Utilizing Time-lapses to Analyze Root and Upper Biomass Correlation

Harrison Qu

Bettendorf, Iowa

2016 Borlaug-Ruan International Intern

Centro Internacional de la Papa

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Personal Background:

Growing up, I was not exposed to very much agriculture. I was boring in Atlanta, Georgia, but I spent the majority of my childhood in Iowa. My parents come from China, and they both came from non-agricultural background, which is one of the reasons I was never involved in agriculture. Growing up in Iowa, I have always been close to agriculture and loved it, even though I never actively participated in it.

All my life, I have been trying to make sense of the world around me. Charts, graphs, and sheets of data—that would put most of my friends straight to sleep—leave me feeling recharged and invigorated. The schools I went to never put an emphasis on the importance of agriculture. My school does not have any agricultural programs, such as FFA or 4-H. As a result, I was very involved in academics and other extracurriculars.

Service and helping others has always been one of the most important things in my life. I believe that giving back to the community is one of the most important things I can do and thoroughly enjoy volunteering and making others happy. Since fourth grade, I have been involved in many volunteering activities. One current example is Teens 4 Tomorrow, a philanthropy group that is given an annual grant of $10,000 to distribute to local non-profit organizations. First, we identify several significant problems within our community, then interact with multiple qualifying non-profit organizations, and finally distribute the grant based on the nonprofit's' impact on the identified problems. I served as a board member for this group for the last two years, moving up to Student Assistant this year—a position that requires responsibility, leadership, and commitment.

I also take part in my school's Service Learning Advisory Committee. Only a select number of students were chosen for this committee, and we serve to ensure that the students better the community by completing their service learning hours. I have learned many of the traits required to be a good citizen and a hard worker because of my commitment to my community as well as my school. In addition to all of my extracurriculars, I also volunteer at local retirement homes every Saturday by giving cello concerts. There are always new people each week, so I have learned how to interact with and assist strangers.

In an effort to help others around the world who are less fortunate than I am, I participated in the World Food Prize Iowa Youth Institute, where I investigated climate volatility in Kenya and proposed potential solutions to fight for their drought problems. It was at the Iowa Youth Institute where I became extremely motivated and inspired to join the fight against hunger. The Borlaug-Ruan International Internship was the perfect way to combine both my love for science and my love for helping others. My passion and research for this issue awarded me the opportunity, as an international Borlaug-Ruan intern, to conduct independent research at the

International Potato Center in Peru in hopes of making an impact in the lives of those affected by food insecurity.

I feel very at home in a lab or in a cornfield, and enjoy getting my hands dirty and witnessing results firsthand. My love for science has led me to spend many summers conducting research in a variety of areas. For the past few years, I have been interning in a couple different labs around the Midwest. Two summers ago, I interned in a biology lab at Bradley University. I conducted research on the genome sequence of Tetrahymena thermophila, sequencing and annotating its extremely intricate protein sequences. While interning at Bradley University, I learned about the work ethic required to be a researcher in a scientific lab. This includes being very detail-oriented and not rushing through the current task at hand, but instead taking the time to verify that every detail is correct.

More recently, through the Secondary Student Training Program, I researched independently at the University of Iowa to help create a more realistic and therefore my accurate simulator experience for the National Advanced Driving Simulator participants. I researched simulated lane changes performed by simulated vehicles. I was given existing data to analyze and create codes for; the codes graphed the data and made comparisons between the variables, creating parameters for a new way of modelling lane changes. The codes I wrote using MATLAB processed the existing data on lane changes and took information from the data points. Eventually, I came up with a new way of modeling lane changes in the simulator as a sigmoid function. This internship taught me how to work and solve problems independently while still meeting deadlines and expectations.

Through all my research work and activities I did this summer in Peru, I have developed a strong work ethic and skill set. Always striving to be the best in everything I do, I take pride in giving my best effort and managing my time well. The research skills that I learned from my previous internships combined with the interpersonal skills I gained from my service to my community allowed me to make the most of my internship. The Borlaug-Ruan International Internship allowed me to further help others through my passion for science, to experience first-hand Norman Borlaug's dream, and to continue the fight against hunger.

Lima, Peru:

Peru has a population of around 30 million people, with 8.5 million people living in the capital of Lima. Peru borders the Pacific Ocean and is home to a wide variety of different crops. The terrain changes greatly across Peru; there are rainforest, beaches, deserts, and mountains. Due to the number of people living in Lima, it is split into 43 different districts. Lima always has 100% humidity, so the temperature is always a constant 50 degrees Fahrenheit during the winter. Because of the humidity, it never rains in Lima and slight changes in temperature feel much larger. I spent most of my time in La Molina, one of the districts of Peru. The International

Potato Center is in La Molina, so I was able to explore that district of Lima. However, I moved to different houses, so I was also able to explore other districts of Lima. I lived in both Miraflores and Barranco as well, which are both bigger cities.

My view from the Barranco apartment I was living in:

I was also able to travel to Cieneguilla and Callao, other districts of Lima. In addition to exploring Lima, I was able to travel south to Ica, which is located in a desert.

The International Potato Center:

The International Potato Center, also known as Centro Internacional de la Papa (CIP) in Spanish, was founded in 1971. CIP is a global research institution; it currently has 20 other offices in a variety of countries in Latin America, Asia, and Africa, with the headquarters located in Lima, Peru. The center was established to develop sustainable solutions through root and tuber research to problems such as poverty, hunger, and decreasing amounts of natural resources.

The   International

Potato Center (CIP) focuses on alleviating world hunger through sustainable solutions. One of the main visions of CIP is "Roots and tubers improving the lives of the poor." Through this vision, CIP is able to increase food security, gender equality, and the general well-being of those in developing countries. The International Potato Center has very innovative studies and advanced research, which allows it to further its mission.

CIP is involved in the CGIAR (Consortium of International Agricultural Research Centers) Consortium. The CGIAR Consortium focuses on increasing food security, nutrition, and human health, while working to decrease rural poverty. Similar to CIP, CGIAR researches way to fight the degradation of natural resources, such as more sustainable management.

The mission of CIP is to "work with partners to achieve food security, well-being, and gender equity for poor people in root and tuber farming and food systems in the developing world. We do this through research and innovation in science, technology, and capacity strengthening." The values of CIP are excellence, dignity and respect, empowerment, integrity, staff diversity, innovation and creative, and partnership. During my internship, it was evident that CIP strives to follow these values and to achieve their mission.

Genetics and Crop Improvement:

During my internship in Peru, I was assigned to work under Dr. Awais Khan. I was assigned to the genetics and crop improvement program. Within this program, I was working with the abiotic stress team. Abiotic stress means subjecting potato plants to different stresses, such as drought or salinity. Although I was working with the abiotic stress team, I was working on a separate project. My project was to create a way to record measure the correlation between root and upper biomass growth. The reason I worked with the abiotic stress team was because of the future implications of my project. Researchers will eventually be able to see how different stresses affect the growth of the root and upper biomass, and how they correlate.

Objectives of the Project:

There has always been a question as to if there is a correlation between root growth and upper biomass growth. How do potato/sweet potato plant roots change over time? Is there a correlation between the changes in upper biomass (leaves and shoots) of the potato plant and the root growth? I was given the task to create a system for predicting root growth and corresponding upper biomass growth. Prior to this experiment, there was no method for looking at the correlation between potato plant roots and upper biomass growth.

To better understand root growth, upper biomass growth, and the correlation between the two, time lapses were utilized. I analyzed three different genotypes of sweet potato: Wild Sweet Potato, Beauregard, and Tanzania; I used three plants of each genotype, 9 plants in total. The time lapses allow researchers to see the root/upper biomass changes over weeks in a matter of

seconds. Another objective of the project was to set up a system with root properties (root angle, number, etc.) to help researchers predict plant performance and behavior. Another system will also be created to show the correlation between the root properties and the leaf properties. This will be done analyzing each frame of the time-lapse manually or with an image analysis software. Researchers are able to better understand the relationship between upper biomass and root growth in potatoes and sweet potatoes.
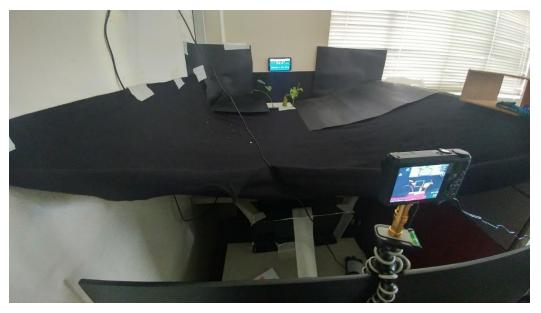
The Abiotic Stress Team:

The abiotic stress team consisted of Lorena Guardia, Jorge Vega, Rudy Cruz, Maria David, Monica Santayana, Veronica Mosquera, and Dr. Khan. Each member of the team had their own individual projects they were working on. Since my project was mostly independent research, I did not collaborate with many people. However, whenever I needed anything, they were readily available to help. I was also able to work on different projects while waiting for my time-lapse to finish. I spent around two weeks assisting coworkers with cross-pollination, the removal of stamen from potato plants, and recording different genotypes of potato into the field book.

Personal Contributions:

I was responsible for designing a way of accomplishing the objective and testing if the method was successful. How the experiment was set up consisted of filling a glass tank with potato plants at the very top of the tank. I used black cloth and paper to cover the part of the glass where the roots were visible, so that the roots did not have any exposure to light. The leaves of the plants were subject to light, so they were not covered in any way. Underneath the cloth, there was a Raspberry Pi camera that took pictures of the roots. The Pi camera used an IR light

lamp to see the roots. A Canon S100 camera was used to take pictures of the leaves. Both cameras took pictures at given intervals that were used in a time-lapse.

The time-lapse displayed the date and time in the bottom right. This experiment was performed for both young, potato sprouts and sweet potato cuttings. After obtaining all the pictures from the time-lapses, I used different software to edit and combine them. From the time-lapse, changes within the roots/leaves over time were seen very easily.

Materials:

- Raspberry Pi
- Wireless Mouse
- Wireless Keyboard
- Computer Monitor
- Strong Tape
- Raspberry Pi Camera Add-On
- Canon S100 with SD Card (Preferably 8 gb or more)
- CHDK Program
- Tripod
- Black Paper
- Black Cloth
- Black Spray Paint
- (Optional: Heater)
- Infrared Lamp
- Water Tank (Or Glass with Water)
- Styrofoam
- Sponge
- 3 Genotypes of Potato Plants, 3 of each Genotype
- Irfanview 64 Program
- GoPro Studio
- Movavi Video Editor 11

Methods:

Raspberry Pi Setup

1. Set up the Raspberry Pi
2. Connect the computer monitor to the Raspberry Pi
3. Connect the wireless mouse and keyboard to the Raspberry Pi
4. Connect and configure the Pi Camera
5. Create a time-lapse script using Python
6. Click the Menu button in the top left
   a. Go to Programming
   b. From the Programming Menu, click Python 2 (IDLE)
   c. Type the following code

```
from picamera import PiCamera
from time import sleep
camera=PiCamera()
camera.resolution=(1024,768)
for i in range (100000):
        camera.capture('Name-{0:5d}.jpg'.format(i))
        sleep(1800)
```

6. Set the desired camera resolution (it is 1024,768 in this example)

7. The i value (which is 100000 in this example), is the amount of pictures the Raspberry Pi will take.
8. Next to camera.capture, the name of the time lapse will be placed (it is "Name" in this example)
9. After the name of the time lapse, {0:5d} specifies how many digits there will be in the number. For example, if it was {0:3d}, the numbers would go 000, 001, 002. If it was {0:4d}, it would go 0000, 0001, 0002.
10. Finally, to set the intervals that the time lapse will take pictures, enter the number (in seconds) after "sleep." In the example code, the time lapse would take a picture every 1800 seconds, which is 30 minutes.

Canon S100 Setup

1. Load the SD card into computer (preferably 8 gb or more)
2. Download the program "CHDK" for the S100.
3. Place the program onto the SD card
4. Load the SD card into the camera
5. When the camera is completely off, hold down the play button.
6. Push the MENU button and scroll down until FIRMWARE UPDATE.
7. Select FIRMWARE UPDATE and confirm.
8. The camera is now using the CHDK software.
9. For the clearest and most focused photos, make sure the camera is in auto mode.
10. Hit the play button to access the CHDK menu.
11. Scroll down to "Script (program your camera)"
12. Under "Intervalometer", scroll down to "= interval (sec)" and set the intervals between pictures (in seconds).

Experiment Setup

1. Measure the length and width of the top of the water tank.
2. Cut styrofoam into those dimensions.
3. Create 9 holes, all in a line, on the styrofoam.
4. Spray paint the styrofoam black. This is to make sure no light is going to the roots. Spray multiple thin coats.
5. Take 3 cuttings from 3 different genotypes, 9 cuttings in total.
6. Cut the cuttings so that they are very similar in length.
7. Use thin layers of sponge to wrap the part of the cutting that will be inside the hole. Make sure the cutting is tightly held by the hole, so that it will not fall or move.
8. Fill the tank with water and make sure that the bottoms of the cutting are touching the water.
9. Cover the back and the sides of the tank with black paper, folding the paper is most effective. Use masking tape to secure the paper.

10. Cover the top and front of the tank with a black cloth to make sure the all the roots do not come in to contact with light. Use masking tape to secure the cloth.
11. Set up the Raspberry Pi camera underneath the cloth. The Raspberry Pi will be used to take photos of the roots.
12. Set up an infrared lamp underneath the cloth to allow the Raspberry Pi to see the roots. Adjust the lamp to have the most efficient lighting.
13. Place the Canon S100 on the tripod and focus it on the upper biomass.
14. Start both time lapses at the same time. For the Raspberry Pi, save the script (ctrl+s) and hit f5 to start it. (The script can be stopped by closing the Python window that pops up). To start the Canon camera time lapse, simply press the shutter button. (To end the Canon camera time lapse, press the shutter button).
15. End both time lapse programs when growth has stopped or when necessary.

Creating the Time Lapse

1. Create a folder to put the Canon S100 photos into. Create a separate folder for the Raspberry Pi photos.
2. Put the time lapse photos into the folders.
3. Open the program "IrfanView."
4. To add timestamps to the photos
    a. File -> Batch Conversion/Rename
    b. Select "Batch conversion" under "Work as:"
    c. Select "JPG - JPG/JPEG Format" as the "Output format:"
    d. Select the photos to be used in the time lapse and click "Add all"
    e. Check the box marked "Use advanced options (for bulk resize…)"
    f. Click the "Advanced" button in the top left.
    g. Check the box that says "Add overlay text" and click "Settings"
    h. Enter 10 as the X-Coord and 1000 as the width
    i. Enter 10 as the Y-Coord and 100 as the height
    j. To choose where the timestamp goes, select position under "Start Corner"
    k. Enter the code: $E36867(%d/%m/%Y %H:%M) to display the date and time on the timestamp
    l. Select "Transparent" for "Backgr. transparency"
    m. Select "None" for "Text Transparency"
    n. Check the box named "Antialiased text"
    o. The text color, font, and size can be changed to best fit the photos
    p. Select "Right" for "Text alignment"
5. Set the folder where the photos with added timestamp will be placed in
    a. Under "Output directory for result files" -> "Browse" -> Choose where the photos should be put in
6. Click "Start Batch"
7. Open the folder where the photos are saved and organize the photos by date, from oldest to newest. (Right click, scroll to "Sort by", select "Date modified")

8. Open the program "GoPro Studio."
9. Click "IMPORT NEW FILES" on the left.
10. Select all of the photos that will be used in the time lapse.
11. The speed or fps (frames per second) can be changed by clicking "ADVANCED SETTINGS."
12. Click "ADD CLIP TO CONVERSION LIST" and then click "CONVERT ALL."
13. Select "BLANK TEMPLATE" and click "CREATE."
14. Select the video made from the time lapse photos.
15. The video speed or display settings can be changed from the menu on the right.
16. When the video is complete, click "EXPORT" and select HD 1080p as the resolution.
17. Open the program "Movavi Video Editor 11"
18. Choose "16:9" for the movie settings.
19. Add the videos by click "File -> Add new media files..."
20. Set the background as black
    a. "File -> Add new media files…"
    b. Add a picture (jpg) of a black screen
    c. Change the length of the black screen to match the length of the time lapses
21. Add the time lapse videos (of the roots and upper biomass)
22. Select one video and drag it above the black screen
23. Select the other video and drag it above the first video
24. Double click the video, select "Picture in picture" (at the top of the page)
    a. Move or resize the video as needed
25. Double click the other video and repeat step 24
26. If needed, the videos can be cropped by selecting "Crop" under "Tools"
    a. Once the video is cropped, click apply to finalize the crop
27. When the time lapse is ready to be finalized, click "Export" (in the bottom right)
    a. The video type (.mov/.mp4/etc…) can be changed by clicking the drop-down menu
    b. Select "Highest" for the video quality
    c. Browse to where the video will be saved (click the "Browse" button)
    d. Change the name of the time lapse under "File Name"
    e. When all the steps are completed, click "Start"
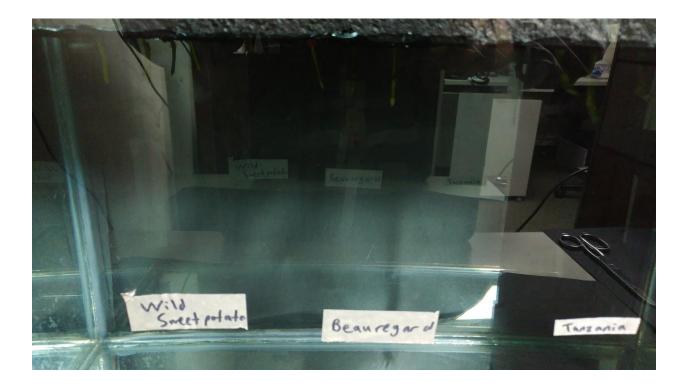28. The completed time lapse showing both the upper biomass and the roots will be created.

Results of Experiment:

The individual time-lapses were combined to show root growth vs. upper biomass. The time lapses allow researchers to see the root/upper biomass changes over weeks in a matter of seconds. However, after letting the plants grow for 4 weeks, I came across a problem. The root growth was very slow; there was not enough growth to analyze. I also did not have enough time to start another time lapse. To see why the roots were not growing, I created a side experiment. In the side experiment, I kept temperature, exposure to light, and the genotypes of plants the

same, and I changed the volume of water the plant roots were in. Surprisingly, the plants grew more in one week than the previous plants grew in four weeks.

The water tank we used in the original experiment was held 10 liters of water. In an earlier experiment involving the water tank, the growth was very slow as well. My experiment made it evident that the water tank was not effective for experiments.

Due to the lack of data, I could not create a system for analyzing and determining correlation between root and upper biomass growth due to the lack of data. Instead, a system used to record the root growth, upper biomass growth, and the correlation between the two over a period of time was created. In the future, this method could help to predict root architectural traits and their role in yield and resilience to abiotic stresses using upper biomass features as proxy by avoiding laborious and destructive digging of roots out of the soil. By having a way to measure root performance by looking at the upper biomass, farmers will be able to find the best time to harvest. Not only does this lead to higher crop yield, but higher quality crops, giving farmers a method of growing more efficiently.

How I was Affected:

My overall experience in Peru made me realize that I can make a difference. Before my internship, I did not realize that as an individual, I would be able to make such a big difference. This internship inspired me greatly, and I plan on making an even larger impact in the future. During my time in Peru, I was introduced to the study of Agricultural Economics by US Embassy workers. In the future, I hope to intern at other research institutions and pursue a career in Agricultural Economics. Growing up in Midwest, I have always been interested in agriculture for its unique characteristic of bringing people together. It was at this internship where I saw agriculture's power of bringing people together. My experience allowed me to dive deeper into food security and agricultural research, and it equipped me with the knowledge and skills necessary to make a difference in the world.

The Borlaug-Ruan International Internship gave me the chance to experiment in different branches of science, as well as pursue my passions for both agriculture and helping others. What most entices me about this internship is its hands-on approach to learning, which feels active and relevant to the current world. I want to be part of the current conversation, to learn from and participate in issues that are affecting the world right now. What I love about the international internship is that the institution is actively engaging with and contributing to the larger world. It is my aspiration to become part of such a vibrant and essential community, to witness close up and experience hands-on the discoveries and developments that will shape the discourse of our country and the future of our planet. This was Dr. Norman Borlaug's dream, and his dream will continue to inspire me and motivate me to fight against hunger.

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