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Studying the Characteristics of Common Red Raspberry Fungal Pathogen, Botrytis Cinerea

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Abstract

In the summer of 2022, I worked as a laboratory assistant on a project studying red raspberry fungus and was based at a Washington State University agricultural laboratory. During this internship I worked mostly in the lab and performed a variety of tasks and procedures primarily involving the fungus, *Botrytis cinerea*. Prior to my employment I set learning targets of improving my lab technique, time management, writing skills, and gaining a better understanding of the research process. All of these goals were achieved, and I was able to apply the skills I learned through university coursework in a practical setting in addition to gaining experience beyond the classroom.

Introduction

This internship took place from June-September of 2022 at Washington State University's Northwestern Washington Research and Extension Center (WSU NWREC) in Mount Vernon, Washington. I was an employee of WSU working under Jeff DeLong, a USDA plant pathologist for the Agricultural Research Service (ARS).

The WSU NWREC was established in 1947 and conducted important research to control diseases infectious to beets and cabbage during WWII ("About WSU"). Recognizing the importance of this research, the community raised funds to support ongoing work in agricultural research at the facility ("About WSU"). Today the NWREC supports a wide variety of agriculture-based projects from entomology to hard cider research ("About WSU"). The facility hosts a USDA ARS laboratory which is where I was an intern.

The USDA's ARS supports thousands of scientists and hundreds of research projects and "delivers scientific solutions to national and global agricultural challenges" ("About ARS"). It was created in 1953 as the main scientific research agency for the USDA. Many scientific breakthroughs in agricultural research have occurred since, through the work of USDA scientists and collaborators ("About ARS").

The purpose of participating in an internship was for me to develop my practical scientific skills to better prepare me for future jobs in the environmental science field. I applied for the ARS internship because the description was a good fit for my learning objectives. It offered a range of tasks and would allow me to have ownership of the parts of the project that I would be working on.

Figure 1: Healthy red raspberry (*Rubus idaeus*) on the cane before harvest. Photo from: Olivia Pells.

Figure 2: *Botrytis cinerea* (gray mold) on a fruiting red raspberry. Photo from: USDA-ARS (J. DeLong).

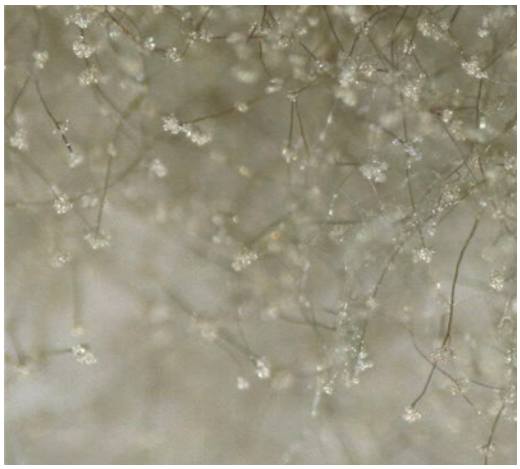

Figure 3: Close up of *Botrytis* conidia and conidiophores. Photo from: USDA-ARS (J. DeLong).

The project I assisted with involved *Botrytis cinerea*; a type of gray mold found on Washington Red Raspberries (Figure 2). *Botrytis* is an ascomycete which means it grows elongated structures called conidiophores which are a type of hyphae used in asexual reproduction. At the end of the conidiophores are spores, or conidia, which, up close, resemble a bundle of grapes (Figure 3). Conidia are asexual spores, but *Botrytis* can also reproduce sexually with structures called sclerotia that are essentially bundles of hyphal tissue (Williamson et al. 2007).

Raspberry production is an important industry in Washington and the state accounts for around 95% of processed raspberry production in the United States ("Red Raspberry"). Red raspberries grow on biennial canes that are vegetative in their first year, then bud, overwinter, and produce fruit in their second year (Figure 1). Raspberries are valued for their nutritional benefits as they are a good source of fiber, vitamin C, potassium, and antioxidants and have been a food source for thousands of years. They also have medicinal value in the sense that they contain compounds such as procyanidins and ellagic acid that are thought to help with cancer prevention and vascular health (Barney et al. 2007).

The overall goal of this ongoing project is to study the genetic characteristics of gray mold populations in raspberry fields in Northwestern Washington to improve management of the pathogen. In order to study the genetic profile of *Botrytis*, it has to be processed to the point

where the DNA can be extracted. This processing involves many steps including transferring the tissue to an agar plate to grow, isolating a single spore of the sample, extracting DNA, and quantifying the DNA.

Studying and managing fungicide resistance in pathogens, especially those that are present on crops used for food is becoming increasingly important. Uncontrolled growth of pathogens like Botrytis can cause massive economic damage and devastate crops (Hanh 2014). Botrytis was one the first fungi observed to be demonstrating resistance to fungicides (Hanh 2014). This is an issue because Botrytis is very widespread, affecting many fruit and vegetable species (Williamson et al. 2007). It also has a lot of genetic diversity which contributes to its ability to adapt when new fungicides are introduced (Hanh 2014).

Internship Responsibilities

As an intern, I assisted with tasks in the field and the laboratory, but the majority of my work was conducted in the lab. At the beginning of the summer my responsibilities mostly involved media preparation and making transfers of botrytis cultures. The media we used most often was a half-strength potato dextrose agar (PDA). I was tasked with measuring and mixing the media, autoclaving it, and pouring it into plates in a biosafety cabinet using aseptic technique. Additionally, I prepared other types of media which included water agar, a sand-oatmeal mixture, a milk solution, and a glycerol solution, the latter three being for sample storage.

Figure 4: Raspberry harvester at a field in Lynden, WA. Photo from: Olivia Pells.

There were a few days where I joined my supervisor in the field. Some of those days were spent visiting farms in Lynden, where our *Botrytis* samples were collected, and some were spent in the fields at the NWREC. Field days in Lynden primarily involved changing spore rods on spore-collecting devices. (A major benefit of these field days was getting to eat fresh raspberries off the cane.) I also had the opportunity to ride on a berry harvester and see what the sorting process looks like (Figure 4). At the NWREC field I assisted with planting and staking various raspberry cultivars for a different, long-term, project.

The lab that we were working in was quite small which required us to be organized in order to prevent it from getting cluttered. A lot of the equipment we used such as pestles, stir bars, and metal instruments were reusable but needed to be sterilized between uses. Other single use plastic equipment such as centrifuge tubes and pipette tips also had to be autoclaved before use.

Sterilizing equipment and maintaining a clean workspace was an important part of this job. Because we were looking at genetic material, it was necessary to try to keep the cultures

separate from each other and to prevent contamination from secondary pathogens. Sanitary measures included wearing gloves, regularly spraying surfaces with 70% ethanol, and sanitizing equipment between uses. Later in the summer we covered the growing trays in sticky plastic wrap to prevent the spread of mites.

Figure 5: *Botrytis* culture growing on a ½ strength PDA plate. Photo from: USDA-ARS (J. DeLong).

The first skill I learned while working with *Botrytis* was transferring cultures from one plate to another. This required the extensive use of a dissecting microscope. I had previously used microscopes in biology classes, but only a handful of times. Through my work on this project I grew competent at adjusting the scope and working at strong levels of magnification. I learned how to identify the parts of the fungal body that were best to transfer and how large of a sample to take. The process of transferring the sample was difficult due to the nature of the fungus. It had a tendency to cling to the tools once it was picked up, which slowed down the process. This required me to refine my technique in order to remain efficient. Sometimes there were dozens of samples that needed to be transferred so working quickly was necessary. There

are a range of tools that can be used to transfer samples. I typically opted for a 20-gauge hypodermic needle tip because it was thin and easy to handle. Between each transfer, the needle had to be cleaned with 99% ethanol and flamed with a Bunsen burner to prevent cross-contamination.

In order to grow a new culture quickly, it is best to transfer a portion of the culture with a lot of conidia as well as some of the hyphal tissue. Under the microscope I would find a viable sample, then transfer it to a new plate of ½ strength PDA. Once the cultures are sporulating (producing spores) the DNA extraction can begin. Extraction was a relatively straightforward but time-consuming process. Typically, it would take at least half of a workday if not a full day. I followed a strict procedure in order to end up with two pellets of genetic material from each isolate. After extraction, the next step was quantification, where we took small drops of water containing the DNA, pipetted onto a plate, and ran it through a TECAN plate reader which generated a numerical value. I would then standardize the DNA values and dilute the remaining DNA so that we could run a polymerase chain reaction (PCR) and look at the genetic makeup of the samples in greater detail.

PCR is a useful tool for many disciplines, especially medicine because it can be used to diagnose certain diseases (Mackay et al. 2002). In short summary PCRs run the DNA through a heat cycling process to break it into segments. On our last set of samples, I learned how to operate the thermocycler and run the PCR procedure. I then ran gel electrophoresis to separate the strands of DNA so we could compare relatedness of the isolates.

Later in the summer we started a new sampling set. This meant that I got to be a part of the entire process from identifying *Botrytis* on a berry to packing isolates into storage. For this round though, we were not single sporing the samples, so it was slightly expedited. I started with the infected berries that my supervisor harvested from the field, then took *Botrytis* samples from those berries and transferred them to ½ strength PDA plates. Once those plates were growing, I transferred them one more time to a clean plate to help prevent contamination

from secondary pathogens. After the transferred samples had grown a substantial amount, it was time to extract the DNA.

Outcomes

The work that I did on this internship was effective in helping me to achieve my learning targets. I learned many new laboratory skills and was able to apply techniques I learned in the classroom in a practical setting. Although I was not working with others much, I did develop a good working relationship with a supervisor. Working independently also taught me to manage my time well and become more efficient with my work.

Working in a professional lab has a different workflow than school labs where you are following a procedure to reach an objective in a discrete time frame. On this project, I had a larger objective and a much larger timeframe in which to accomplish it, and rather than following a set of written instructions for everything, I was able to tailor certain procedures to find what worked best for me. But there is also more room for error with a less cookie-cutter environment. Sometimes mistakes are made, or you run into obstacles like a mite infestation, and data is simply lost. You cannot make data up, so you have to account for a certain margin of error when planning your project. When errors occur that you did not account for, you just have to explain what happened and move on with the rest of your work.

From my interview for this job until my last day, my supervisor was committed to helping me fulfill my educational goals. He had me keep a lab notebook for the summer and record the procedures I was working on. I told him I wanted to learn PCRs, so he made sure that I got to do one. He wanted to ensure that I felt ownership of this project and that I was able to develop my own methods for the tasks I was doing. This was definitely the case. By the end of the summer I felt that if asked, I could run all of the procedures almost entirely independently. I would consider this internship a personal success and that was made possible by having a good supervisor.

Through this internship I grew as a scientist as a person and learned many new skills and laboratory techniques. In the future I see myself spending more time out in the field as opposed to in a lab, simply because I prefer to work outdoors, but I have a level of comfort with lab work now that was not there before.

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Photo Sources

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