Project Identification

1. **Project Title:** Sclerotinia Risk Assessment Tools for Spray Decision Support in Canola
2. **Project Number:** 20200524
3. **Producer Group Sponsoring the Project:** Indian Head Agricultural Research Foundation (IHARF)
4. **Project Location(s):**
   - R.M. of Indian Head No. 156
   - R.M. of of Trampling Lake No. 380
   - R.M. of Star City No. 428
   - R.M. of Antler No. 61
5. **Project start and end dates (month & year):** March 2021 to February 2022
6. **Project contact person & contact details:**

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Objectives and Rationale

7. **Project objectives:**

The objectives of this project are:

1) To demonstrate various tools for assessing Sclerotinia stem rot risk in canola;
2) To assess their value in supporting producers with the decision to spray fungicide for Sclerotinia management.

The tools being demonstrated include the Spornado Sampler, DNA-based petal testing, an online Decision Support Tool (CanolaDST.ca), and the Sclerotinia Stem Rot Checklist from the Canola Council of Canada. These tools have the potential to help producers avoid unnecessary fungicide applications.

8. **Project Rationale:**

The risk of Sclerotinia stem rot infecting a canola crop depends on 1) conditions leading up to flowering being conducive to sclerotial germination and apothecia development, and 2) conditions being favourable for disease development during and after the flowering period. Fungicide use decisions for Sclerotinia management need to be made before visible symptoms are present in the canola crop. Without reliable, accurate, and timely methods and data to measure or predict the presence and abundance of the pathogen, or to measure or predict environmental conditions during crop development, producers often opt for a routine fungicide application as a risk-management strategy.

Many factors have been shown to influence the risk of disease developing, in particular, the amount of moisture leading up to and during flowering, ascospore presence, forecasted weather, canopy density and yield potential, cultivar resistance, and field disease history (Canola Council 2020, McLaren et al. 2004, Turkington et al. 2011). Using these factors, several methods and tools have been developed for Sclerotinia forecasting and risk analysis:

- The Sclerotinia stem rot checklist is recommended by the Canola Council of Canada and is accessible on their website. Points are added for several risk factors and the total score indicates the probability of a positive economic return to a foliar fungicide application. This method includes a limited consideration of the potential for apothecia development and weather forecast.

- The Canola Decision Support Tool (CanolaDST.ca) is a free online-based application that was developed by Weather Innovation Network (WIN) to model Sclerotinia stem rot risk in canola fields based on regional weather data and weather forecasts, and user-inputted agronomic data including specific field location, seeding date, cultivar, field history, and plant density.

While these two tools rely on the prediction of apothecia development, there are a few methods that directly measure the presence and abundance of ascospores:

- Petal testing initially required plating the petals on selective agar to observe if the petals were infected (Turkington et al. 1991). The method was fairly accurate in predicting disease incidence (Turkington & Morrall 1993) but the process was too time-consuming for producers to use practically as a spray decision tool. The development of DNA marker technology to identify and quantify the level of ascospores has made petal testing more practical for producers as the results can be obtained in one or two days (Freeman et al 2002, Ziesman et al 2016). These petal tests are offered commercially as kits from Discovery Seed Labs or from Quantum Genetix.
- Spore trapping samplers capture airborne spores and also effectively use DNA marker technology to assess the presence of ascospores (Freeman et al 2002). The Spornado Sampler uses this technology and is available from 20/20 Seed Labs.

When using tools that only measure the presence and abundance of ascospores, environmental conditions during and after flowering still need to be considered when making the decision to spray. A combination of two or more tools may be the most effective for supporting producers in making the decision to spray.

The technologies being demonstrated in this project have the potential to help producers feel more confident in their decision to spray and may help prevent non-economic fungicide applications. Preventing unnecessary fungicide applications would also have environmental benefits, would help prevent fungicide resistance, and would boost public trust.

Canola Council of Canada. 2020. Canola Encyclopedia. [Online] https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/

Freeman, J., Ward, E., Calderon, C., and McCartney, S. 2002. A Polymerase Chain Reaction (PCR) Assay for the detection of inoculum of Sclerotinia sclerotiorum. European Journal of Plant Pathology. 108: 877-886.

McLaren, D.L., Conner, R.L., Kutcher, H.R., Platford, R.G., Lamb, J.L., and Lamey, H.A. 2004. Predicting diseases caused by Sclerotinia sclerotiorum on canola and bean – a western Canadian perspective. Can J Plant Path 26:489-497.

Turkington, T.K., Kutcher, H.R., McLaren, D., and Rashid, K.Y. 2011. Managing Sclerotinia in Oilseed and Pulse Crops. Prairie Soils and Crops Journal. 4:105-113.

Turkington, T.K., and Morrall, R.A.A. 1993. Use of petal infestation to forecast Sclerotinia stem rot of canola: the influence of inoculum variation over the flowering period and canopy density. Phytopathology. 83:682-689.

Turkington, T.K., Morrall, R.A.A., and Gugel, R.K. 1991. Use of petal infestation to forecast sclerotinia stem rot of canola: Evaluation of early bloom sampling, 1985-1990. Can J Plant Pathol 13:50-59.

Ziesman, B.R., Turkington, T.K., Basu, U., and Strelkov, S.E. 2016. A quantitative PCR system for measuring Sclerotinia sclerotiorum in Canola (Brassica napus). Plant Disease 100:984-990.

Methodology and Results

9. Methodology:

One of the objectives of this project was to demonstrate the risk assessment tools in the field, and so the methodoly will be described in detail for this purpose.

The tools and procedures being demonstrated were designed to be utilized at the field scale.The demonstration was conducted in commercial fields, in cooperation with local producers at each

location (R.M. of Indian Head no. 156, R.M. of Trampling Lake no. 380, R.M. of Star City no. 428, R.M. of Antler no. 61). Producers were asked to leave an unsprayed strip in their canola fields for the purpose of this demonstration. There were three fields at each of the four locations, a total of 12 fields across the province.

Each of the following tools were utilized to assess Sclerotinia stem rot risk in each field, at both optimal spray timing (20-30% flower) and late spray timing (50% flower):

I. Spornado sampler from 20/20 Seed Labs:

The sampler is installed in a location in the field where wind flow is unobstructed and free from road dust. The sampler turns with the direction of the wind, like a weather vane, and collects airborne particles on a filter in the cassette. The cassette is inserted in the sampler 2 to 4 days before the desired crop timing (Figure 1). The lab provides a link where the cassette is registered when inserting and removing it from the sampler. The cassettes are dropped off or couriered to 20/20 Seed Labs in Nisku, Alberta, and the results are available within 24 hours of the lab receiving them. The lab report indicates the level of sclerotinia as “not detected”, “trace levels detected”, or “detected”, and it is suggested to use this information in combination with other weather-based risk assessment tools (Figure 2).

Figure 1. Spornado spore sampler installation and cassette insertion.

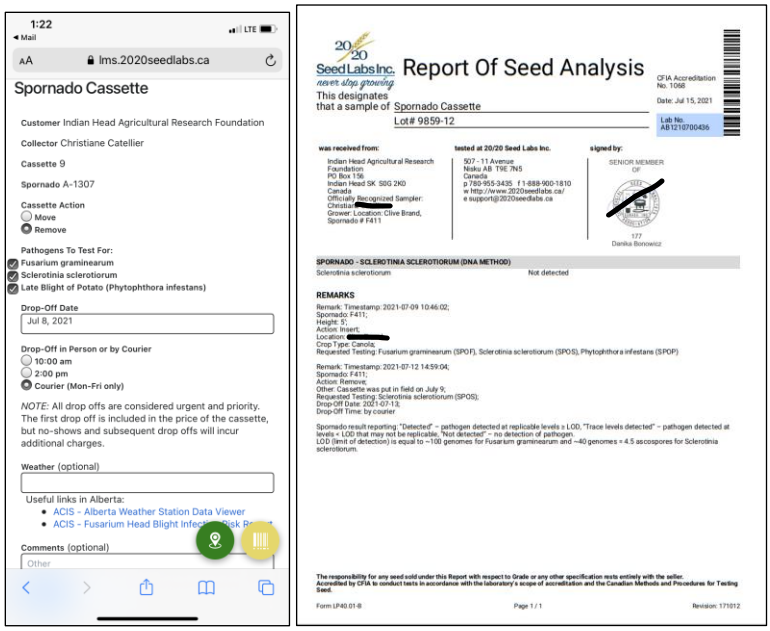


Figure 2. Screen shot of cassette registration and example of lab report provided by 20/20 Seed Labs for each cassette submission.

II. Petal test kit from Discovery Seed Labs:

The kit includes sample vials, forceps, and a submission form. Petals are collected from 8 different plants in 8 different locations in each field. Two petals are collected from each plant, from the lower and upper part of the stem (older and newer petals) (Figure 3). The samples are dropped off or couriered to Discovery Seed Labs in Saskatoon, and results are available within 24 hours of the lab receiving the samples. The user is directed to a Sclerotinia calculator via the Discovery Seed Labs website. The total percent infected petals from the report is entered along with crop density and a weather assessment to obtain an estimate for probable % diseased plants and probable percent yield loss (Figure 4).

Figure 3. Petal collection procedure using Discovery Seed Labs' petal test kit.

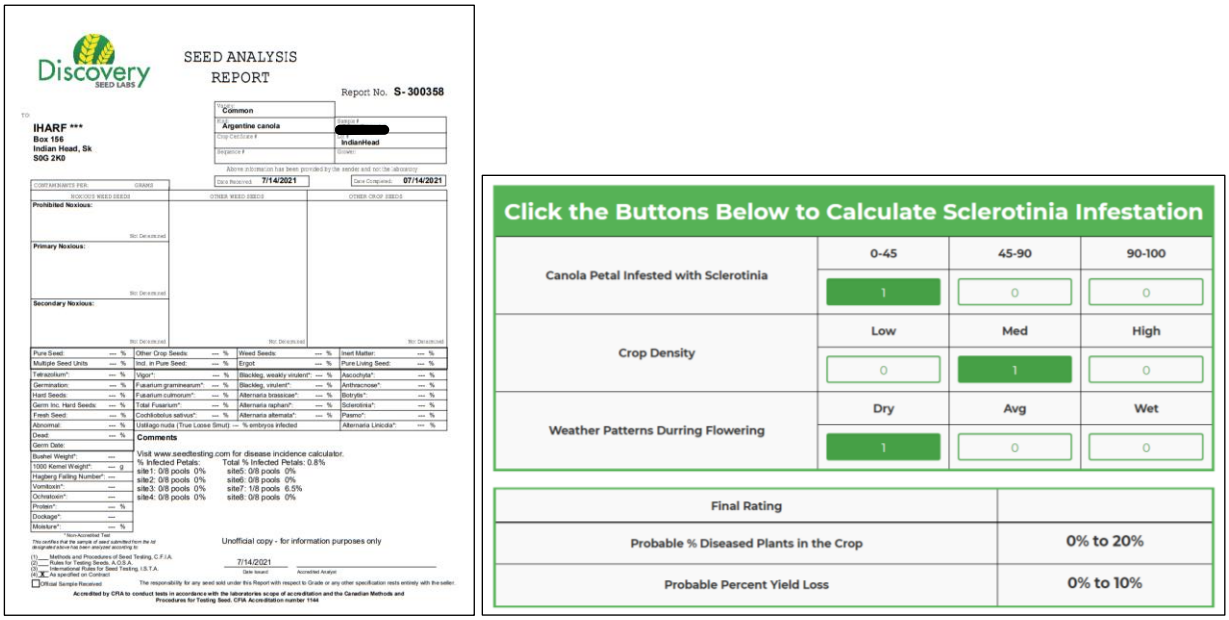
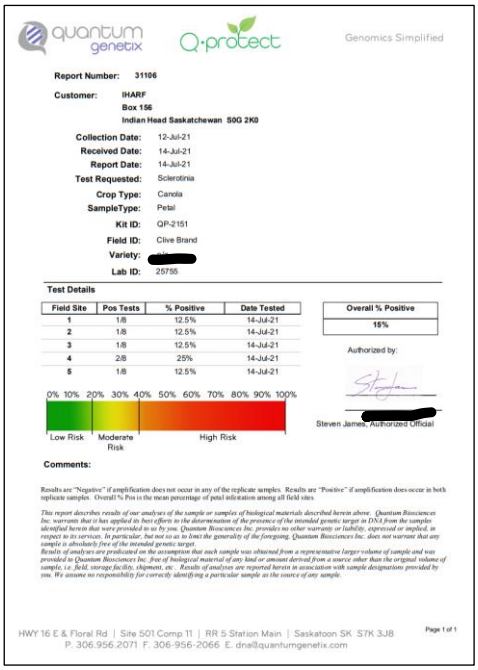


Figure 4. Example of lab report provided by Discovery Seed Labs for each petal test, and online tool for interpreting results and assessing Sclerotinia risk.

III. Q-Protect petal test kit from Quantum Genetix:

The Q-Protect Kit includes sample vials, a sample ID form, instruction booklet, and a set of forceps for collecting petals. Petals are collected from 8 different plants in five different sites in each field. From each plant, three petals are collected from the lower, middle, and top of the plant (mixture of older and newer petals) (Figure 5). The samples are dropped off or couriered to the Quantum Genetix lab in Saskatoon and results are available within 24 hours of the lab receiving the samples. An example of the lab report is shown in Figure 6. An explanation on how to interpret reported results was provided in the instruction booklet. A percent positive sclerotinia presence is translated to a risk level shown on the grid. It is indicated that Q-Protect results of 40% correlate to a yield loss of 7.5% of more and would justify a fungicide application.

Figure 5. Petal collection procedure using Quantum Genetix's Q-Protect Kit.



quantum genetix Q-protect Genomics Simplified

Report Number: 31106

Customer: IHARF
Box 156
Indian Head Saskatchewan S0G 2K0

Collection Date: 12-Jul-21
Received Date: 14-Jul-21
Report Date: 14-Jul-21
Test Requested: Sclerotinia
Crop Type: Canola
SampleType: Petal
Kit ID: QP-2151
Field ID: Clive Brand
Variety: [redacted]
Lab ID: 26755

Test Details

Field Site	Pos Tests	% Positive	Date Tested
1	1/8	12.5%	14-Jul-21
2	1/8	12.5%	14-Jul-21
3	1/8	12.5%	14-Jul-21
4	2/8	25%	14-Jul-21
5	1/8	12.5%	14-Jul-21

Overall % Positive
15%

Authorized by:

Steven James, Authorized Official [redacted]

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

Low Risk | Moderate Risk | High Risk

Comments:

Results are "Negative" if amplification does not occur in any of the replicate samples. Results are "Positive" if amplification does occur in both replicate samples. Overall % Pos is the mean percentage of petal infestation among all field sites.

This report describes results of our analyses of the sample or samples of biological materials described herein above. Quantum Biosciences Inc. warrants that it has applied its best efforts to the determination of the presence of the intended genetic target in DNA from the samples identified herein that were provided to us by you. Quantum Biosciences Inc. provides no other warranty or liability, expressed or implied, in respect to its services. In particular, but not so as to limit the generality of the foregoing, Quantum Biosciences Inc. does not warrant that any sample is absolutely free of the intended genetic target.
Results of analyses are predicated on the assumption that each sample was obtained from a representative larger volume of sample and was provided to Quantum Biosciences Inc. free of biological material of any kind or amount derived from a source other than the original volume of sample, i.e. field, storage facility, shipment, etc. Results of analyses are reported herein in association with sample designations provided by you. We assume no responsibility for correctly identifying a particular sample as the source of any sample.

HWY 16 E & Floral Rd | Site 501 Comp 11 | RR 5 Station Main | Saskatoon SK S7K 3J8
P. 306.956.2071 F. 306-956-2066 E. dna@quantumgenetix.com

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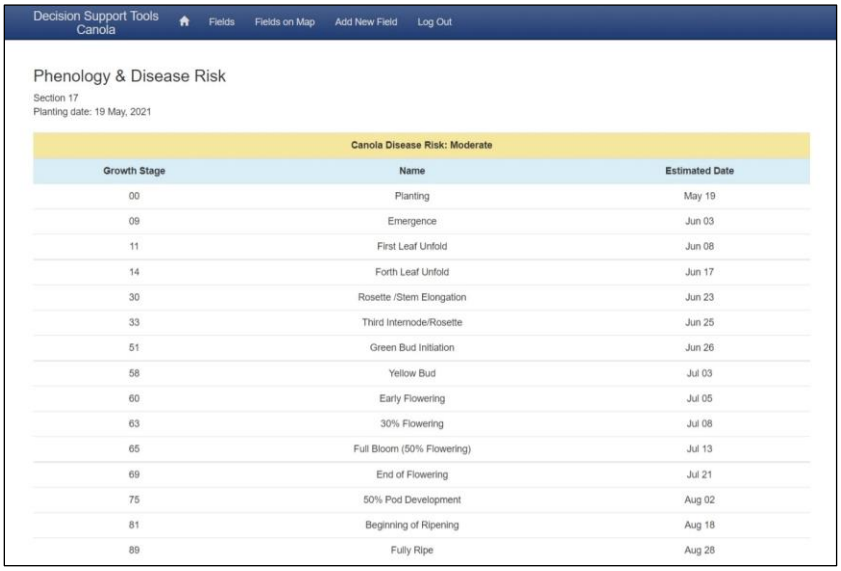
Figure 6. Example of lab report provided by Quantum Genetix for each petal test.

IV. Canola Decision Support tool (CanolaDST.ca):

The free online-based application uses regional weather data and weather forecast from the Weather Innovation Network (WIN) weather stations to model Sclerotinia stem rot risk in canola fields. Users input agronomic data including the specific field location (to relate to the nearest weather station), the cultivar (to assess disease resistance and to predict crop development stage throughout the growing season), seeding date, plant density, disease incidence in the last host crop, and the number of years with host crops. Sclerotinia risk is assessed as low, moderate, or high risk (Figure 7). It is not clear how to interpret the differences between the risk categories.



Decision Support Tools Canola | Fields | Fields on Map | Add New Field | Log Out

Phenology & Disease Risk

Section 17
Planting date: 19 May, 2021

Canola Disease Risk: Moderate		
Growth Stage	**Name**	**Estimated Date**
00	Planting	May 19
09	Emergence	Jun 03
11	First Leaf Unfold	Jun 08
14	Forth Leaf Unfold	Jun 17
30	Rosette /Stem Elongation	Jun 23
33	Third Internode/Rosette	Jun 25
51	Green Bud Initiation	Jun 26
58	Yellow Bud	Jul 03
60	Early Flowering	Jul 05
63	30% Flowering	Jul 08
65	Full Bloom (50% Flowering)	Jul 13
69	End of Flowering	Jul 21
75	50% Pod Development	Aug 02
81	Beginning of Ripening	Aug 18
89	Fully Ripe	Aug 28

Figure 7. Web browser view of Canola Decision Support tool risk assessment model result for a single field.

V. Sclerotinia stem rot checklist:

Points are added for risk factors including the number of years since the last canola crop, disease incidence in last host crop, crop density, rain in the last 2 weeks, weather forecast, and regional risk for apothecia development (Figure 8). The score indicates the probability of a positive economic return to a foliar fungicide application. A score of 30 to 35 would be the point at which producers would want to consider an application.

Sclerotinia Stem Rot Checklist

(For each risk factor, circle the risk points that apply to your field).

RISK FACTOR	POSSIBLE ANSWERS	RISK POINTS
NUMBER OF YEARS SINCE LAST CANOLA CROP	More than six years	0
	Three to six years	5
	One to two years	10
DISEASE INCIDENCE IN LAST HOST CROP	None	0
	Low (1 to 10%)	5
	Moderate (11 to 30%)	10
	High (31 to 100%)	15
CROP DENSITY	Low	0
	Normal	5
	High	10
RAIN IN THE LAST TWO WEEKS	Less than 10 mm (0.4")	0
	10 to 30 mm (0.4 to 1.2")	5
	More than 30 mm (1.2")	10
WEATHER FORECAST	High pressure	0
	Variable	10
	Low pressure	15
REGIONAL RISK FOR APOTHECIA DEVELOPMENT	None found	0
	Low numbers	10
	High numbers	15

TOTAL RISK POINTS FOR ALL RISK FACTORS =

Figure 8. Sclerotinia Stem Rot Checklist.

10. Results

Results of each of the tools are provided for each of the 12 fields at 20-30% flower (Table 1) and 50% flower (Table 2). At both timings, all of the tools generally predicted a low disease risk overall. A stem rot check list value over 35 indicates a more significant risk, and there were a few fields at this level. There was only one field assessed as moderate disease risk with the decision support tool. Petal test results from Quantum Genetix were all within the low risk category. Percent infection values from Discovery seed labs petal test were all in the lowest bracket in their calculator. Only one sample came back with trace levels for the Spornado samples. Results were very similar between the two timings.

Table 1. Sclerotinia risk assessment values for each field at optimum spray timing (20-30% flower) for each of the tools evaluated in the project.

Field	SSR checklist	canolaDST	Quantum Genetix % Positive	Discovery Seed Labs % Infected Petals	Spornado
Indian Head 1	30	Low	2.5	0	Not Detected
Indian Head 2	25	Moderate	5	0	Not Detected
Indian Head 3	20	Low	2.5	0.8	Not Detected
Melfort 1	35	Low	5	0	Not Detected
Melfort 2	25	Low	2.5	0	Not Detected
Melfort 3	30	Low	10	0	Not Detected
Redvers 1	40	Low	10	0.8	Not Detected
Redvers 2	40	Low	10	0	Not Detected
Redvers 3	45	Low	7.5	0	Not Detected
Scott 1	15	Low	7.5	4.8	Trace levels
Scott 2	15	Low	0	0	Not Detected
Scott 3	25	Low	12.5	1.6	Not Detected

Table 2. Sclerotinia risk assessment values for each field at late spray timing (50% flower) for each of the tools evaluated in the project.

Field	SSR checklist	canolaDST	Quantum Genetix % Positive	Discovery Seed Labs % Infected Petals	Spornado
Indian Head 1	35	Low	15	0.8	Not Detected
Indian Head 2	35	Moderate	0	0	Not Detected
Indian Head 3	30	Low	2.5	0	Not Detected
Melfort 1	35	Low	5	0	Not Detected
Melfort 2	25	Low	2.5	8.4	Not Detected
Melfort 3	30	Low	0	0	Not Detected
Redvers 1	40	Low	7.5	0	Not Detected
Redvers 2	40	Low	2.5	-	Not Detected
Redvers 3	45	Low	5	1.6	Not Detected
Scott 1	25	Low	5	0	Not Detected
Scott 2	15	Low	2.5	0	Not Detected
Scott 3	35	Low	5	0	Not Detected

Sclerotinia stem rot disease incidence was assessed in each field at 40-60% seed colour change. The proposed methodology included an assessment of how accurate each of the tools were at predicting disease incidence. There was zero sclerotinia found in any of the fields in the study, so it was not possible to correlate disease levels with any of the risk assessment data.

Yet, the second objective of the project was to assess the value of the tools in supporting producers with the decision to spray fungicide for sclerotinia management. Using either the petal tests or spore sampler, low to no spores detected would be a strong indicator of low disease pressure on its own, regardless of the results of the SSR Checklist or Decision Support Tool. At the same time, the stem rot checklist and decision support tool did sometimes show higher risk levels, which would make the decision process more uncertain, and so these tools would be less helpful on their own. In years where there would be a greater level of spores produced, then the checklist and decision support tool would likely be of greater value in combination with a lab tests showing the level of spores detected. One minor observation with the Decision Support Tool was that the predicted crop stage was not always accurate and could affect the risk assessment. For example, in Indian Head there was one

field with delayed emergence and so the predicted crop stage was more advanced than what was seen in the field at the time of flowering. Emergence date was an optional data entry point and may have corrected this issue.

The proposed methodology suggested a basic economic analysis using the information collected at each spray timing to predict potential yield loss, and comparing this to actual yield loss. Without any actual yield loss from sclerotinia stem rot in any of the fields, the only variable was the cost of a fungicide application compared to no fungicide application, and the cost of the tools themselves, which is minimal. The Q-protect kit from Quantum Genetix is $320, and the petal test kit from Discovery Seed Labs is $199, both include the lab tests. The Spornado Sampler initially costs $490, which includes 1 cassette and lab test. The sampler is a one-time cost but additional cassettes and lab tests are $120. There are also courier costs unless the samples can be dropped off at the labs.

In regards to the usefulness of the tests in helping producers with the decision to spray, the main observation from this project was the importance of timing. For planning and logistics reasons, especially with larger operations, the decision to spray must be made at least a few days or more before the date of fungicide application. Thus, if the crop is to be sprayed at the optimum timing of 20-30% flower, samples should be submitted and results obtained prior to the crop reaching this stage. Courier time is significant; depending on location, there may be an additional day required for samples to be received at the labs, and also couriers do not generally operate over the weekend.

In the end, it was difficult to determine how useful the tools were in helping producers make the decision to spray during the very dry conditions experienced in 2021. Many producers had already made the decision not to apply fungicide prior to receiving results.

Disease prediction models from digital agriculture providers would be an interesting addition to this project. Different providers use different data for their models, which can include a combination of regional weather data, remotely sensed and satellite data, and data from local in-field sensors like soil moisture and leaf wetness. The increase in the availability of data from farm-based weather stations could possibly increase the accuracy of these models but it would interesting to assess the various models either way.

Extension activities

The demonstration was highlighted at the SERF Field Tour on July 15, 2021, and on WARC's twitter account in July 2021. A summary of the demonstration was presented orally at the Agri-ARM Research Update on January 13, 2021. A recording of the presentation will be available on the Agri-ARM website.

11. Conclusions and Recommendations

Spore detection methods were all successful in predicting low risk of Sclerotinia development in 2021. In such a low disease pressure year, the spore detection methods would likely have been especially valuable in confirming that a fungicide application was not necessary.

The Decision Support Tool also mainly showed low disease risk, but the Sclerotinia stem rot checklist did show higher values in some cases. Without any knowledge of the level of spores detected, the probability of making the correct decision not to spray would be lower if using only the stemrot checklist. The Stem rot checklist appears to be more useful in combination with spore detection.

It would be beneficial to repeat the field demo to potentially capture higher disease risk and be able to re-assess the tools under higher disease pressure.

Supporting Information

12. Acknowledgements

This project was supported by the Agricultural Demonstration of Practices and Technologies (ADOPT) initiative under the Canadian Agricultural Partnership bi-lateral agreement between the Saskatchewan Ministry of Agriculture and the federal government. Discovery Seed Labs, Quantum Genetix, 20/20 Seed Labs, and Sporometrix provided in-kind support. The collaborative involvement of local producers in each location was important and appreciated.

13. Appendices

None.

Abstract

14. Abstract/Summary

Fungicide use decisions for Sclerotinia management rely on accurate and timely methods and data to predict the probability of disease development and yield loss. Tools that have been developed for Sclerotinia forecasting and risk analysis include the Spornado spore sampler, DNA-based petal testing, an online Decision Support Tool (CanolaDST.ca), and the Sclerotinia Stem Rot Checklist. The objective of this study was to demonstrate these tools under field production conditions and to assess their value in supporting producers with the decision to spray fungicide for Sclerotinia management. The tools were demonstrated in three fields at four locations (Indian Head, Melfort, Scott, and Redvers). There was no sclerotinia stem rot observed in any of the fields in 2021. Spore detection methods (Spornado and petal testing) were successful in predicting low risk of Sclerotinia development, and would be valuable in confirming that a fungicide application was not necessary. The Decision Support Tool also mainly showed low disease risk, but the Sclerotinia stem rot checklist indicated higher risk values in some cases. Without any knowledge of the level of spores detected, the probability of making the correct decision not to spray would be lower if using only the stemrot checklist. Timing was identified as an important aspect affecting the usefulness of the spore detection tools for spray decision support.

Finances

15. Expenditure Statement

Provided in attached spreadsheet.