The wheat stem rust pathogen, *P. graminis* f.sp. *tritici* (Pgt), has a complex life cycle that includes 5 spore stages, and two unrelated hosts. The main hosts of Pgt are wheat and barley, and the alternate hosts are species in the *Berberies* (Barberry) and *Mahonia* genus.

The spore stage that we see in a wheat fields is the urediniospores. This is the stage that I will be describing to answer your questions.

Growing Spores in a Lab

- The lab at University of Minnesota uses a BSL level 3 lab to handle spores, but the fungus itself is classified as BSL-1. Is the BSL level 3 lab used because Pgt is such an environmental hazard?

  The BSL-3 facility is used for foreign Pgt races. Although stem rust is a common disease of wheat in the US, some foreign Pgt races carry virulence to relevant stem rust resistance genes widely used in North American cultivars. BSL-3 facility is a safe place for working with these races. All the foreign races are used in the BSL-3 facility and our team works with them only in the winter months (December – March). Working with wheat stem rust does not have any environmental hazard.

- What other environmental considerations are there for working with Pgt?

  The environmental conditions in a BSL-3 facility do not differ to the ones used in a regular laboratory. The main difference, in addition to the restricted access and isolation, is the use of negative pressure that helps preventing the movement of spores outside of the facility.

- How do you attain wheat rust spores to study?

  If properly handled, rust spores can be increased and stored for decades. Rust spores collected in a field can be increased by inoculating them on a susceptible wheat line. After two weeks of incubation and disease development, the spores (urediniospores) can be collected, desiccated for 5-6 days, and then stored in a -80oC freezer. Desiccation is a critical stem. If spores are stored wet, they will not survive in storage.

- How are spores germinated in a lab environment without directly using wheat plants?

  Spores can be germinated on water agar plates (Spray sporer over water agar). But after germination (development of germ tube), spores will stop growing. Rust spores need plant living tissue for appresorium formation and finally development of haustoria (feeding structure) inside the living cell.

- How are temperature, humidity, light, and other environmental factors involved in germination?

  For spore germination, humidity is the essential factor. Spores need a period of 12-14 hours at 100% humidity for germination to happen on plant tissue. Also, the optimum temperature range for Pgt spores germination is 15-24 oC.

- If spores are germinated in a lab, how much can they germinate before they die because they aren't growing on a true wheat plant?

  If the spores are derived from a recent collection from plants (fresh spores), all of them will be able to germinate in water agar plates. In you heavily load your agar plate with rust spores, some will not germinate, as there will be some spore competition.

Resistance Factor Interactions

- Are most stem rust resistance factors in wheat assumed to interact directly with their corresponding effectors in Pgt?
- Where are resistance genes and avirulence factors in wheat generally thought to interact? If they interact, how do avirulence factors enter the wheat cells?

  Check this paper:

  Periyannan S, Milne RJ, Figueroa M, Lagudah ES, Dodds PN. 2017. An overview of genetic rust resistance: from broad to specific mechanisms. PLoS Pathog 13:e1006380. doi:10.1371/journal.ppat.1006380

Current Research

- Which resistance genes in wheat do you work with most often? Have most of these resistance genes been sequenced?

  Because of the type of work I do, I have to work with all the stem rust resistance genes available. The focus of my research is to race type and characterize stem rust samples collected from all over the world, focused on places where the pathogen is causing epidemics or outbreaks. After race type the isolates (using the 20 differentials set), I characterize these races against the other stem rust resistance genes that are available. In particular genes that are widely used in agriculture and genes that have proven to be widely effective.

  I also work with durum (tetrapoid) wheat and its relatives, looking for new sources for stem rust resistance. I have to work a lot with *Sr13* as it is the most important source of stem rust resistance in not only North American, but also in durum wheat growing worldwide. *Sr13* gene has been cloned, and molecular markers are available for its use.

- Where in the world is having the most issues with stem rust right now? In these areas, is stem rust the biggest threat to crops or are there other issues and diseases that are even worse?

  Wheat stem rust is considered now as a re-emerging disease, having outbreaks and epidemics in East Africa, Europe, and Central Asia. Severe epidemics occurred in Ethiopia (2013-14), Kazakhstan and South Siberia (2015-16), outbreaks in Germany (2013), Italy (2016), and Sweden (2017). After the Ug99 occurrence and spread (1998), new races with critical virulences have been occurring that have been posing a threat to both bread and durum wheat worldwide.

- What resistance genes are currently being studied the most and are most relevant and promising, even if they haven't been deployed yet?

  This is not an easy question to answer. So far, seven stem rust resistance genes have been cloned: *Sr13, 22, 33, 35, 45, 50*, and APR gene *Sr57* (*Lr34, Yr18*).

  There are some promising genes like *Sr47*, *Sr26*, *Sr33*, *Sr32*, *Sr40*

- What is the best way to find information about virulence of a stem rust race to resistance genes that aren't included in the nomenclature system?

  Once you identify new races, you have to evaluate them for virulence to these genes that are not included in the nomenclature system. These evaluations require that you have these genes in single-gene stocks.

- Once new resistance genes are found, what is the process of implementing them into commercial wheat lines?

  It will depend a lot on where this gene is coming from. If the gene is derived from a wild relative (many effective stem rust resistance genes are derived from wild relatives), lots of work has to be done to facilitate: 1) genome incompatibility and chromosome pairing, and 2) to reduce the linkage drag and simultaneous introgression of deleterious traits. Cloning of these genes and wheat transformation are the best approach to incorporate these genes in cultivated wheat.

  Genes from cultivated wheat origin are easier to breed for. Can be incorporated in breeding programs by direct crosses into breeding lines.

The availability of molecular markers linked to these genes is essential for incorporating these genes in wheat breeding programs. Mostly when you intend to combine (pyramid) multiple resistance genes in your cultuvars.

- How are different strains of stem rust differentiated in the lab?

  Isolates of the wheat stem rust pathogen are race typed using a set of 20 differential lines (each one carrying a single stem rust resistance gene). Based on the virulence/avirulence pattern of the isolate on these genes we are able to assign a race. We use a five-letter code system that was developed by Roelfs and Martens (1988) modified by Jin et al. (2008) to further delineate races in the TTKS race group.

  Roelfs, A.P., and J.W. Martens. 1988. An international system of nomenclature for Puccinia graminis f. sp. tritici. Phytopathology 78:526-533.

  Jin, Y., L.J., Szabo, Z.A. Pretorius, R.P. Singh, R. Ward, and T. Jr. Fetch. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92: 923-926.

- What are some of the most promising pathways for achieving durable resistance against wheat stem rust?

Two main approaches for achieving durable resistance:

1) use a combination of adult plant resistance (APR) genes that are not race specific and provide a high level of stem rust resistance. APR genes acting alone will not provide sufficient level of resistance buy, when used in combinations of 4 or more, will provide in the field adequate protection.

2) The most recent approach is to develop genetically modified gene cassettes. These cassettes include multiple effective *Sr* genes that have not been widely used in agriculture. Good candidates for being used in cassettes are genes derived from wild relatives. Cassettes can be generated using major and APR genes, as well as genes conferring resistance to different pathogens.

- The specific resistance gene that we are working with is Sr35. Is there anyone else that would be helpful for us to get in contact with who has worked with this gene?

  Have you contacted the group that cloned *Sr35*?

  Saintenac, C.; Zhang, W.; Salcedo, A.; Rouse, M.N.; Trick, H.N.; Akhunov, E.; Dubcovsky, J. Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. *Science* **2013**, *341*, 783–786