Crocidolomia, Cabbage, and Culture

Borlaug~Ruan Internship 2016

The World Vegetable Center

Shanhua, Taianan, Taiwan

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Table of Context

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I. Acknowledgments

My first and foremost thanks go out to Norman Borlaug for his commitment to feeding the world. I, one day, hope to follow in his footsteps in helping billions of people.

I would also like to formally thank the World Food Prize Foundation (WFP) for providing an opportunity of a life time. My relationship with WFP began in October 2015 when I was selected to be a Global Youth Institute Scholar. Since then, WFP has welcomed me into their family and I am extremely grateful for that. Also, thank you to the WFP for providing programs like these that empower the youth to be agents of change.

Thank you to Ambassador Quinn for supporting the Borlaug ~ Ruan interns and making the Borlaug Dialogue possible.

Thank you to Ms. Lisa Fleming for being like a second mom while I was abroad. You were always happy to guide me and provide valuable advice.

Thank you to Dr. Srinivasan Ramasamy for welcoming me into your lab and teaching me the critical skills of leadership and team work while having fun.

Thank you to Ms. Mei-ying Lin for being an amazing mentor and friend. Your sense of humor and laughter never cease to cheer me up.

Thank you to all World Vegetable staff members and researchers that welcomed me into their lab and provided hands on learning activities for me.

Thank you to the cafeteria staff for all your accommodations, especially, thank you to Ms. Helen Chen for always making sure that I have eaten enough, and sometimes more than enough!

To my new friends, the memories we have made together are ones I will never forget, but always cherish.

I could not have spent these two months away without the support of my loving mother. Thank you for all that you do.

As my time here are the World Vegetable Center comes to a close, I will forever be grateful for the opportunities that I have had and the people that I have met.

THE WORLD FOOD PRIZE

World Vegetable Center

II. Introduction

A. Personal Remarks

Nihao and hello, I'm Priyanka Bonifaz, a 12th grader at Barrington High School in Rhode Island. My personality and passions are built upon my early childhood experiences. I am a fanesca of two vibrant cultures, Ecuador and India. I was born in Ecuador and have been raised single handedly by my extraordinary mother. At an early age I knew I had to embrace adversities as blessings in disguise. As my family moved across the globe in my early childhood, exposure to different cultures instilled in me deep compassion and connectivity to humanity and desire to serve the underserved population.

Becoming a Borlaug ~ Ruan Intern has provided me with an immense platform to become a leader and to take action towards fighting global food insecurity. This incredible opportunity allowed me to open my heart to new experiences. While in the process of learning how to help the world feed itself, I have made lifelong friends and partnerships.

B. The World Vegetable Center

I was placed at the World Vegetable Center in Tainan, Taiwan. The World Vegetable Center (WorldVeg) is an international research institute that works to alleviate poverty and food insecurity in the developing

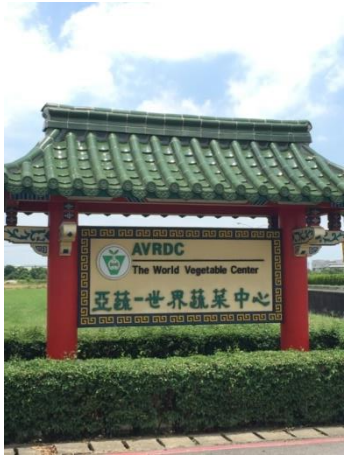

Figure 1. Welcome sign for WorldVeg

world. The institute works to achieve this goal by increasing crop production, promoting nutritionally dense foods, and uniting countries to combat malnutrition.

It is known that vegetable production is parallel with creating income and job security for local smallholders such as farmers. The center emphasizes the importance of micronutrients to ensure that adults and children are receiving the necessary nutrients to live a successful, healthy life. The center also ensures food sustainability through practices of diversifying crop systems. Additionally, the Center focuses on breeding to improve vegetable lines and nutrient dense foods (World Vegetable Center).

Figure 2. World Vegetable Center administration building on campus

III. Research Project

A. Abstract

Crocidolomia pavanana are one of the leading causes of *Brassica oleracea* devastation worldwide. The larvae of the moth destroy the entire plant by eating at the growth center.

Neem oil is a known insect repellent and pest management tool. It will be used as our insect repellent treatment for the common cabbage plant.

B. Introduction

Brassicas are an important group of vegetables as they are annually cultivated over 2.4 billion hectares globally. However, there have been severe production losses due to insect pests including *Crocidolomia pavonana* Zeller (Lepidoptera: Crambridae), also known as cabbage head caterpillar. *C. pavonana* is one of the major pests of crucifers globally. The larvae destroy the entire plant by feeding on the growth center, inhibiting the development of the plant. Neem oil is a known insect repellent and pest control tool in pest management programs and will be used as our treatment. Neem is also a desirable pest control tool as it is a natural and safer component in integrated pest management.

If neem oil is successful in intoxicating and interrupting the development and behavioral patterns in *C. pavonana*, then neem oil treatment can be deemed as a viable method for Brassica pest management.

C. Methodology

Figure 3. Cabbage vegetable and treatments

The first part of the experiment consists of 3 different treatments: 1) check (no treatment), 2) foliar spraying of neem on plant, 3) drenching soil with neem oil. Each treatment will have 10 plants in five replicates, with a total of 30 plants. After treating the plants, 25 larvae will be released. The mortality of larvae and pupae and development time was observed and recorded for two weeks or until pupation. At the end of pupation, the adult emergence percentage was recorded. The next part pertains to the attractant, which are the pheromones produced and released by the female moths to attract the males moths for mating. From the surviving adult moths from treatments 1, 2, and 3 of the previous experiment, 15 adult moths were reared in acrylic cylinders and the duration of their mating behavior was observed and recorded.

The reason for experimenting both with net and cylinder rearing is because in previous trails we were just using net rearing and there was high morality in all treatments, including the check. A possible cause of death could be the humidity and lack of room for aspiration, as a result both the plants and insects suffered. The data listed below is from our fifth trial, which we compared the use of net and cylinder rearing.

Figure 4.Left: net cage rearing. Right: cylinder rearing

D. Results

Net Cage Rearing

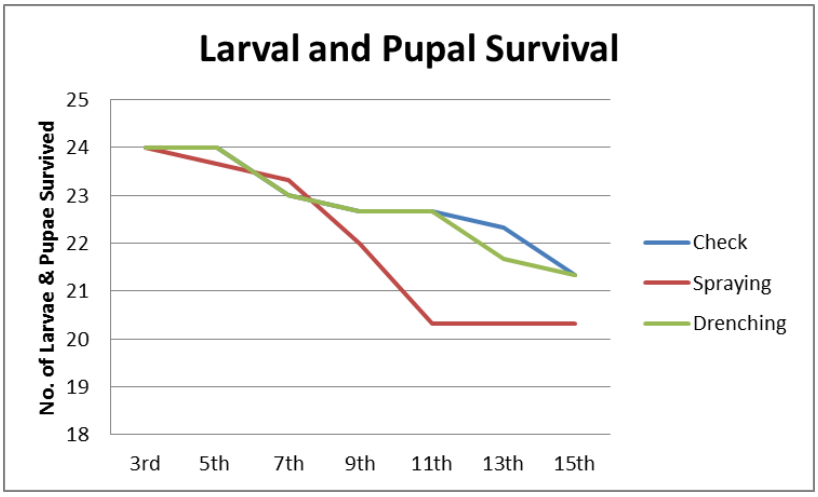

Figure. 5

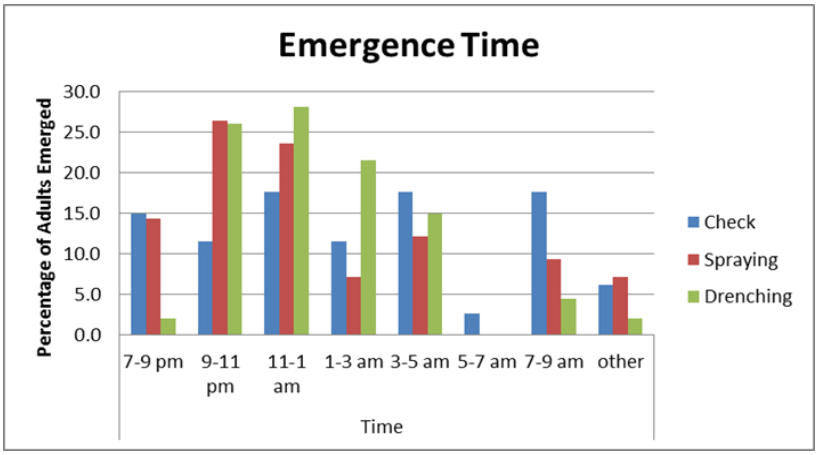

Figure. 6

Treatment	Larval period (days)	Larval mortality (%)	Pupal period (days)	Pupal mortality (%)
Check	9.79±0.18	14.7±12.9	10.40	47.2±4.7
Spraying	9.81±0.26	18.7±16.2	10.30	31.8±15.5
Drenching	9.80±0.26	14.7±12.9	10.6	29.6±6.7
F value	**0**	**0.12**	**0.88**	**2.38**
P value	**0.9965**	**0.8865**	**0.4624**	**0.1729**

Figure. 7: no significant difference as p value is greater than 0.05

Cylinder Rearing

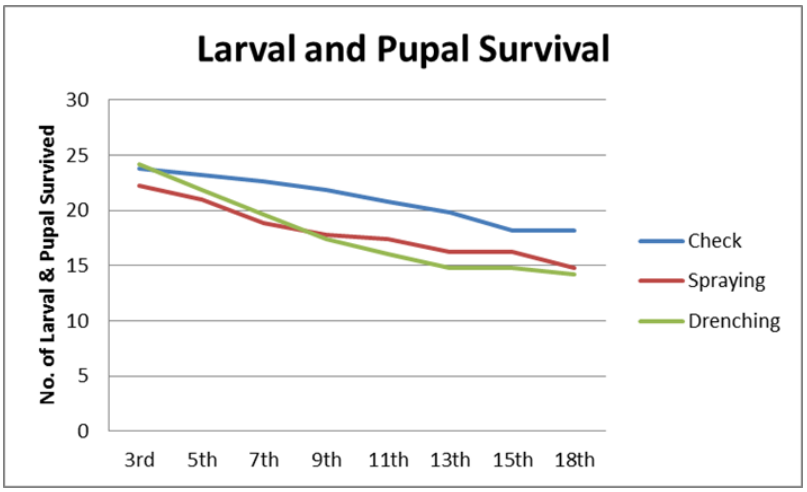

Figure. 8

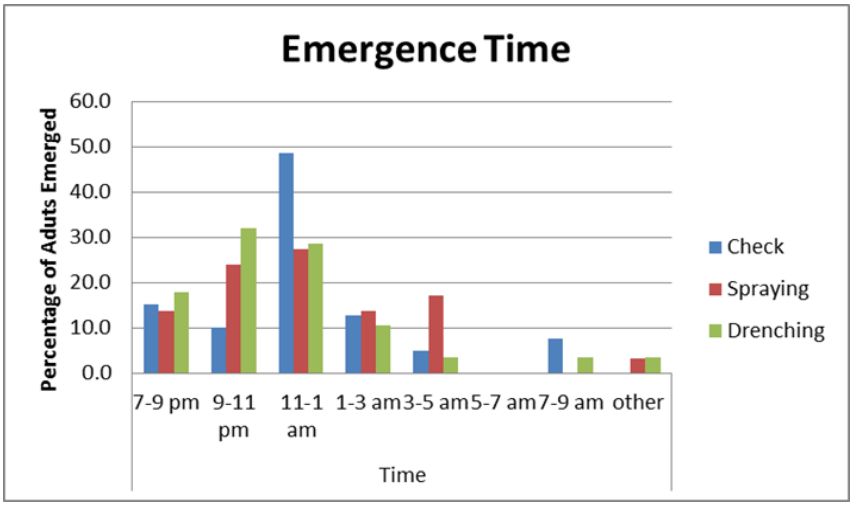

Figure. 9

Treatment	Larval period (days)	Larval mortality (%)	Pupal period (days)	Pupal mortality (%)
Check	11.0±1.2	27.2±15.8	10.28	57.4
Spraying	11.8±0.9	40.8±14.8	10.24	62.2
Drenching	11.8±0.8	43.2±14.3	10.2	60.5
F value	**1.1**	**1.7**	**0.12**	**0.2**
P value	**0.3627**	**0.2242**	**0.8898**	**0.8179**

Figure. 10: no significant difference as p value is greater than 0.05

Treatment	Mating Duration	Mean (min)
Check	00:15-00:49AM	41.7
	00:48-01:40 AM	
	01:22-02:01AM	
Spraying	00:20-01:15AM	53.3
	01:10-01:55AM	
	01:20-02:20AM	
Drenching	02:25-03:10AM	43.3
	01:30-02:20AM	
	01:35-02:10AM	

Figure. 11

Figure 12. Check treatment (day 3)

Figure 13. Spray treatment (day 3)

Figure 14. Drench treatment (day 3)

E. Discussion

Although neem is a known repellent, in our assay, neem oil did not significantly affect the *Crocidolomia* population. Our assays were geared towards observing the effects of neem oil on the biology of the insect. However, we can conclude from the data recorded above that neem does not affect the biology of the insect.

As shown in net cage rearing, spraying treatment did have a slightly higher morality than the other two treatments in figure 6. However, we can conclude that statically all the treatments are equal, as proven by p values. Additionally, drenching seems to have lower emergence times at 7 pm to 9 pm and then higher emergence times during 11 pm to 1 am. This can be attributed to the early or delayed pupa reposes to the emergence time. We can also justify that the neem did not affect the emergence time based on the p value, and also through the fact that all the treatments were almost equivalent around 3 am to 5 am.

Continuing with net cage rearing in figure 7, we noticed that the larval period was a slightly longer in spraying or drenching treatments. We can assume that this was caused by a phenomenon called chronic effect, which explains a small

effect over a long period of time. This chronic effect can explain why some of the insects did not feed properly on the plants with treatment, causing the accumulation of the neem to diffuse or fade away, thus not affecting the biology of the insect. Additionally, it is suspected that the high humidity in lab setting did not allow plants to transpire. If the plants cannot transpire properly, then the plant and insect will not survive, thus making it an unsuitable environment for the project.

Cylinder rearing did provide a higher mortality rate for the treatments. This can be attributed to greater circulation of air in the tube and less humidity, creating a more comfortable environment for the plant and insect. This greater comfortability allowed for the insect to feed more on the plant, thus possibly affecting the biology of the insect. Using Analysis of Variance (ANOVA), it was determined, through the f value, that neem treatment was not significant in figure 10. Although there was a greater morality, as seen in figure 10, the p value of figure 8 indicated that the neem treatment effects were not significant. Our f statistic in our ANOVA test was smaller than our f value; therefore we can reject our null hypothesis. We cannot solely rely on the f value; as a result we also considered the p value, which indicates the probability of obtaining a result greater than observed. If the p value is less than or equal to 0.05 (5%), then there is a significant difference among the treatments. If it is higher than 0.05, then the mean values are not statistically different among the treatments. Therefore in figure 7, 47. 2 of the check and 29.6 drenching for the pupal mortality are statistically the same. Similarly, in figure 10, 27.2 and 43.2 are statistically the same, although the latter number is a bigger number than the former. Therefore, based on the statistical analysis, neem treatment did not have significant effects over the check.

Additionally referring to the emergence time for the pupa, it can be observed that there is no obvious impact of neem. As stated early, the increase and

decrease in percentage of adults emerged could be a factor of early or delayed emergence depending on the internal cycle of the moth. When analyzing the mating behavior in figure 11 of both the net caged rearing and the cylinder rearing it can be observed that there is no significant difference. The check and drenching treatments were almost the same statistically. This is due to the time of translocation of drenched neem into the plant system. Since the concentration of the neem is particularly low in commercial oil, it is not greatly absorbed into the plant system. Therefore, the drench treatment behaved almost like the check treatment during mating behavior analysis. Also it can be observed from the data that the spray treatment has a greater mating duration. We cannot conclude that neem caused this factor because if neem had an effect on the biology on the insect, then the pheromone production would have been lowered. In this case the mating duration increased, therefore the pheromone production may have increased. This can be said because mating behavior is an indirect analysis of pheromone activity in insects. Although my hypothesis was proven incorrect, there are many factors that can be improved in our project.

A. Humble Recommendations

There were limitations to my project, a major one being time. If only there was unlimited time to perfect the art of our research. However, this is part of the scientific process.

In future studies, I recommend purchasing pure neem oil and diluting the concertation to cater to the experiment. Additionally, seasons play a role in the outcome of the project. *Crocidolomia* thrives in the winter and spring seasons. We conducted this experiment during the most humid time of the year. I recommend conducting the same assays, but in a more suitable environment. This includes less humidity, larger cylinder tube, and greater concentration of neem.

Increasing the neem concertation and conducting this research in the winter might yield greater moralities in spraying and drenching treatments. Additionally, we might be able to tract the biological processes (larval period → emergence time → mating behavior) more effectively as we would have clearer results of the effects of the treatments at the first stage.

IV. Specializing my Scientific Perspective

A. Shadowing in Different Departments

During my time outside of the insectary, Dr. Srinivasan Ramasamy and Ms. Lin were kind enough to organize meetings and shadowing opportunities with various researchers. This was an incredible experience for me to better understand different career options.

I am extremely interested in medicine and global health. But those are broad fields and there are many specialties within those fields so shadowing different professionals provided me the chance to narrow down my passions. I shadowed researchers in Entomology department, Biotechnology department, Bacteriology department, Nutrition department, Pepper and Tomato Breeding departments, Impact Evaluation department, Virology department, Genebank department and Global Technology Dissemination department.

My first exposure was in Entomology department with Dr. Senthil Kumar. Dr. Kumar showed me how to conduct gel electrophoresis and DNA isolation. We conducted genomic DNA extraction of thrips using Gene 8 kit. Also we conducted polymerase chain reaction (PCR), DNA amplification technique, and then gel electrophoresis, DNA separation technique. We conducted genomic DNA isolation, PCR (amplify cox-1 universal mitochondrial gene) and then gel electrophoresis to know if the gene product size is 700 base pair.

I, then, worked with the Biotechnology department. Interns explained to me how they are using biomarkers to identify relationships between different gene strands. My friend Hoa Le, an intern this summer, showed me how to design guide RNA (gRNA). gRNA is a short synthetic RNA that guides in Cas-9 binding. She also showed me how Crispr Cas9 works. Crispr Cas9 allows you to edit the sequences of any genome. Crispr is based on a natural system used by bacteria to protect them from infection by viruses. When the bacterium detects the presence of virus DNA, it produces two types of short RNA, one of which contains a sequence that is identical to the invading virus. The two RNAs form a complex with the protein Cas9, an endonuclease (type of enzyme that can cut DNA). When gRNA finds the virtual genome, the Cas9 cuts the target DNA, disabling the virus. Our last stop was the transgenic lab where we saw various plants that have been inserted with DNA from another organism (figure 14). This is a method that is used to improve crops. Learning about these techniques is really helpful as I am interested in pursuing medicine, and bioinformatics could potentially be an advantage in the conducting cutting edge research.

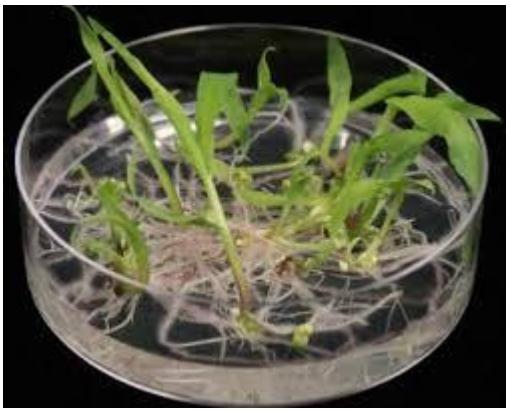

Figure 14. Transgenic plant

In Bacteriology department, I participated in inoculum preparation and inoculation in lab and greenhouse. The first day I learned how to culture the bacteria *Ralstonia solanacearum*, which is a prevalent disease in Solanaceous plants such as tomatoes, eggplant and potatoes. On the second day, I learned how to prepare the bacterial suspension of *R. solanacerum*. After preparing the suspension from the bacteria that I cultured the day before, we went to the lab to see how inoculation was conducted. Lab workers made slices into the soil to break up the roots of plants such as eggplant and tomatoes. Then they added 30 ml of the

prepared suspension to the soil. They will then observe if the plant is resistant or susceptible to the disease.

In Pepper Breeding, I had the opportunity to learn how to conduct hybrid cross pollinations (figure 15). This process is conducted by taking the stigma of a male fertile plant and applying the pollen to a mother plant. The next generation of plants will determine whether the mother plant is fertile or sterile, all depending on the genotype and phenotype of the plant.

Figure 15. Cross pollinating pepper plants in green house

Figure 16. Learning how to stalk tomato plants

During my time in Tomato Breeding department, I had a chance to learn how to transplant plants. I also had a chance to go out into the fields and learn how to stalk tomato plants so that they can grow tall with support (figure 16). I learned the difference between indeterminate and determinate plants. Indeterminate tomato plants can grow up to 12 feet and produce fruit until the weather becomes too cold. Determinate tomato plants can only grow a compact height of 4 feet and stop growing when fruit sets on the top bud.

In Impact Evaluation, I learned the various techniques of assessing the productivity of WorldVeg methods in different countries. There are three

techniques that workers in Impact Evaluation observe and record data for. The first method is tracking the productivity of seeds in different countries. Trainees will ask farmers in developing countries several questions about WorldVeg seeds. These questions are about productivity and the consumer market. Similar questions will be asked for the other two techniques of home/school gardens and Integrated Pest Management (IPM). Once all the data has been collected through questionnaires, the trainees will come back to the World Vegetable Center to analyze data and to better understand the farmer's point of view.

During my time in Virology department, I went out to the field to watch inoculation of the virus Cucumber Mosaic in pumpkin plants (figure 17). This virus has devastated the population of pumpkin plants in Asian regions. The purpose of inoculating the plant with the virus is so that researchers can create susceptibility to such diseases. After learning how to place the inoculum on the plants by breaking the veins, I went to the green house. Within the greenhouse, there were lines of plants that were inoculated with different virus.

Figure 17. Inculcating pumpkin plants with cucumber mosaic virus

In the Nutrition department, I observed vitamin C analysis. We used a spectrophotometer to determine the vitamin C concentration in cowpea plants. The lighter the color appears in the solution the lower the vitamin C concentration is.

The World Vegetable Center has a large collection of seeds. All seeds are stored in the Genebank department. The Genebank grew into a facility that had the ability to aid in research and development on a global level. The facility contains 442 vegetable species, enabling them to conserve vegetable biodiversity and

provide the supply necessary for researchers to fulfill their project requirements. In addition, Genebank has a genetic information website called AVGRIS that provides information on various vegetable.

In my last but not certainly least visit, I went with Global Technology Dissemination department to a fern field in Shanhua. The field was very large and beautiful. The man that owns the field takes care of it all by himself. Yearly, he earns 7 million NTD, which is equivalent to 200,000 USD. After the farm visit, I went to the green house to learn how to graft (figure 18). We used chili pepper but one of the most common plants that are grafted together is tomatoes as the scion and eggplant as the rootstock. This is because tomatoes are not resistant to floods but eggplants are resistant to floods. Grafting increases the survival of plants, controls soil borne diseases, and increase harvest periods.

Figure 18. Graphing chili peppers plants.

V. Exploring the Culture and Country

WorldVeg became a home away from home for me. I am very grateful to have meet people such a welcoming group of people. I interacted and became close friends with people from Malaysia, Indonesia, Taiwan, America, Austria, Hungary, China, India, Papua New

Figure 19. My dear friends

Guinea, Nepal, Egypt and Korea. Talk about a diverse group of people from all walks of life!

One of my favorite memories was when all the interns gathered in the kitchen to make meals together and catch up after a long work day. In our common room there were two small couches and one very large TV. After cooking our fried noodles with vegetables, everyone would pile onto the couches to root for their favorite country during the Olympic Games.

Figure 20. Sunset in front of train station in Tainan City

Aside from hanging out on campus, the WorldVeg provided bicycles for all interns and staff members. So on Friday nights we would ride our bikes to local restaurants in Shanhua or to the night markets. Sometimes we took the train to Tainan City to go to Dream Mall or to a restaurant in the city.

Besides our fun adventures in the Tainan area, interns and I organized trips to go explore more of the country. I had the opportunity to travel with mentors and interns to Kenting, Taipei, Sun Moon Lake in Nantou, and Kaohsiung.

Figure 21. At the southernmost tip of Taiwan

This was my first experience being far away from home for a long of a period of time. Living by myself and collaborating with professionals for two months instilled me the confidence for building lasting and empowering relationships.

VI. Final Remarks

I distinctly remember Ambassador Quinn telling our parents to say goodbye to us as we will be transformed individual when we return from our international internships. He could not have closer to the truth with this statement.

During my time in Taiwan, I was able to do a lot of reflecting on my experiences. Despite learning a plethora of lessons, there were three key things that stood out to me the most.

1. Overcoming barriers:

At first, I was overwhelmed by the language barrier. The majority of people in Taiwan speak Mandarin and very little English. At times, I would feel excluded from conversations; however I quickly learned not to take it personally. I learned that it all depends on how I decided to look at the situation. I also learned to be patient when people were explaining something to me. Despite the language barrier, my mentor and I tried our best to consistently check up on each other to ensure that we were on the same page. This experience has taught me to be patient and understanding with others.

2. Be patient with the scientific process:

Scientific research does not always follow your terms and conditions. It is important to remember that it is okay to make mistakes and grow from them. For example, my mentor and I had to conduct five trials for my research project.

During the four other trials, we came across some complications; however, this forced me to critically think about how we can improve each trial. This is a tool that I will value as I plan to continue my commitment to scientific research in the future.

3. Making the best of your experiences:

There were times where I was feeling homesick and isolated. However, I remembered a conversation that my mom and I had over opportunities that cross our paths. It is easier to feel overwhelmed over a circumstance, than it is to find a silver lining. I was always taught the latter. I decided to make the best of this incredible opportunity by getting out of my comfort zone and learning to not take myself so seriously. One of my most profound experiences, that reminded to make the best of my situation, was when I was feeling isolated in my dorm room. WorldVeg provides single dorm rooms for interns. This was a new experience for me as I am used to sharing a room when I travel. Most days the Wi-Fi was spotty, and at the moment I had a choice whether to wallow in my room or take this as a sign from the universe to go out and explore my surroundings. Life is an adventure, right? So I would change into gym clothes and knock on all my friend's doors with a basketball on my hip, encouraging them to not wallow over the Wi-Fi situation. Once I began playing basketball on the court, interns from all over campus would join in.

The Borlaug ~ Ruan Internship taught me something extremely valuable: the power of choice. I learned to distinguish between feeling down and possibly of turning a situation around. This realization will permeate through all aspects in my life.

A. Stepping Stone to Global Food Security

I have always dreamed of a world where families do not have to worry about where their next meal is coming from. I no longer want to dream, instead I want actively contribute to this movement.

I believe the most profound realization I had during my internship was when I shadowed different professionals. I am grateful to all of the researchers who, at the same time, participated in specializing and broadening my view of the scientific world. In addition to learning about the difference specialties involved in research as future career options, I also witnessed the interconnectedness of these departments. During these moments, I saw how integrated the various units were! The issue of global insecurity does not fall on the shoulders of one individual, but it is a collaborative effort.

During the time that I spent in the various departments, I noted how passionate and knowledgeable researchers were about their subject fields. This was an important observation because I realized that each and every staff member and intern were there for the same reason: to eliminate global food insecurity. Despite people working in different departments, everyone worked together to work to achieve this one goal. A goal that takes time and cooperation, but something that must be done because food security is not a privilege, it is a human right.

VII. Works Cited

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