

## Final Internship Report, August 2013

### Research on *Rhizoctonia* infection in onion and pea crops in the Pacific Northwest USA

Throughout my internship I communicated and worked mostly with Dr. Dipak Sharma Poudyal, who worked under the supervision of Dr. Tim Paulitz and Dr. Lindsey du Toit on the project of *Rhizoctonia* spp. infecting pea and onion bulb crops in the Pacific Northwest. *Rhizoctonia* is a soil-borne fungal pathogen that impacts many different plants. Until 2001, little was known about *Rhizoctonia* infecting field peas in the inland Pacific Northwest when pea crops are grown in rotation with cereal crops, either cereal cover crops (to control erosion) or cereal cash crops. *Rhizoctonia* is now well established as a general root pathogen of pea, but in rotation with cereal crops on very sandy soils, it is a relatively new disease that has developed resulting in patches of stunted pea plants, with the patches ranging from a few feet to greater than 30 feet. The fungus was isolated from pea plants grown on a farm southeast of Lewiston, ID (Paulitz, 2002). For more than a decade, *Rhizoctonia* has also been known to infect onion, but the situation that leads to *Rhizoctonia* causing similar stunted patches in onion crops rotated with cereal cover crops on very sandy soils is a new phenomenon in the Pacific Northwest (du Toit et al. 2012). Scientists at WSU and USDA-ARS have been working on finding out the cause of this fungal pathogen called *Rhizoctonia*. They suspected that the previous cereal crop could serve as a host for *Rhizoctonia*, which then transferred to the onion crop that was planted in the spring. They received funding from the Washington State Specialty Crops Block Grant to work on identifying the disease and how to manage it. My responsibilities were to assist and conduct research in the lab, greenhouse and in the field with this research on *Rhizoctonia* in pea and onion crops, and to learn plant pathology research techniques.

When I first started, I went through safety training in the lab and in the greenhouse. I learned what to do and what not to do. The way I framed my responsibilities was to show up on time, be well rested, dressed appropriately, and to be prepared (bring my lunch/drinking water and have a pen in my pocket). The way

I initiated resolution was to get up to speed as soon as possible to relieve some of the responsibilities of my direct supervisor. Anytime you start a new job, there is a learning curve. About 'doing what is right', I felt was giving 100% every day so that those paying for this research were getting the most for their money.

My work first started in the lab. Most of the lab work focused on preparing the *Rhizoctonia* inoculum for the greenhouse experiments. Some of the techniques I had to learn were how to do the preparation work for the experiments. The first item was the pathogen that we used to inoculate the plants. To prepare the inoculum, small plugs (about 5 mm<sup>3</sup>) were taken from isolates growing on potato dextrose agar (PDA) in petri dishes. Those small plugs were then transferred to new petri dishes with PDA. The isolate then grew until it filled the dish. During that time, Erlenmeyer flasks filled with oat seed that had been soaked in water were sterilized by autoclaving them twice, with a 24 hour break between autoclavings. After autoclaving, the oats were re-distributed by shaking the flasks. Once the oats had cooled to room temperature after the second autoclaving, the isolate of *Rhizoctonia* was transferred into the flasks with the oats. Then, on a weekly basis, the oats were shaken again to re-distribute the mycelium. After about four weeks, the inoculum was ready. The inoculum was then taken out of the flasks and dried for 24 hours in a laminar flow hood, and then stored in bags at 4°C in the dark. I also learned how to protect the cultures we were growing from contamination. All transfers of the cultures were done in a laminar flow hood with all of the tools used to make the transfers (forceps, scalpels, etc.) dipped in a 95% solution of ethanol. The tools were then placed above a small flame to burn off the ethanol and kill any microorganisms present on the tools. Whenever the lid or cover to any container was removed to make a transfer in the laminar flow hood, the lid was put back immediately after the transfer to limit the amount of time the fungal culture was exposed to the air. Then, most containers were taped shut with Parafilm to keep airborne contaminants out but also allow gas exchange that is necessary for this aerobic fungus to grow.

The greenhouse work then began. The greenhouse experiments were done to test different hypotheses before testing in field trials. Techniques I learned in the greenhouse included the steps used to inoculate the plants. The inoculated oats were ground with a coffee grinder, and sifted through a 1-mm pore size sieve. The ground up oat seed/inoculum was then mixed with pasteurized soil at 1% or less by weight, depending on the experiment. The inoculum was mixed based on weight, not volume, because soil volume can fluctuate. The inoculated soil was used to grow half of the plants in the experiment. For the other half of the experiment, the plants were grown in non-inoculated control soil. The soil used in the control part of the experiment was not inoculated to be able to distinguish how the healthy plants grew compared to the infected plants. One of the details documented in the research was the amount of *Rhizoctonia* present in the soil. Because *Rhizoctonia* does not produce asexual spores that are easily counted, it is a little more difficult to identify the quantity of *Rhizoctonia* put into the soil. The way we identified how much *Rhizoctonia* was in the soil was by colony forming units (CFU). This was done by dilution of a sample of the ground inoculated oat seed in deionized (DI) water. Ten-fold dilutions were done for 3 or 4 repetitions. Then an aliquot of each dilution was spread on PDA in a petri dish to count how many colonies formed. I was unfamiliar with all of these techniques until I began work in this internship. I was excited to learn about all these techniques used in plant pathology, and why they are used.

If for any reason something went wrong in the process, checks were put in place to make sure that contamination (any other disease causing microorganism than the desired pathogen) had not occurred. If contamination had occurred, all the materials were discarded, resulting in lost time and materials invested up to that point in the preparation of the experiment. The materials could be replaced but the time lost could not be replaced. This causes serious setbacks for the scientists because they are always operating on a tight and efficient schedule. Quality and precision are of utmost importance, not quantity. In most projects, the scientists are funded by grants and are expected to deliver regardless of such circumstances. They cannot simply report a list of excuses. At the same time, even if every experiment goes as planned,

scientists do not always find the answers to what they were looking for. They simply find what it is not, or what doesn't work, and report the work objectively. Even though this is progress, the scientist(s) may feel some disappointment. The solution is to think like Thomas A. Edison- "I have not failed. I've just found 10,000 ways that won't work". So, it is their ethical responsibility to gather as much information as possible regarding their research to be profitable. The research may result in more effective ways to suppress or avoid disease outbreaks, increase the yields for the farmer, and/or reduce the amount of chemicals used therefore reduce any potential negative impacts on the environment.

Field work done was carried out to identify what is actually happening with the pathogen in growers' field situations, which may alter the direction of the research or confirm that the researchers are on the right track. Some of the field work done this summer (2013) was to: 1) identify the mycorrhizal fungi present in onion roots and soil sampled from onion crops on certified organic and conventional farms, 2) measure yield losses due to *Rhizoctonia* in onion bulb crops, and 3) time cover crop herbicide spray to the cereal cover crop grown in rotation with conventional onion bulb crops to determine if this might influence the amount of disease that develops from *Rhizoctonia* in the onion crop. The benefits that might result from the field work would be to possibly apply some commercially available mycorrhizae to onion crops at seeding, and reduce the amount of fungicide needed to control *Rhizoctonia*. It is also beneficial for farmers, researchers, and others to know what percentage of their onion yield is lost due to this disease. This helps put into perspective the significance of the disease. Knowing the best timing of spraying the cover crop with an herbicide could help reduce the green bridge between the cover crop and the cash (onion) crop, reducing the impact of *Rhizoctonia* on the latter.

I realized early on that communication was going to be difficult in this internship. I had very little experience in the lab so all of the vocabulary was new to me. I also had very little understanding of plant pathology so I was unfamiliar with the techniques used. I realized very quickly that the field of plant pathology is so vast that I was only seeing the tip of the iceberg. This is going to be a life-long learning experience. In many cases I would have to ask a lot of questions and sometimes I was embarrassed to ask.

After some time I began to feel more comfortable asking questions, therefore learning more and becoming more independent and confident. Even the tiniest details were vitally important. On one occasion I did one of the steps in the process incorrectly, and it ended up costing us the entire experiment. After the fact I asked myself what went wrong in the communication process so I would never make that mistake again. I would also say that sometimes we learn more from our mistakes than what we get right. Besides verbal communication, I also realized that written communication was even more important. With the hundreds of samples that we took, one could easily get off track by mislabeling something and no one would know the difference between the samples, thereby ruining the data. Periodic checks were built into the procedures to avoid this. All data and information about each experiment were documented promptly in a lab notebook. After all the data were collected it would then be entered onto a spreadsheet to be analyzed. Data entry also has to be double-checked before we could begin to analyze it. In the end we have to write a report to communicate the findings. This final part of research is the most important - communication. You can do all the research you want but if you are unable to communicate the work and results effectively in writing, it does the world no good.

I don't think I've ever been this close to the "front lines" of working with a real world problem. It was great to be out in the field with these scientists, observing them as they would ask the growers a lot of questions about the current issues they are dealing with in search of the smallest details that might make a difference. My main goal this summer was to find out if plant pathology is the career I would like to follow. I have come to a definite conclusion that it is.

Paulitz, T.C. 2002. First report of *Rhizoctonia oryzae* on pea. Plant Disease 86: 442-442.

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