Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A complete statement of expenses should be included. In the event of major changes within the budget, supporting notes should be included. The report should capture a complete summary of activity for the final year and an overview of the entire project.

Project Title: Verticillium Stripe - The Disease Management

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Sheau-Fang Hwang, Professor	University of Alberta	PI
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Stephen Strelkov, Professor	University of Alberta	Co-Investigator
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Project Start Date: April 1, 2019 **Project Completion Date:** March 31, 2024

Reporting Period: April 1, 2019 to March 31, 2024

CARP Project Number: 2019.34

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition, a Final Extension Report is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: ___March 31, 2024_____

_____ Ahead of Schedule _____ On Schedule __Behind Schedule _X____ Completed

Comments: The original scheduled end-date was March 31, 2023. The project has fallen slightly behind schedule due to the maternity leave of the graduate student. We are thankful for getting the approval of extending the project until March 2024.

2. Abstract/Summary - Maximum of one page. This must include project objectives, results, and conclusions for use on the Funders' websites. (refer to template)

Verticillium stripe (VS) is a potential threat to canola production in Canada, because microsclerotia can persist in the soil and spread readily on farm equipment and contaminated seed. The disease has resulted in devastating crop losses in Europe, where it is often known as Verticillium wilt. Based on a 2015 CFIA national survey, the pathogen (*Verticillium*) appears to occur throughout the Prairies and beyond. Widespread canola production in Canada has the potential to favor rapid increase of the disease, and the tight rotations practiced by western Canadian producers may allow for *Verticillium* inoculum to build up rapidly in the soil.

Verticillium infection results in leaf and stem chlorosis, wilting, early ripening and necrosis as the disease progresses. *Verticillium longisporium* (Stark) Kara., Bainbr., & Heale was first discovered in canola (*Brassica napus* L.) in Manitoba in 2014, and surveys in 2015 found the pathogen in Alberta, British Columbia, Saskatchewan, Manitoba, Ontario and Quebec. The first case of Verticillium wilt caused by *V. dahliae* Kleb. was confirmed in Alberta in 2016.

Both species of fungi are capable of producing disease symptoms such as early ripening, yellowing, stunting and wilting in canola (Fig. 1). Hot, dry conditions are favoured by both species. In general, the symptoms produced by *V. longisporium* are more severe. Dark stripes appear on the stems and necrosis occurs, followed by a shredded appearance in dead plant tissue. Very long-lived microsclerotia are produced in the stem cortex that can remain dormant for over a decade. *V. longisporium* is usually restricted to cruciferous hosts. Yield losses of up to 35% have been observed with a 60% disease incidence. In contrast, *V. dahliae* affects a broad range of hosts and attacks weeds and volunteer herbaceous plants. Little is known of the potential for yield losses under Canadian conditions.

The project is designed to mitigate the threat posed by VS to sustainable canola production through a variety of research activities. First, an evaluation of yield losses associated with VS is important to help farmers and industry predict the impact of this disease and determine whether or not control measures are warranted. Second, improved inoculation techniques can facilitate further research into VS, allowing researchers to evaluate control measures more efficiently and to more effectively screen genetic material for resistance to the pathogen. Third, knowledge of the resistance of canola cultivars to this disease aids in the identification of potential management strategies, including an assessment of genetic resistance as a potential management tool. Fourth, the observation of both verticillium stripe and blackleg in the same fields, and even on the same plants makes determination of how they interact a top priority.

This research has been an integrated and collaborative approach to addressing the major research priorities around the new disease, Verticillium stripe, in Canada. It includes four specific objectives:

1) Measure yield loss
 - The effects of inoculum density on disease intensity was evaluated.
 - A model to relate disease severity to yield loss was developed.
2) Disease development
 - The effects of inoculation at various growth stages of canola and using various techniques was determined.
3) Evaluation of canola genotypes for resistance to Verticillium stripe
4) Determination of the interacting effects of verticillium stripe and blackleg.

3. Introduction – Brief project background, rationale, and objectives.

Verticillium stripe, caused by the fungus *Verticillium longisporum*, was first found in canola in Canada in 2014. A subsequent survey in 2015 revealed that this disease is present in all provinces from Quebec to British Columbia, but is most frequently found in Manitoba (Canadian Food Inspection Agency 2018). There is much that is not known about this disease in Canada. Nonetheless, it has been present and studied for many years as "Verticillium wilt" in Europe (Karapapa et al. 1997), where it has been shown to cause significant yield loss in both spring and winter types of *Brassica napus*.

Based on information regarding the disease on winter oilseed rape production in Europe, it was determined that *V. longisporum* has significant potential to negatively impact the Canadian canola industry, both in terms of direct yield losses in the field as well as potential effects on market access. Yield reductions in winter oilseed rape appear to be highly dependent on the disease severity in infested fields (Dunker et al. 2008). Anecdotal reports of 50% yield losses or greater have appeared regarding fields in eastern Saskatchewan and Manitoba and yield losses of 10-50% have been reported in Sweden (Rimmer et al 2007). In Canadian canola production, there are no effective chemical control measures available and there is no information on resistance to the pathogen in commercial cultivars.

Verticillium stripe and wilt of canola are monocyclic diseases and inoculum production occurs over the course of the growing season. Disease symptoms include leaf chlorosis, premature leaf loss, internal stem discolouration, early ripening, stunting, and as the disease the progresses, it also causes necrosis, and shredding of the stem tissue. Dark unilateral striping develops on the main stem approximately 3-4 weeks prior to harvest; this is followed by the formation of fungal microsclerotia below the epidermis and in the stem pith. The stem of the infected plants peels off to reveal these tiny black pepper-like microsclerotia. Incorporation of the long-lived microsclerotia into the soil completes the disease cycle (Heale and Karapapa 1999; Depotter et al. 2016).

This project is an integrated and collaborative approach to addressing the major research priorities around the new disease, Verticillium stripe, in Canada.

Project objectives:

1. Determine if there is yield loss and extent of yield losses from Verticillium stripe.
2. Determine the effects of growth stage and inoculation techniques on infection.
3. Evaluation of brassica genotypes against Verticillium stripe.
4. Determine interacting effects of Verticillium stripe and blackleg

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

1) Measurement of yield loss:

- Effects of inoculum density of Verticillium on disease severity and seed yield:

Grain inoculum of *V. longisporum* was produced on wheat kernels. The wheat kernels were soaked in tap water for 24 h and then autoclaved in mushroom spawn bags on consecutive days for 2 h at 121°C. Mycelial plugs from 3 to 4-week-old PDA cultures were used to inoculate the wheat kernels, which were incubated in the dark at room temperature for 4 weeks at room temperatures and were shaken periodically to ensure complete colonization of the grain. After incubation, the infested grain was air dried, then ground through a 1-mm mesh screen. The dry, milled inoculum was mixed with sand prior to seeding. Treatments were replicated four times and arranged in a randomized split plot design. The canola cultivars '45H31' and 'CS 2000' were served as main-plots and five different inoculum densities (0 mL, 50 mL, 100 mL, 150 mL, and 200 mL per 6-m row) were served as sub-plots. Two field trials were conducted (Location A and B) near Edmonton in 2020 and 2021. At the end of the growing season, At the end of September, 10 plants in each plot were randomly uprooted and bagged. Each plant was examined for verticillium stripe symptoms on the main stem and the amount of microsclerotia colonization was assessed **(Table 1, Fig. 1),** and plant height was measured. The plots were harvested to collect seed yield data.

Table 1. Verticillium stripe disease severity scale

Rating	Symptoms
0	None
1	Stunting and senescence; discolouration with dark unilateral stripe on main stem
2	Less than 25% of microsclerotia colonized on stem cortex; stem discolouration
3	Up to 75% of main stem colonized by microsclerotia; stem epidermis shredded; pod loss; plant lodging
4	Entirely necrotic with microsclerotia present; stem epidermis shredded; pod loss; plant lodging

Fig. 1. Scale of disease rating for verticillium stripe with a pictorial representation below.

- A model to relate disease severity to yield loss:
Regression analysis was performed to estimate the yield loss resulting per unit increase of disease severity.

2) Disease development:
- The effects of inoculation at various growth stages of canola:
Canola was seeded every week over four weeks into 450-mL cups (2 seeds/cup) filled with soil-less potting mixture to generate plants of different ages. A virulent isolate of Verticillium was used to inoculate the plants using the optimum method determined below and at a density based on the results of the above study. The plants were assessed for symptom development and rated for disease severity as described previously.
- The effect of inoculation techniques on Verticillium stripe development.
Canola seedlings were germinated in Petri dishes or in sand and inoculated by dipping the roots into a spore suspension of Verticillium, inoculated with microsclerotia, or placing an agar plug of the fungus in contact with the lower stem. The inoculated seedlings were grown to maturity, evaluated and plant growth will be compared with a non-inoculated control.
3) Evaluation of canola genotypes for Verticillium stripe resistance:
Canola cultivars were used to screen against Verticillium stripe using the inoculation technique developed above. The single-spore isolate VL43 of *V. longisporum* was grown on Petri dishes (9-cm diameter) filled with potato dextrose agar (PDA). Cultures were incubated under darkness in room temperature for 28 days before harvesting conidial suspension. Ten milliliters of sterile distill water was added to each Petri dish and a sterile inoculating loop was used to gently dislodging the spores. The spore suspension was filtered through four layers of sterile cheesecloth to remove mycelial fragments. The spore concentration was estimated using a haemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA) and adjusted to 1×10^6 spores mL^{-1} with sterile distilled water. The plants were grown in 25-cm diameter pots. The experiments were arranged in a split-plot design which were replicated four times with inoculation or non-inoculation as

the main plots and canola varieties as the sub-plots. The soils/roots were inoculated with spore suspension/microsclerotia. Seedling emergence, plant height, internal discoloration, disease symptoms and disease severity were recorded.

4A) Field experiments for blackleg and Verticillium stripe interactions:

Field trials to evaluate the effect of blackleg/Verticillium stripe interactions on canola yield were conducted over 2 years at two sites located at the Crop Diversification Centre-North. The canola hybrids '45H31'and 'CS2000'were included in the experiments, which were arranged in a split-plot design with four replicates. Each plot consisted of four rows, 6 m in length and 1.5 m in width with 0.25-m spacing between the rows. Adjacent plots were separated by a 1 m buffer zone, with 2 m between replicates. Each row was seeded with 0.7 g of canola as described above. Grain inoculum of *L. maculans* and *V. longisporum* was applied at various ratios in the different treatments: *L. maculans* grain inoculum applied at 200 mL/row; *V. longisporum* grain inoculum at 200 mL/row; a 3:1 mix of *L. maculans* (150 mL/row) and *V. longisporum* (50 mL/row); a 1:1 mix of *L. maculans* (100 mL/row) and *V. longisporum* (100 mL/row); and a 1:3 mix of *L. maculans* (50 mL/row) and *V. longisporum* (150 mL/row) inoculum. Control treatments did not receive any inoculum. The grain inoculum was applied at seeding time by placing it in the seeder with the seed. Experiments were seeded on 17 May 2020 and 18 May 2021.

To assess the impact of blackleg and Verticillium stripe interactions, 15 plants from each plot were carefully collected with a shovel and placed in paper bags. Plant samples were visually examined for the presence of pycnidia of *L. maculans* and microsclerotia of *V. longisporum*. Horizontal and vertical sections of the stems were made with a bypass pruner for comparison of blackleg and Verticillium stripe symptoms. The remainder of each plot was harvested with a small plot combine using a straight cut header, and the seed was weighed to determine overall yield. The percentage yield reduction was calculated relative to non-inoculated controls. Experiments were harvested at maturity on 13 Oct. 2020 and 22 Sept. 2021. The plants were rated for blackleg severity on a 0–5 scale and for Verticillium stripe severity on a 0-4 rating scale.

4B) Greenhouse experiments for blackleg and Verticillium stripe interactions:

Greenhouse experiments were conducted with the canola cultivars '45H31' and 'CS2000'. The experiments were arranged in a split-plot design with four replicates using plastic containers (40.9 × 28.2 × 15.0 cm) filled with Sunshine® mix #4 potting medium. Two rows were seeded per container, at a rate of 20 seeds per row, and grain inoculum was placed along with the seeds. Inoculation treatments included: *L. maculans* grain inoculum applied at 20 mL/row; V. longisporum grain inoculum applied at 20 mL/row; a 3:1 mix of *L. maculans* (15 mL/row) and *V. longisporum* (5 mL/row); a 1:1 mix of *L. maculans* (10 mL/row) and *V. longisporum* (10 mL/row); and a 1:3 mix of *L. maculans* (5 mL/row) and *V. longisporum* (15 mL/row). Control treatments were not inoculated. The experiment was repeated. All the plants in each container were rated for blackleg severity on a 0–5 scale and Verticillium stripe severity on a 0-4 scale as described below. Horizontal and vertical sections were made for identification of blackleg and Verticillium stripe. Emergence counts were taken 14 days after seeding. Seed yields were weighed and recorded.

4C) Disease assessments:

Plants were rated for blackleg severity on a 0-5 scale, where: 0 = no infection; 1 = lesion area < 25% of the cross-section area of the crown; 2 = lesion area 25-50% of the cross-section area of the crown; 3 = lesion area 51-75% of the cross-section area crown; 4 = lesion area 76-100% of the cross-section area of the crown; and 5 = plant dead. Verticillium stripe severity was assessed as described above.

4D) Statistical analysis:

Statistical analysis was conducted using R: A Language and Environment for Statistical Computing (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2013). To establish the relationship between blackleg severity and pod number and seed yield, regression analysis was performed. The Akaike information and Bayesian information criteria were used for selection of the best model for the data. Adjusted R2 values and the F test were used to examine compatibility of the regression. Residual data were tested for normality with the Shapiro–Wilk test in R shapiro.test stats. Regression equations were generated to evaluate the losses in pod number and seed yield with increasing disease severity. The yield of plants with no blackleg symptoms was used as a point estimate, with different yield data points at each disease se-verity transformed into yield percentages relative to canola yield with no disease. Regression analysis was performed to estimate yield loss percentage per unit increase in disease severity. To examine blackleg and

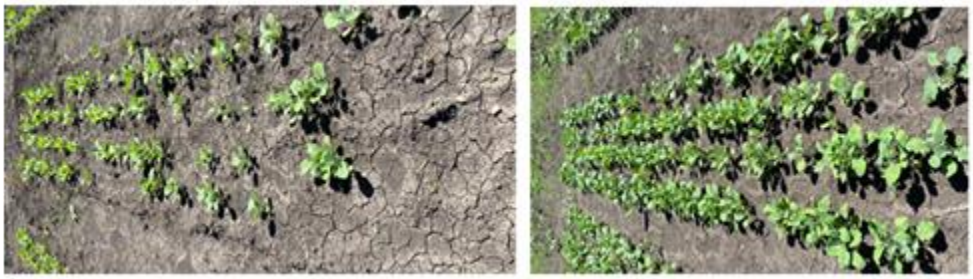
Verticillium stripe interactions, canola hybrid was considered as a fixed effect, and replication and site-year and their interaction as random effects. Analysis of variance was performed. Least significant difference comparisons were used to determine whether disease severity and seed yield differed among concentrations.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

1) Measurement of yield loss:

In 2020, the germination rates were much lower in the inoculated plots than in the non-inoculated control at both sites four weeks after seeding. Half-sided yellowing of leaves was observed in the field at both sites in early July as an early symptom of Verticillium stripe **(Fig. 2)**. Symptoms and signs on the mature canola plants included discolouration and shredding of the stem, the presence of microsclerotia, and necrosis. No symptoms of Verticillium stripe were observed at maturity on plants in the non-inoculated plots at either site (and hence they received ratings of 0). In general, mean disease severity increased as the inoculum level increased for both cultivars at both sites.

Fig. 2. Seedling germination in Verticillium-inoculated plots (left) and non-inoculated plots (right).



At Site 1, the severity of Verticillium stripe in all of the inoculated plots was significantly greater than in the non-inoculated plots for both canola hybrids **(Table 2)**. However, at Site 2, there was no significant difference in disease severity between the non-inoculated control and the low inoculum plots for either hybrid **(Table 2)**. The most severe Verticillium stripe on 'CS2000' (mean ratings = 2.0-2.5) and '45H31' (1.6-2.0) at Site 1 developed in the medium-low, medium, and high inoculum treatments. Likewise, at Site 2, the most severe Verticillium stripe was also observed in the medium-low, medium, and high inoculum treatments (mean ratings = 0.8-1.7 on '45H31' and 1.4-2.1 on 'CS2000'). These values were significantly greater than observed in the low inoculum treatments **(Table 2)**. Disease was generally more severe at Site 1 vs. Site 2. A trend of decreasing single plant yield with increasing inoculum was observed at Site 1 in 2020, particularly for 'CS2000'. For this hybrid, the mean single plant yield in the non-inoculated control treatment at Site 1 was 6.5 g, significantly greater than the yields obtained in the low (2.6 g), medium-low (2.3 g), medium (2.1 g), and high inoculum treatments (2.4 g) **(Table 2)**. At Site 2 in 2020, there was no significant difference in mean seed yield per plant between treatments for either canola hybrid. Significant reductions in total plot yields were not detected at either site, even in the highest inoculum treatments **(Table 2)**.

Due to the lack of precipitation throughout the entire growing season in 2021 **(Fig. 3)**, no visible early-season symptoms were observed at either site. Disease severity at maturity on both hybrids remained mild. Additionally, weak symptoms of Verticillium stripe (mean rating = 0.1) were noted in the non-inoculated plots of '45H31'. No significant ($p \leq 0.05$) differences in disease severity were detected in either hybrid, although the numerically highest mean disease severities were found in the medium-low inoculum treatment for both '45H31' (0.4) and 'CS2000' (0.3) **(Table 2)**.

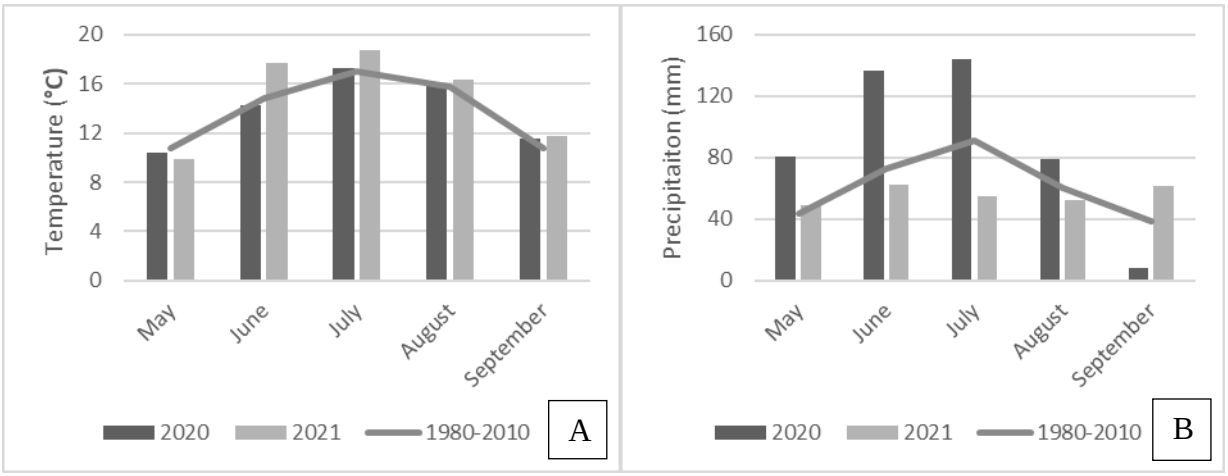


Fig. 3. Average monthly precipitation (A) and temperature (B) in 2020 and 2021 vs. the 30-year average (1980-2010) at the St. Albert Research Station, University of Alberta (A).

Regression analysis indicated that the relationships between disease severity and seed yield per plant at the two sites were best described by quadratic equations **(Fig. 4A, B)**. In the case of hybrid '45H31', the regression model was $y = 3.5 - 0.33x - 0.12x^2$, with the expected average seed yield ranging from 0.26 g to 3.5 g per plant at Site 1 **(Fig. 4A)**. At Site 2, the expected average seed yield ranged from 0.792 g to 1.8 g per plant with a regression model $y = 1.8 + 0.052x - 0.076x^2$ **(Fig. 4B)**. In the case of the hybrid 'CS2000', the regression model at Site 1 was $y = 4.6 - 1.3x + 0.08x^2$ and the expected average seed yield ranged from 0.68 g to 4.6 g per plant. At Site 2, the regression model for this hybrid was $y = 2.7 - 0.023x - 0.14x^2$ with the expected average seed yield ranging from 0.368 g to 2.7 g per plant **(Fig. 4A, B)**.

In 2021, no significant differences in mean seed yield per plant were noted between inoculum treatments with the exception of the non-inoculated control (6.2 g) vs. the high inoculum treatment (13.2 g) for 'CS2000' **(Table 2)**. The mean seed yield per plant was 6.0 g and 6.2 g for '45H31' and 'CS2000', respectively, in the non-inoculated controls. This was not significantly ($p \leq 0.05$) different from the seed yields per plant for these hybrids in the low ('45H31' = 7.5 g, 'CS2000' = 8.0 g), medium-low (8.9 g, 8.7 g), medium (9.1 g, 9.6 g), or high inoculum treatments (9.3 g, 13.2 g) **(Table 2)**. No trends were observed for total plot yield for either of the hybrids, and any differences between treatments were not significant **(Table 2)**.

As was found in 2020, the relationships between disease severity and seed yield per plant in 2021 were also best explained by quadratic equations. For hybrid '45H31' at Site 1, the regression model was $y = 10 - 7.1x + 1.3x2$, with the expected average seed yield ranging from 0.4 g to 10 g per plant. At Site 2, the expected average seed yield ranged from 0.8 g to 7.4 g per plant with a regression model of $y = 7.4 - 6.7x + 1.5x2$ (**Fig. 5A, B)**. In the case of 'CS2000' at Site 1, the regression model was $y = 9.6 - 5.9x + 0.93x2$ and the expected average seed yield ranged from 0.27 g to 9.6 g per plant. At Site 2 for this hybrid, the regression model was $y = 10 - 8.6x + 1.8x2$ with the expected average seed yield ranging from 0.4 g to 10 g per plant **(Fig. 5 A, B)**.

In 2021, the regression models for percentage yield loss per plant vs. disease severity were $y = 1.2 + 68x - 13x2$ and $y = 2.3 + 89x - 20x2$ for '45H31' at Sites 1 and 2, respectively **(Fig.5 C, D)**. For 'CS2000', the models were $y = -0.3 + 61x - 9.5x2$ at Site 1 and $y = 1.5 + 85x - 18x2$ at Site 2 **(Fig. 5 C, D)**. Both hybrids showed yield losses exceeding 50% at disease severities ≥ 1 at both sites in 2021. At Site 2, plants with a disease severity of 2 showed a greater percentage of seed yield loss than those with a severity rating of 3 for both hybrids **(Fig. 5D)**.

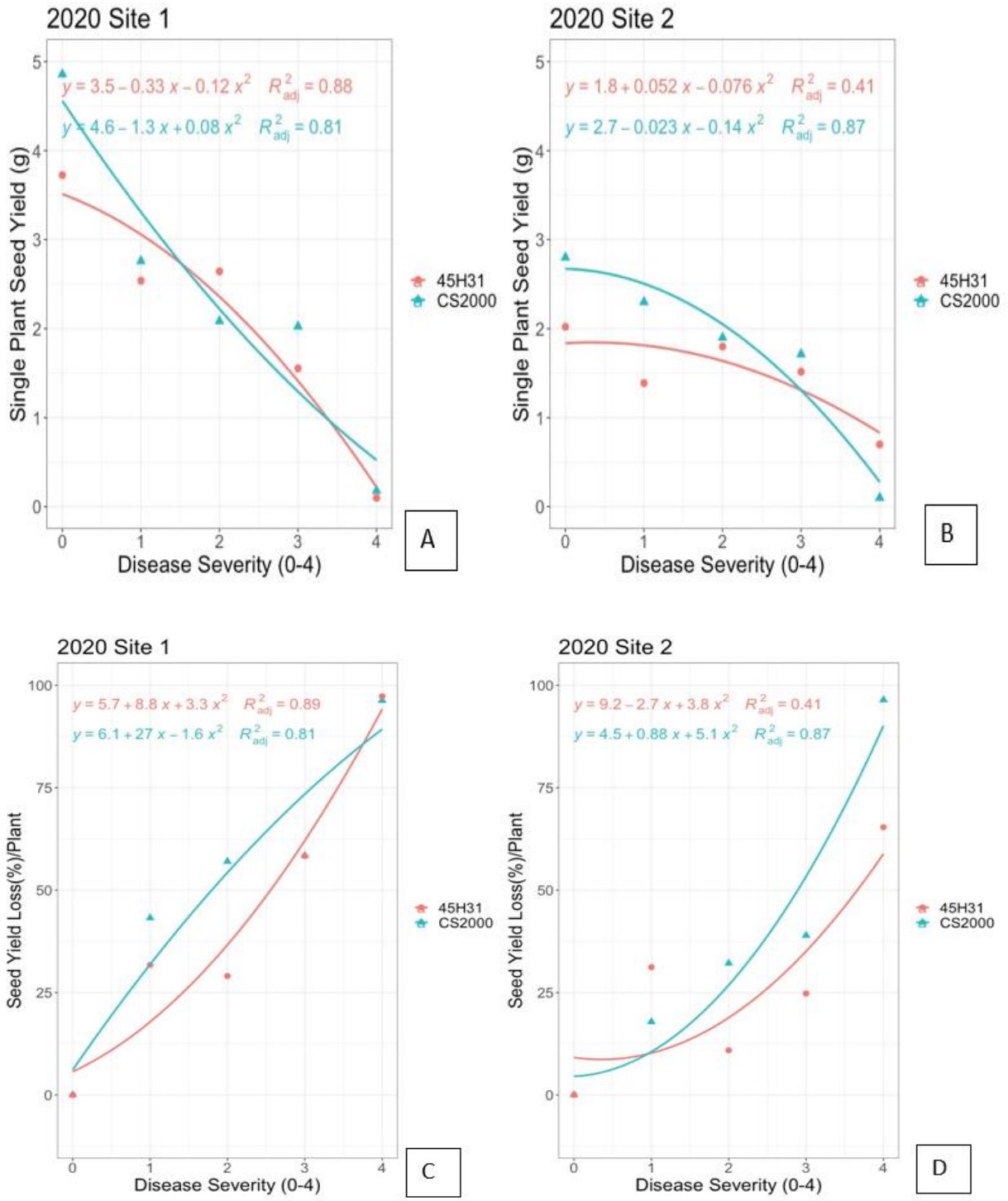


Fig. 4. Relationship between Verticillium stripe severity and single plant seed yield (A, B) and yield loss (C, D) in the canola hybrids '45H31' and 'CS2000' under field conditions at the St. Albert Research Station, University of Alberta, in 2020. Each point represents the mean of four (A, C) or three (B, D) replicates. Verticillium stripe severity was assessed on a 0–4 scale. The yield loss data were estimated using the y-intercept in the equation averaged over the replicates. The data points were transformed into a percentage of the maximum yield in (C, D).

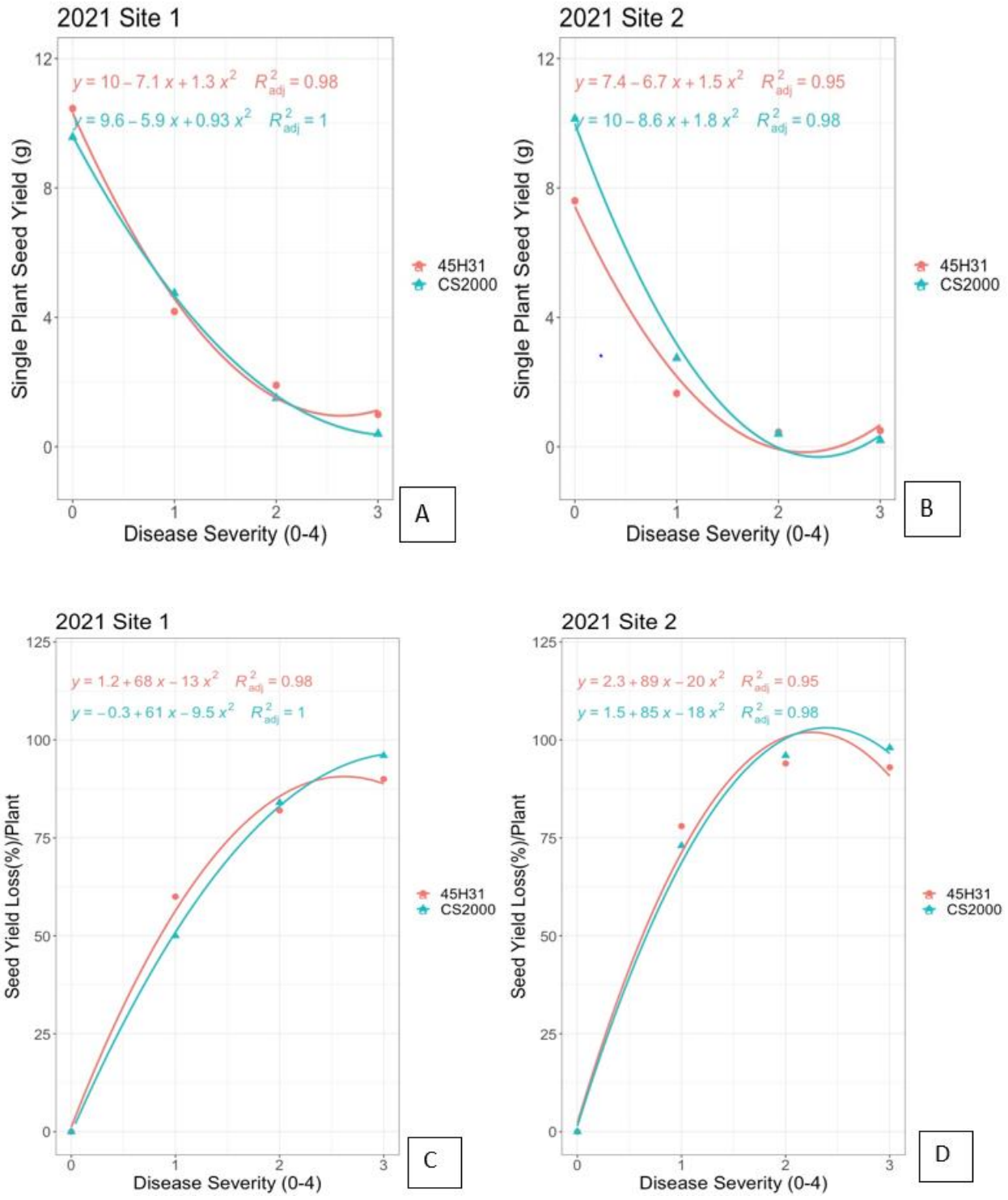


Fig. 5. Relationship between Verticillium stripe severity and single plant seed yield (A, B) and yield loss (C, D) in the canola hybrids '45H31' and 'CS2000' under field conditions at the St. Albert Research Station, University of Alberta, in 2021. Each point represents the mean of four (A, C) or three (B, D) replicates. Verticillium stripe severity was assessed on a 0–4 scale. The yield loss data were estimated using the y-intercept in the equation averaged over the replicates. The data points were transformed into a percentage of the maximum yield in (C, D).

Table 2. Mean Verticillium stripe severity, mean single plant yield, and mean plot yield of the canola hybrids '45H31' and 'CS2000' in field experiments with different quantities of *Verticillium longisporum* inoculum in 2020 and 2021.

Hybrid	Treatment	Disease Severity		Mean single plant yield (g)		Mean plot yield (g)	
		2020	2021	2020	2021	2020	2021
	Control	0.0 A	0.1 a	4.2 AB	6.0 a	752 A	387 a
	Low	0.3 AB	0.3 a	2.8 ABC	7.5 a	637 A	320 a
'45H31'	Medium-Low	0.7 ABC	0.4 a	2.1 BC	8.9 a	576 A	339 a
	Medium	1.1 BCD	0.1 a	1.7 C	9.1 ab	596 A	408 a
	High	1.4 CD	0.1 a	1.8 C	9.3 ab	646 A	398 a
	Control	0.0 A	0.0 a	4.7 A	6.2 a	564 A	406 a
	Low	0.8 ABCD	0.0 a	2.4 ABC	8.0 a	797 A	360 a
'CS2000'	Medium-Low	1.3 BCD	0.3 a	2.2 BC	8.7 a	782 A	454 a
	Medium	1.9 D	0.1 a	1.6 C	9.6 ab	662 A	353 a
	High	1.5 CD	0.1 a	2.3 BC	13.2 b	690 A	485 a

Note: Field plots were located at two sites in the St. Albert Research Station, University of Alberta. Treatments refer to the relative amount of *V. longisporum* grain inoculum applied to the plots. Verticillium stripe severity was assessed on a 0–4 scale. Means in a column followed by the same letter do not differ based on the LSD at $p \leq 0.05$.

2. Effects of growth stage and inoculation techniques on infection:

Verticillium longisporum was grown on potato dextrose agar plates at room temperature for 14 days and the colonies were used for inoculum preparation. A conidial suspension was prepared by adding 10 mL of sterile distilled water to the plate, rubbing a glass rod over the colony and filtering the suspension through a 4-layer sterile cheesecloth.

Seeds of the canola cultivars 'Westar' and 'CS2000' were sown in Petri plates. Roots of 1, 2 and 3-week-old seedlings were inoculated by dipping into a spore suspension of $1x10^7$ spores/mL for 45 min. The seedlings were transplanted into 450-mL cups filled with soil mixture, four plants per cup, 15 replicate cups for each cultivar and each seedling stage. Early stage disease severity was recorded at 7 and 10 DPI and a disease severity rating at harvest was based on 10 randomly selected plants for each treatment. Plant heights were recorded at 14 DPI, mortality was recorded at 21 and 35 DPI and the plants were harvested at 98 DPI and the yield was determined by weighing total seeds harvested from each plant.

Symptoms increased and plant height decreased with age at which the seedlings were inoculated **(Fig. 6).** Plant mortality and reduction in yield were greater for plants inoculated at 3 wk that at 1 wk. This may have been due to greater transplant shock for older seedlings or greater total root area exposed to inoculum at 3 wk vs. 1 wk. Stunting of plants and leaf yellowing were observed in five-week-old 'Westar' inoculated with VL at both one and two weeks after seeding. Higher seedling stage DS was observed in 'Westar' inoculated at two weeks after seeding compared with 'Westar' inoculated at one week after seeding. For 'Westar' inoculated at three weeks after seeding, Verticillium stripe symptoms were present at 28 DPI. Stems were shredded and cracked and microsclerotia colonized the stem cortex. Necrotic plants were observed. Plant height was significantly reduced as inoculated at later growth stage.

For both cultivars, the greatest DS occurred in plants inoculated at three weeks after seeding. However, there was no significant difference in DS between inoculation at one week after seeding and at two weeks after seeding. For 'CS2000', DS was 1, 1.2, and 2.1 for plants inoculated at one, two, and three weeks after seeding, respectively. For 'Westar', DS was 2.9, 2.6, and 3.1 for plants inoculated at one, two, and three weeks after seeding, respectively. Seed yield declined significantly with later infection date in 'CS2000', especially for plants inoculated at two or three weeks after seeding compared with non-inoculated control. Seed yield reduced by 27% and for inoculation at three weeks after seeding compared with inoculation at one week after seeding. However, there was no significant difference in seed yield reduction among plants inoculated at one, two, and three weeks after seeding in 'Westar' even though a significant decline in seed yield was observed between non-inoculated and inoculated plants.

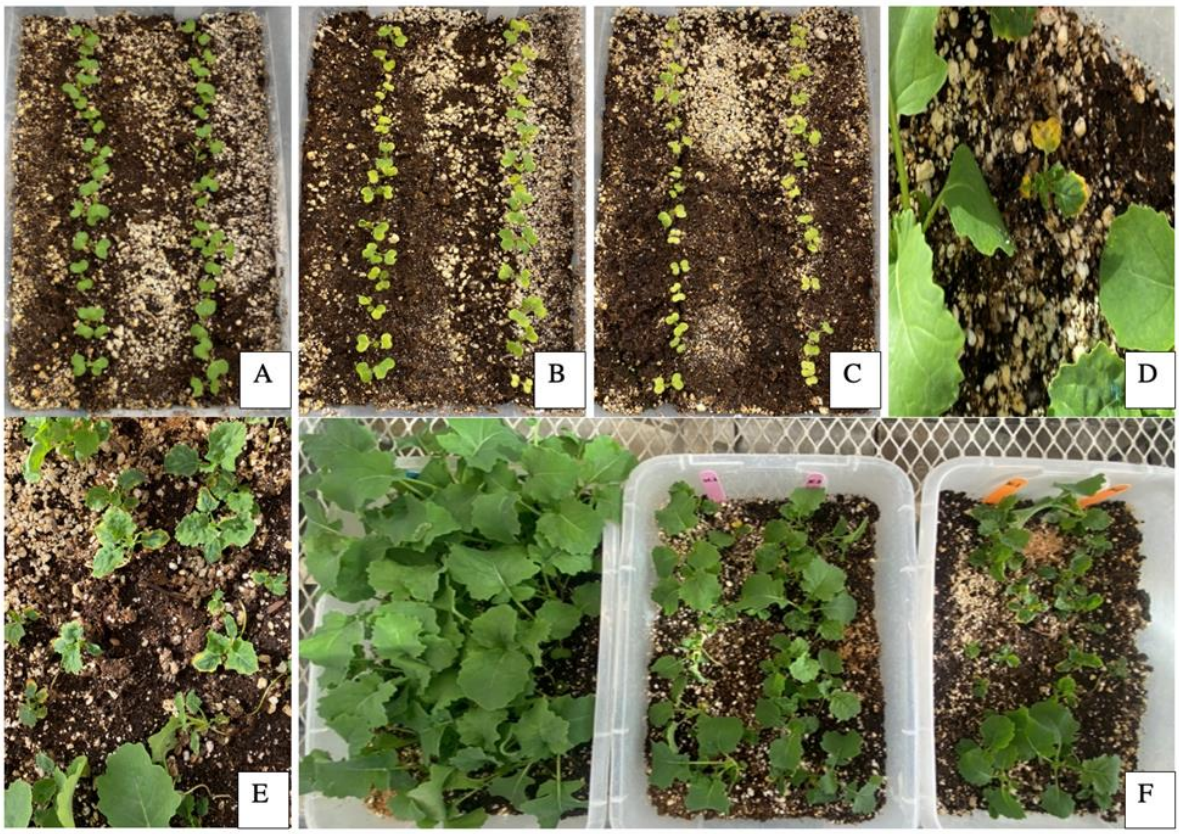


Fig. 6. Symptoms of infection by Verticillium longisporum on canola at the seedling stage inoculated by the root-dip method. (A). Non-inoculated control 7 days post-inoculation (DPI). (B). Appearance of seedlings at 7 DPI with a low concentration of inoculum. (C). Appearance of seedlings at 7 DPI with a high concentration of inoculum. (D, E) Close-ups showing yellowing of true leaves and plant stunting at 14 DPI. (F). Appearance, from left to right, of the non-inoculated control, low inoculum treatment and high inoculum treatment at 21 DPI.

3) Evaluation of canola genotypes for resistance to Verticillium stripe

Inoculum was produced as described in the field study. The experiment was carried out in a split block design. 'Westar', '45H31', 'CS2000', 'D3155C', '74-47BL' and '45H29' were sown in 470-mL cups, initially filled with sunshine mix soil, then 50 mL of infested grain-sand inoculum was added on the top of the soil prior to seeding. The ratios of grain: sand was adjusted to 1:10, 1:5, 2:3 and 4:1 (vol:vol). The soil mixture treated with non-infested grain-sand inoculum (1:5 vol:vol) was used as the control. Six seeds were sown directly in grain-sand inoculum per beer cup followed by the addition of 15 mL Sunshine mix on top of the grain-sand inoculum. Five cups were assigned to each treatment. Germination rate was measured 12 days after planting, while plant height was evaluated on 19 days after planting. Disease severity will be assessed later. Shoot weight and root length/weight are yet to be evaluated.

Twelve days after planting, seeds planted in grain-sand ratios of 2:3 and 4:1 showed no emergence. Thus, data were not collected for these two treatments. Germination rate was evaluated on 12 days after planting **(Figure 7)**. *V. longisporum* inhibited seed germination with 'Westar' most severely affected and '74-47BL' least affected. This fungus also affected plant height significantly in all cultivars except 'Westar'. Disease symptoms at the early growth stage will be examined 28 days after planting.

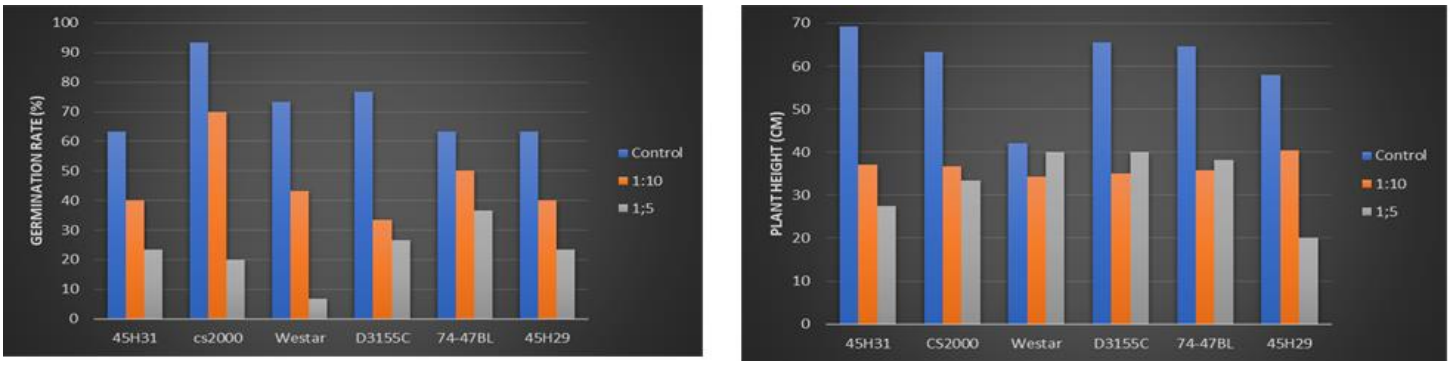


Fig. 7. Germination rate and plant height (19 days after planting) of six canola cultivars inoculated with three concentrations of *V. longisporum* inoculum.

4) Field experiments for blackleg and Verticillium stripe interactions

Mean blackleg disease severity ranged from 0.1 to 1.6 on '45H31', and from 0.0 to 1.3 on 'CS2000', at the two sites over two years **(Table 3).** On '45H31' in 2020, the most severe blackleg (1.3-1.6) at site 1 was observed in treatments inoculated with V. longisporum alone or with a 3:1 or 1:1 mix of *L. maculans* and *V. longisporum*; in the *L. maculans* alone treatment, the blackleg severity (1.0) was significantly lower than in the 1:1 mix of pathogens. At site 2 in 2020, the most severe blackleg (1.2-1.5) on '45H31' developed following inoculation with the 3:1 and 1:1 mixes of *L. maculans* and *V. longisporum*, while the lowest disease (0.1) was observed on the control. The L. maculans alone and 1:3 mix of *L. maculans* and *V. longisporum*, and *V. longisporum* alone treatments developed intermediate blackleg severities (0.7-1.0). On 'CS2000' in 2020, all inoculated treatments developed blackleg severities ranging from 0.8 to 1.3 at the two sites, which was significantly greater than the severity (0.1) on the non-inoculated control **(Table 3).** In 2021 at site 1, the most severe blackleg (1.5) on '45H31' developed on the *L. maculans* alone treatment, and the mildest blackleg was observed on the control (0.5) and 1:3 mix of *L. maculans* and *V. longisporum* (0.3) and *V. longisporum* alone (0.7) treatment; the disease severity on the other treatments was intermediate (Table 2). Similar trends were observed for '45H31' at site 2 and 'CS2000' at sites 1 and 2 in 2021; the most severe blackleg developed on the *L. maculans* alone treatment, on which dis-ease was generally higher than most other treatments, although the mildest symptoms did not always occur on the non-inoculated control **(Table 3).**

The mean Verticillium stripe severity ranged from 0.0 to 2.2 on the hybrid '45H31' and from 0.0 to 2.0 on 'CS2000' at the two sites over two years **(Table 4).** At site 1 in 2020, the numerically most severe Verticillium stripe (0.5) on '45H31' was observed in the V. longisporum alone treatment, although this was not significantly greater than the disease (0.4) that developed following inoculation with the 3:1 and 1:1 mixes of the pathogens. However, Verticillium stripe on the V. longisporum alone treatment was significantly more severe than on the control (0.0), 1:3 mix of *L. maculans* and *V. longisporum* (0.2), and L. maculans alone (0.3) treatments for '45H31' at site 1 in 2020. At site 2 in 2020, there were no significant differences in Verticillium stripe severity on this hybrid **(Table 4).** In the case of 'CS2000' at both sites in 2020, the most severe Verticillium stripe (0.6-0.7) was observed in treatments inoculated with *V. longisporum*, while the mildest disease was found on the control (0.1-0.2) and *L. maculans* alone (0.2-0.3) treatments **(Table 4).**

In 2021 on '45H31' at site 1, the most severe Verticillium stripe (1.0-1.2) was observed on treatments inoculated with 3:1, 1:1, 1:3 mixes of *L. maculans* and *V. longisporum*, as well as on the *V. longisporum* alone treatment at site 1. The no inoculum control (0.5) and L. maculans alone (1.1) treatments were significant different from other treatments. While the L. maculans alone treatment had a high numerical value (1.1) relative to all other inoculated treatments, the low standard devia-tion resulted in significant differences. At site 2 in 2021, the most severe Verticillium stripe was observed on the 1:3 mix of L. maculans and V. longisporum (1.8) and V. long-isporum alone (1.9) treatments. The mildest Verticillium stripe was observed on the control (0.6) and *L. maculans* alone (1.1) treatments (Table 3). In the case of 'CS2000', the most severe Verticillium stripe was observed on the 1:3 mix of *L. maculans* and *V. longisporum* (1.2) and the *V. longisporum* alone (1.6) treatments. The mildest Verticillium stripe was observed on the control (0.2) and *L. maculans* alone (0.4) treatments at site 1 in 2021. At site 2, the control (0.0) and L. maculans alone (0.2) treatments had the lowest Verticillium stripe and significant different from other treatments (1.5-2.0) **(Table 4).**

The mean seed yield was similar in the two hybrids, ranging from 0.9 to 2.5 t/ha on '45H31' and from 0.9 to 2.8 t/ha on

'CS2000', and was significantly greater in 2021 than in 2020 **(Figure 8a and 8b).** However, the mean seed yield was not significantly different among treatments for either hybrid in either year.

Table 3. Blackleg severity (0-5) on the canola hybrids '45H31' and 'CS2000' following inoculation with Leptosphaeria maculans (Lm) and/or Verticillium longisporum (Vl) alone and in various combinations under field conditions.

Treatment[1]	2020				2021			
	Site 1		Site 2		Site 1		Site 2	
	'45H31'	CS2000	45H31	CS2000	45H31	CS2000	45H31	CS2000
Control	0.4d [2]	0.2B	0.1b	0.1B	0.5c	0.2C	0.6bc	0.0C
Lm alone	1.0bcd	0.9A	0.7ab	0.8A	1.5a	1.1A	1.0a	1.2A
3 Lm: 1 Vl	1.3abc	0.9A	1.2a	1.0A	1.0ab	0.6B	0.4bc	0.4BC
1 Lm: 1 Vl	1.6a	1.0A	1.5a	0.8A	0.6ab	0.6BC	0.3b	0.6AB
1 Lm: 3 Vl	1.1cd	0.9A	0.9ab	0.9A	0.3bc	0.5B	0.4bc	0.3BC
Vl alone	1.5ab	1.3A	1.0ab	1.2A	0.7c	0.3BC	0.6c	0.4BC

[1] Lm alone = Lm applied at 200 mL inoculum/row; 3 Lm: 1 Vl = 3:1 mix of Lm (150 mL/row) and Vl (50 mL/row); 1 Lm: 1 Vl = 1:1 mix of Lm (100 mL/row) and Vl (100 mL/row); 1 Lm: 3 Vl = 1:3 mix of Lm (50 mL/row) and Vl (150 mL/row); Vl alone = Vl applied at 200 mL/row inoculum.

[2] Data were collected over four site-years in Edmonton, AB, Canada, and are the means of four replications. Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test.

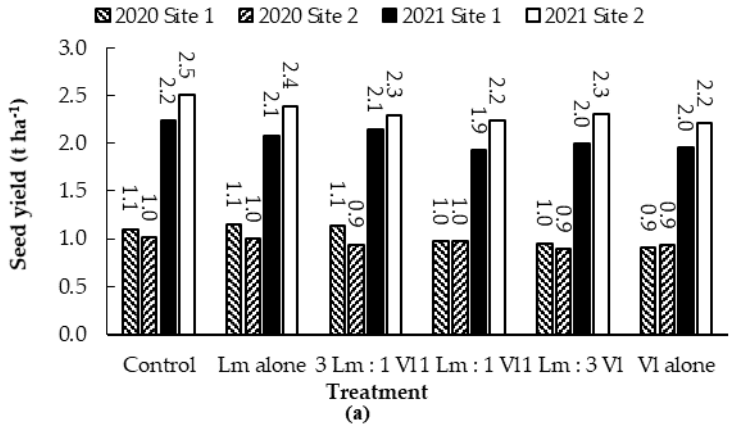
Table 4. Verticillium stripe severity (0-4) on the canola hybrids '45H31' and 'CS2000' following inoculation with Leptosphaeria maculans (Lm) and/or Verticillium longisporum (Vl) alone and in various combinations under field conditions.

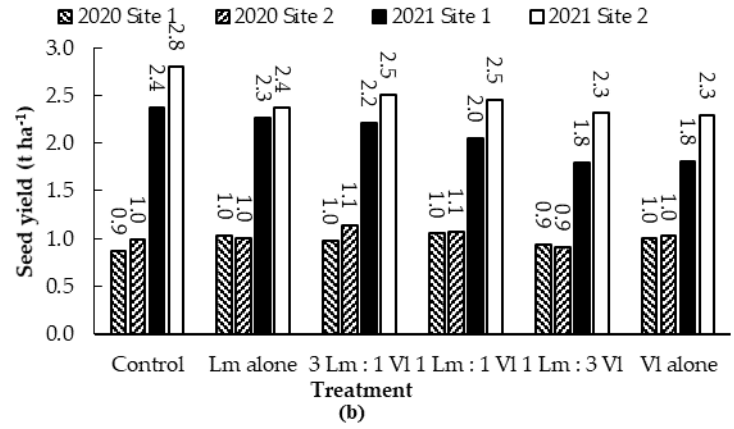
Treatment[1]	2020				2021			
	Site 1		Site 2		Site 1		Site 2	
	'45H31'	'CS2000'	'45H31'	'CS2000'	'45H31'	'CS2000'	'45H31'	'CS2000'
Control	0.0c [2]	0.1C	0.0a	0.2B	0.5b	0.2C	0.6c	0.0B
Lm alone	0.3b	0.3BC	0.0a	0.2B	1.1a	0.4C	1.1c	0.2B
3 Lm: 1 Vl	0.4ab	0.3ABC	0.4a	0.3AB	1.0a	0.5BC	2.2b	1.5A
1 Lm: 1 Vl	0.4ab	0.6AB	0.2a	0.2AB	1.0a	0.8ABC	1.7b	1.8A
1 Lm: 3 Vl	0.2bc	0.5ABC	0.2a	0.2AB	1.1a	1.2AB	1.8a	1.6A
Vl alone	0.5a	0.7A	0.3a	0.6A	1.2a	1.6A	1.9a	2.0A

[1] Lm alone = Lm applied at 200 mL inoculum/row; 3 Lm: 1 Vl = 3:1 mix of Lm (150 mL/row) and Vl (50 mL/row); 1 Lm: 1 Vl = 1:1 mix of Lm (100 mL/row) and Vl (100 mL/row); 1 Lm: 3 Vl = 1:3 mix of Lm (50 mL/row) and Vl (150 mL/row); Vl alone = Vl applied at 200 mL/row inoculum.

[2] Data were collected over four site-years in Edmonton, AB, Canada, and are the means of four replications. Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test.

Figure 8. Mean seed yield of the canola hybrids '45H31' (a) and 'CS2000'(b) under field conditions. Data were collected over two years (2020 and 2021) in Edmonton, AB, Canada, following inoculation with *Leptosphaeria maculans* (Lm) and *Verticillium longisporum* (Vl) alone or in various combinations (3:1, 1:1, 1:3). Values represent the mean of four replications for each year. Mean seed yields were not significantly different according to the Tukey-Kramer test (P > 0.05) among any of the treatments.





5) Comparison of symptoms and signs on canola

While the symptoms and signs of blackleg and Verticillium stripe were superficially similar, they could readily be distinguished with careful examination, even when they occurred together. The microsclerotia of *V. longisporum* were much smaller than the pycnidia produced by *L. maculans*, and were greyer in color **(Figure 9a).** Due to their larger size, individual pycnidia could be discerned more easily, and were generally more darkly pigmented than the microsclerotia. Moreover, while both *V. longisporum* and *L. maculans* caused a vascular discoloration visible in cross-sections of the crown or base of the stem, the staining associated with blackleg was darker (black) and more discrete than the grey, more diffuse staining resulting from Verticillium stripe **(Figure 9b).**

Longitudinal sections of the stem further served to distinguish the two diseases. In the case of blackleg, the vascular discoloration was restricted to the lower stem, affecting the cortex and epidermis **(Figure 10a);** in the case of Verticillium stripe, symptoms extended up the stem, with a hollow, darker centre **(Figure 10b).** In cases where the two pathogens occurred together, longitudinal sections revealed a hollow and darker centre together with black discoloration of the cortex and epidermis **(Figure 10c).** Infection by *V. longisporum* also was usually associated with some shredding of the stem.

Figure 9. Pycnidia of *Leptosphaeria maculans* (lower portion of stem) and microsclerotia of *Verticillium longisporum* (upper portion) occurring on the same canola stem (a). Cross-sections of canola stems showing the discoloration caused by Verticillium stripe (left) and blackleg (right) (b).

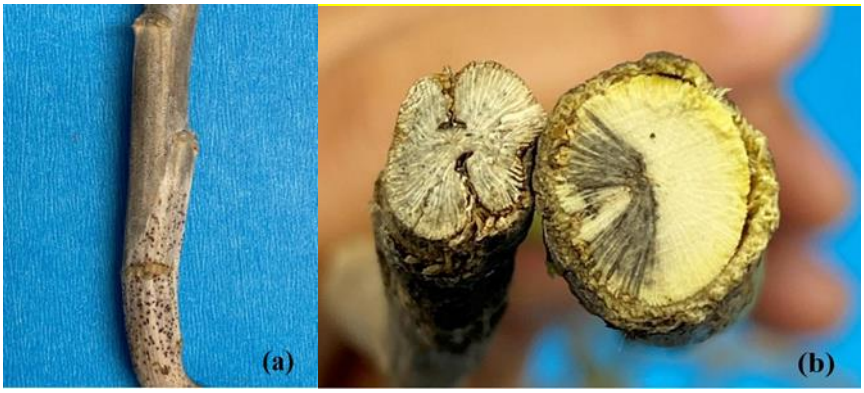


Figure 10. Longitudinal sections of canola stems infected by *Leptosphaeria maculans* (a), *Verticillium longisporum* (b), and both pathogens (c).

6)Greenhouse experiments for blackleg and Verticillium stripe interactions

Emergence ranged from 41.6 to 94.7% and from 51.9 to 95% in the canola hybrids '45H31' and 'CS2000', respectively, 14 days after seeding in the greenhouse experiments **(Table 5).** For both hybrids, percent emergence was highest (~95%) in the control (non-inoculated) treatments. In the case of 'CS2000', the emergence in all of the treatments that received any inoculum (regardless of the ratio of *L. maculans* and *V. longisporum*) was similar (51.9-63.8%). In contrast, for '45H31', the lowest emergence was observed in the *L. maculans* only treatment and in the 3:1 mix of *L. maculans* and *V. longisporum* (41.6-42.2%), followed by the 1:1 and 1:3 *L. maculans/V. longisporum* mixes and the *V. longisporum* only treatment (54.7-57.2%).

The mean blackleg severity ranged from 0.0 to 1.3 on '45H31', with no blackleg detected (severity of 0.0) in either the no inoculum control or *V. longisporum* only treatment. On this hybrid, the greatest blackleg severity (1.3) was obtained with the 3:1 mix of *L. maculans* and *V. longisporum*, followed by the L. maculans only (0.9) and 1:3 *L. maculans/V. longisporum* (0.6) treatments **(Table 5).** On 'CS2000', blackleg severity ranged from 0.0 to 1.0, with no symptoms of the disease detected on the non-inoculum control or *V. longisporum* only treatment. The most severe blackleg on 'CS2000' was obtained with the 3:1 and 1:1 mixes of *L. maculans* and *V. longisporum*, as well as with the *L. maculans* only treatment (severities of 0.8 to 1.0) **(Table 5).** The mean Verticillium stripe severity ranged from 0.0 to 1.9 on both '45H31' and 'CS2000', with no Verticillium stripe detected in the no inoculum control or *L. maculans* only treatment for either hybrid **(Table 5).** On '45H31', the highest Verticillium stripe severity was observed in the *V. longisporum* only treatment, followed by intermediate severities (1.0 to 1.3) in the 3:1, 1:1 and 1:3 *L. maculans/V. longisporum* treatments. On 'CS2000', the most severe (1.6-1.9) Verticillium stripe was observed in any treatment that included *V.*

longisporum, regardless of the ratio or whether or not *L. maculans* was included (although there seemed to be a numerical increase in severity as the proportion of *V. longisporum* increased) **(Table 5).**

Under greenhouse conditions, mean seed yield ranged from 1.5 g to 3.9 g per plant on '45H31' and from 1.2 g to 2.3 g per plant on 'CS2000' **(Table 5).** For '45H31', the lowest yields were observed in the non-inoculated control and *V. longisporum* only treatments, followed by the 1:3 mix of **L. maculans/V. longisporum**. The highest yields were obtained in the *L. maculans* only and 3:1 *L. maculans/V. longisporum* treatments; yield in the 1:1 *L. maculans/V. longisporum* treatment was intermediate. Similar trends were observed for 'CS2000' **(Table 5).** The lowest yields were observed in the non-inoculated control and V. longisporum only treatment, and the highest was recorded in the L. maculans only treatment; yields in the various mixes of *L. maculans* and *V. longisporum* were intermediate **(Table 5).** Symptoms and signs of Verticillium stripe and blackleg in the greenhouse resembled those described above for the field experiments.

Table 5. Seedling emergence, blackleg severity, Verticillium stripe severity and seed yield of the canola hybrids '45H31' and 'CS2000' following inoculation with *Leptosphaeria maculans* (Lm) and/or *Verticillium longisporum* (Vl) alone and in various combinations under greenhouse conditions.

Treatment[1]	Emergence (%) [2]		Blackleg severity (0-5)		Verticillium stripe severity (0-4)		Yield (g plant^{-1})	
	'45H31'	'CS2000'	'45H31'	'CS2000'	'45H31'	'CS2000'	'45H31'	'CS2000'
Control	94.7a	95.0A	0.0d	0.0C	0.0c	0.0B	1.5c	1.2B
Lm alone	41.6c	52.5B	0.9b	0.8AB	0.0c	0.0B	3.9a	2.3A
3 Lm: 1 Vl	42.2c	52.5B	1.3a	1.0A	1.0b	1.6A	3.8a	2.0AB
1 Lm: 1 Vl	54.7b	62.2B	0.9bc	0.8AB	1.0b	1.7A	3.4ab	1.8AB
1 Lm: 3 Vl	57.2b	63.8B	0.6c	0.6B	1.3b	1.8A	2.7b	1.7AB
Vl alone	55.6b	51.9B	0.0d	0.0C	1.9a	1.9A	1.6c	1.4B

[1] Lm alone = Lm applied at 20 mL inoculum/row; 3 Lm: 1 Vl = 3:1 mix of Lm (15 mL/row) and Vl (5 mL/row); 1 Lm: 1 Vl = 1:1 mix of Lm (10 mL/row) and Vl (10 mL/row); 1 Lm: 3 Vl = 1:3 mix of Lm (5 mL/row) and Vl (15 mL/row); Vl alone = Vl applied at 20 mL/row inoculum.

[2]Data are the means of four replications in each of two repeats of the experiment, which were combined as they were not significantly different ($P > 0.05$); means in a column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P \leq 0.05$).

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

Conclusions:

Refined methods for the inoculation of V. longisporum on canola were developed. Collectively, these represent an important tool for screening germplasm and evaluating resistance phenotypes in this crop. Symptoms of Verticillium stripe were observed at the seedling stage as early as 2 weeks following root dip inoculation under greenhouse conditions. A 0-6 disease assessment scale was proposed to evaluate disease severity in seedlings, as most studies have focused on older plants. In addition, a 0-4 scale was developed for the evaluation of Verticillium stripe at the adult stage.

The capacity to assess disease at different stages of plant growth is important for distinguishing between different types of resistance (such as quantitative vs. qualitative) that may be active at different times, and provides a quantitative measure of disease progression. Moreover, through the evaluation of different inoculum types—a conidial suspension applied by the root-dip method and grain inoculation—and comparisons of the timing of inoculation, this work

established a foundation for more accurate evaluation of host resistance.

The relationship was established between Verticillium stripe severity and canola yield under field conditions in western Canada. Prior to this research, there was a notable absence of information quantifying V. longisporum-induced yield losses in canola. Such data are critical for estimating the potential threat posed by this pathogen, particularly considering the significant role of canola in the Canadian economy (Canadian Canola Growers Association 2022). The results from these experiments, which were conducted over 2 years with two hybrids, indicated that the relationship between disease severity and yield was most accurately described by second-degree quadratic equations. In 2020, in the first year of the study, both cultivars experienced yield losses exceeding 60% at one of the sites when Verticillium stripe severity was > 3 on a 0-4 scale. In the second year of the study in 2021, single plant seed yield losses surpassed 50% for both hybrids at both sites when the disease severity was ≥ 1. These results suggest that that V. longisporum has the potential to cause significant yield losses in canola, even when disease levels are relatively mild.

Recommendations:
Root dip inoculation methods for inoculating the seedlings can be time-consuming and labor-intensive. As such, future studies could explore how to streamline seedling inoculation for large-scale screening of germplasm. The coordinated testing of much larger numbers of Brassica genotypes from seed banks and breeding programs could help to identify effective resistance for deployment in the Canadian Prairies and beyond.

The quantification of yield losses, as determined by the severity of Verticillium stripe, underscores the potential impact on productivity of *V. longisporum* infection in Canadian canola hybrids. Further exploration of the influence of this pathogen on canola yields, including field-level assessments and evaluation of a wider selection of hybrids, could lead to a more robust yield loss model to aid growers in establishing action thresholds for disease management.

Blackleg and verticillium stripe were shown to increase blackleg severity when combined, but verticillium stripe was not exacerbated by the presence of blackleg. Future studies could examine the effects of inoculum timing on their combined effects.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

Refereed scientific papers: *corresponding author

- Cui, J., S.E. Strelkov, R. Fredua-Agyeman, and S.F. Hwang*. 2023. Development of optimized Verticillium longisporum inoculation techniques for canola (Brassica napus). Can. J. Plant Pathol. 45: 92-102. https://doi.org/10.1080/07060661.2022.2120913
- Wang, Y., S.E. Strelkov and S.F. Hwang*. 2023. Blackleg yield losses and interactions with Verticillium stripe in Brassica napus in Canada Can. Plant 2023, 12, 434. https://doi.org/10.3390/plants120304 34.

Oral and poster presentations & abstracts:

- Strelkov, S.E., Hwang, S.F., Manolii, V.P., Aigu, Y., Hollman, K., Fox, N., Storfie, E., Cui, J., Botero, A, and Wang, Y. 2024. Clubroot & Verticillium stripe: Soilborne canola diseases. Lakeland Agronomy Update, March 12, 2024, Ashton, AB. Oral presentation 25 people attended
- Hwang, S.F., S.E. Strelkov, and R. Fredua-Agyeman. 2023. Research update on Verticillium stripe of canola in Alberta. Oral presentation at Verticillium Workshop organized by CCC. Dec. 4, 2023. Calgary, AB. 80 people attended
- Wang, Y., Hwang, S.F., and S.E. Strelkov. 2023. Blackleg and Verticillium stripe interactions in canola. Oral presentation at WCC/RRC meeting. Feb. 6, 2023. 50 people attended (online)
- Wang, Y., Strelkov, S.E., Fredua-Agyeman, R., and Hwang, S.F. 2022. Exploring resistance to Verticillium longisporum in Brassica genotypes. In: Annual Meeting of the Canadian Phytopathological Society, July 4-8, 2022. Virtual Conference
- On Feb. 25, 2022, at the request of Kaeley Kindrachuk (Agronomy Extension Specialist), Dr. S.F. Hwang accommodated Golden Media House to photograph live plant material in the greenhouse at the University of

Alberta to produce a Verticillium Stripe Video Project from SaskCanola.

- Dr. S.F. Hwang and Y.X. Wang were interviewed by Golden Media House on Feb. 25, 2022.
- Y. Wang, S.E. Strelkov, and S.F. Hwang. 2022. Interactions of Verticillium with blackleg. Oral presentation at WCC/RRC Pathology Subcommittee Meeting, Feb. 1, 2022. (virtual conference)
- Y. Wang, S.E. Strelkov, and S.F. Hwang. 2022. The next challenge: Verticillium stripe – Evaluating disease, host resistance and interactions with blackleg. Oral presentation at the Verticillium Stripe Workshop organized by SaskCanola. Feb. 10, 2022 (Webinar)
- Cui, J., Hwang, S.F., and Strelkov, S.E. 2022. Comparison of inoculation techniques for Verticillium longisporum on canola. Oral Presentation. Tri-Society Virtual Conference, July 5-9, 2021. Can. J. Plant Pathol. 44: 279; https://doi.org/10.1080/07060661.2021.2009254
- Cui, J., Hwang, S.F., and Strelkov, S.E. 2022. Yield Losses in canola caused by Verticillium longisporum. Poster Presentation. Tri-Society Virtual Conference, July 5-9, 2021. Can. J. Plant Pathol. 44: 279; https://doi.org/10.1080/07060661.2021.2009254
- Hwang, S.F., S.E. Strelkov, and J. Cui. 2021. Update on Verticillium research of Canola in Alberta, Canada. Oral presentation at Top Notch Farming Webinar (SaskCanola's winter extension meeting), Jan. 22, 2021. Over 150 people attended (online).
- Hwang, S.F. and Strelkov, S.E. 2020. Verticillium stripe management in canola. COUNTRY-GUIDE.CA, Western Edition/ November 2020. P. 35-38.
- Hwang, S.F., S.E. Strelkov, and J. Cui. 2020. Update on Verticillium research of Canola in Alberta, Canada. Oral presentation at Verticillium Research Update/ VBL Steering Group Meeting, Edmonton, AB, April 3, 2020.
- Cui, J., Hwang, S.F., and S.E. Strelkov. 2019. Impact of Verticillium dahliae on Canola Yields: A Preliminary Assessment. Poster presentation at 40th Annual Meeting, Plant Pathology Society of Alberta, Lacombe, Alberta. November 4-6, 2019.

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

The support by the Funders has been acknowledged in the manuscripts prepared below:

- Cui, J., S.E. Strelkov, R. Fredua-Agyeman, and S.F. Hwang*. 2023. Development of optimized Verticillium longisporum inoculation techniques for canola (Brassica napus). Can. J. Plant Pathol. 45: 92-102. https://doi.org/10.1080/07060661.2022.2120913
- Wang, Y., S.E. Strelkov and S.F. Hwang*. 2023. Blackleg yield losses and interactions with Verticillium stripe in Brassica napus in Canada Can. Plant 2023, 12, 434. https://doi.org/10.3390/plants120304 34.

In oral presentations, the speaker verbally thanked the Funders for their support and a slide at the end of the talk listing the Funders. In poster presentations, an "Acknowledgements" section was included on the poster, which lists each Funder.

9. Literature Cited

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10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

- Ji Cui successfully completed her M.Sc. based on these research results for her MSc. Thesis.
- Yixiao Wang as a Ph.D. student for this project (started Sept. 2020) who uses some of these results for her doctoral dissertation.

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. An invoice for each Funding Agency

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	_X_ Yes – this version can be posted ___ Yes – a modified version will be sent ___ No
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	_X Yes ___ No

Please send an electronic copy of this completed document to:

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