2013F075R Development of a rapid quantitative detection method for sclerotinia stem rot inoculum to aid disease risk assessments and fungicide spray decisions.

Funding Partners: Alberta Canola Commission, and Western Grains Research Foundation

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Background:

Sclerotinia stem rot, caused by *S. sclerotiorum*, is one of the most important biotic stresses influencing long-term production and economic stability for canola producers. The wide host range of the pathogen, its production of wind-borne ascospores, and the sporadic nature of disease development limit the effectiveness of many management strategies. Stem rot on canola is managed primarily by the routine application of fungicides, typically without any indication of disease risk. To reduce the negative economic and environmental effects associated with the application of fungicides, a reliable stem rot forecasting system is desirable. Although producers and industry have access to existing risk assessment tools, such as weather-based forecast maps, checklists and petal testing, there has been limited acceptance or widespread use of these tools. Weather maps are regional and not field-specific forecasts, while checklists are qualitative in nature. Petal testing is the only available risk assessment tool providing a direct assessment of inoculum levels, but is under-used due to the time required from sample collection to availability of results. The development of a quantitative PCR-based system to accurately measure the amount of *S. sclerotiorum* inoculum on canola petals could overcome some of these limitations, helping to provide forecasts for disease risk while taking into account environmental conditions.

Objectives:

a) Development and refinement of a rapid quantitative method for pathogen detection in canola flowers

b) Understanding of the relationship between the amount of pathogen on the petals and final stem rot levels in commercial fields

c) Assessment of correlations between pathogen detection, weather-based forecasts and final stem rot levels

What we did:

A quantitative PCR (qPCR)-based assay was developed for measuring the amount of *S. sclerotiorum* DNA in canola petals, to enable rapid and accurate estimates of infestation levels. The assay targets a 70-bp region of a single-copy gene encoding the hypothetical secreted protein ssv263. The specificity and sensitivity of the assay were evaluated.

The relationship between petal infestation levels and final stem rot incidence in canola was explored in two additional studies. In the first study, conducted over 2 years, petal infestation was compared with disease incidence in 34-35 commercial canola fields distributed across Alberta, Saskatchewan and Manitoba. In the second study, these parameters were compared over 3 years in 9-11 fields located in central Alberta.

Three key results:

The qPCR assay was highly specific, and did not amplify DNA from any of a number of closely related fungi and other pathogens of canola. It also did not amplify DNA of the host plant. These

results indicate that the assay is highly specific for *S. sclerotiorum* and can be used to estimate pathogen biomass in canola petals. The assay also is highly sensitive, with a limit of detection of 8.0×10^{-4} ng *S. sclerotiorum* DNA.

When the qPCR assay was used to quantify *S. sclerotiorum* in field-collected canola petals, considerable variation was observed in the amount of petal infestation in different fields and at different crop stages, indicating the importance of assessing petal infestation and risk potential for a particular field as opposed to an assessment of risk based on regional conditions.

The incidence of stem rot generally was found to increase with increasing petal infestation. The strength of the relationship varied across the study years, and was strongest when canola petals were analyzed at full bloom and in years when disease pressure was high.

Take home message for the industry:

The qPCR assay may serve as the basis for a risk assessment system, as well as representing a useful tool for the study of the epidemiology of Sclerotinia stem rot of canola. It can quantify the level of petal infestation, thereby providing a measure of disease risk when timely fungicide application decisions need to be made. It is important to emphasize, however, that a forecasting system based on qPCR quantification of petal infestation should be linked to environmental conditions, as well as cropping history, seeding date and crop canopy conditions, which may influence stem rot development and the need to spray a fungicide.

Value to industry:

It is estimated that up to 20% of canola acres are sprayed for stem rot each year at an estimated cost of $55 million. Spraying is typically required when disease incidence approaches 20%; however, surveys suggest that only 10-20% of fields actually have at least 20% infected plants. Routine use of risk assessment tools like qPCR-based detection and quantification of *S. sclerotiorum* will help rationalize fungicide application. Even just a 10% reduction in acres sprayed will reduce the amount of fungicide applied in the prairie ecosystem by as much as 44 million grams of active ingredient annually. The potential return per dollar invested as part of this project could be as much as $80, based on annual cost savings related to lower chemical and application costs; this excludes environmental benefits.

Value to the team:

This project served as the basis for one graduate student program at the Ph.D. level. The student obtained expertise in experimental design, statistical analysis, molecular biology, plant pathology and epidemiology. The project also enabled the training of two students enrolled in the Women in Science, Engineering, Scholarship and Technology (WISEST) Program, as well as three undergraduate summer students.