The Use of Mustards as a Biofumigant to Manage Verticillium Wilt of Potato in Manitoba

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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 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 in regionally with all cultivars of mustards, especially with a specialty-type mustard bred for biofumigation called 'Caliente Rojo'. The project's overarching goal is 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 'Andandte', 'Caliente Rojo', and 'Cutlass'. Additional studies examined field-scale mustard biofumigation to verify *Verticillium* CFU/g soil before biofumigating, mustard biomass at the time of biofumigation, and then the final *Verticillium* CFU/g one month after biofumigating. The conclusions of this project will scientifically reinforce growers' efforts with evidence to effectively and economically manage Verticillium wilt of potato for their entire operations.

Objectives and Deliverables

1. [Haider Abbas] Characterize agronomic practices for mustard cultivars ‘Andandte’, ‘Caliente Rojo’, and ‘Cutlass’ necessary to achieve maximum biomass to theoretically maximize glucosinolate production.
    a. Practices to target: planting date (Mid July, Late July, Aug 1, Mid Aug), flea beetle control, minimum inputs (irrigation, N+S fertilization) needed to achieve max biomass, seedbed preparation (stubble type, chaff spreading, best seed-to-soil contact ratio)
    b. Deliverables
        i. Develop list of recommended and experimentally verified practices to successfully use mustard biofumigants as part of program to manage Verticillium wilt in Manitoba
        ii. Improve recommendations for the inevitable question of “does this process work with other mustards?”
        iii. Develop experimental evidence to make the call for Canada-bred mustards for biofumigation (if existing mustards will not suffice)

2. [Zack Frederick] Evaluate whether mustard biofumigation with “Caliente Rojo” reduces *Verticillium dahliae* soil CFU and/or Verticillium wilt of potato
    a. Deliverables
        i. Implement and validate the applicability of a real-time *Verticillium dahliae* quantification tool for soil testing
        ii. List approximate number of acres planted, and practices used to grow the crop
        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.
        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
            2. Severity of black dot symptoms
            3. Severity of Rhizoctonia symptoms
        v. Calculate cost of use for reduction in Verticillium CFU/g or Verticillium wilt

Methods

Objective 1

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. Caliente Rojo, Cutlass, and Andante.

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 growing season, fall rye (variety: Bono), and winter wheat (variety: wildfire) were seeded to produce stubble crop prior to mustard seeding. Plot area was 6 m^2 with a length of 5 m, and width of 1.2 m. After harvesting the top 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 (July 26, and August 09), 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.

Objective 2

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 1-3 inches 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.

Manitoba Pest Surveillance Initiative (PSI) received eighty soil samples in small zip bags provided by MHPEC Inc. in 2019 for inoculum quantification. The original soil samples were ground to fine powder. 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 by Guillaume et al. (2011) 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. The both parties of PSI and MHPEC were satisfied the model validation and agreed to their application on the real soil samples. The model was MSVd = 4*10^(9.019 – 0.2721*Ct) for *V. dahliae*. 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:

Objective 1:

A significant flea beetles' infestation rate was observed throughout the grown season in the 2019 planting year. An area of 1 m^2 was harvested to analyze biomass production in each variety. In addition to CMCDC, 2 more local sites were selected to collect data points from off-site for observation purpose.

1). CMCDC On-site:

Fall Rye: Biomass production from all varieties was significantly different from each other. No sufficient biomass was generated in the Date 2 of Caliente Rojo, and Cutlass varieties due to the high infestation rate of flee beetles.

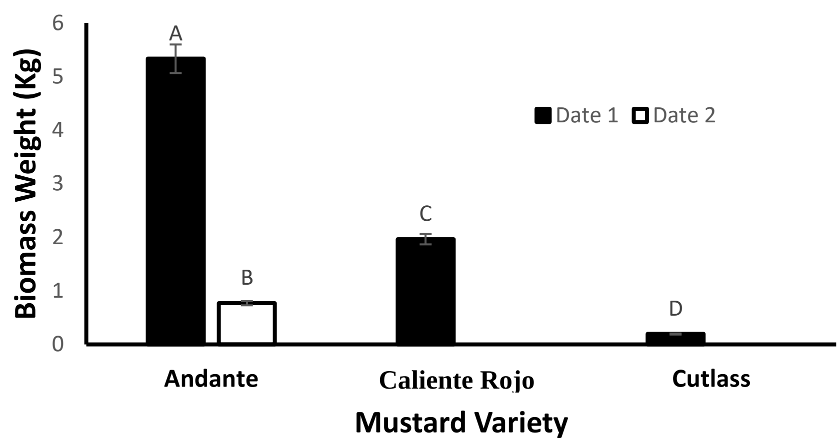


Fig. 1 Mustard verities seeded on Fall Rye (Variety: Bono) Stubbles

Winter Wheat: Biomass production of seeding Date 1 & 2 of Andante variety was significantly different from each other. No sufficient biomass was generated in the Date 1 & 2 of Caliente Rojo, and Cutlass varieties due to the high infestation rate of flee beetles.

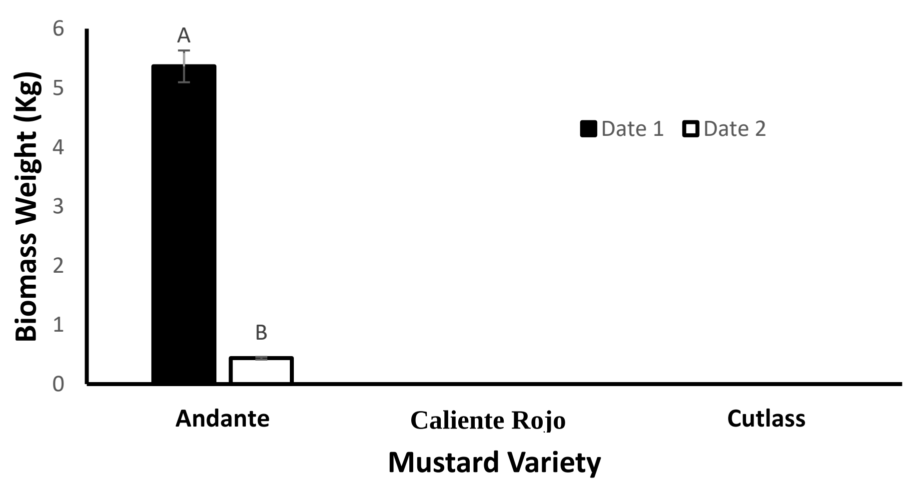
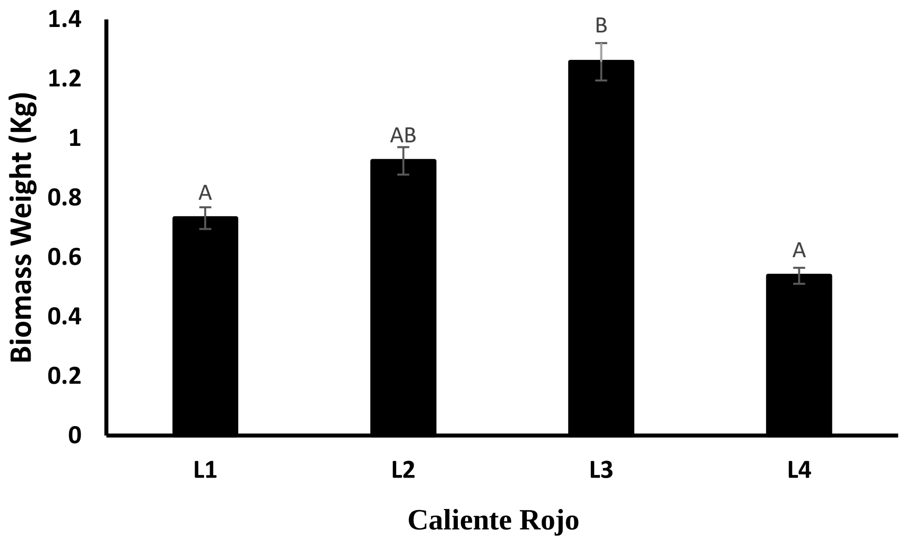


Fig. 2 Mustard verities seeded on Winter Wheat (Variety: Wildfire) Stubbles

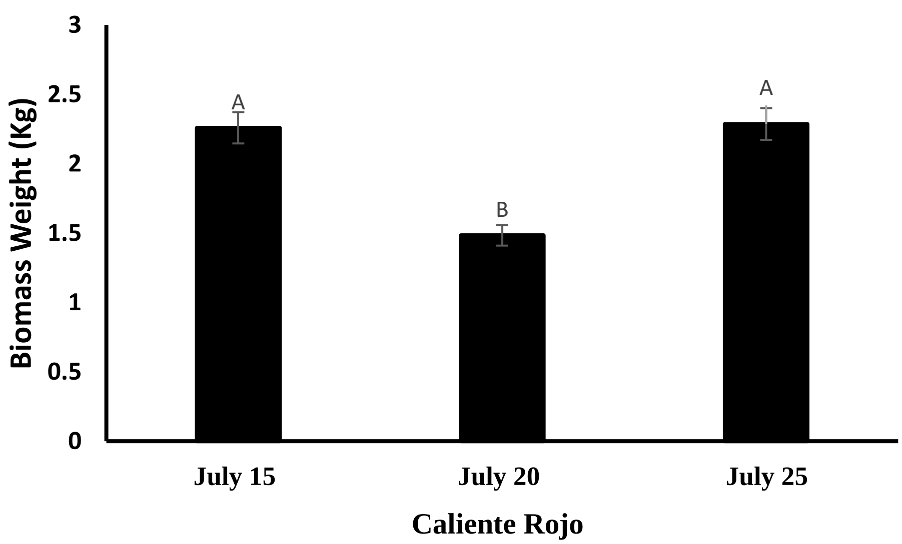
2). Field MB-3

Data was collected at 4 different sites (Locations 1-4) with same treatment but different land features.



3). Field MB-1

Mustard was seeded at 3 different dates. No significant difference in biomass production was observed in Date 1 and Date 3. However, Date 2 was significantly different from Date 1, and Date 3.



Objective 2

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).

Designation	Planting Date	Biofumigation Date	Irrigation Status	Flea Beetle Damage	Cold Damage
MB-1	20-Jul	N/A	Irrigated	Moderate to destroyed	Did not survive
MB-2	29-May	23-Jul	Dryland	Minor	N/A
MB-3	01-Aug	28-Oct	Irrigated	Minor to moderate	Minor to moderate
MB-4	26-Jul	N/A	Irrigated	Did not survive	N/A

There was insufficient replication in the first year to provide a strong dataset for statistical analyses. At last two more years of study are planned to produce more robustly replicated data. *Verticillium dahliae* counts from fields that were not biofumigated in 2019 will not be shown to simplify the results.

Site MB-2 had sufficient accumulation of microsclerotia to possess Verticillium wilt hotspots of sufficient magnitude that the grower and consultant are aware of the disease. Established literature suggests that between 5-30 *V. dahliae* microsclerotia are required to infect a susceptible potato plant. The exact sites that were subject to biofumigation have been sampled annually since 2017, when the field was last in a potato crop. The areas assigned for plots have increased from approximately 136 to 360 microsclerotia from 2017 to 2018. A simple two tailed t-test would lead us to conclude there is a significant difference (P=0.0077) between the number of *V. dahliae* microsclerotia pre- and post-biofumigation, although the power is low because of inferior replication and tremendous variability in the response of microsclerotia count. Any appearance of a difference between the means for the non biofumigated plots is nonsignificant by the same test (P=0.6391) and would likely be the error associated with sampling. It also possible that the significance of the biofumigation treatment is more due to this same error as opposed to actual biofumigation. This is where additional replication is necessary, and depending on the critiques of the community, additional proofs might be required.

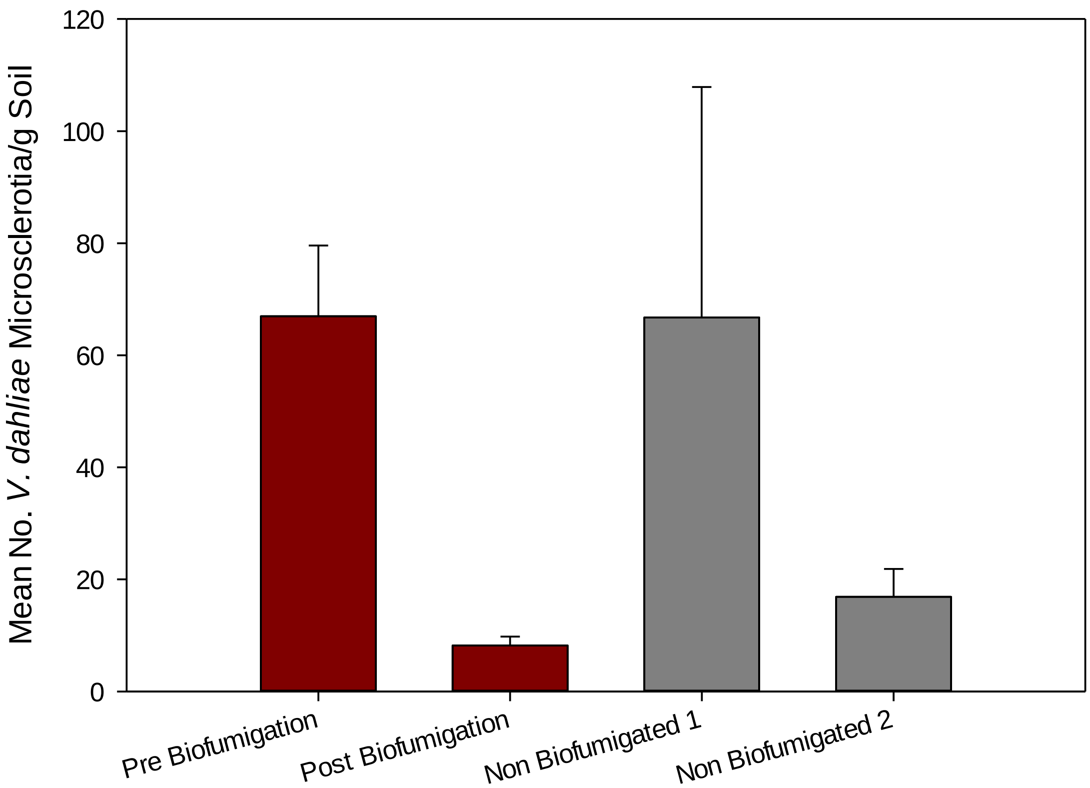


Fig 1. The comparison of *V. dahliae* microsclerotia observed for site MB-2 before and after biofumigation for plots subjected to biofumigation (red) and plots receiving no biofumigation (gray).

Site MB-3 had, on average, a low count of microsclerotia. Established literature suggests that between 5-30 *V. dahliae* microsclerotia are required to infect a susceptible potato plant. No samples from the pre-biofumigation set exceeded the minimum threshold required to cause disease, although the same was not true for the post-biofumigation samples (Fig. 2). Regardless of being over threshold, this field has lower Verticillium pressure than similar fields studied in Manitoba, where a *Verticillium* hotspot can have 200-300 microsclerotia in the same soil sample (data not shown). A simple two tailed t-test would lead us to conclude there is no significant difference (P=0.6395) between the number of *V. dahliae* microsclerotia pre- and post-biofumigation, although the power is low because of inferior replication. Any appearance of a difference between the means would likely be the error associated with sampling.

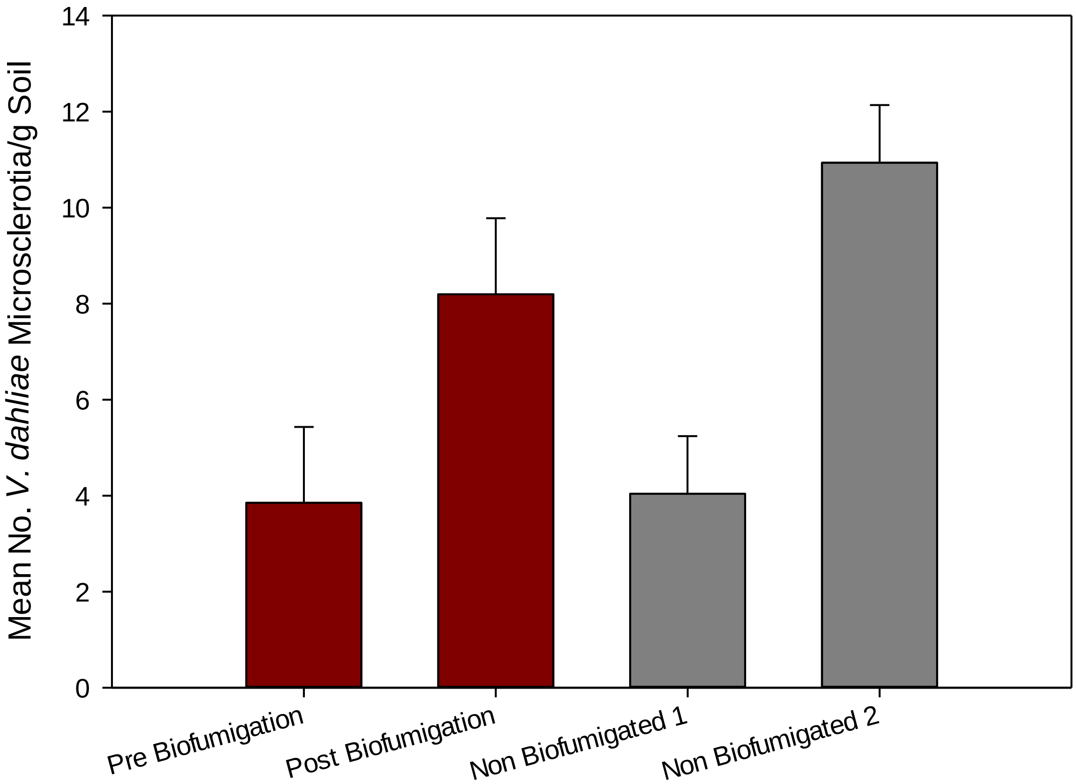


Fig 2. The comparison of *V. dahliae* microsclerotia observed for site MB-3 before and after biofumigation for plots subjected to biofumigation (red) and plots receiving no biofumigation (gray).

Conclusions

Objective 1

When managed properly mustard offers another tool to help growers control soilborne pests and diseases. It is important to strictly follow the outlined cultural practices to have any chance of success using mustard as a biofumigant. A high infestation rate of flea beetles was observed in the study areas which effected the capacity of biomass production of mustard varieties, highlighting a potential change that needs to be made for growing mustards in Manitoba. Proper chopping of plant material and soil incorporation is of utmost importance. Although mustard is a remarkable biofumigant, it could have other benefits that is expected from any other cover crop such as; prevention of soil erosion, recycling of soil nutrients, improved soil structure and maintaining soil organic matter. Interestingly, there are other crops that show possible biofumigation effect such as but not limited to; buckwheat, pearl millet, Sorghum-Sudan grass, rape seed and oil seed radish. CMCDC will test the biomass production from the mustard varieties during the 2020 planting year again with more added treatments. For this purpose, crops of fall rye, and winter wheat were seeded as a stubble crop in the fall of 2019.

Objective 2

Although only two fields survived to biofumigate, useful observations were still gathered to add to the collection of information that the project leads have amassed so far. Superficially, it appears as if biofumigation did work to reduce *V. dahliae* microsclerotia in one field in 2019. More fields and years of study are necessary to assert if the biofumgiation process can achieve the objective to control Verticillium wilt of potato in Manitoba.

Additional anecdotal observations were also recorded in 2019. Chaff spreading is necessary on rye and wheat fields before seeding mustard because a thick mulch reduces soil to seed contact and reduces germination and growth, leading to mustard that is at the cotyledon stage after a month and a few inches tall after two months of growth. Flea beetle damage was severe in 2019, but markedly less so in fields that were not in Carberry or had stubble to protect mustard seedlings. Even a rigorous insecticide program did not afford the same protection as the presence of stubble. It was also surprising to see that a dryland field was so effective in 2019 to raise a mustard crop using only precipitation and two flea beetle insecticide treatments, granted a crop of rye was lost to plant the mustard in May. Growers and consultants have also expressed interest in whether mustard biofumigation has any control of powdery scab, can build organic matter, or can reduce wind erosion.

References

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

Manitoba Mustard Biofumigantion Recommendations [2020]

Planting

PLANTING DATE

- Still the subject of ongoing research.
    - Original August planting date (with late October biofumigation) doesn't seem compatible with Manitoba's wet falls and extended potato harvests
    - 2020 experiment will focus on early June planting date with late August biofumigation date
        - Early June flea beetle damage expected to be less. Goal to have true leaves up before flea beetles begin to feed. Crucifer flea beetles thought to be more damaging than striped flea beetle in 2019.
        - Late August biofumigation date precedes bulk of potato harvest
- Seed treatment will be an ongoing area of experimentation in 2020. Seed treatment needed for flea beetle protection

DIRECT SEEDING

- Seeding rate between 6-10 lbs/ac depending on amount of residue
- Can be done in both heavy and light residue situations
- Makes for great seed to soil contact
- Caliente Brand seeds can be planted using main hopper or small seed attachment
- Drilling into dry soil and over circle tracks can be damaging to equipment. Cutting or filling tracks prior to planting helps

BROADCAST

- Seeding rate between 8-12 lbs/ac depending on amount of residue
- Use cheaper, dry fertilizer while simultaneously sowing
- After broadcasting, a pass with a undercutter, packer, harrow, or other tool will be required to ensure better seed to soil contact

FERTILIZER

- For maximum bio-fumigation potential, 120-150 units of available N are needed. Apply up to 90 units at seeding.
- Working in residue prior to planting may require more fertility due to nutrient tie-up
- For maximum growth, 25-30 units Sulphur are also recommended

PACKING

- Seed to soil contact critical, more is better
- Multiple packer types will work: Schmeiser/Ring Packer, Tire Packer/Roller, Coil Packer
- Harrows can also be used: Spring tooth Harrows, Rolling Harrows
- Vertical tillage/cutter tools can be utilized in heavy residue

GENERAL

- When growing a Biofumigant crop behind wheat or other grasses, the use of a grass herbicide is highly recommended because volunteer grains compete for water and nutrients
- Mustard is likely to be tolerant to salinity stress
- It is possible that mustard biofumigation reduces wind erosion, but this depends on wind speed, particle size, frequency of wind, and duration of winds.
- Allowing the plants to enter full bloom before biofumigation is not a bad thing – glucosinolate concentration is high in petals, levels in leaves won't drop until petal fall as the plant sheds older leaves. Don't let the plant get beyond petal fall before biofumigating.

IRRIGATION

- Shortly after seeding, multiple 1/4" shots of water are required to allow seeds to germinate
- In season, these bio-fumigant crops can use up to 2" or more per week
- When these plants are stressed for water, they will bolt and flower early (not what we want)

Incorporation

- The highest GL concentration is right at flowering, but these levels will hold for 2-3 weeks in cooler fall temps with adequate moisture
- As soon as flowers start dropping, so does the GL concentration
- For many areas, an August 5th planting date means middle to end of October for incorporation. This also avoids overlapping w/ harvest
- Ensure good soil moisture at incorporation to allow for rapid release of AITC's
- Extremely important to macerate plant tissue
    - Glucosinolate and enzyme are found in different parts of cell
    - Shredding thoroughly will allow for higher concentrations of AITC release and improved disease/pest/weed suppression
    - Flails tend to work better than mowers due to better mulching abilities.
    - Rears MFG makes a pul-flail that can be customized for more blades and higher arbor speeds, allowing for optimal AITC release
- **Flail the mustard – do not macerate plant tissue using any other way**
- We recommend residue is incorporated within 10-15 minutes of chopping. Sooner is better.
- Up to 80% of AITC can volatilize in the first 20 minutes after being chopped
- The most important rule for incorporating is to stay as close to the flail chopper as possible
- The use of a heavy offset disk or rototiller is recommended

- In extremely heavy residue, double disking is an option, but make second pass as close to first pass as possible
- Pull a heavy packer behind incorporation tool to seal soil
- If possible, a shot of water will help seal the soil surface

CAUTION

When planting directly after bio-fumigant incorporation, wait 10-14 days before planting

The AITC's released during bio-fumigation can be very phytotoxic

When planted too closely to incorporation, the AITC's have affected the germination of crops from corn and peas, to apples and potatoes

Good moisture at incorporation will speed up the release of AITC's

Flea beetle damage may be extreme without stubble protection. Foliar insecticides may be needed in certain situations, although exact thresholds have not been established.