Interaction dynamics between *Verticillium longisporum* and *Leptosphaeria maculans* in Canola

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Abstract

Co-occurrence of both *Verticillium longisporum* (Verticillium stripe) and *Leptosphaeria maculans* (blackleg) recently reported many times in canola across the Canadian Prairies.

Co-infection by these pathogens may lead to complex interactions, influencing pathogen fitness, host defense, and disease outcomes.

Dual culture assays were conducted using eight LM and eight VL isolates on mixed PDA + V8 and canola extract-amended V8 media.

Results showed isolate-specific variations in competitiveness between the two pathogens.

Depending on the isolate combination and media used, both inhibitory and stimulatory effects on growth were observed relative to single-culture controls.

These findings demonstrate measurable interaction dynamics between the two pathogens, suggesting that nutrient or host-derived factors can influence their growth behavior.

OBJECTIVES

- To investigate competitiveness and interaction dynamics of *V. longisporum* and *L. maculans* under controlled experimental conditions, both *in vitro*, and in canola plantlets
- To study whether *V. longisporum* and *L. maculans* compete within the host, and how these interactions shape disease severity and persistence under controlled environmental conditions.

MATERIAL & METHODS

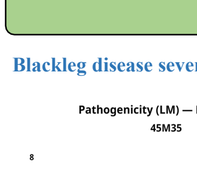
- VL and LM isolates, exhibiting a range of aggressiveness levels, collected from several agricultural fields in Manitoba and Alberta were used to screen for the interaction of VL and LM *in vitro* and *in planta*.

Pathogen	Isolation Code	Location
Verticillium longisporum	DAOMC243377	Kelburn
	DAOMC251897	Manitoba
	CO1	Morden
	CO2	Morden
	VL 43	Edmonton
	MB 2	Manitoba
	MB4	Manitoba
	MBCA001	Manitoba
Leptosphaeria maculans	MB10-025D	Manitoba
	MB10-079A	Manitoba
	MB10-083A	Manitoba
	MB10-116D	Manitoba
	MB10-182B	Manitoba
	MB10-191A	Manitoba
	Blg 97.17	Alberta
	Blg87.19	Alberta

MATERIAL & METHODS

Comparative evaluation of VL & LM aggressiveness in canola

Westar & 45M35 seedlings (10 days old) → LM - cotyledon inoculation → 7 dpi → Disease rating

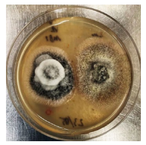


Westar & 45M35 seedlings (21 days old) → VL – Root dip inoculation → Transferring into bigger pots → Flowering stage → Disease rating

Assessing *in vitro* competitive and coexistence dynamics of VL and LM dual culture assays

PDA and V8-mixed media

Canola plant extract incorporated media



Co-culturing LM & VL

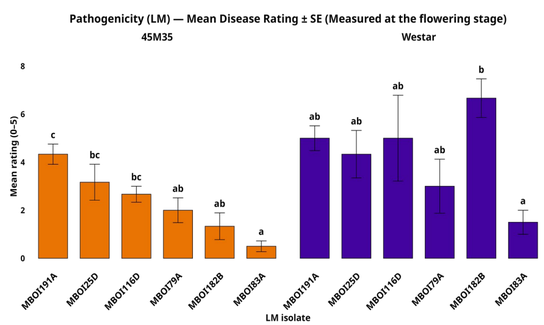
Recording colony diameters at 3rd, 5th, 7th and 10th days

% inhibition → Statistical analysis

Harvesting mycelia - both pathogens in dual assays freeze drying for future gene expression analysis

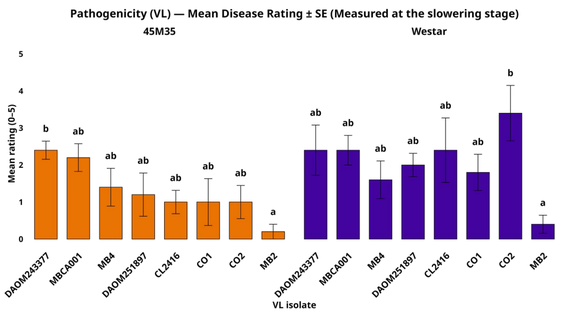
RESULTS

Blackleg disease severity *in planta*



Mean disease ratings (± SE) for LM isolates on canola cultivars Westar and 45M35 at flowering stage (n = 5). Ratings follow Cui et al. (2023) scale 0–9.

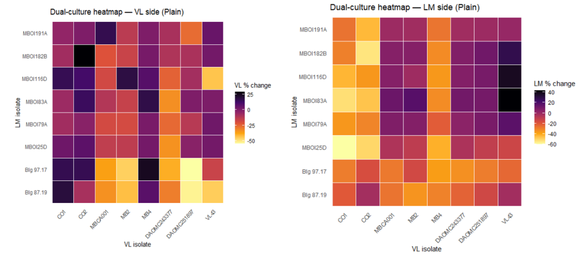
Verticillium stripe disease severity *in planta*



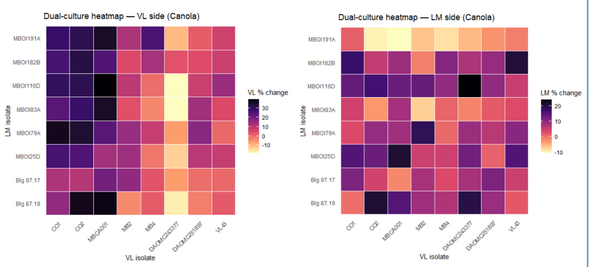
Mean disease ratings (± SE) for VL isolates on canola cultivars Westar and 45M35 at flowering stage (n = 5). Ratings follow Cui et al. (2023) scale 0–9.

Assessing *in vitro* competitive and coexistence dynamics of VL and LM dual culture assays

- Dual-culture assays involving 64 combinations showed distinct differences in antagonistic potential between LM and VL across both V8+PDA and Canola plant extract incorporated media media. Here we are presenting only two VlxLm combinations:



Mean percentage inhibition of colony growth in *L. maculans* (LM)–*V. longisporum* (VL) dual cultures on plain PDA.



Mean percentage inhibition of LM and VL isolates grown on canola-amended PDA.

RESULTS

Pathogenicity screening: Pathogenicity assays on 45M35 and Westar revealed clear differences in aggressiveness among isolates. For *L. maculans* (LM), MBOI191A was the most aggressive and MBOI83A the least. For *V. longisporum* (VL), DAOMC243377 (on 45M35) and CO2 (on Westar) were the most aggressive, while MB2 was the least across both cultivars.

Dual inoculation in planta: The highly aggressive and less aggressive isolates identified in the pathogenicity screening were subsequently used in dual inoculation assays to evaluate combined effects on disease expression in canola. In 45M35, the pairing MBOI191A × DAOMC243377 resulted in the highest mean disease rating, while MBOI83A × DAOMC243377 produced the lowest. In Westar, MBOI83A × CO2 caused higher disease intensity, whereas MBOI191A × MB2 showed the lowest severity.

Dual inoculation in Plain PDA and V8 mixed medium: Strong isolate-specific antagonism was observed between LM and VL. On the VL side, the combinations CO2 × MBOI182B and MB4 × Blg97.17 produced the greatest inhibition, while DAOMC251897 × Blg97.17 and DAOMC251897 × Blg87.19 showed minimal inhibition. On the LM side, VL43 × MBOI83A and VL43 × MBOI116D exhibited the highest growth inhibition, whereas CO2 × MBOI25D and CO2 × MBOI182B demonstrated the least inhibition. These findings confirm that the interaction strength varied among isolate pairings.

Dual inoculation in Canola-amended PDA medium: When the medium was Canola extract, inhibition was notably reduced. On the VL side, the most inhibition occurred in MBC001 × Blg87.19, MBC001 × MBOI116D, and MBC001 × MBOI191A, while DAOMC243377 × MBOI116D and DAOMC243377 × MBOI83A exhibited minimal inhibition. On the LM side, the combinations DAOMC243377 × MBOI116D, MBC001 × MBOI25D, and CO2 × Blg87.19 produced the highest inhibition, whereas MBC001 × MBOI191A and CO2 × MBOI191A showed the least. The reduction in inhibition compared with plain mixed media suggests that host-derived metabolites mitigate antagonistic effects, promoting partial coexistence between the two pathogens.

CONCLUSION

The combined results demonstrate that *Leptosphaeria maculans* and *Verticillium longisporum* display considerable variation in aggressiveness, and their interactions are shaped by both isolate identity and environmental context. Dual inoculation in canola produced moderate, non-additive disease levels, suggesting that co-infection is largely competitive rather than synergistic. In vitro assays confirmed strong antagonism under nutrient-rich conditions, while the addition of canola extract reduced inhibition, indicating that host-derived compounds can moderate competition. Together, these findings reveal that the coexistence of *L. maculans* and *V. longisporum* within canola is governed by a balance between pathogen competitiveness and host-mediated modulation.

FUTURE DIRECTIONS

- Further work will quantify fungal biomass and transcriptomic responses during co-infection.
- This connects to your ongoing research trajectory.
- To assess co-infection outcomes *in planta* and in soil, including quantification of pathogen biomass and effector gene expression during dual infections.
- To elucidate whether *V. longisporum* and *L. maculans* tend to compete or coexist within the canola host, and how these interactions may shape disease severity and persistence under field conditions.

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ACKNOWLEDGEMENTS

University of Manitoba · University of Alberta · NSERC CRSNG · Manitoba Canola Growers · SASK OILSEEDS · SaskCanola · mitacs · WGRF Advancing Agriculture through Research · canola council of canada

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