

## **Stem rust in perennial ryegrass seed crops: epidemiological and genetic research at USDA-ARS**

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### **Abstract**

In production of perennial ryegrass seed in the Northwest USA, stem rust is the primary disease constraint in terms of management cost and potential yield loss. The USDA-ARS research program on this problem is focused on improved decision making for fungicide use, and tools for genetic improvement. Data from 10 years of field and greenhouse research was used to construct an epidemiological model. This model is based on the effects of weather (temperature, leaf wetness) on plant growth and pathogen activity, and includes the effects of two major fungicide classes (triazoles and strobilurins) on disease development, particularly on the spread of disease within a plant. The model is implemented as a decision aid on a publicly-accessible internet site. Users enter scouting data and select a location for weather data to be loaded into the model. The website displays the model results, including the relationship of modeled disease development to an action threshold. The decision aid has performed well in large-scale field demonstration plots across 10 location-years, providing an average economic advantage of \$39 per acre (range: \$0 to \$140) compared to standard fungicide programs. In genetic research, a perennial ryegrass mapping population was constructed from a cross between resistant and susceptible parents. The progeny (193 individuals) were tested by inoculating with two different pathotypes of the stem rust fungus, in separate experiments. Using a genetic map of these parents constructed with SSR and RAD markers, QTL analyses were conducted. The goal of this genetic research is to produce genetic markers for marker-assisted selection of stem rust resistance, as well as to provide information about genomic organization of disease resistance in perennial ryegrass.

### **Stem rust on perennial ryegrass and tall fescue in the Pacific Northwest USA**

In perennial ryegrass (*Lolium perenne*) and tall fescue grown for seed, the economically most important disease in major seed production areas of the United States is stem rust caused by *Puccinia graminis* subsp. *graminicola* (Welty & Azevedo, 1984). Perennial ryegrass seed yield losses up to 98% due to stem rust damage have been recorded (Pfender, 2009a). The disease is also a significant production constraint in New Zealand, where yield losses of 35% were documented (Hampton, 1986). Since the 1980s, there has been a research program at the USDA-ARS Forage Seed Research Laboratory in Corvallis, OR, USA. Starting in the late 1990s the research focused on developing an epidemiological model that could be used in a decision

support system to optimize fungicide use in disease management. Within the last few years another component, stem rust resistance genetics in *Lolium*, has been added to the effort.

### **Epidemiological research and fungicide decision support system**

The goal of the epidemiological work at USDA-ARS has been to quantify the effects of environmental factors on disease development, and to summarize that knowledge in the form of a mathematical model. The model is intended to depict the linked disease cycle components, and is driven by weather data that can be collected with automated weather stations in the field.

#### *Stem rust epidemic model*

We determined that the probability of infection, given the presence of stem rust inoculum, can be calculated from temperature and leaf wetness occurring during nighttime hours and in the few hours after sunrise. If the leaves are wet and temperature is greater than 2C, the fungus spores can germinate and grow. At cold temperatures, a long period of wetness is required for adequate growth to cause infection, but the required wetness duration is shorter at warmer temperatures. The temperature X wetness duration must be adequate during both the dark period (for spore germination) and the early morning period (for penetration into the plant). We derived an equation for calculating infection probability from overnight and morning weather data recorded in the field (Pfender, 2003). After the fungus has successfully penetrated the plant, some time is required for the infection to develop sufficiently to produce the next generation of spores. The duration of this time period between infection and production of new spores (the latent period) is determined primarily by temperature. The latent period duration can be calculated by accumulation of heat units (e.g. degree days) (Pfender, 2001). The processes of infection and the latent period form the core of the stem rust epidemic model.

An additional important aspect of stem rust development is the ability of the fungus to spread from infections on the sheath to the stem or flower that is extending from within the infected sheath (Pfender, 2004). This disease spread occurs because the pustule on the sheath is producing spores on the inner sheath surface as well as the outer surface. The result is that a single infection on the flag sheath, for example, can produce hundreds of infections on the inflorescence head and stem. Our observations indicate that up to 80% of the infections that appear on ryegrass stem sections at the height of an epidemic are the result of this process. The amount of disease due to this within-plant component is dependent not only on the frequency of lesions on sheaths, but also on the rate of extension of the internodes. Therefore the epidemic model includes a component for stem elongation (Pfender, 2006), which is driven by heat units. The full epidemic model includes infection probability, latent period and within-plant spread. The model works on a daily time step, and the amount of inoculum at the beginning of each day is proportional to the amount of disease (sporulating pustules) at the end of the previous day.

Although within-plant spread seems to be a general feature of stem rust in perennial ryegrass, we have noted that varieties differ in the prominence of the sheath lesions that act as sources for the

process. In the turf varieties we have worked with the sheath pustules are usually prominent, and it is easy to see the relationship between the sheath pustule and the resultant stem lesions. However in some forage varieties such as Linn, pustules may be relatively inconspicuous on the outside surface of the sheath although they are sporulating on the inner surface. In these cases it is only with some difficulty that one can trace the origin of extensive stem lesions (including those underneath a sheath) to the source lesion on the sheath. This difference among varieties has important implications for disease scouting.

The effectiveness of fungicides is an important factor to include for the epidemic model to be used as a decision support for disease management. We measured protective and post-infection ("kick-back") activity of azoxystrobin and propiconazole fungicides acting against infections on treated plant surfaces, and derived equations for efficacy as a function of time between infection and fungicide application (Pfender, 2006). We also noted that the strobilurin suppressed the within-plant spread process of stem rust by preventing sporulation from the inner surface of sheath lesions, whereas the propiconazole had very little such activity. Therefore, if a fungicide is applied when sheath lesions are beginning to shed spores onto the enclosed stem internodes, very different fungicide efficacies will be apparent for the two fungicide types starting about a latent period later. The stem rust model accounts for the differing activities of fungicides as affected by the timing of tiller extension (Pfender, 2009c).

#### *Stem rust decision aid*

For use as a decision aid, an action threshold is required. The threshold should be based on the relationship between disease severity and yield loss. We determined that the best predictor of yield loss due to stem rust is the severity of disease during the 2 to 3 weeks centered on anthesis. A damage function was derived that estimates yield as a function of healthy plant area duration (total plant area minus diseased plant area) (Waggoner, & Berger, 1987) during this time window (Pfender, 2009a), and the action threshold for the decision aid is set such that significant yield loss is avoided.

The decision aid is currently implemented on a publicly available internet web site. Users of the site choose a geographical source for weather data input, and enter information for plant growth stage, their most recent disease scouting results and any fungicide applications. A simulation is run with this information and results are displayed as a graph of outputs running from March 1 to the current day. The graph shows daily infection probabilities and two categories of simulated disease severities. One line is plotted for visible disease, and another for the total number of infections, including latent infections that have not yet become visible. The threshold criterion is compared with the total infection level (visible plus latent). The graphical output also shows the timing and estimated effects of fungicide applications.

An important aspect of information delivery for the decision support system is the use of representative weather data for the user's location. Currently we are using a limited number of

weather stations in grass seed fields; the stations have communications gear and automatically transmit newly-acquired data to our laboratory computer every morning for incorporation into the season-long weather database for that location. To obtain better geographical coverage, there is an ongoing multidisciplinary, multi-state effort to produce relatively high-resolution (~1 km grid) weather estimation for parts of the western U.S. The weather estimates are produced from the 60-km gridded US National Weather Forecast, processed through climatological and physical process models. Our goal is to use this 1-km weather data, including 7-day forecast, as the weather data source for the stem rust decision support system.

Rigorous validation of the simulation model is in progress, but has not yet been completed. However, the model has been tested in large-scale replicated plots in grower fields over the last 5 years, 2 locations per year (Pfender et al., 2009). In these 10 location-years use of the decision support produced economic results (revenue from seed yield minus fungicide costs) at least as good as current standard practice. In three location-years there was no significant difference between results of standard practice and decision-supported practice, and in five location-years there was a saving on fungicide costs with no yield penalty. In two cases there were yield increases, one of which was achieved with fewer fungicide applications (but different timing) than the standard treatment.

### **Genetics of stem rust resistance in *Lolium***

In field and greenhouse trials we determined that the cultivar 'Kingston' (PGG Wrightson Seeds, New Zealand) typically has a lower level of stem rust than other varieties we tested. To gain some insight into genetics of stem rust resistance, we created a mapping population by crossing two plants (resistant and susceptible) that we selected from 'Kingston' after repeated stem rust testing under controlled conditions. A population of 193 F1s from this cross was mapped.

#### *Mapping*

Linkage maps for the male and female parents of the mapping population were constructed with SSR (simple sequence repeat) and RAD (restriction-site associated DNA) (Baird et al., 2008) markers. Tall fescue SSR markers, previously developed (Saha et al., 2006) by researchers at the Samuel Roberts Noble Foundation (Ardmore, OK, USA) were screened against parental DNA and genotyped on the progeny by M. Saha. Additional SSR markers, also run at the Noble Foundation, were originally developed for *Lolium* by other research groups (Gill et al., 2006). Population-specific RAD markers were developed from parental DNA and genotyped on the progeny by Floragenex (Eugene, OR, USA). For RAD library preparation, *Lolium* genomic DNA was digested with SbfI and fragments were ligated to P1, a modified Solexa adapter (Illumina, Inc.). After PCR amplification, libraries were run on an Illumina Genome Analyzer II located at the University of Oregon. Raw Solexa data were processed using Floragenex custom programs. In the RAD marker development phase, sequence data from each parent were grouped into highly similar sequences with no more than a two-bp mismatch allowed. Sequence

groupings from the parents were then compared to identify alleles from SbfI-tagged loci, expected to segregate in three configurations in an F1 population: testcross (heterozygous SNP in one parent, homozygous in the other), cut-site testcross (heterozygous SNP in one parent, absence of sequence in the other presumably caused by a nucleotide polymorphism in the SbfI site), and intercross (heterozygous SNP in both parents). F1 genotypes were scored based on comparison of F1 sequences with the parental marker panel.

Maps were assembled for each parent from the F1 segregation of co-dominant and male- and female-specific SSR and RAD alleles, using JoinMap 4 software and CP population type codes (Kyazma, Wageningen, Netherlands). We used the test for independence LOD score, which is not affected by segregation distortion, to group markers into seven linkage groups for each map (significance level of 6.0 LOD).

### *Phenotyping*

Disease phenotypes were determined in inoculation assays conducted in a greenhouse, with bulk inoculum (field-collected, genetically mixed) or single-pustule isolates (genetically uniform). We had previously demonstrated pathotype specificity in stem rust of *Lolium perenne*, by purifying and increasing two different, single-pustule isolates of the pathogen (Pfender, 2009b). Isolate 101 is avirulent on one of the mapping population parents, and resistance is inherited as a single dominant gene that is heterozygous in the resistant parent. Isolate 106 is virulent to some degree on both parents. Phenotypes were scored as number of pustules per plant. There were three replicate (cloned) plants per F1 individual in each experiment, and each experiment was conducted at two different times. QTL analysis was conducted in MapQTL5 for the male and female parent maps. Kruskal-Wallis analysis and automatic cofactor selection were used to choose cofactors for use in MQM analysis.

### *QTL Results*

There is a distinct location for resistance to pathotype 101, on linkage group 7. The peak, with LOD scores of 10 to 28, is located at about 40cM on both male and female maps. The same peak can be seen in the QTL analysis of the bulk-inoculum experiments. QTL analyses of data from phenotypes produced by pathotype 106 lack the peak at 40cM on LG 7, but have a significant peak on LG 1 on the male and female maps. A peak at the same LG1 location is seen in all QTL results for the bulk-inoculum phenotypes as well, although the peak appears at a slightly displaced position on the male map for one of these experiments. Whereas the resistance to isolate 101 (on LG7) has a pathotype-specific qualitative resistance phenotype, the resistance to isolate 106 is of a quantitative nature. Other possible QTL locations are: LG2 where there are distinct peaks (but less than genome-wide significance level for LOD score) for several experiments on the female map, and LG6 where consistent, but similarly non-significant, peaks occur in the male and female maps for several experiments.

These findings are the first report of linkage-group locations for qualitative and quantitative stem

rust resistance loci in *Lolium perenne*. The use of RAD markers, which have defined sequences, will make it possible to make probes for the markers that are associated with these QTL peaks.

## References

- Baird, N., Etter, P., Atwood, T., Currey, M., Shiver, A., Lewis, Z., Selker, E., Cresko, W. & Johnson, E. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* 3(10): e3376.
- Gill, G.P., Wilcox, P., Whittaker, D., Winz, R., Bickerstaff, P., Echt, C., Kent, J., Humphreys, M., Elborough, K. & Gardner, R. (2006). A framework linkage map of perennial ryegrass based on SSR markers. *Genome* 49: 354-364.
- Hampton, J.G. (1986). Fungicidal effects on stem rust, green leaf area, and seed yield in 'Grasslands Nui' perennial ryegrass. *New Zealand J. Exp. Agric.* 14:7-12
- Pfender, W.F. (2001). A temperature-based model for latent period duration in stem rust of perennial ryegrass and tall fescue. *Phytopathology* 91:111-116.
- Pfender, W.F. (2003). Prediction of stem rust infection favorability, by means of degree-hour wetness duration, for perennial ryegrass seed crops. *Phytopathology* 93:467-477.
- Pfender, W.F. (2004). Role of phenology in host susceptibility and within-plant spread of stem rust during reproductive development of perennial ryegrass. *Phytopathology* 94:308-316.
- Pfender, W.F. (2006). Interaction of fungicide physical modes of action and plant phenology in control of stem rust of perennial ryegrass grown for seed. *Plant Disease* 90:1225-1232.
- Pfender, W.F. (2009a). A damage function for stem rust of perennial ryegrass seed crops. *Phytopathology* 99:498-505.
- Pfender, W.F. (2009b). Demonstration of pathotype specificity in stem rust of perennial ryegrass. *Phytopathology* 99:1185-1189.
- Pfender, W.F. & Eynard, J. (2009c). Field assessment of a model for fungicide effects on intraplant spread of stem rust in perennial ryegrass seed crops. *Phytopathology* 99: 696-703.
- Pfender, W.F., Mellbye, M.E., Silberstein, T.B. & Gingrich, G.A. (2009). Five year field evaluation summary of the USDA stem rust model for perennial ryegrass. In 2008 Seed Production Research at Oregon State University, USDA-ARS Cooperating. Department of Crop and Soil Science Ext/CrS. 128. p. 26-28. 2009.
- Saha M.C., Cooper J.D., Mian, M., Chekhovskiy K & May G.D. (2006) Tall fescue genomic SSR markers: development and transferability across multiple grass species. *Theo Appl Genetics* 113: 1432-2242

Waggoner, P.E. & Berger, R.D. (1987). Defoliation, disease, and growth. *Phytopathology* 77:393-398.

Welty, R.E. & Azevedo, M.D. (1994). Application of propiconazole in management of stem rust in perennial ryegrass grown for seed. *Plant Dis.* 78:236-240.