Developing Whitefly Resistance in Domesticated Tomato Plants

Hailey Hampton

The World Food Prize

2017 Borlaug-Ruan Internship

AVRDC
The World Vegetable Center

The World Vegetable Center

Shanhua, Taiwan

Table of Contents

Acknowledgements

Thank you to my internship supervisors, Dr. Peter Hanson, Dr. Mohamed Rakha for supporting and mentoring me in this project.

A very big thanks to Lisa Fleming, who arranged everything and made sure all the interns were safe and taken care of.

I appreciate the World Food Prize Global Youth Institute for this opportunity and the continued support.

Also, thank you to the interns and employees of AVDRC, WorldVeg. They made me feel welcome at all times, and ensured that I could have a fulfilling time my first time abroad.

Finally, a thank you to my family, who packed me up, drove me to the airport, and who are still willing to proofread my papers.

Abstract

Developing Natural Defenses Against Pest Insects in Tomato, Eggplant, and Pepper Plants

Selected primarily on the factors of yield, growth and fruiting rates, and quality of produce, the vast majority of domesticated tomato, eggplant, and pepper cropping plants exhibit levels of parasite vulnerability that can severely diminish harvest and profitability without the use of commercial insecticides.

Several undomesticated varieties of these cropping species contain much higher levels of parasitic resistance but have poor crop yields. Research to date has determined that there exists a correlation between the presence of higher trichomes and higher acyl sugars to parasitic resistance

Through the use of selective breeding, it is possible to cross strains of undomesticated cropping plants with domestic varieties to produce offspring with the traits of both. The question we face is: can cross breeding two species of a crop plant, one domestic and one undomesticated, result in offspring that bears produce of a domestic crop while retaining the natural defenses (e.g. acyl sugars and trichome patterns) of the undomesticated varieties. This method can take years, if not decades to produce viable offspring. By careful selection and backcrossing, however, it is in fact possible. Using tests in choice and non-choice pest settings, we are able to observe which plant varieties contain the optimal trichome patterns or acyl sugar quantities. We hope to develop successful varieties of domestic cropping plants that contain natural defenses against pests in the field, thereby saving viable food crops and assisting the farmers in saving money.

Personal Background

I was raised in rural Idaho near a town called Mackay. In Mackay, there's a population of under 500 people. I lived most of my life in the valley, seeing friends, making up things to do, and attending the local high school until I graduated in 2016. Like a lot of kids my interests and career plans drifted as I grew up. It wasn't until I entered high school and joined FFA that I stumbled upon what would fascinate me and inspire me to learn more. The high school had a greenhouse that stood unused. As a freshman I asked my advisor to let me plant a few cucumbers inside, I didn't know that it was a hydroponic greenhouse, or even what hydroponics were. However, that moment caused a landslide of events that eventually led me to where I am now; studying Plant Science Research at Utah State University.

In my junior year of high school, while I was still careening around the learning curve of owning a small business and raising 2 ½ tons of tomatoes hydroponically, my FFA advisor handed out copies of "The man who fed the world" and posed the challenge to the 5 of us in his Plant Identification class to write a research paper on the topic of food insecurity for a chance to attend a conference called "The World Food Prize". I thought it sounded like an interesting experience, and with the world's best proofreader, my dad, I wrote a paper on the topic of Egypt's depleting wheat supply with the proposed solution of phasing in Hydroponics and Aquaponics. A week or so after turning my paper in, my advisor informed me he had submitted it to the Global Youth Institute. Months afterwards, I was told I had been selected to attend the conference as a delegate.

Going to Iowa was an adventure in of itself. Even though I had been away from home before, I had never stayed for long, and I was still very new to flying. While at the conference, I was a bit star struck to be in a room with so many people whose lives were dedicated to agriculture and hunger relief work. My eyes were opened while listening to debate panels, top researchers, and government officials talk about the problems and solutions we were implementing in modern agriculture. I also had an amazing time with the service project, where we set up assembly lines to make boxes of food to donate. But more than all of that, I loved listening to my group leader talk about her experience as a Borlaug-Ruan intern. She got to fly to India, stay with a host family, do research, and immerse herself in an entirely new culture for an entire 2 months. To a girl who had graduated in a class of 6, this was an opportunity that was almost hard to grasp. I made it my goal to become a Borlaug-Ruan intern and the result was a life experience I will appreciate forever.

This is my research paper compiled from what I learned on my own Borlaug-Ruan internship spent at The World Vegetable Center located in Shanhua, Taiwan on the project of selective breeding for insect resistance. Although I only got to see the project for a brief window of time, I look forward to seeing how it develops and impacts the world around us.

The AVDRC- The World Vegetable Center

The World Vegetable Center, formerly AVRDC or Asian Vegetable Research and Development Center, was founded in 1971 in Shanhua, Taiwan. Its mission is to overcome malnutrition and facilitate good health by increasing the production, quality, consumption, and profitability of nutritious and health-promoting vegetables. Since its foundering, the Center has spread to 5 additional regional offices throughout South Asia and Africa, and employs people from many different cultural backgrounds around the globe.

The center not only focuses on research and development, but also has the world's largest public-sector vegetable gene bank. This allows them to not only supplement their own research, but also supply both common and native vegetables to other research facilities and farmers. They also partner with Europe, the United States, Japan, South Korea, and Australia. These collaborations allow them to further research and build bridges that span the world, bringing together scientists in the pursuit of improving lives.

Research Background and Objectives

The whitefly is a family of insects that thrives in tropical, subtropical, and less predominately in temperate habitats (1). Main host plants of the whitefly include vegetable and ornamental plants, leading to infestations in farms, greenhouses, and gardens. The mechanical damage caused by them feeding is nothing to the secondary damage inflicted by their ability to transmit viruses and diseases between host plants. Because the majority of feeding, mating, and oviposition occurs on the underside of leaves, it can be difficult for pesticides to be effective against them. With over 1500 species of whiteflies worldwide (1), the threat they pose to crops is considerable.

Certain species of plants have shown to have a natural defense to whiteflies and other small pest insects. These defenses are called trichomes and acyl sugars. Trichomes are defined as “fine outgrowths or appendages on plants, algae, lichens, and certain protists.” (2) There are two ways trichomes can defend the plant from pests; Biochemical defense, and physical defense. Using a physical defense, glandular and non-glandular trichomes simply act as a shield, prevents insects from reaching the surface of the plant, or interfering with feeding and oviposition. A biochemical defense uses glandular trichomes to release compounds that may poison or repel insects. These compounds are called acyl sugars. Not all plants that have trichomes and produce acyl sugars produce the types that are optimal for repelling or killing insects, however. Many domesticated varieties of common vegetables are plants that no longer have the necessary trichome types, and therefore have lost much of their natural defenses.

Tomato species S. pimpinellifolium and S. galapagense are wild strains native to South America that produce small fruits and generally have a small yield. Compared to their domesticated cousins, they are highly undesirable for use in agriculture. However, they have displayed a strong natural insect resistance.

The objective of this experiment is to combine the insect resistant traits of the wild strains S. pimpinellifolium and S. galapagense with the production of domesticated varieties. In this case, the primary domestic plant used is S. lycopersicum. The process used to combine the two is called “Backcrossing”. The process of backcrossing is detailed as “This method works by crossing the transgenic inbred line with an elite inbred line of choice. In the following step, the breeder crosses the selected transgenic offspring back to the elite inbred again. This process of crossing back to the elite line (backcrossing) is repeated until the offspring has 99+% elite genes”. (3)

In order to reach the objective, multiple lines of S. pimpinellifolium and S. galapagense will be cross bred with S. lycopersicum, and tested for insect resistance. Upon creation of a viable plant with insect resistance, said plant will be cross bred again with S. lycopersicum. This process will be repeated until the goal of 99+% elite genes is obtained.

Responsibilities and Experimental Process

During this experiment, my involvement centered around testing for insect resistance. In order to progress to a new generation, the leaves have to be sent off for a detailed analysis. Before that, however, data is needed on mortality and egg rates of whiteflies exposed to the plants. This is done in two different ways; choice and non-choice experiments. In this case, Choice and non-choice relate to the perspective whiteflies have in their choice of which plant to attempt to oviposition and feed on.

Before beginning either trial, prospective plants are bred carefully through manual pollination. The fruits are allowed to grow before being harvested for seeds. Once the seeds are propagated, the plants are grown in a greenhouse until they are 5 weeks old. They are then removed and relocated to growth chambers. There, the temperature and light can be monitored as the trials go forward. The plants are given the opportunity to acclimate to the new environment, and have their labels and positions carefully recorded.

Non-Choice

To begin the non-choice trial, 5 pairs of whiteflies are captured in test tubes, which are sealed with parafilm. This way, it can be ensured that there are 5 males and 5 females per test tube. The plants are fitted with two whitefly cages with supports on upper leaves. The cage is positioned so that the small hole opening is on the underside of the leaf, where whiteflies typically prefer to feed and oviposition. The tube of 10 whiteflies is then carefully placed with the tip inside the cage opening, and the whiteflies are blown into the cage. The tube is removed, and a cork is swiftly put in its place, sealing the whiteflies inside. Therefore, the plant now has 2 cages with 10 whiteflies in each, for a total of 20 whiteflies attached to its upper leaves.

The cages are left undisturbed for 3-4 days, at which point the leaves they are attached to are removed, labeled, and brought back to the lab. There the cages are opened, and a count is taken and recorded of the whiteflies that are alive and dead. The leaves are saved to be examined under a microscope to gather a count of the whitefly eggs.

Choice

In the Choice trial, plants or either the same species or a mix, such as different tomatoes, peppers, and eggplants, are place in a tent made of tightly woven netting. The tent mesh is broad enough to allow light and air to enter, while also tight enough to prevent the whiteflies from escaping. The whiteflies are still captured according to the process above, with 20 captured per plant. However, instead of being released into cages, the whiteflies are instead released into the tent, allowing them to travel between the plants inside. After 3-4 days, the excess whiteflies are carefully vacuumed out, and the plants are removed. The entire plant is then very carefully examined under a microscope to gather an egg count without damaging the plant or the eggs. After this is recorded, they are returned to the tent.

Several more days are allowed to pass, before they are again removed and examined, this time for pupae. Both full pupae, those still containing young whiteflies, and broken pupae, those that have already hatched, are given their own count. The plants are returned and left for a few more days, before finally being examined for larva.

Conclusion

This experiment will last years, if not decades. However, with the skills I learned and experience I gained, I can wholeheartedly say that I am extremely optimistic about this experiment's conclusion. The data gathered varied between the species tested, but some data sets showed a very promising trend. This experiment procedure has been used successfully in the past, and with the research in the hands of researchers as competent as those at WorldVeg, I await the news of a successful outcome.

Experience

While in Taiwan, I kept a blog that was updated at least once a week about my experiances

6/17/17

After ~33 hours of travel, I arrived at Wenchang Village at around 1:30 AM local time. The trip went well, with the only hiccup being a delayed flight in Vancouver led to a missed connection in Hong Kong.

From there, I learned my checked bag had gotten lost in Vancouver, but I had packed everything I would need in case this happened in my carry on, so as long as it was recovered in a few days, I would be just fine. Happy to see that the driver who would be taking me the rest of the way had not left, I climbed into his car and drove the last hour. I was quietly ushered into my room, which is surprisingly large with plenty of space to store things, and bid goodnight. While settling in, I learned that the current occupants of the room, large cockroaches, were still around. It took a few rounds with a raised shoe before I felt safe enough to turn out the lights.

After a day to settle in, I met with an energetic woman named Aileen. She led me on a rapid tour of the two nearby buildings, which turned out to be offices, auditoriums, and a lab where I will be working. After a maze of hallways, I was eventually left with my supervisor, Dr. Mohamed Rakha, who told me about their research.

From there I was guided to a lab just down the hall where I was introduced to Hank, one of my new coworkers. I helped him count whiteflies living on eggplant leaves, and we talked about video games, bugs, travel, and the price of college. After lunch, I helped count the whitefly eggs under a microscope. For the duration of the trip, Hank would be the person I spoke to the most.

That weekend, I went to the first night market of my trip. The night markets I went to were very different from each other, but all had strange foods, arcade games, and more people and mosquitos than I had ever seen. The night markets were also perhaps the harshest contrast between life in America and life in Taiwan. I was always glad to have others with me when trying to navigate the crowded stalls in a storm of new senses.

After I got adjusted to my new schedule I could go to the lab and help take data from whiteflies, and then either explore the local area or read and listen to a tape that taught mandarin. When I asked what I should do in Taiwan for the short time I was there, I always got the same answer: "Try the food!". Hank frequently brought in weird snacks and desserts that had about a 50% rate of being enjoyable to me, but I thought it was nice of him, and he thought it was funny to see my face. Some of the more memorable things I ate were strange fruits like durian, lots of seaweed, and different teas.

While there, I also got some experience with grafting. It was kind of a crash course, but I've always wanted to try and get a successful graft. Most of them failed, but a few were ok and one looked great. I also got to see what hydroponics in Taiwan looked like from a practical agriculture perspective. We drove up into the mountains where they use greenhouse, and got to see massive hydroponic pepper farming in action. Because of the relatively high altitude and position of the mountains, they didn't have to worry about overheating or sunburn like they would have lower in the valleys.

During the times I traveled around Taiwan, I mostly went south. I was able to see a fish market on the coast (and see the ocean for the second time I could remember), visit beaches, explore Tainan, see the daylilies blooming on 60 rock mountain, and immerse myself in the night life. Saying goodbye to the people I met the was difficult, because even though the other interns and I joked that if any of us were ever back in Taiwan, Korea, India, Thailand, America, or Malaysia we would visit, but I think we all knew we would probably never see each other again. Still, I don't regret meeting them or having the experiences I did.

Photos

Whiteflies on the underside of a cabbage leaf. Choice experiment tent

Whiteflies on underside of leaf, choice whitefly tent

Clip cage positioned on a pepper leaf

Dead adult whiteflies and eggs

Whitefly Pupae

Trichrome effects

Parameters	Trichome type[z]			
	Type I	Type IV	Type V	Type VI
Choice bioassay				
Adult WF 3 days after infestation	-0.04[ns,y]	-0.51**	0.42**	-0.12[ns]
Adult WF 19 days after infestation	-0.15[ns]	-0.60**	0.49**	-0.02[ns]
Eggs (no.)	-0.20**	-0.52**	0.42**	-0.03[ns]
Nymph (no.)	-0.21**	-0.60**	0.49**	-0.01[ns]
Puparium (no.)	-0.18*	-0.63**	0.53**	-0.04[ns]
No-choice bioassay				
Adult mortality (%)	0.44**	0.49**	-0.50**	-0.14[ns]
Eggs (no.)	-0.14[ns]	-0.31**	0.15[ns]	-0.03[ns]

Type-trichome densities were evaluated using a 0-3 visual scale as described in Materials and Methods.

[y] *and ** indicate significance at $P \leq 0.05$ and $P \leq 0.001$, respectively

Data curtesy of Dr. Mohamed Rakha

Time in Taiwan









(1) https://www7.dict.cc/wp_examples.php?lp_id=1&lang=en&s=silverleaf%20whitefly
(2) Just the Facts Textbook- Genetics and Genomics of Cotton: Biology, Genetics
(3) http://agbiosafety.unl.edu/education/backcross.htm