After a long 24 hours flying I finally reached the airport in Kaohsiung, Taiwan. Kaohsiung is the second largest city in Taiwan, situated on the south western coast. It is about an hour by car from The World Vegetable Center. Lydia, the dormitory services manager, kindly agreed to come with the driver to pick me up. She seemed quite shocked at my height. Hemali, the 2007 Borlaug-Ruan Intern to AVRDC, was shorter than the average Taiwanese, but very spunky and obviously well loved by all of the AVRDC staff.

My plane arrived at the airport near midnight, so my first views of Taiwan were in the dark. The 45 minute car ride seemed unreasonably long, because I was very tired and already homesick. There were very few streetlights and no exterior lights on the buildings, so I couldn't see anything except for the dark outlines of the buildings.

Figure 15. The view from my AVRDC dormitory.

The campus looked just like the pictures I had seen, but the fact that I was going to be living in a tropical country with palm trees didn't really hit until I woke up the next morning and looked outside my window (**Figure 15.**). The climate is certainly different from that of Iowa, but the hot, humid, and rainy weather of June and July was sure familiar. However, Iowa does not come with geckos that climb on your walls (and eat the mosquitos!) or fields of pineapple, papaya, and avocado.

I had the chance to travel around the island of Taiwan nearly every weekend of my internship. Some weekends we only went to the nearby towns of Chaiyi or Tainan, and other weekends we took longer trips to places like Alishan (a mountain tourism resort) and Taipei.

Figure 16: Dragon Boat Races in Tainan.

On the first weekend that I was in Taiwan I got to attend the Dragon Boat races held in Tainan (**Figure 16.**), which are traditionally held on the fifth day of the fifth lunar month. The whole festival reminded me a lot of the state fair, complete with food vendors selling goodies such as squid on a stick, 90 degree weather, and 100% humidity. The actual race is 300m long and takes about one hour and 45 minutes to complete. The AVRDC raced in the international division, but there were also other divisions for groups such as schools and senior citizens.

We ended up placing fifth (out of six) in the first race which did not officially qualify us for the finals. The first and fourth place teams left before the finals started, though, so we were called on to compete anyway. We ended up pulling ahead in the second round to take third place. (We couldn't officially get first or second because the other two teams weren't there.) Our competition consisted of teams from English teaching schools and a group of Vietnamese factory workers.

Figure 17 : A flower market in Taipei during my last weekend in Taiwan.

During my time in Taiwan I was able to see a lot of the island's western coast and interior, and for my last weekend Dr. Rachael Symonds and Jennifer Clifford went with me on a final trip to Taipei. Right after work we caught a cab to the high speed rail station in Tainan. The high speed train goes from the northern tip of the island all the way to the southern edge and can go as fast as 300 kilometers an hour. Our train left Tainan at six o'clock and we made it to the hostel in Taipei before 8:45. The same trip by regular train would have taken nearly six hours.

In Taipei we visited a few different night markets, the National Taiwan Museum, jade and flower markets (**Figure 17.**), and of course, Taipei 101. The city of Taipei is actually situated in a valley, so we were able to take the metro to the edge of metropolitan area and take a gondola ride into the mountains. There we sat at a café, ate waffles, and drank tea while looking over the brilliantly lit city.

VI. Exploration of a Culture

Just like any young traveler, I had a few weeks of culture shock. The food was not how I had imagined it and everyday life was interesting. Nap time? For everyone? I liked that, but some of the other cultural differences were a lot harder to adjust to.

Before my summer in Taiwan I had never considered myself a picky eater. I enjoy trying new things and thought I was going to be really brave and adventurous about all of the cultural differences. As I walked through the Shanhua night market (**Figure 18**), I found myself questioning this decision. The stalls had French fries and papaya smoothies, as well as shrimp balls on a stick, fermented tofu, and roasted duck head. I did try the shrimp balls and the fermented tofu, but I couldn't quite bring myself to eat a whole duck head.

Figure 18. The Shanhua Night Market which is held every Tuesday, Friday, and Sunday evening.

While public transportation in Taiwan is very popular, scooters and motorcycles pack every sidewalk and intersection (**Figure 19**). It is also quite common to see entire families of four or a mom with her son and their dog on one scooter. Helmets are required, but the ones that most Taiwanese wear truly envision the term "brain bucket." It took a while for me to stop cringing every time I saw one of these helmets, especially because I witnessed at least half a dozen accidents during my stay.

Figure 19. Scooters line the streets in Kaohsiung.

The overabundance of scooters is just one of the contributors to the large amount of air pollution in Taiwan. A massive amount of over-packaging also takes place. EVERYTHING comes in a bag. A single drink bought at a juice stall will get its own bag. A box of Pocky from the 7-11 or Family Mart will get its own bag. Recycling this is really popular on the AVRDC campus, but it is hard to tell how much takes place throughout the rest of the country. Recycling is a step in the right direction, but in elementary school every student is taught that recycling is just one of the three R's; reducing and reusing are just as important.

Shiatsu massage is an ancient Asian form medicine using fingers to release the build-up of chi in the neck, arms, and legs. It is also supposed to correct the

Figure 20. Experiencing the wonders of shiatsu.

imbalances of the body. Scientifically it is pushing the crystallized toxins out of your lymph nodes and down to your fingers and toes where it is released.

Every week a group of the AVRDC staff living on campus goes to a small shop in Shanhua (that also doubles as a hair salon and living room) for a "massage." This term, massage, usually brings to mind something very relaxing and soothing, but shiatsu can actually be quite painful (**Figure 20**). As you enter the room the smell of menthol is almost overpowering. Everything in the shop happens in the one room. While I was getting my massage there was usually another lady getting her hair washed and five to ten people from AVRDC and Shanhua watching me.

VII. Conclusion

Before leaving for our respective countries, Ambassador Quinn talked briefly with us about what we could expect to get from this once in a lifetime experience. He said we would come back different people. People forever changed in a way that can only come from international experience.

At the time I thought I knew what he was talking about. I had traveled to Europe for two weeks in 2007. There I learned to deal with, airport mazes (and delays), language barriers, the wonders of public transportation, and cultural differences.

But what I experienced in Taiwan was completely different. The language barrier was ten times more restraining than it was in Europe, because I could not sound out Chinese characters. I could not say English words and hope that they were the same or similar in Chinese.

After spending a few weeks back in Iowa, the changes that took place over the summer are very obvious to me. My social skills improved exponentially. Everyone wanted to know about my school, the Borlaug-Ruan International Internship, Iowa, and my family. I explained the Iowa caucuses to a Filipino man and the severity of the floods back home. At first it was a little overwhelming. I was nervous about being the center of attention, but now I can talk to just about anyone about anything.

I have always known that I would work in science. FFA helped me get into agriculture, and plant science was obviously my strongest area. International development has always interested me, and through the World Food Prize Youth Programs I was able to put these passions together and explore a potential career.

I am now a freshman studying agronomy at Iowa State University, and I love it. My focus is in plant breeding, and I am considering a minor or secondary major in international agriculture or horticulture.

When I am walking around Iowa State's campus or working in the dining center. I find myself starting conversations with the international students. It's incredibly interesting to hear where they are all from and how they got the chance to study at ISU. Their grasp of English varies from surprisingly limited to better than my own, but all are willing to try and communicate their experiences here and their lives back at home.

Being a part of the Borlaug-Ruan International Internship program has truly been the experience of a lifetime. It has given me a new perspective of the world, allowed me another taste of the wonders of traveling, and given me more confidence in myself. Very few high school students are allowed the chance to participate in an international internship, and I will be forever grateful for the experience. It has, most definitely, changed me.

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