The Use of Biofumigant Mustards to Manage Verticillium Wilt of Potato in Manitoba 2019-2022

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Abstract

Biofumigation describes the elimination or suppression of soilborne pests, pathogens and weeds by gases emitted from buried biomass from members of the Brassicaceae family (e.g., brown mustard, oriental mustard, radish, etc.). Biomass is pulverized and incorporated into moist soil to combine with oxygen and ultimately convert glucosinolates into degradation products such as isothiocyanates. The process has been developed and experimentally validated as a control measure of Verticillium wilt of potato in the United States and Europe. However, the methods of growing the mustard crop and the effectiveness of the process to reduce Verticillium wilt in Manitoba have yet to be validated regionally with all cultivars of mustards. The overarching goal of this study was to explore a way to economically manage Verticillium wilt of potato in Manitoba using a mustard crop as a biofumigant green manure to kill *Verticillium* propagules in soil and/or suppress the disease. More specifically, experiments were conducted to determine agronomic inputs to maximize biomass of mustard cultivars 'AC Volcan', 'Caliente Rojo', 'Cutlass', 'AAC Brown 18', and a male sterile hybrid. Additional studies examined field-scale mustard biofumigation to verify *Verticillium* CFU/g soil before and after biofumigating, as well as mustard biomass at the time of biofumigation. These experiments demonstrate that these mustards can be grown to sufficient biomass levels to theoretically achieve biofumigation, and that the practice is a viable disease management strategy. The conclusions of this project will scientifically reinforce growers' efforts to reduce Verticillium wilt with evidence to support choices to effectively and economically manage the disease for their entire operations. These experiments also provide use extension results and a blueprint for

Introduction

Potatoes are an economically critical crop to Canada and are found in every province. In 2020, 23% of production was in Manitoba, 22.5% in Alberta, 20.1% in Prince Edward Island, 11% in New Brunswick, 12.4% in Quebec, 7.2% in Ontario, 2.1% in British Columbia, 1.3% in Saskatchewan, 0.3% in Nova Scotia, and 0.1% in Newfoundland and Labrador (Statistics Canada 2022). Statistics Canada (2022) reported that, in 2020, the value of Canadian potato farm receipts was approximately $1,352,569,000. Of that total value, $281,405,000 (or approximately 20.8%) derives from Manitoba alone.

One of the most economically significant diseases of potato has been Potato Early Die (PED) disease complex, which occurs through the combined impacts of pathogenic fungi (*Verticillium dahliae* Kleb and *Colletotrichum coccodes* Wallr) and a nematode (*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven) (Lees et al. 2003; Molina et al. 2014; Powelson and Rowe 1993; Rowe 1985). The fungal components of this complex can inflict a 40-50% yield reduction in potatoes (Powelson and Rowe 1993) and has been noted to have upwards of 90% disease severity in Manitoba (Molina et al. 2014). PED has also been attributed as a main influence for marketable potato yield in Manitoba, more so than weed pressure or declining soil quality (Mohr et al. 2012). Furthermore, Frederick et al. (in press) reinforced that damage from Verticillium wilt, expressed as both disease severity and soil microsclerotia counts frequently made the topmost significant variables responsible for yield variability for total yield, dollar value, and tuber profile sizes 85-170 grams, 283-340 grams, 170-340 grams, and 340 grams or greater. Frederick et al. (in press) explains that a plausible explanation for the significance of Verticillium to yield variability is that higher levels of microsclerotia in the soil or disease expression in plant can be attributed to premature senescence, robbing the plant of bulking days in late summer that manifest in decreased total yield and smaller tubers. This combination of less weight and small tubers is inherently less valuable to growers and incurs further financial loss when contract bonuses are not met.

Management of Verticillium wilt is made difficult by the wide host range of the pathogen, the buffering capacity of soil to pesticide, the costs and environmental impacts associated with successful pesticide programs, the need for potato rotations to be shorter than the lifespan of *V. dahliae* microsclerotia, and the relative susceptibility of the many potato cultivars to Verticillium wilt (Pegg and Brady 2002). One possible management tool is the use of a specific green manure for biofumigation, which specifically involves the breakdown of plant metabolites in soil to produce volatile compounds that are toxic to many soil microorganisms (Sawar et al. 1998). Mustards were identified by multiple authors as a potential Verticillium management tool, amongst other possible green manures (Chen et al. 2022; Davis et al. 1996; Larkin et al. 2011; Larkin and Halloran 2014; McGuire, 2003). Successful biofumigation hinges on the mustard producing sufficient glucosinolate and overall biomass, speed and depth of residue burial, and environmental conditions (particularly the amount of free moisture and oxygen) (Chen et al. 2022; Mattner et al. 2008; McGuire, 2003). However, each author has noted that successfully growing mustard for maximum biofumigant effect has required changes to the agronomic recommendations and grower practices by region, necessitating local adaptation of discoveries made in other potato growing regions. For example, Larkin and Halloran (2014) in Maine suggested the use of mustard cultivar 'Caliente 199' following a grain crop and preceding a potato crop in the northern United States, with an early August planting date. McGuire (2003) showed that, in Washington, planting a successful mustard crop could occur as late as nid-August.

An additional problem has been noted in the use of biofumigant mustards: their use takes land out of production, costs time and some money to implement, and does not directly generate revenue (Larkin and Halloran 2014). This problem requires researchers and extension agents to demonstrate that the investment made in biofumigation is profitable in terms of the improvements to yield and quality of the subsequent potato crop. A possible solution was offered by Larkin and Halloran (2014) in the use of a mustard in the grain rotation preceding the potato crop, where the ground is open for the two-month biofumigant crop after the grain is removed by August of the year following potato. Another solution was shown in Sexton et al. (2007) where the barley crop was replaced with mustard and the economic benefit to potatoes generated approximately $300/ha more than the barley crop would have provided, albeit in a different year and treating a different disease. McGuire (2003) estimated that the cost of the green manure (seed, fertilizer, herbicide, irrigation, equipment/staff time) in Washington was $114/acre at the time, compared to metam sodium fumigation (37.5 gal/acre) at $140/acre. McGuire (2003) also reported there were no statistically distinguishable differences in the yield and value of potatoes subjected to either treatment in three replicated experiments, indicating that the mustard treatment was the most cost-effective option. Conversely, Chen et al. (2022) reported mixed responses with potato yield to biofumigation and no significant impact of biofumigation treatment with mustard cv. 'Centennial Brown' on visual symptoms of PED in New Brunswick. Chen et al. (2022) also reported that biofumigation did not significantly reduce *V. dahliae* population density in multiple trials and hypothesized that the result may have been different if environmental conditions had been more favorable for growing mustard in the years of study.

Several authors have noted additional benefits to biofumigation from nitrogen credits to soil health (McGuire, 2003; Larkin and Halloran 2014). One possibly underexplored benefit could be reductions to wind erosion. Intensive agriculture involving frequent tillage operations in Manitoba, among other prairie provinces and states, carries increased risks of soil erosion (Samson and Knopf 1994) that can manifest most visibly as wind erosion (Zarrinabadi et al. 2022). A device called a sediment sampler (see below) has been employed in Manitoba to demonstrate that the practice of land rolling in soybean production may increase the risk of wind erosion, as measured by the amount of soil moved and particle size, but that this risk is unlikely to create measurable increases in soil loss and ultimately decrease soybean yield (Zarrinabadi et al. 2022). These devices have wider applicability to answer questions relating to risks of soil erosion in potato production, especially when the practice of adding green manures increases the amount of tillage passes that fields are subjected to.

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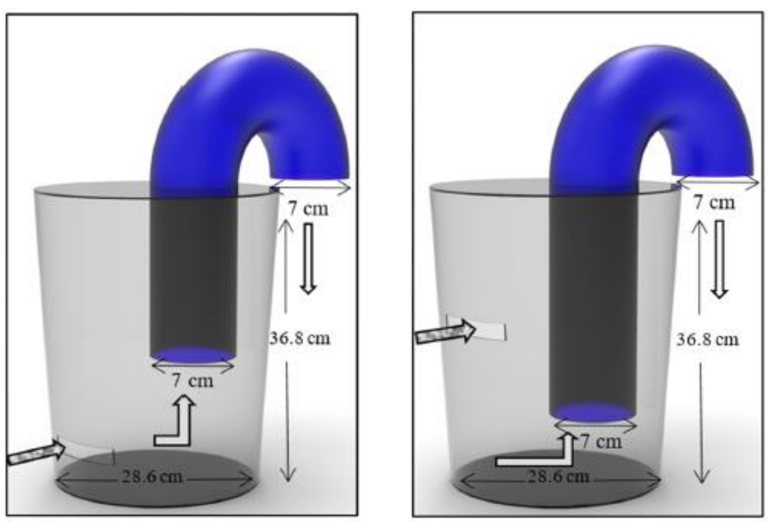

(a) (b)

Fig. 3. (a) the sampler with inlet height at 5-cm, and (b) the sampler with inlet height at 20-cm [A sealed solid concrete block, fiberglass filter and coarse mesh on the inlet and outlet not shown].

Objectives and Deliverables

Evaluate whether mustard biofumigation with “Caliente Rojo” and “AAC Brown 18” reduces *Verticillium dahliae* soil CFU and/or Verticillium wilt of potato (subsequent potato rotation after mustard)

1. Implement and validate the applicability of a real-time *Verticillium dahliae* quantification tool for soil testing
2. List approximate number of acres planted, and practices used to grow the crop
3. Individual grower will have comparison of numbers of Verticillium propagules at three timings: 1) before mustard biofumigation 2) one-month post-biofumigation and 3) post-potato production.
4. Verticillium wilt symptoms ratings will occur in the potato rotation to document visual reduction of disease, possibly as response to Verticillium wilt
5. Calculate cost of biofumigant use for the reduction in *Verticillium* CFU/g or Verticillium wilt (e.g. cost-benefit analysis)

Conclusions

Deliverables

i. Implement and validate the applicability of a real-time *Verticillium dahliae* quantification tool for soil testing
    a. Completed in 2019. PSI is running qPCR markers developed by) for *V. dahliae* every year of study.

ii. List approximate number of acres planted, and practices used to grow the crop
    a. Completed every year. Since 2019, 100 acres of biofumigant mustard are planted across the province and new recommendations are developed annually. These numbers have risen to 200-300 acres in 2021 and 2022. (see supplementary file on recommendations for current best practices, mustard recommendations are published on mbpotatresearch.ca)

iii. Individual grower will have comparison of numbers of Verticillium propagules at three timings: 1) before mustard biofumigation 2) one-month post-biofumigation and 3) post-potato production.
    a. Completed in 2022 – more fields could be added for additional analysis with other mustards targeting other pathogens or wind erosion control
    - Refining mustard biofumigation technique for Verticillium wilt yield variability management. Data supports using Caliente Rojo (High Performance Seeds) to manage Vertcillium wilt on the field scale, working better method to measure cost effectiveness of the model because of the spatial variability associated with Verticillium wilt. Weakness in the method

is that the Caliente mustard requires irrigation to get lots of biomass in Manitoba. Working with new mustard cultivars from Mustard 21 to see if a dryland mustard suitable for growers without dedicated irrigation. This requires verification that the glucosinolate profile in any other mustard compares to Caliente to ensure the efficacy of the method is preserved with other mustards.

iv. Disease ratings will occur in the potato rotation to document visual reduction of disease, possibly as response to Verticillium wilt
   1. Severity of Verticillium wilt symptoms (primary focus)
   2. Severity of common scab, propensity for wind erosion (new focus)
   3. Severity of black dot or rhizoctonia symptoms (secondary focus – if observed and if time)
      a. Ongoing – New grower interest in dryland mustard, propensity for dryland mustard to manage wind erosion and/or common scab. Common scab test requires refining in 2023 – it appears that Streptomyces in Manitoba are different enough from Ontario that PCR techniques from that province do not easily translate to here.
      b. A new collaboration has been fostered with David Lobb to improve the measurement devices for wind erosion. The first data will likely come in 2023 for mustard fields from 2022.

v. Calculate cost of use for reduction in Verticillium CFU/g or Verticillium wilt
   - A soil test of 40 CFU/g (common in Carberry) is estimated to lose the grower $11.30 per acre, 100 CFU/g is estimated to lose the grower $79.10 per acre, and a soil test of 300 microsclerotia is estimated to lose the grower $305.10 per acre.
   - An estimate for the cost of the mustard is |$200-250 per acre, making mustard biofumigation theoretically profitable with sufficient levels of Verticillium in the soil

Methods

Evaluation of Agronomic Recommendations for Growing Mustard for Biofumigation

Pest Control:

When using mustard or any other crop as a biofumigant, it is important to know the targeted pest(s) and its life cycle. The biofumigant crop should be incorporated when the pest is present in the upper soil profile (15 to 20 cm).

Seeding Date:

Seeding date should be based on the targeted pest. Mustard should be seeded about 60 days before pest will be present in the field as mustard should be incorporated into the soil before seed production begins. Seeding date should be planned accordingly in order for the crop to have reached maximum biomass at time of incorporation. Depending on variety and growing conditions, it takes about 60 to 70 days to attain maximum biomass production.

Varieties:

Mustard comes in many varieties but not all are equally as effective when it comes to biofumigation. Some mustard varieties produce more glucosinolates compared to others. In fact, some varieties have been bred for the sole purpose of biofumigation, for example, the "Caliente". Caliente grows quickly and is typically used in spring or late summer, bred specifically for biofumigation as it contains very high levels of glucosinolates. At CMCDC, we are testing all varieties i.e. 'AC Volcan', 'Caliente Rojo', and 'Cutlass'.

For The Best Results:

- **(i)** **pH** of the soil should be above 5.5. If the field has a pH lower than 5.5 the biofumigation process might not be successful. For optimal results, the pH of soil should be as close to 7 as possible.
- **(ii)** **Biomass and glucosinolates** are factors that are fundamental to the success of biofumigation.
- **(iii)** **Fertilizer** Nitrogen is important to the production of biomass and sulfur is crucial for the production of glucosinolates. Nitrogen is applied depending on the field's history. The rate of sulfur should be adjusted in relation to the chosen nitrogen rate in a 6:1 ratio. For example, if 100 lbs/ac of nitrogen is applied then the suggested amount of sulfur to be applied would be 17lbs/ac.

Soil Incorporation:

The following considerations should be taken into account, when incorporating the mustard crop into the soil.

- Mustard crop should be incorporated into the soil before it has reached full bloom.
- Incorporation process should be done when soil has a good level of moisture. Do not incorporate mustard when the soil is dry.

- Mustard must be incorporated IMMEDIATELY after mowing, 80% of the fumigant gas will be released in the first 20 minutes after mowing.

After incorporation, the field should be rolled and packed to trap the fumigant gas in the soil. Finally, once the **incorporation process is complete**, leave the field undisturbed for 14 days to ensure that all the plant material can break down.

In the fall of 2018, and 2019, fall rye (variety: Bono), and winter wheat (variety: wildfire) were seeded to produce stubble crop prior to mustard seeding. Plot area was kept 6 m^2 with a plot length of 5 m, and width of 1.2 m. After harvesting the grain material of fall rye and winter wheat crop, three different mustard varieties were seeded at two different dates with an interval of two weeks. In the 2019 growing season, July 26, and August 09 were first and second seeding dates for mustard, respectively. However, mustard was seeded on July 24, and August 07 in the 2020 growing season. The mustard seed was treated with a seed treatment product called 'Gaucho 600' in the 2020 growing season ensuring protection of the mustard plant against pests from the time of sowing well into the growing period. However, no seed treatment was applied in the 2019 growing season. Herbicides and insecticides were applied when needed. All the other agronomic practices were carried out in accordance with standard mustard production guidelines.

The field-scale experiment had two components in two separate field years: the mustard biofumigant crop and the potato crop that followed.

Mustard biofumigant crop:

The grower provided the mustard cv "Caliente Rojo" seed, fertilizer, and water for seedlings. The grower seeded, watered, and raised the crop. The principal investigator will retrieve all relevant planting info from grower (date, depth, irrigation, fertility, conditions, stubble, texture, costs of inputs).

Fields were generally selected based on previous experience with Verticillium wilt for a field variability study from 2015-2019, although a few fields were selected because of grower willingness to test mustard biofumigation. The experiment was set up only in one quarter section of field to reduce soil variability between plots. A single field was the unit of replication. A quadrant of the field was selected for experimentation to reduce variability in soil conditions, and the exact area selected depended on the known distribution of *Verticillium* CFU from a previous field variability project (data can be retrieved from mbpotatoresarch.ca from the project by the same name). Each plot was 10m wide x 12m long, and four plots of biofumigated and four plots of non-biofumigated crop area were left bare per field (expecting to lose at least one because we may not know Verticillium distribution ahead of time).

Plots were geolocated for return to the plot after biofumigation, and the equipment recorded an average of 20 cm deviation at the time of sampling. A large plot size was selected to avoid the criticism that non-fumigated plots were in close enough proximity to be bio fumigated anyway. Strips of the field were to be bare for non-biofumigated strips. Some growers offered to not plant certain sections to create non-biofumigated strips, while other fields had bare spots created by hand after germination. Each plot (biofumigated and non) had two sampling points. The attempt was made to sample medium to high Verticillium areas and collect from center of the plot, with a few meters between sampling points. Each sampling point consists of two 0-10 cm composite samples. With eight strips per field and two sampling points a strip, there will be 16 sampling points per field. With four fields per year, that is 64 samples. There will be two collection dates (before biofumigation, three weeks after biofumigation) or 128 samples each year. For two years there will be a total of 256 samples. Verticillium counts were determined from 0-10 cm soil samples before biofumigation, just after the grower plants the mustard in late July. Biomass was recorded by harvesting all above-ground plant matter within one square meter from three random locations within a plot and immediately recording the weight in kilograms. Post-biofumigation sampling was done by returning to the same geolocated sampling points and sampling 0-10 cm one month after biofumigation, when biofumigant activity has ceased.

The soil samples were ground to fine powder to prepare them for DNA extraction and eventual *V. dahliae* quantification. Two sub-samples of 0.25g each were taken from each ground soil sample after it was well mixed between each sub-sampling. DNA was extracted from the sub-samples using DNeasy PowerSoil Kit (QIAGEN) following the manufacturer's instruction. Two extracted DNA samples were combined and mixed as the stock DNA to represent the original soil sample for the next step. The target DNA was amplified using the qPCR markers developed for *V. dahliae.* A model was developed and validated based on the relation of the numbers of microsclerotia per gram soil and threshold cycle threshold (Ct) of DNA amplification. Both parties of PSI and MHPEC were satisfied the model validation and agreed to their application on the real soil samples. The first assessment of the effectiveness of biofumigation will be through the comparison of microsclerotia pre and post biofumigation, using the biomass measurement as an approximate measurement for "dose".

Potato crop:

The final assessment for the effectiveness of biofumigation will be the reduction of Verticillium wilt in potato and/or the continued reduction of *V. dahliae* microsclerotia during the potato rotation that follows mustard. There will be one more verticillium testing date the following year after biofumigation on the potato rotation. There are 64 samples (eight strips x two points/strip X four fields) for three years, or 192 samples, making a total of 448 samples in total for both the potato and mustard component of the field study. This sample from the potato rotation will be from the same geolocated plots as the mustard crop year and will be from 0-10 cm in depth. The *V. dahliae* from these soils will also be quantified using the same method as before. These samples will be collected in mid August, and a 10m row of potato plants over each sampling point will be rated for percentage wilt severity from 0-100%. If applicable, ratings for black dot, rhizoctonia, or other disease symptoms and signs will be rated for severity (0-100%).

Results:

Six field sites were established in 2020 with generally one field site per grower cooperator (Table 1, below). 'Caliente Rojo' seed was treated with 16 cwt/acre of Gaucho in 2020, which when coupled with generally lower flea beetle pressure in 2020 than 2019, led to all mustard fields surviving until the desired biofumigation date. The absence of heavy snow or rain at the desired dates of biofumigation also allowed all fields to progress as originally planned. Three irrigated fields in 2020 excelled in biomass production and produced mustard crops that were over 5 feet tall (MB-7, MB-8, MB-10), which was a first for the project. These fields generally had seed drilled at higher rates of 10 lbs per acre and were planted in early June and biofumigated in mid-July. These fields required fairly frequent irrigation in the first two weeks after planting and needed a total of 6-9 inches of water depending on the sand content of the soil. The sandiest soils in Shilo seem to need 3 extra inches of water and 130 lbs more nitrogen to achieve the same biomass result as in the Carberry area. Shilo may even need an increase of nitrogen to 180 lbs N, with most being applied through frequent (weekly) fertigation events that put down approximately 30 units of N because of the sandy soil's propensity to leach.

Four field sites were established in 2019 for study with one field site per grower cooperator. Two sites did not survive to biofumigate (MB-1 and MB-4) due to three feet of snow in mid September and extreme flea beetle pressure, respectively. MB-3 did not have sufficient growth to successfully biofumigate (average of 3-5 inches plant height). MB-2 was the only site with several feet of biomass with about 3-4 feet of mustard in wetter, high organic matter areas and 1-2 feet in the sand ridges (data not shown). An additional 6 fields were added in 2020, all of which survived until biofumigation.

Year	Designation	Planting Date	Biofumigation Date	Irrigation Status	Flea Beetle Damage	Cold Damage
2019	MB-1	July 20	N/A	Irrigated	Moderate to destroyed	Did not survive
2019	MB-2	May 29	July 23	Dryland	Minor	N/A
2019	MB-3	August 1	October 28	Irrigated	Minor to moderate	Minor to moderate
2019	MB-4	July 26	N/A	Irrigated	Did not survive	N/A
2020	MB-5		October 14	Irrigated	Minor	N/A
2020	MB-6		October 14	Irrigated	Minor	N/A
2020	MB-7	June 3	July 14	Irrigated	Minor	N/A
2020	MB-8	June 3	July 15	Irrigated	Minor	N/A
2020	MB-9	June 6	July 23	Dryland	Minor	N/A
2020	MB-10			Irrigated	Minor	N/A

Table 1 – All relevant planting, biofumigation, irrigation status, flea beetle damage, and cold damage information relevant to raising the mustard crops in each field that participated in the study in the early years of 2019 to 2020. 2020 is when the first large mustard crops were observed, and fertility data was recorded.

Field #	Year	Seeding Dates	Seeding Rate	Total NPKS	Upfront	Fertigation	Total Irrigation
MB-10	2022	10-Jun	6.00	140-0-0-30	120-0-0-25	2.26 gal/ac 15-0-0-20 5.44 gal/ac 28-0-0-0	7.2 total for both crops
MB-10	2022	28-Jul	6.00	120-0-0-25	120-0-0-25		
MB-11	2021	Aug 14-19	3-3.5 15" row spacing	130-0-0-25	70-0-0-15		3.05
MB-12	2021	06-Aug		150-0-0-40	135-0-0-40	15-0-0-0	
MB-13	2021	18-May	7.99	180-30-30-45	120-30-30-30	3 apps 20-0-0-5 ea.	~25" total for both crops
MB-13	2021	15-Jul	7.01	80-0-0-25	80-0-0-25	-	
MB-14	2021	06-Jun		95-0-0-19			0
MB-15	2021	Aug 14-19	3-3.5 15" row spacing	130-0-0-25	70-0-0-15		3.05
MB-16	2021	Aug 14-20	3-3.5 15" row spacing	130-0-0-26	70-0-0-15		3.05
MB-18	2021	17-May	8.00	180-30-30-45	120-30-30-30	3 apps 20-0-0-5 ea.	~25" total for both crops
MB-18	2021	19-Jul	7.22	80-0-0-25	80-0-0-25		

Table 2: Mustard commercial collaborator fertility data. Generally, the mustard crops benefitted from a more advanced fertility program, at least 7 inches of irrigation (depending on rain, most of the irrigation required to germinate seed), and a seed treatment and/or follow up insecticide programs in years where flea beetle pressure was high.

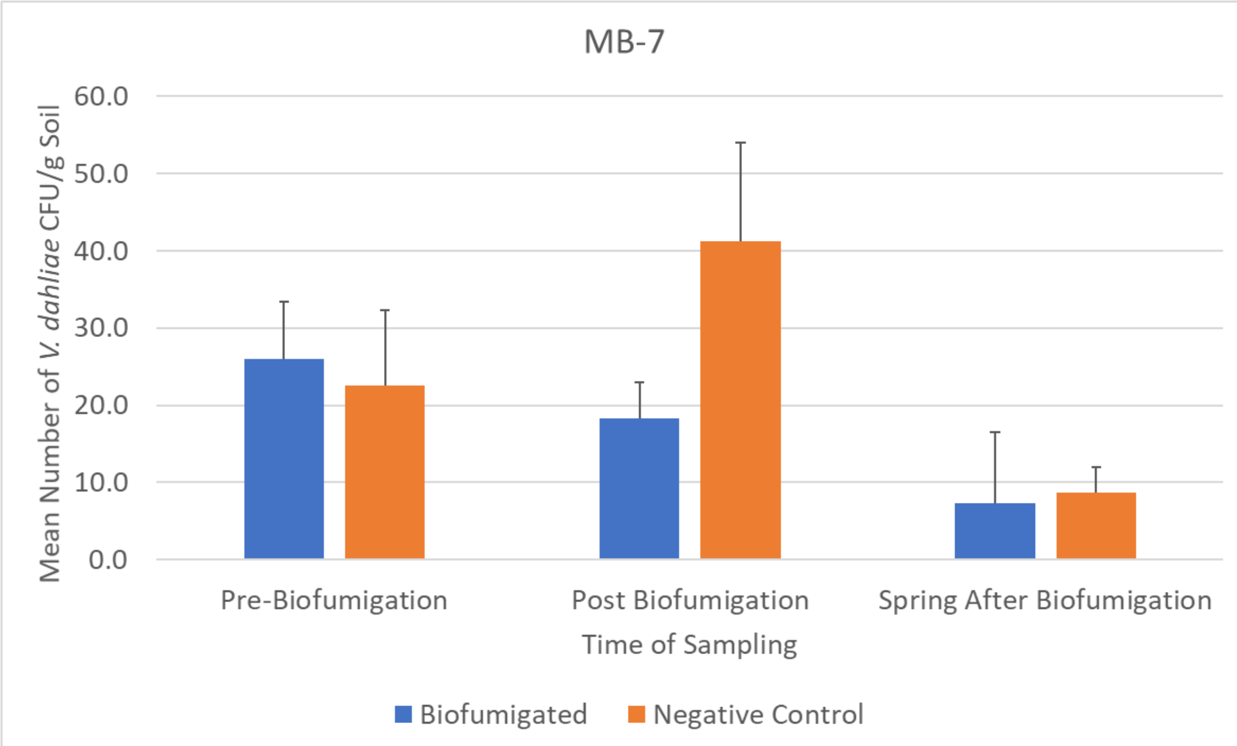

Fig. 1: The number of *V. dahliae* microsclerotia per gram of field soil (y-axis) observed within replicated strips (four per treatment) for field MB-7. Given the standard error whiskers overlap for both treatments pre-biofumigation, both sets of plots started with approximately the same amount of microsclerotia per gram of soil. Post biofumigation, it appears that biofumigated plots generally had fewer microsclerotia per gram of soil than the non-biofumigated, negative controls. In the following spring, after the potato crop was planted, it appears that both sets of plots had fewer microsclerotia per gram of soil. It is important to note that, in this particular case, only the upper ranges of observed microsclerotia were over the 10 threshold to cause disease in a potato. While fall tillage and spring planting disrupts soil and moves it around, which changes the number of microsclerotia observed in the soil, this one field does support the idea that mustard treatment could reduce the number of microsclerotia in the soil. More fields across more years would be needed to provide the complete set of evidence needed to make such a claim.

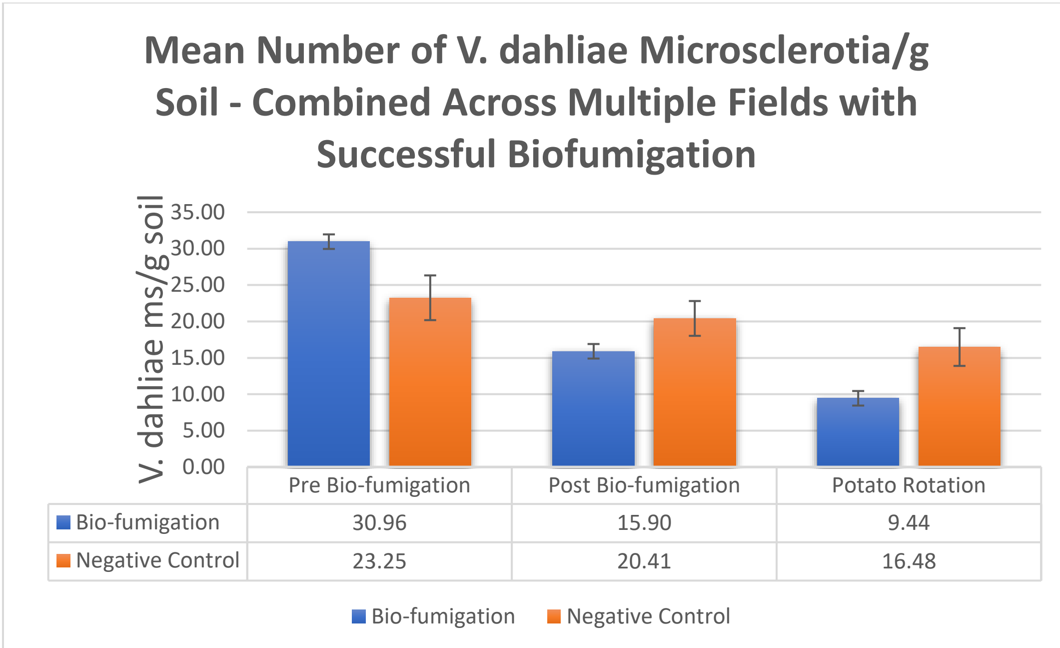

Fig. 2 The number of *V. dahliae* microsclerotia per gram of field soil (y-axis) observed within replicated strips (four per treatment) for four fields MB-7, 8, 10, and 16. These fields were chosen because the mustard achieved sufficient biomass and there were more than five *V. dahliae* microsclerotia per gram of soil to observe a treatment effect. Given the standard error whiskers overlap for both treatments pre-biofumigation, both sets of plots started with approximately the same amount of microsclerotia per gram of soil. Post biofumigation, it appears that biofumigated plots generally had fewer microsclerotia per gram of soil than the non-biofumigated, negative controls. In the following spring, after the potato crop was planted, it appears that both sets of plots had fewer microsclerotia per gram of soil. It is important to note that, in this particular case, only the negative controls consistently had a number of microsclerotia were over the 10 threshold to cause disease in a potato. While fall tillage and spring planting disrupts soil and moves it around, which changes the number of microsclerotia observed in the soil, this one field does support the idea that mustard treatment could reduce the number of microsclerotia in the soil.

Works Cited

Bilodeau, G. J. et al. 2011. Development of an assay for rapid detection and quantification of *Verticillium dahliae* in soil. Phytopathology 102: 331-343.

Chen, D., Zebarth, B. J., Goyer, C., Comeau, L. P., Nahar, K., and Dixon, T. 2022. Effect of Biofumigation on Population Densities of *Pratylenchus* spp. and *Verticillium* spp. and Potato Yield in Eastern Canada. American Journal of Potato Research 99: 1-14.

Davis, J.R., O.C. Huisman, D.T. Westermann, S.L. Hafez, D.O. Everson, L.H. Sorensen, and A.T. Schneider. 1996. Effects of green manures on verticillium wilt of potato. Phytopathology 86: 444–453.

Larkin, R.P., C.W. Honeycutt, and O.M. Olanya. 2011. Management of Verticillium wilt of potato with disease-suppressive green manures and as affected by previous cropping history. Plant Disease 95: 568– 576.

Larkin, R. P., and Halloran, J. M. 2014. Management effects of disease-suppressive rotation crops on potato yield and soilborne disease and their economic implications in potato production. American Journal of Potato Research, 91: 429-439.

Lees, A. K., and Hilton, A. J. 2003. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. Plant Pathology, 52: 3-12.

Mattner, S.W., I.J. Porter, R.K. Gounder, A.L. Shanks, D.J. Wren, and D. Allen. 2008. Factors that impact on the ability of biofumigants to suppress fungal pathogens and weeds of strawberry. Crop Protection 27: 1165–1173.

McGuire, A.M. 2003. Mustard green manures replace fumigant and improve infiltration in potato cropping system. Crop Management doi:10.1094/CM-2003-0822-01-RS.

Mohr, R. M., Irvine, R. B., Mohammad, K., McLaren, D. L., Monreal, M. A., Moulin, A. P., and Derksen, D. A. 2012. Potato Rotation Study-Carberry, Manitoba (1997-2010). Prairie Soils and Crops, 5: 36-41.

Molina Tirado, O. I. 2016. Identification of Verticillium Species and Control Methods. [Doctoral Dissertation, University of Manitoba]. University of Manitoba Libraries Myspace < https://mspace.lib.umanitoba.ca/handle/1993/32945>

Pegg, G. F., and Brady, B. L. 2002. *Verticillium wilts*. CABI Publishing. Pages 124-249

Powelson, M.L., and R.C. Rowe. 1993. Biology and management of early dying of potatoes. Annual Review of Phytopathology 31: 111–126.

Rowe, R.C., Riedel, R.M., and Martin, M.J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75: 412–418.

Samson, F., Knopf, F., 1994. Prairie conservation in North America. Bioscience 44: 418–421. https://doi.org/10.2307/1312365.

Sarwar, M., J.A. Kirkegaard, P.T.W. Wong, and J.M. Desmarchelier. 1998. Biofumigation potential of Brassicas. III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil 210: 103–112.

Sexton, P., A.B. Plant, S.B. Johnson, and J. Jemison. 2007. Effect of a mustard green manure on potato yield and disease incidence in a rainfed environment. Crop Management, 6: 1-6.

Statistics Canada. 2022. Potato Market Information Review 2015-2020. Published by Agriculture and Agri-Food Canada, Horticultural Sector. <https://www.agr.gc.ca/eng/canadas-agriculture-sectors/horticulture/horticulture-sector-reports/potato-market-information-review-2015-2016/?id=1500402297688>

Zarrinabadi, E., Lobb, D. A., Koiter, A. J., and Goharrokhi, M. 2022. Assessment of the effects of land rolling on wind erosion and crop growth in soybean production in the Red River Valley, Canada. Soil and Tillage Research, 222: 105439.