The Use of Mustards as a Biofumigant to Manage Verticillium Wilt of Potato in Manitoba 2019-2020

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>
> **Objective 2: [Zachary Frederick] Funds generously provided to MHPEC by Simplot Canada II, McCain Foods, and the Keystone Potato Producers Association**

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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 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', and 'Cutlass'. 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. 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.

Objectives and Deliverables

1. [Haider Abbas] Characterize agronomic practices for mustard cultivars ‘AC Volcan’, ‘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. Verticillium wilt symptoms ratings will occur in the potato rotation to document visual reduction of disease, possibly as response to Verticillium wilt
        v. Calculate cost of biofumigant use for the reduction in *Verticillium* CFU/g or Verticillium wilt (e.g. cost-benefit analysis)

Conclusions

Objective 1

Planting date, presence of cereal's stubbles and seed treatment significantly impacted mustard yield and characteristics. The early seeded mustard planting date had the highest yield, population, height, and early season vigor. On the other hand, the late seeded mustard planting date had the lowest yield, population, height, and early season vigor.

The mustard grown in this trial did not produce as much biomass as commonly seen in producers' field in the Carberry area, where mustard has become a popular biofumigant. It is possible that more mustard biomass is needed to have a stronger impact on subsequent potato plantings. In addition, growers have experimented with rolling, packing, or irrigating freshly incorporated mustard to help create a seal over the soil surface and increase release of biofumigants in the soil. It is possible that other techniques may be more effective at using mustard as a biofumigant. Additional research is needed to continue developing best agronomic practices for this pest control measure.

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 treated mustard varieties planted at four seeding dates (June 1, June 15, July 02, and July 15) during the 2021 planting year again. For this purpose, cereal crops of fall rye, and winter wheat were seeded as a stubble crop in the fall of 2020.

Objective 2

1. 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 Guillaume et al. (2011) for *V. dahlia* 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 - 300 acres of biofumigant mustard are planted across the province and new recommendations are developed

annually (see supplementary file on recommendations for current best practices)

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. Ongoing – more fields are needed for completed analysis

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
      a. Ongoing – this process is a year behind point iii, meaning more fields are needed to complete the analysis

v. Calculate cost of use for reduction in Verticillium CFU/g or Verticillium wilt
   a. Will be calculated once a large biomass crop has been successfully demonstrated to reduce *V. dahliae* microsclerotia in soil and/or Verticillium wilt in the subsequent potato crop.

Although only two fields survived to biofumigate in 2019, 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.

All fields survived to biofumigate in 2020, however the amount of biomass and levels of *V. dahliae* CFU differed dramatically between fields. Superficially, it appears as if biofumigation did work to reduce *V. dahliae* microsclerotia, but the analysis must include more fields from a variety of soil types and varying pressures of Verticillium wilt. The data set is too narrow at present to definitively state that mustard biofumigation works as intended to reduce Verticillium wilt in Manitoba, especially given the amount of sampling error showing up in the standard error of each figure.

Additional anecdotal observations from 2020 suggest the fields with the most mustard biomass 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. These changes to our guidelines should allow more growers to hit the biomass targets and are very timely and helpful as the project progresses into identifying whether biofumigation reduces Verticillium wilt by giving us more fields where success was likely to occur.

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.

Suggested Changes to Mustard Growing Recommendations

- Grower for MB-10 put on more fertilizer more frequently than recommended with most of it being fertigated on Jun 11, 23, Jul 28. He thought he still ran out of nitrogen and could have gotten more biomass. He strongly suggests moving the recommendation up to 180 lbs N and that fertilizer needs to be spaced out and fertigated on.
- I also learned today that grower for MB-5 and MB-6, who had knee-high mustard this year, put most of his fertilizer on as urea preplant (220 lbs), and I bet it leached and part of the problem we saw was lack of nitrogen because the soil has a very high propensity to leach. He did fertigate once.
- Grower for MB-10 put on 9 inches of water on his mustard for the June-July crop and 10 inches of water on the Aug-Oct crop. Both hit the biomass targets very well, but 9 inches of water isn't going to get us many growers.
- Grower for MB-10 is backing off of the 2 year idea because of common scab problems. I take this as a good thing. The grower is thinking of growing mustard in problem spots during the canola rotation, which will preserve the 3-year rotation in Shilo AND is irrigated with enough water to get the 9 inches.
- Grower for MB-10 suggests a new Bayer seed treatment called Buteo that is new this year. It's supposed to be good against both crucifer and striped flea beetles and could outperform senator. Senator did well in grower fields this year but poor Haider still had a lot of flea beetle pressure at CMCDC in Carberry.
- Grower for MB-7 and MB-8 but down 6 and 5 inches of irrigation, respectively, with most going down in the first two weeks. Drilled seed at 10 lbs per acre. Put down 70 units of N and 15 units of S in less sandy soil and fertigated until 130 units N put down and 25 units of S.

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. ‘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.

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 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 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 both years. 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:

2019-Growing Season:

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.

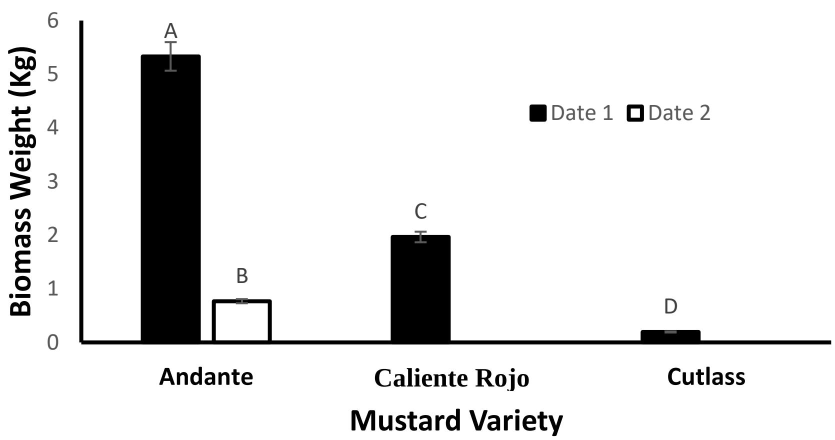


Fig. 1 Mustard verities seeded on Fall Rye (Variety: Bono) Stubbles

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.

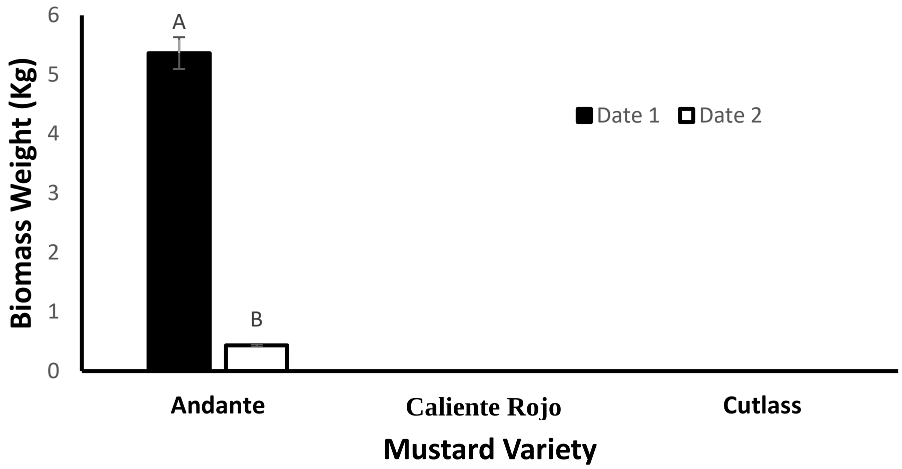
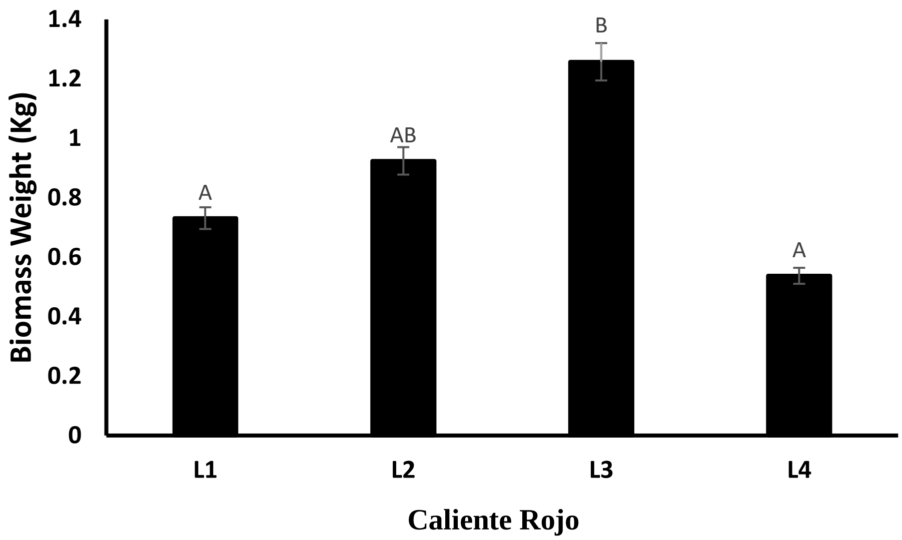


Fig. 2 Mustard verities seeded on Winter Wheat (Variety: Wildfire) Stubbles

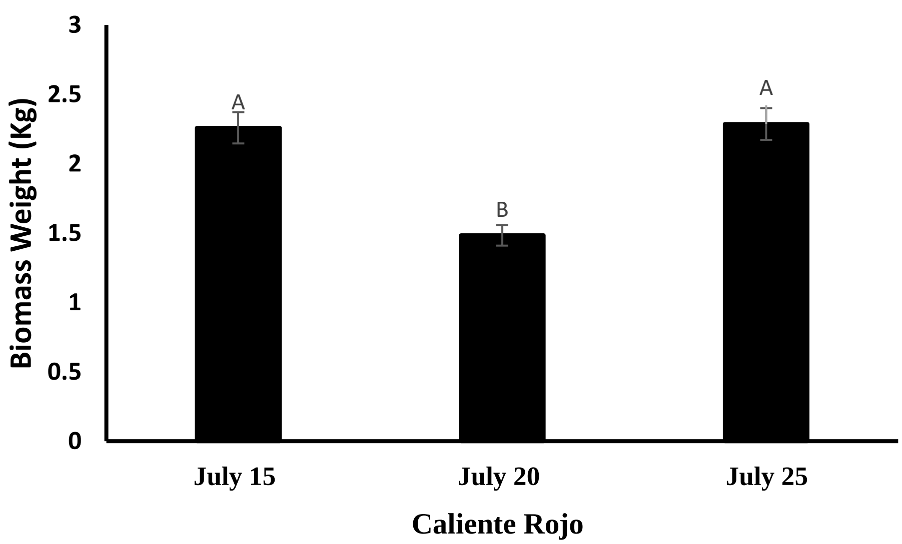
2). Field MB-3

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.



2020-Growing Season

Treatments

<u>Primary Treatments</u>

- Seeding on Cereal Stubbles (Fall Rye, and Winter Wheat)
- Seeding on Non-stubble land

<u>Secondary Treatments</u>

- Seeding dates (2 dates)
- Seed Treatment (Treated vs non-treated)

Results

Fall Rye Stubbles

Date 1 vs Date 2:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the seeding date 1 compared to the seeding date 2 for all mustard varieties within the 1 m^2 harvested area (Fig. 1, 2, & 3).

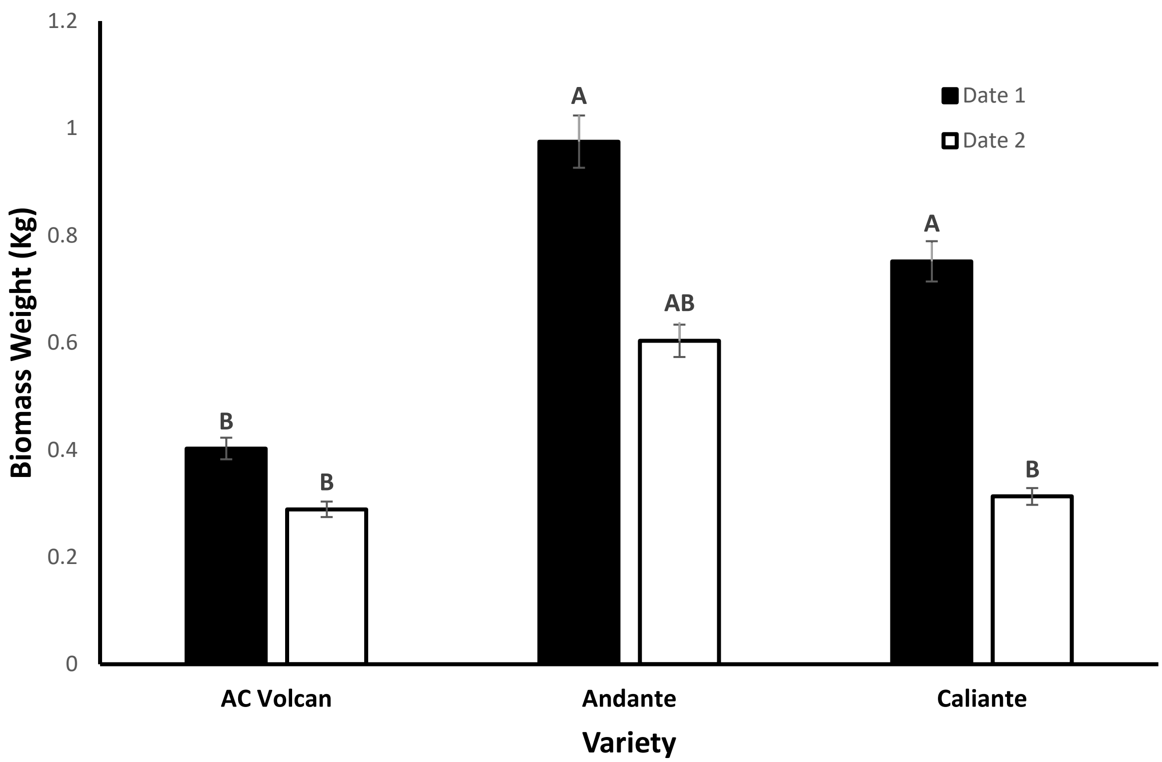


Fig. 1 Biomass of mustard varieties seeded on Fall Rye Stubbles – Seeding dates comparison

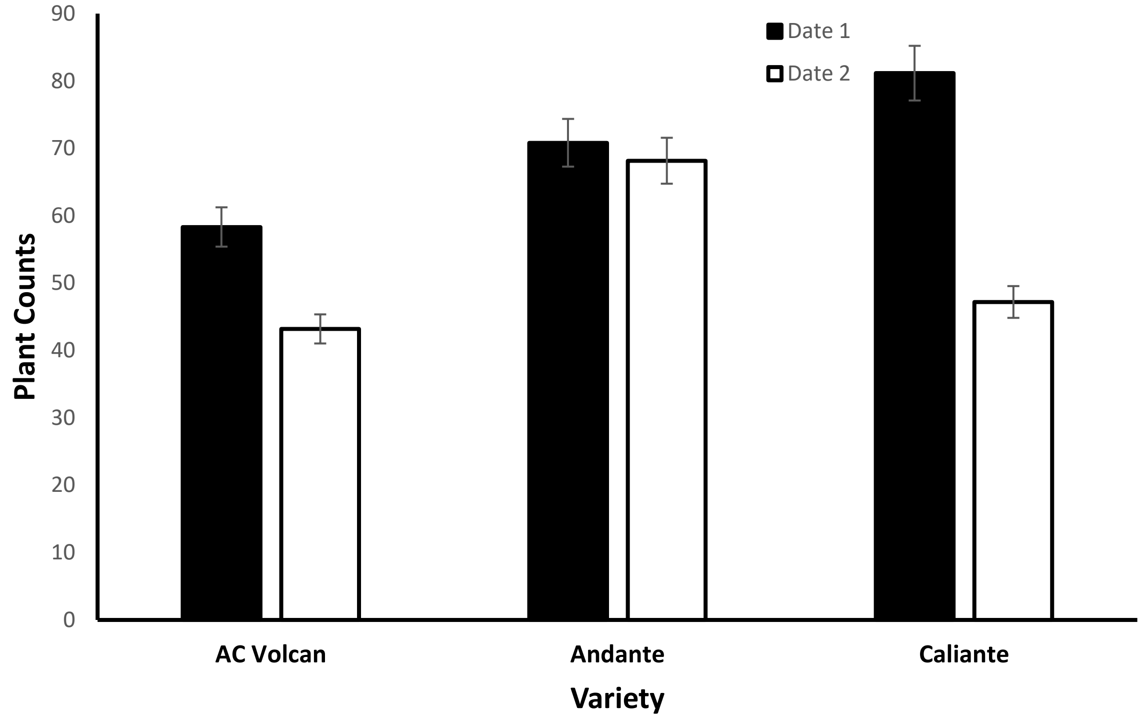


Fig. 2 Plant Counts of mustard varieties seeded on Fall Rye Stubbles – Seeding dates comparison

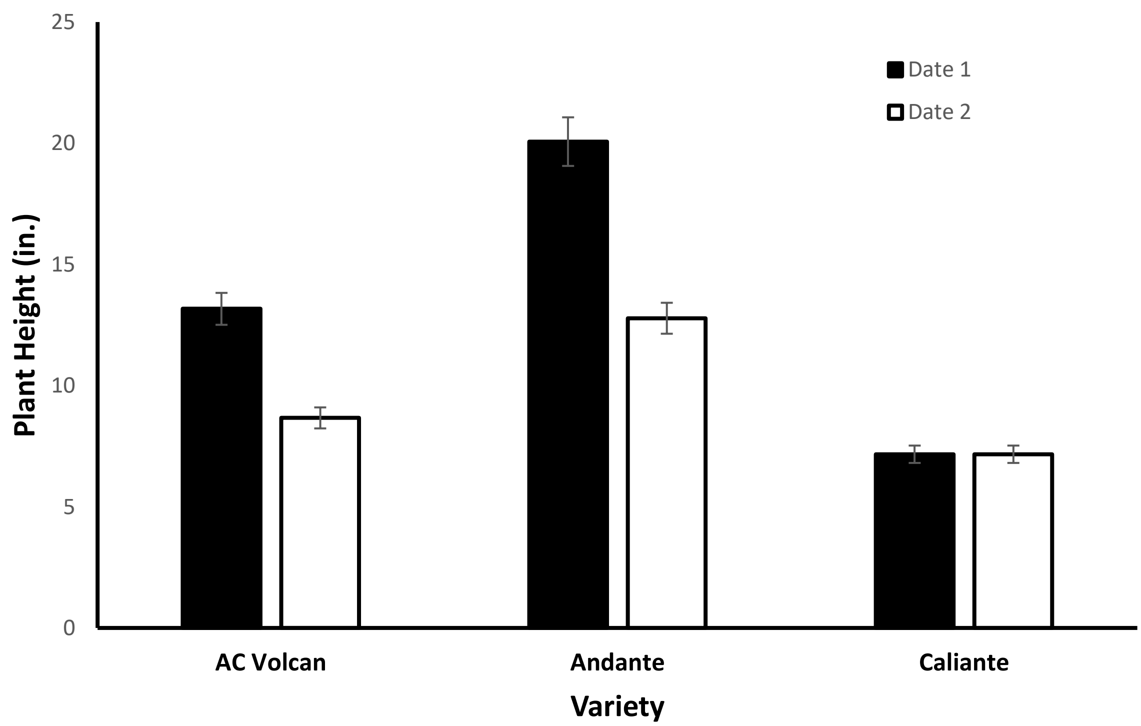


Fig. 3 Plant Heights of mustard varieties seeded on Fall Rye Stubbles – Seeding dates comparison

Treated Seed vs Non-treated Seed:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the treated seed treatment compared to the non-treated seed treatment for all mustard varieties within the 1 m^2 harvested area (Fig. 4, 5, & 6).

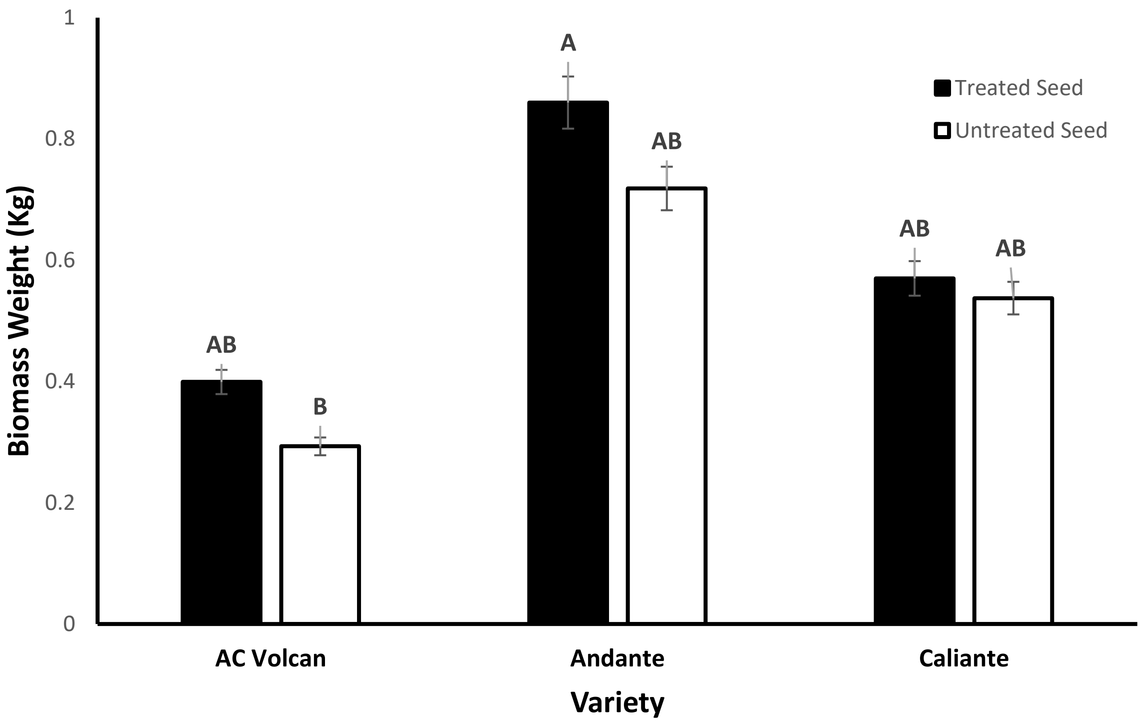


Fig. 4 Biomass of mustard varieties seeded on Fall Rye Stubbles – Seed treatment comparison

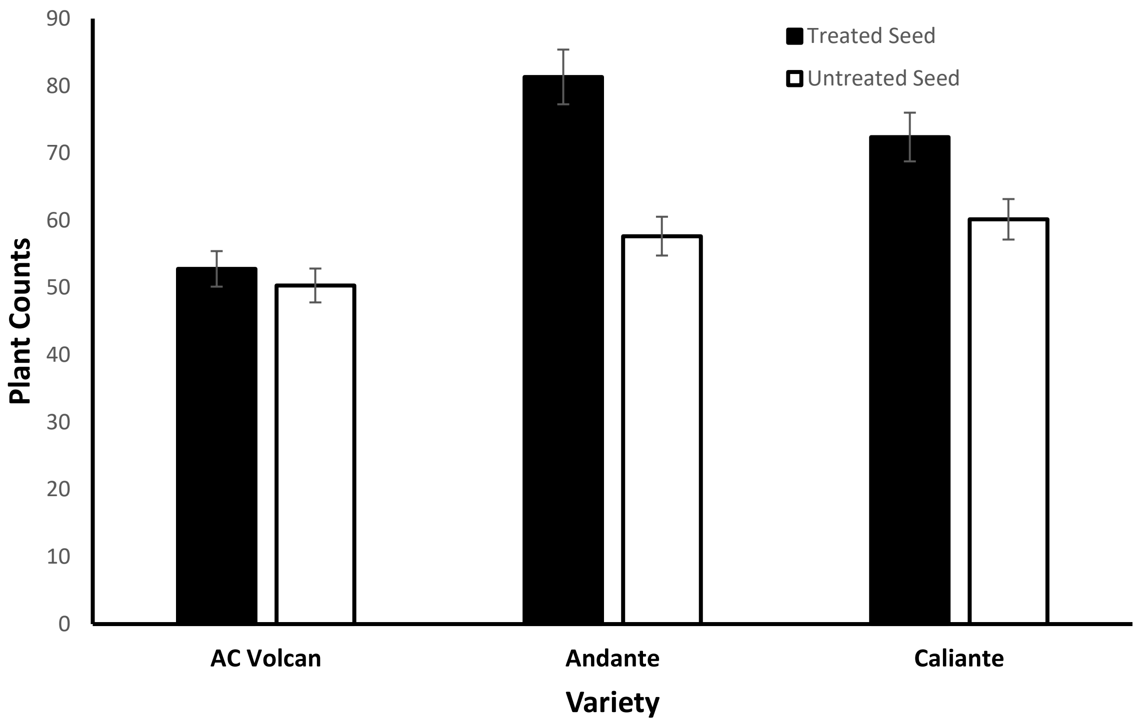


Fig. 5 Plant Counts of mustard varieties seeded on Fall Rye Stubbles – Seed treatment comparison

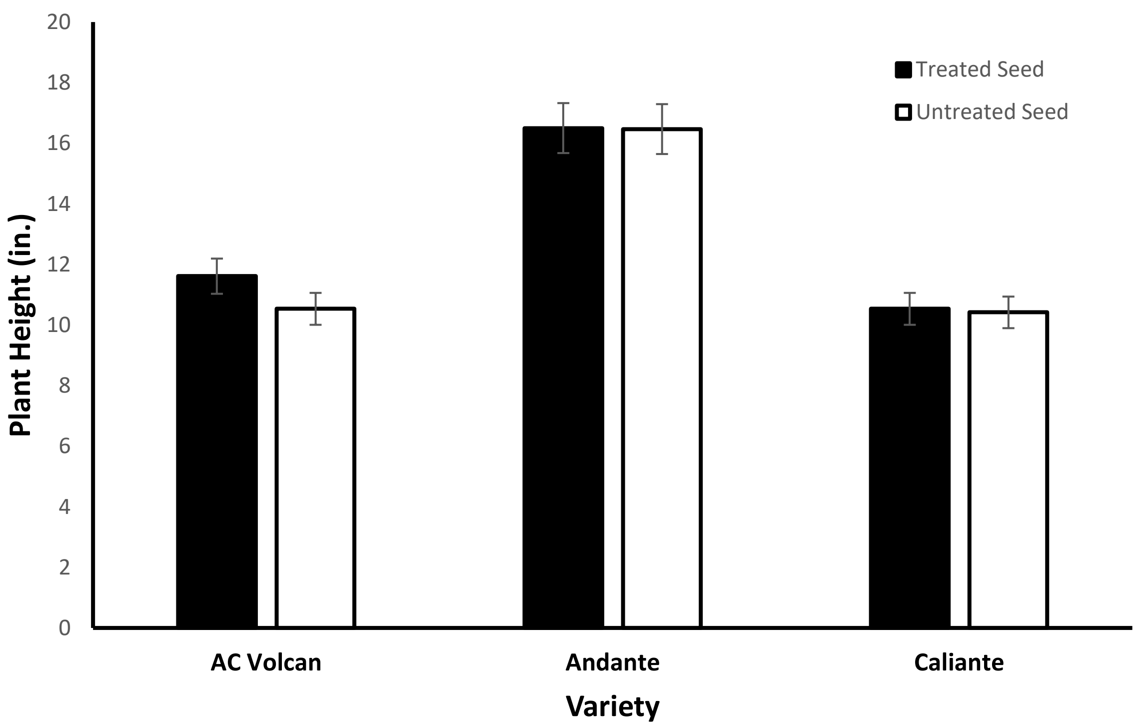


Fig. 6 Plant Heights of mustard varieties seeded on Fall Rye Stubbles – Seed treatment comparison

Winter Wheat Stubbles

Date 1 vs Date 2:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the seeding date 1 compared to the seeding date 2 for all mustard varieties within the 1 m^2 harvested area (Fig. 7, 8, & 9).

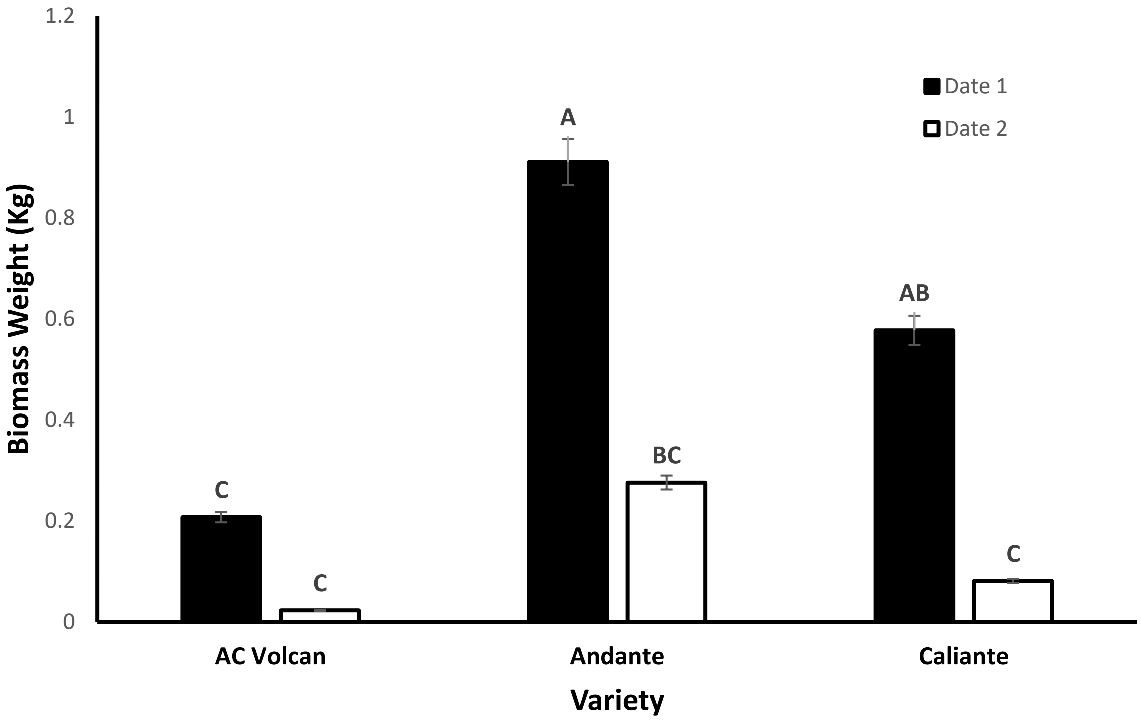


Fig. 7 Biomass of mustard varieties seeded on Winter Wheat Stubbles – Seeding dates comparison

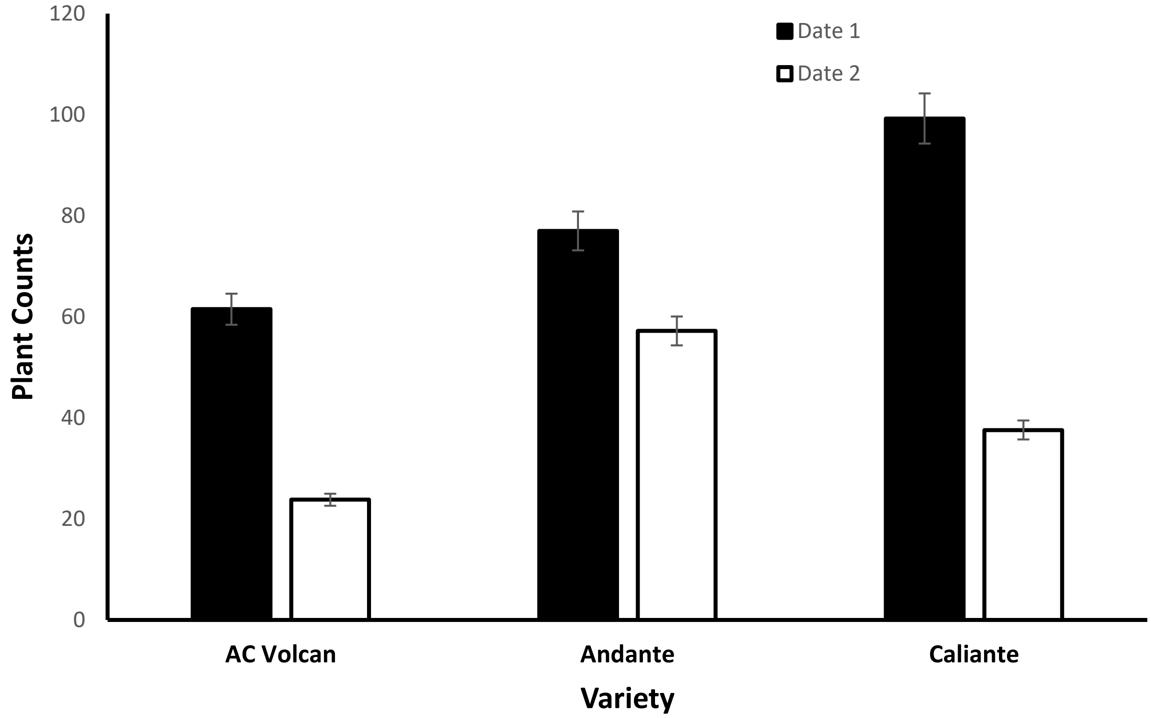


Fig. 8 Plant Counts of mustard varieties seeded on Winter Wheat Stubbles – Seeding dates comparison

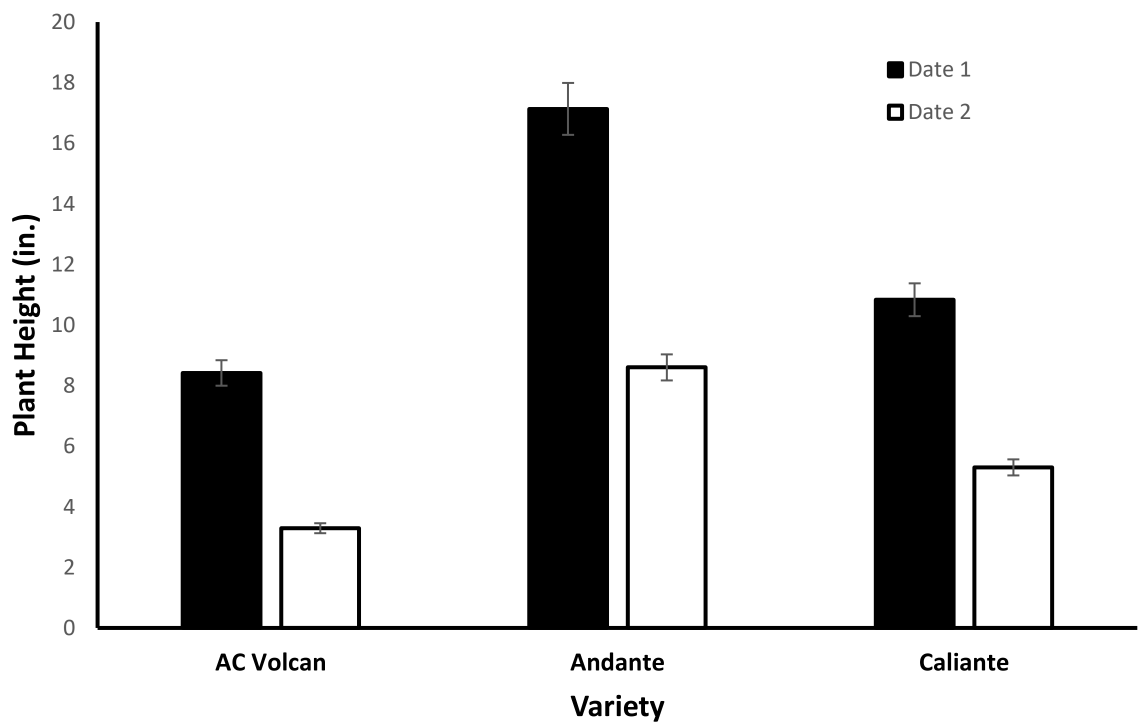


Fig. 9 Plant Heights of mustard varieties seeded on Winter Wheat Stubbles – Seeding dates comparison

Treated Seed vs Non-treated Seed:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the treated seed treatment compared to the non-treated seed treatment for all mustard varieties within the 1 m^2 harvested area (Fig. 10, 11, & 12).

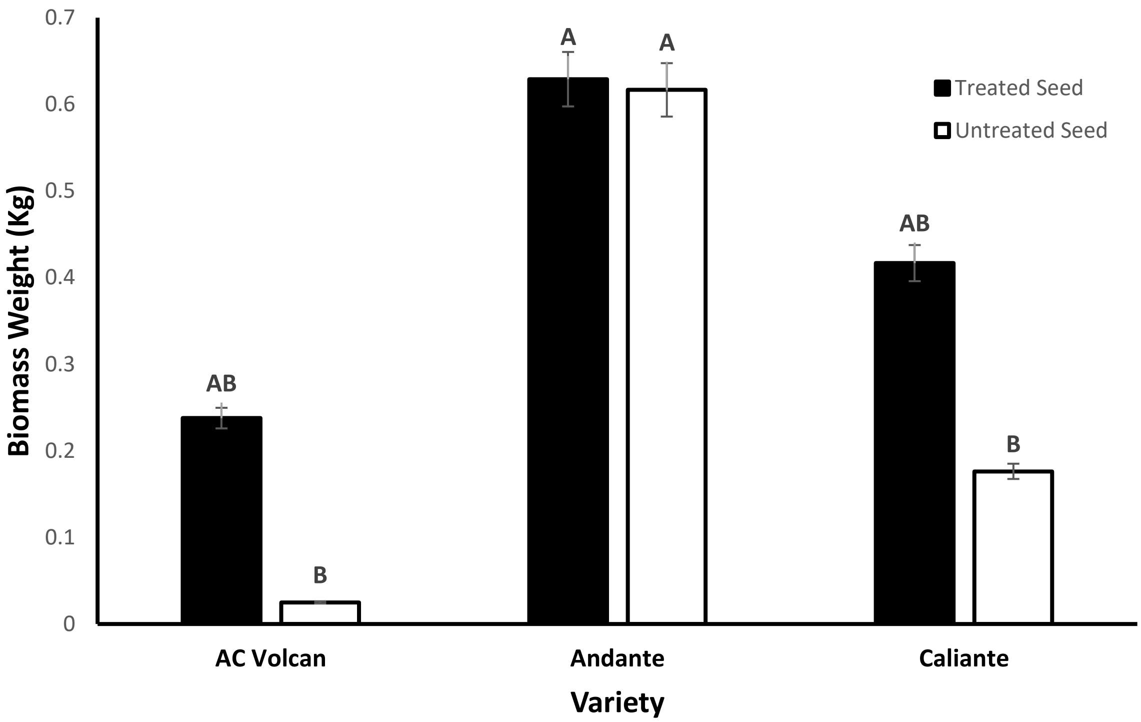


Fig. 10 Biomass of mustard varieties seeded on Winter Wheat Stubbles – Seed treatment comparison

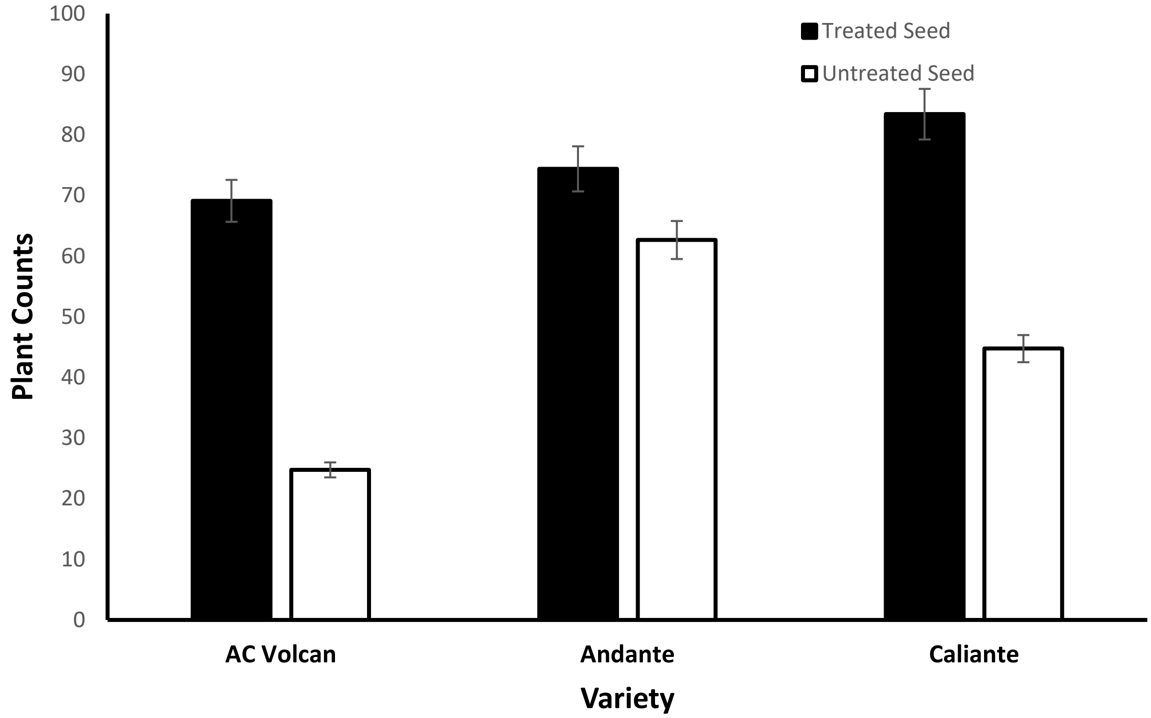


Fig. 11 Plant Counts of mustard varieties seeded on Winter Wheat Stubbles – Seed treatment comparison

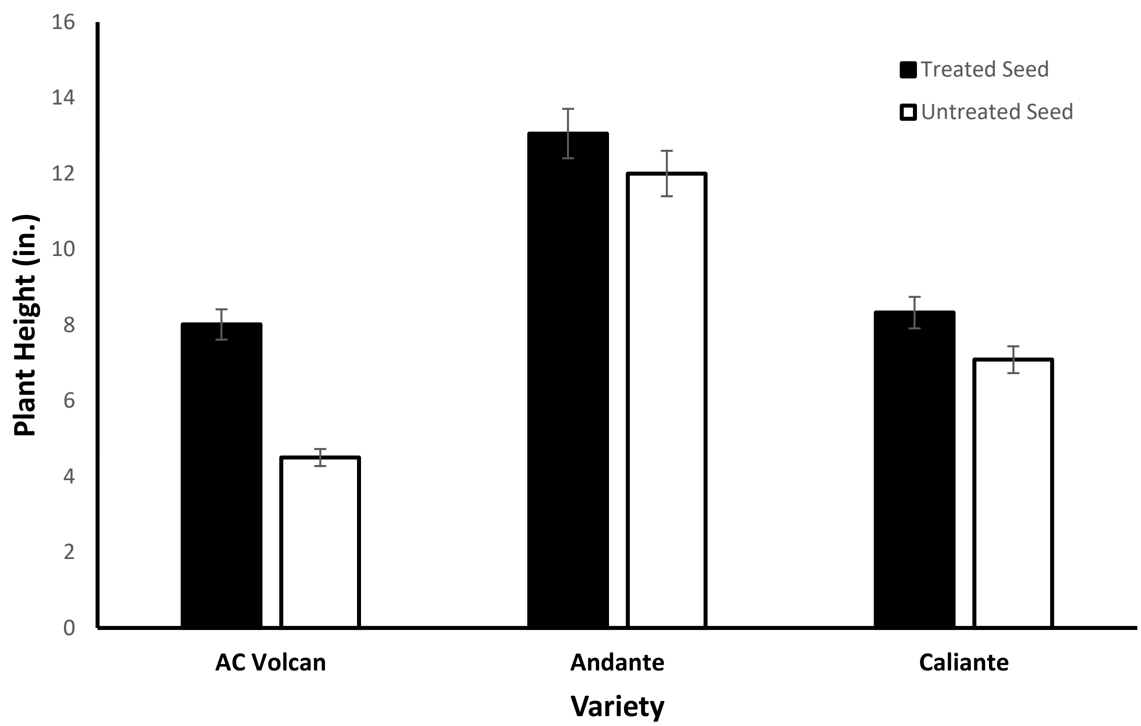


Fig. 12 Plant Heights of mustard varieties seeded on Winter Wheat Stubbles – Seed treatment comparison

Non-stubble Land

Date 1 vs Date 2:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the seeding date 1 compared to the seeding date 2 for all mustard varieties within the 1 m^2 harvested area (Fig. 13, 14, & 15).

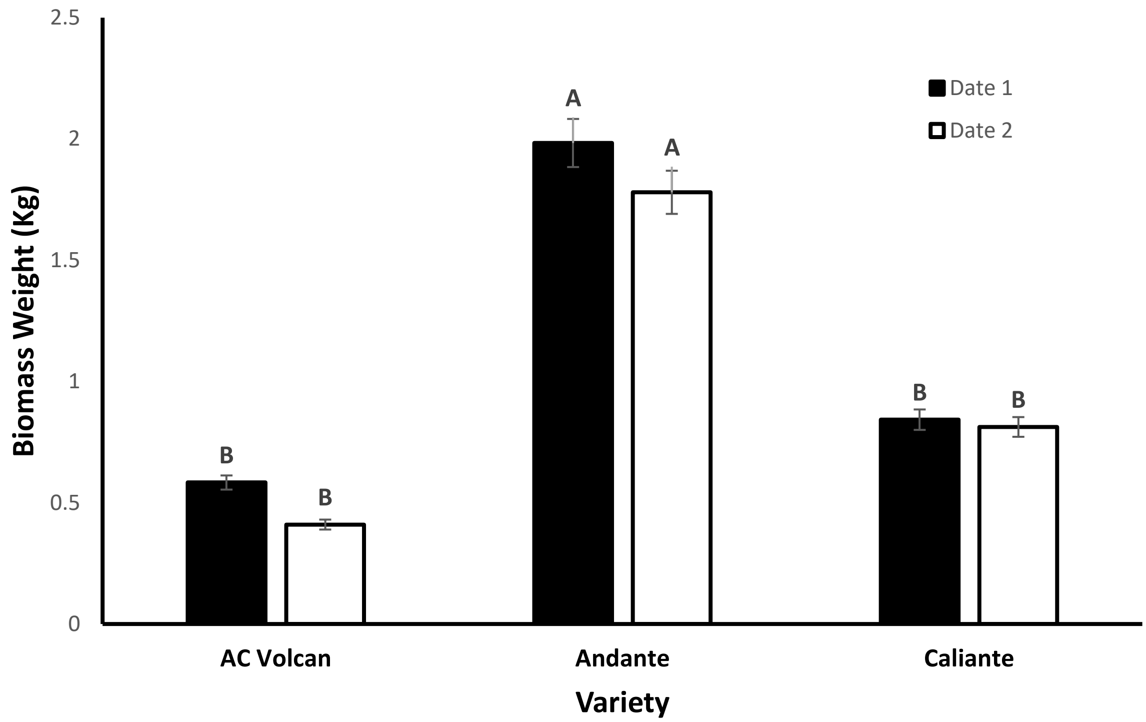


Fig. 13 Biomass of mustard varieties seeded on Non-stubble land – Seeding dates comparison

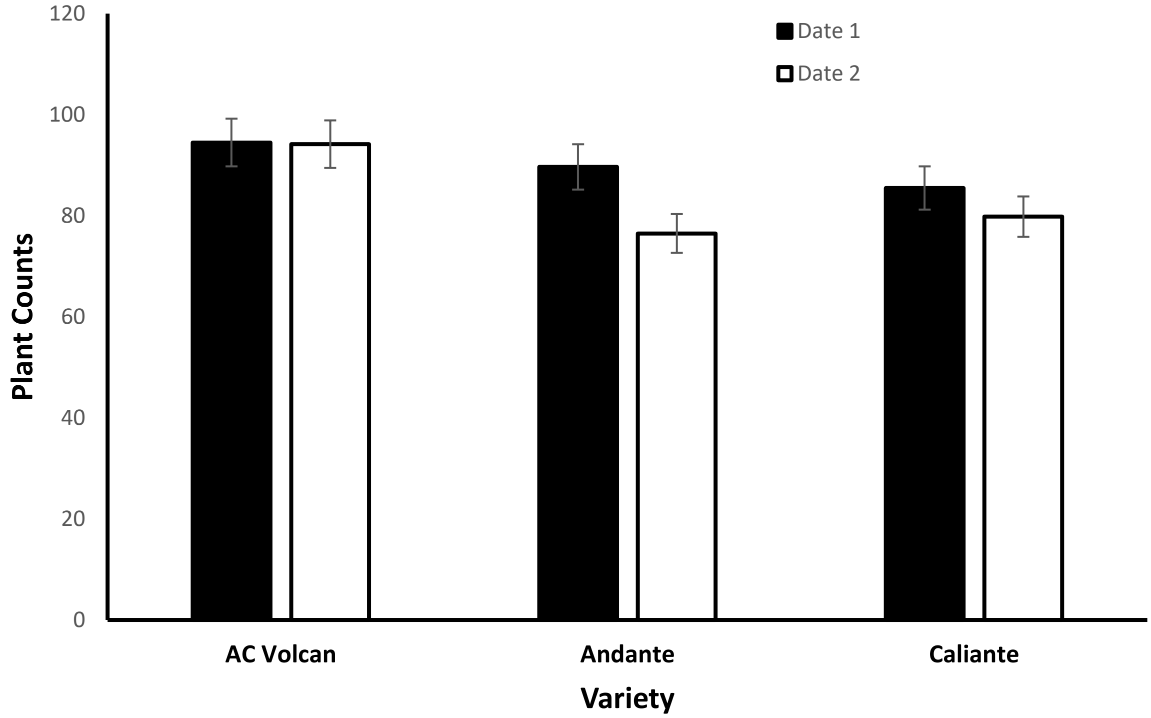


Fig. 14 Plant Counts of mustard varieties seeded on Non-stubble land – Seeding dates comparison

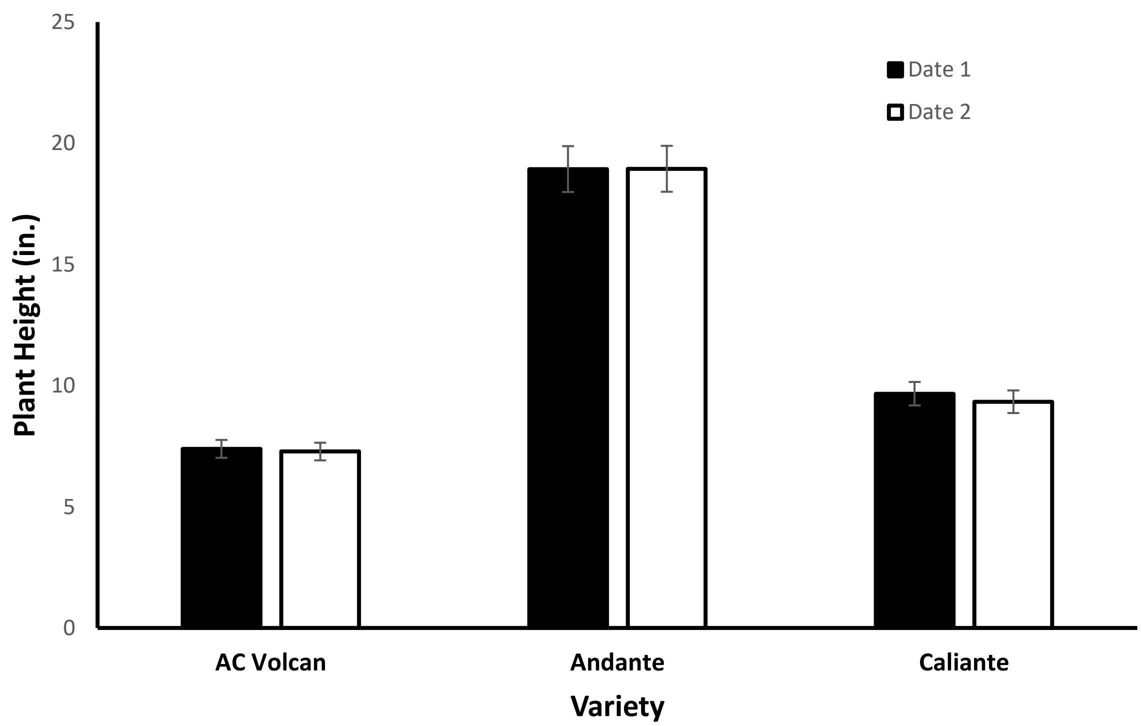


Fig. 15 Plant Heights of mustard varieties seeded on Non-stubble land – Seeding dates comparison

Treated Seed vs Non-treated Seed:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the treated seed treatment compared to the non-treated seed treatment for all mustard varieties within the 1 m^2 harvested area (Fig. 16, 17, & 18).

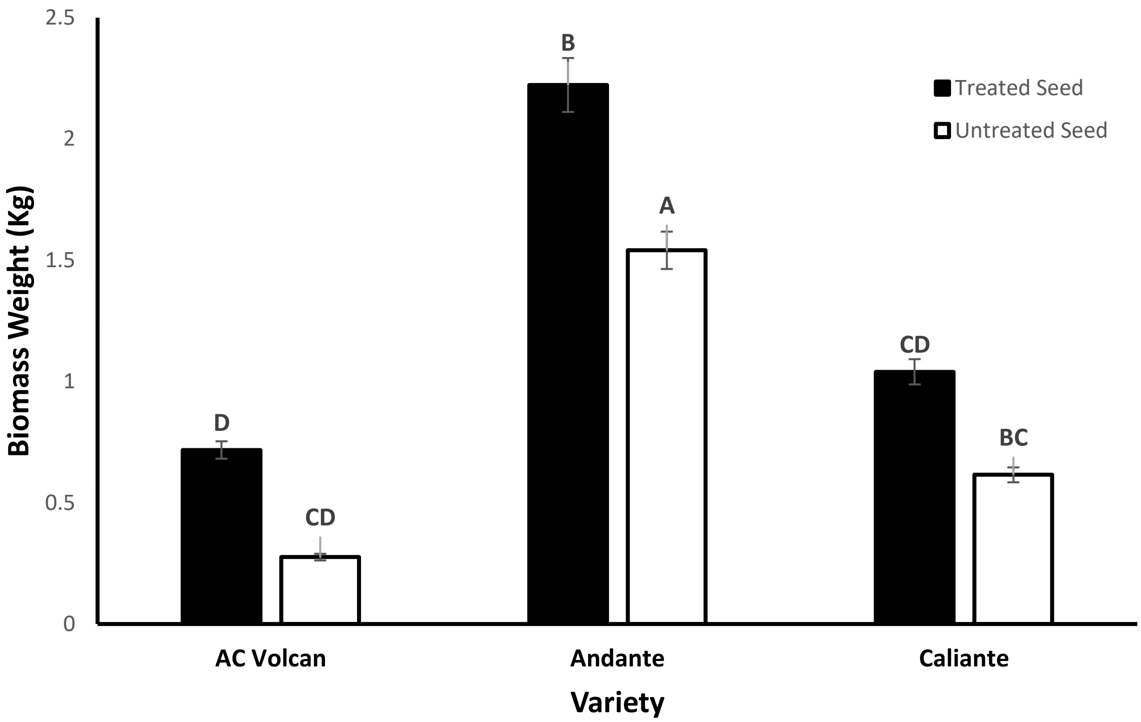


Fig. 16 Biomass of mustard varieties seeded on Non-stubbles land – Seed treatment comparison

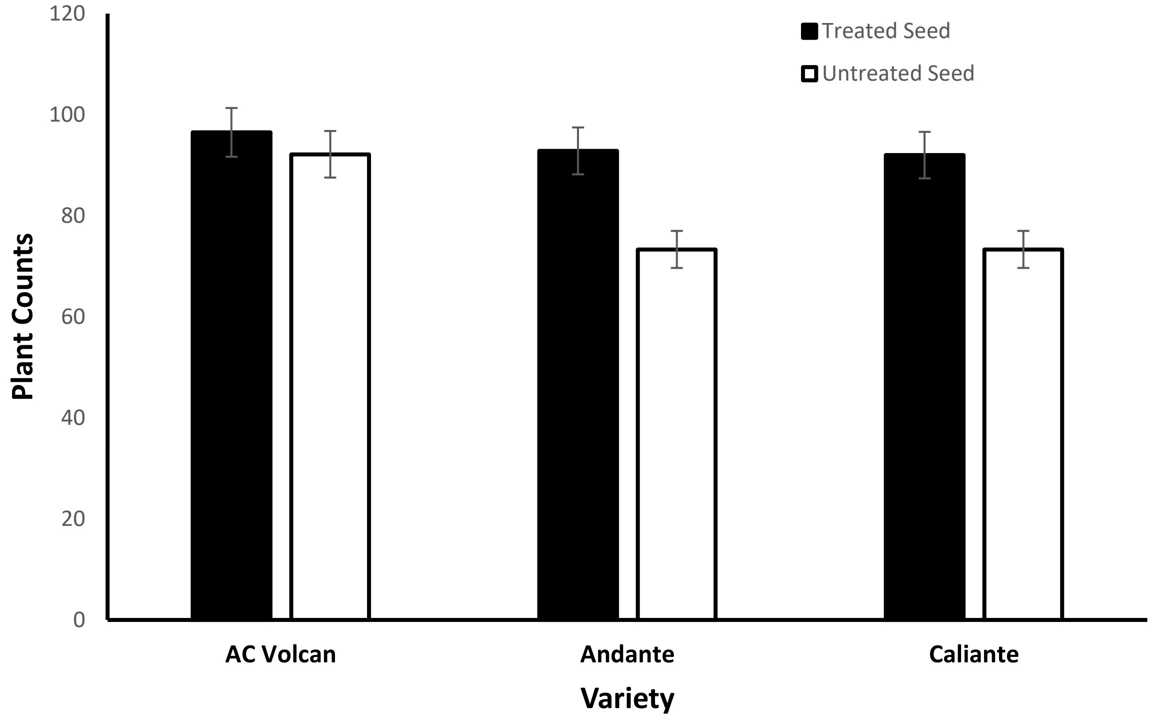


Fig. 17 Plant Counts of mustard varieties seeded on Non-stubbles land – Seed treatment comparison

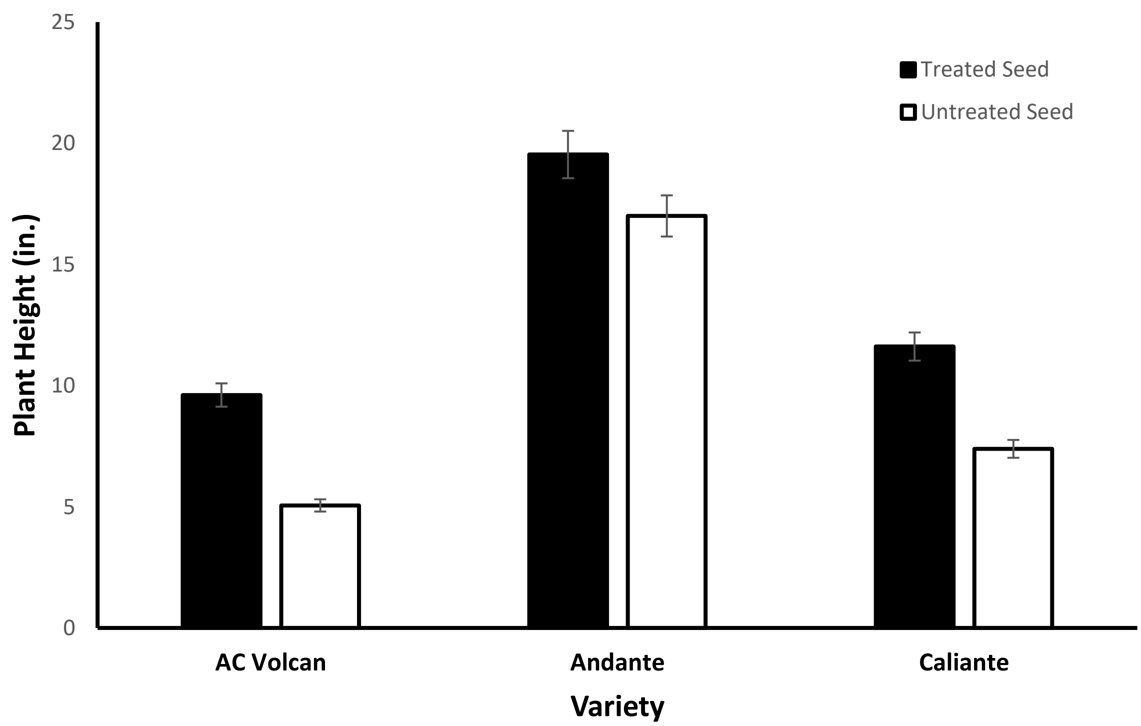


Fig. 18 Plant Heights of mustard varieties seeded on Non-stubbles land – Seed treatment comparison

General Comparison

Date 1 vs Date 2:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the seeding date 1 compared to the seeding date 2 for all mustard varieties within the 1 m^2 harvested area (Fig. 19, 20, & 21).

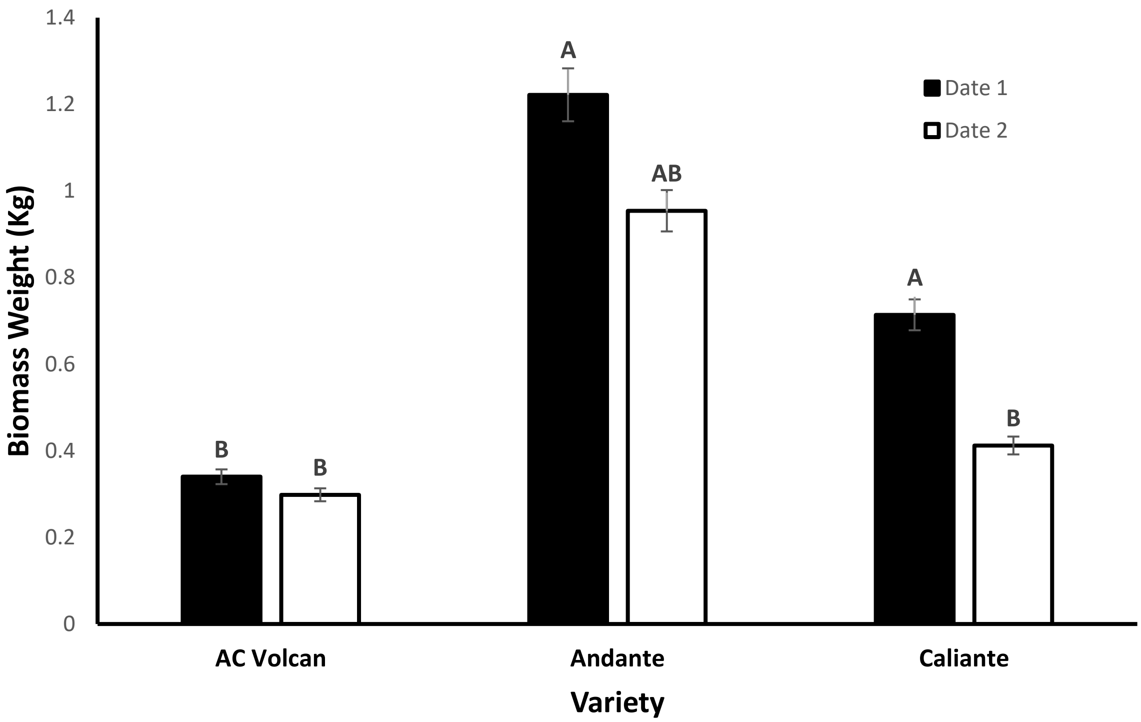


Fig. 19 Biomass of mustard varieties – Seeding dates comparison

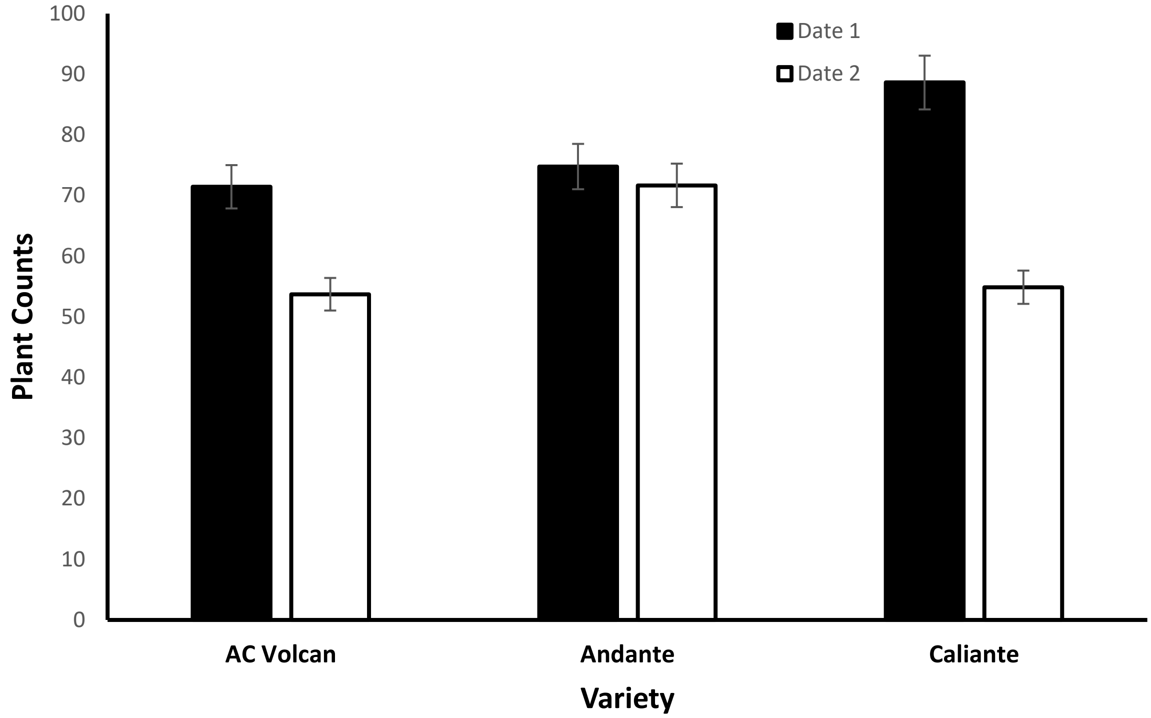


Fig. 20 Plant Counts of mustard varieties – Seeding dates comparison

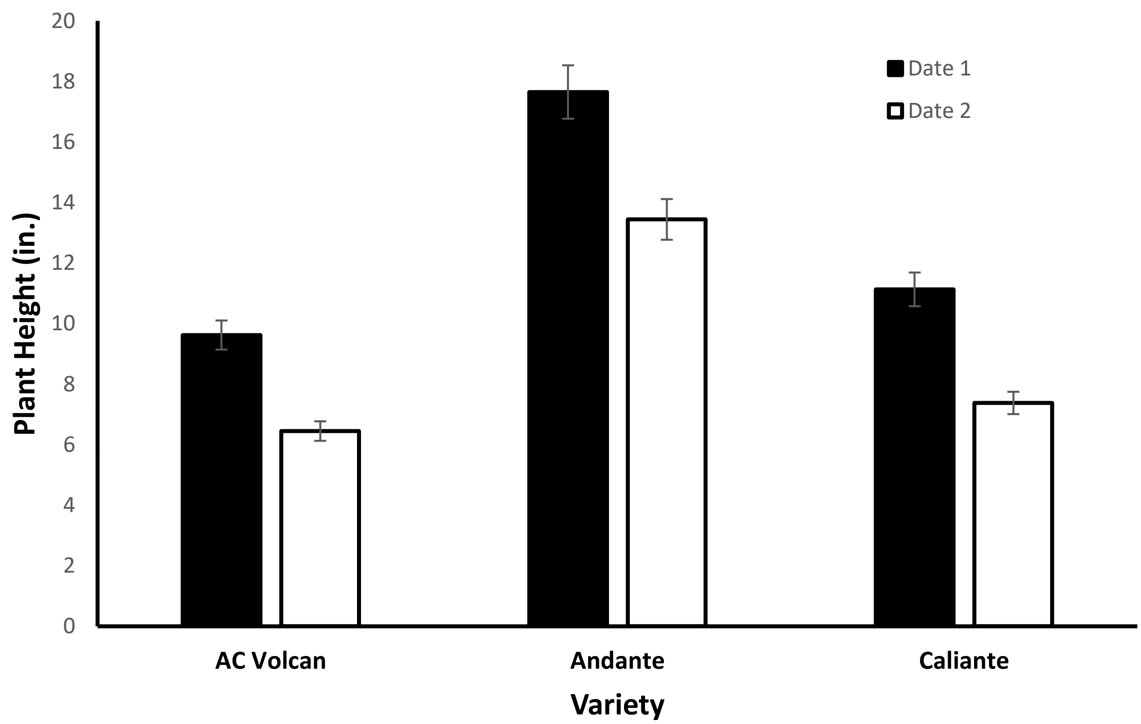


Fig. 21 Plant Heights of mustard varieties – Seeding dates comparison

Treated Seed vs Non-treated Seed:

A relatively higher proportion of biomass, plant counts, and plant height was observed in the treated seed treatment compared to the non-treated seed treatment for all mustard varieties within the 1 m^2 harvested area (Fig. 22, 23, & 24).

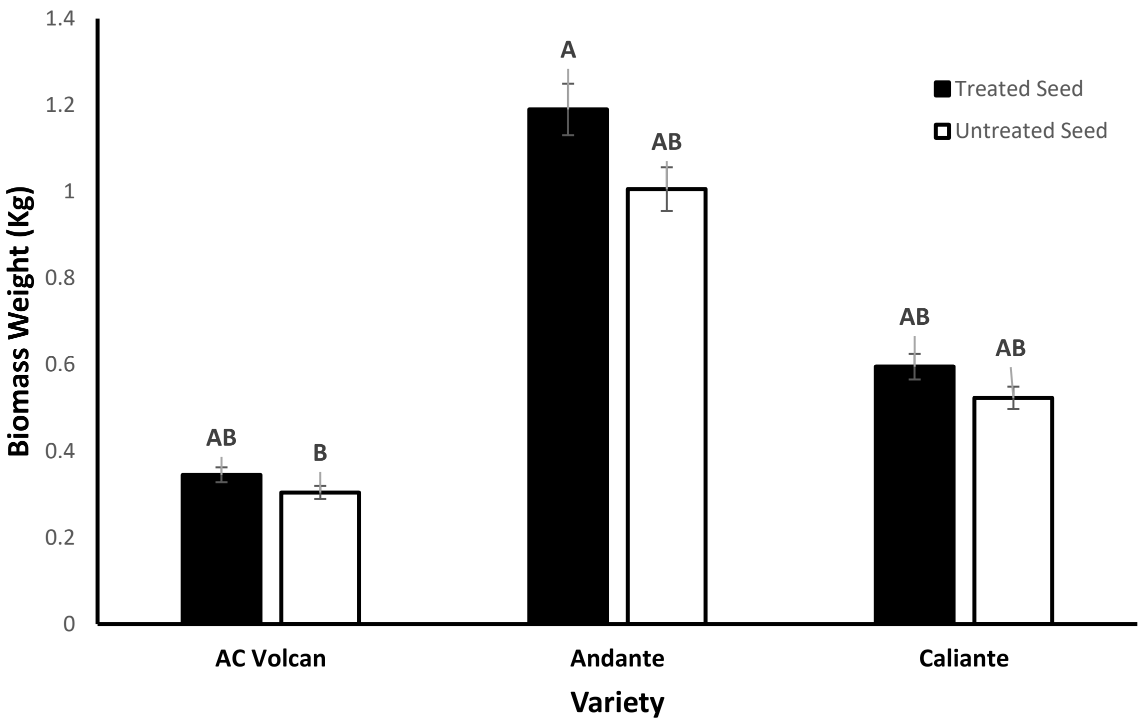


Fig. 22 Biomass of mustard varieties – Seed treatment comparison

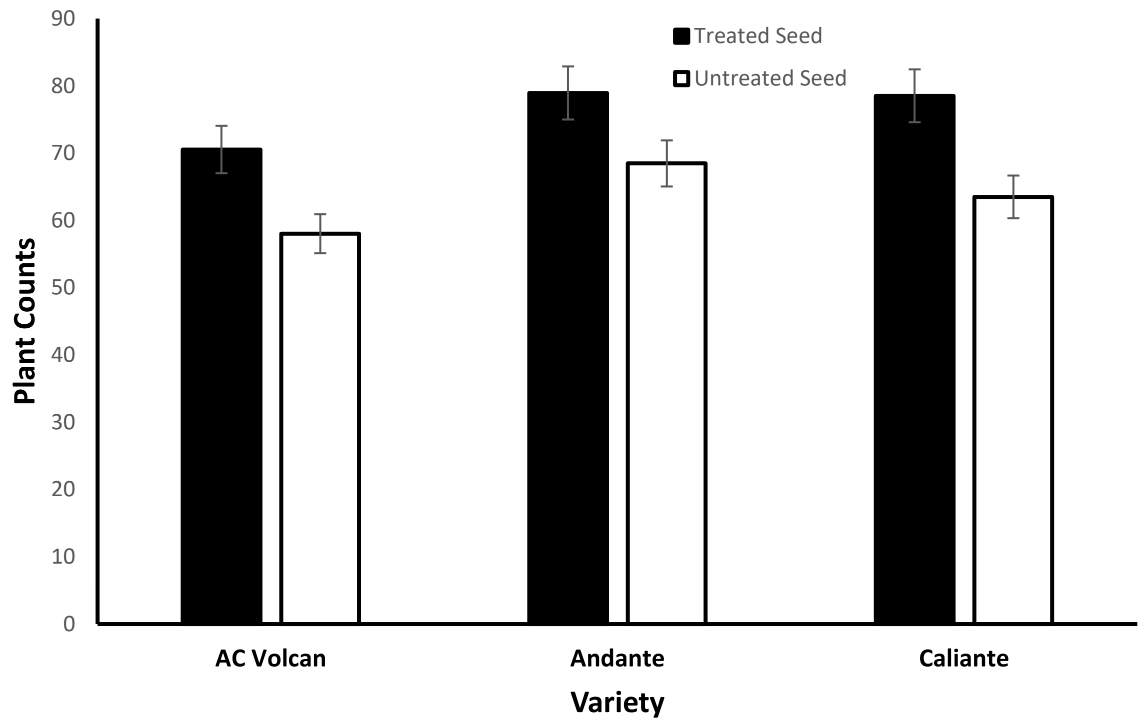


Fig. 23 Plant Counts of mustard varieties – Seed treatment comparison

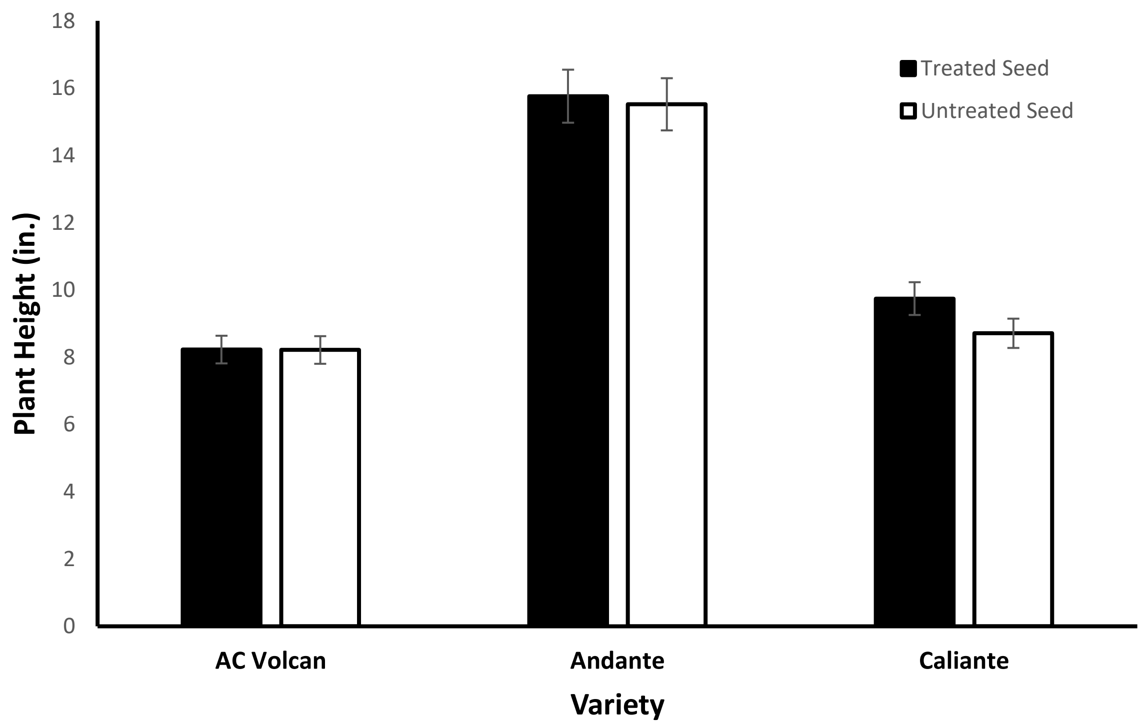


Fig. 24 Plant Heights of mustard varieties – Seed treatment comparison

Data collected from Producers' Field:

Field Name: MB-8

Data was collected at 4 different sites (Locations 1-4) with same treatment but different land features. Variability in biomass density, plant counts, and plant heights is shown in Fig. 25, 26, & 27. The producer seeded the treated Caliente variety of mustard and an area of 1 m^2 for observations and analysis.

Average Biomass: 2.78 Kg

Average Plant Counts: 142

Average Plant Height: 35 in.

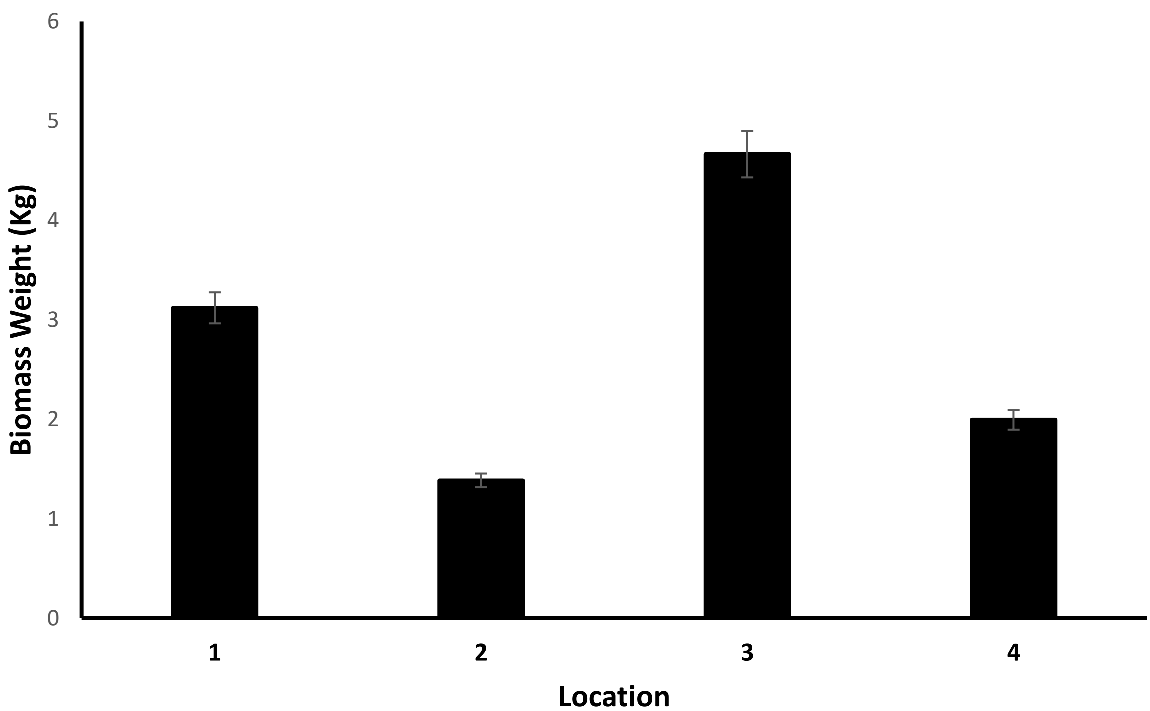


Fig. 25 Biomass weight at MB-8

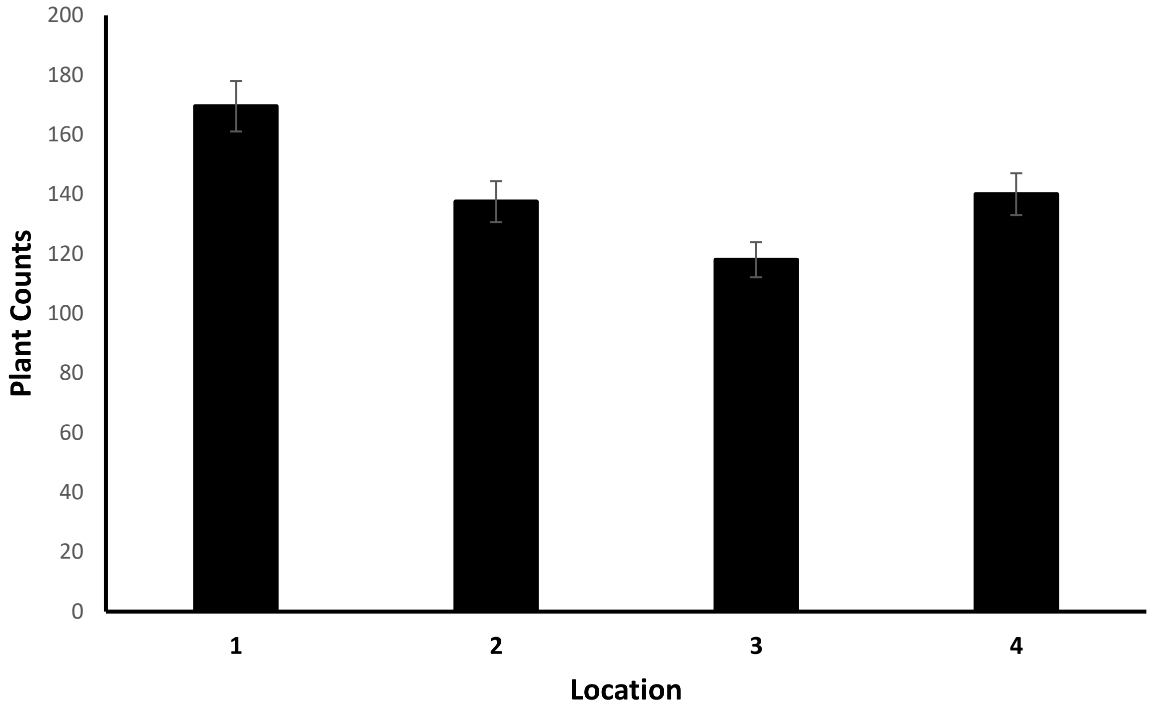


Fig. 26 Plant Counts at MB-8

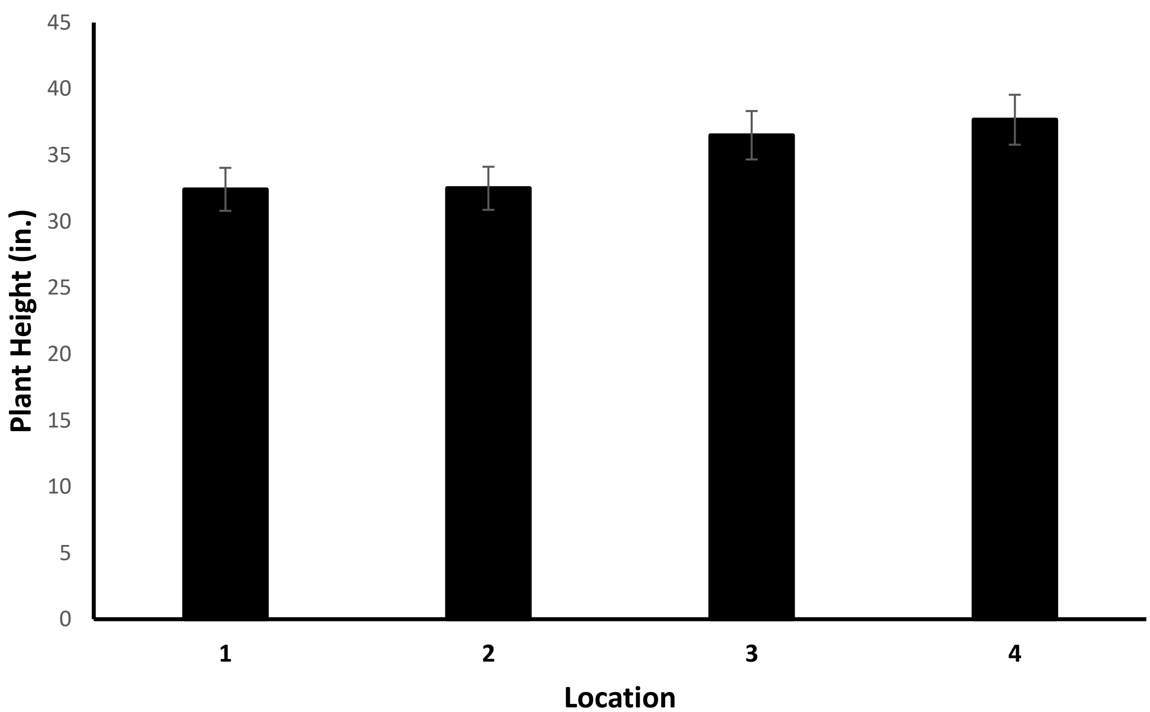


Fig. 27 Plant Heights at MB-8

Field Name: MB-7

Data was collected at 4 different sites (Locations 1-4) with same treatment but different land features. Variability in biomass density, plant counts, and plant heights is shown in Fig. 28, 29, & 30. The producer seeded the treated Caliente variety of mustard and an area of 1 m^2 for observations and analysis.

Average Biomass: 1.5 Kg

Average Plant Counts: 88

Average Plant Height: 14 in.

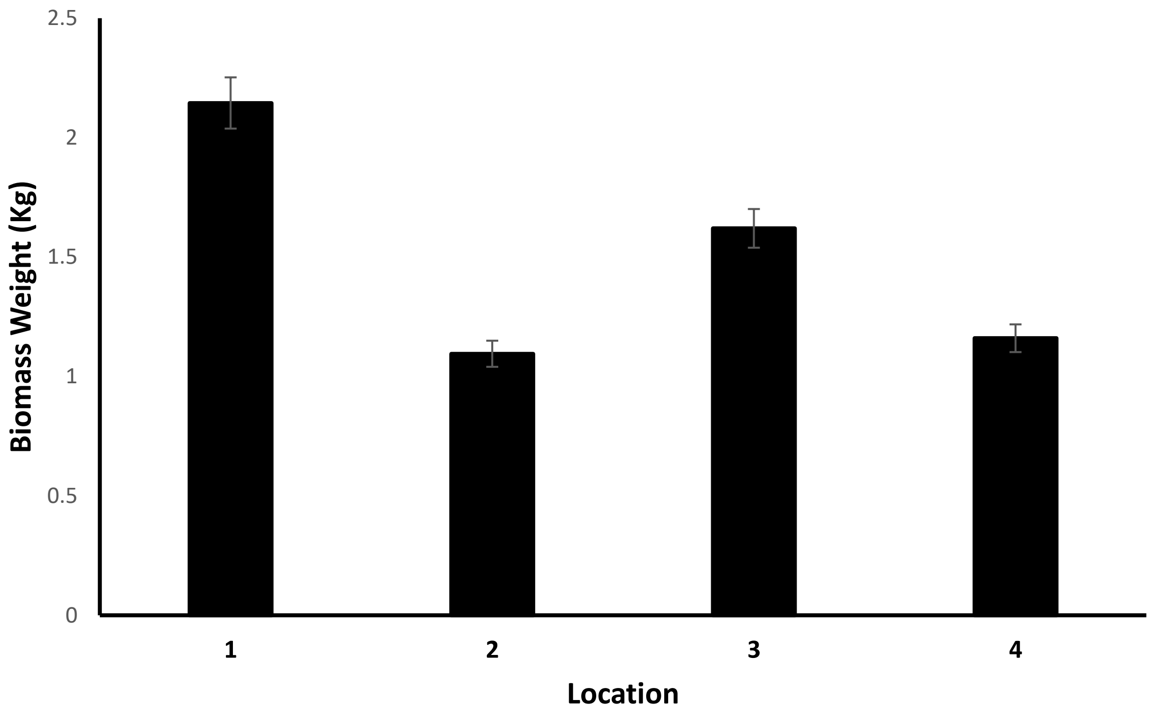


Fig. 28 Biomass weight at MB-7

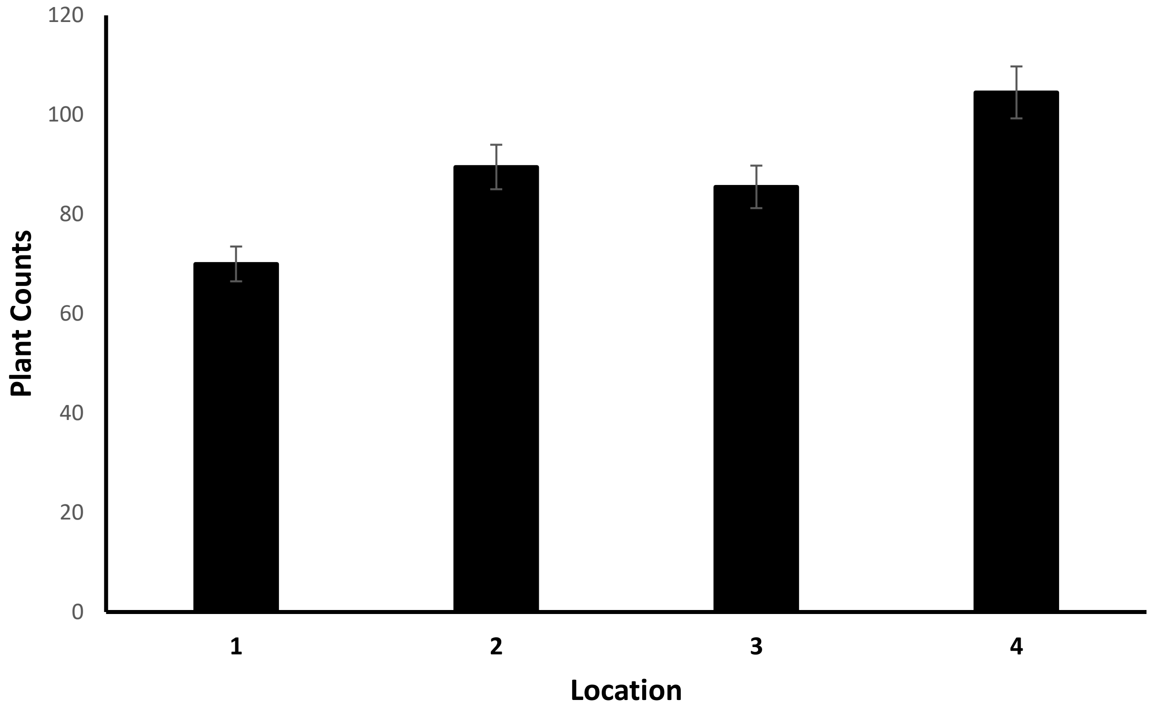


Fig. 29 Plant Counts at MB-7

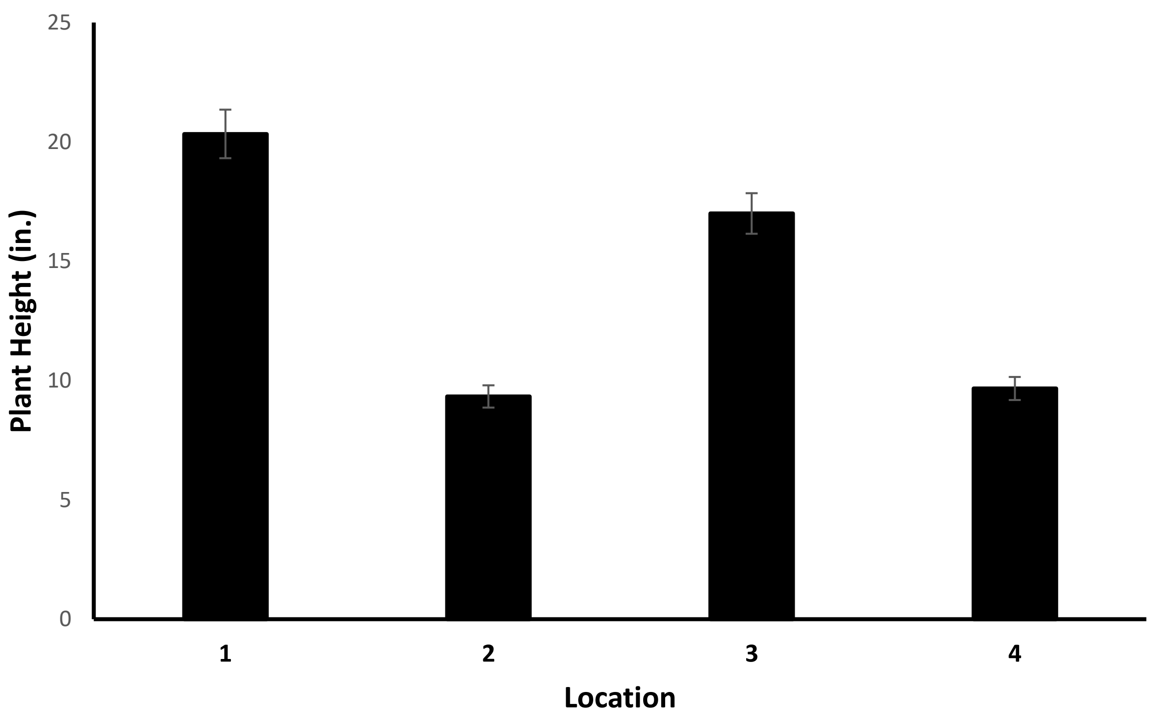


Fig. 30 Plant Heights at MB-7

Field Name: MB-10

Data was collected at 4 different sites (Locations 1-4) with same treatment but different land features. Variability in biomass density, plant counts, and plant heights is shown in Fig. 31, 32, & 33. The producer seeded the treated Caliente variety of mustard and an area of 1 m^2 for observations and analysis.

Average Biomass: 1.84 Kg

Average Plant Counts: 217

Average Plant Height: 23 in.

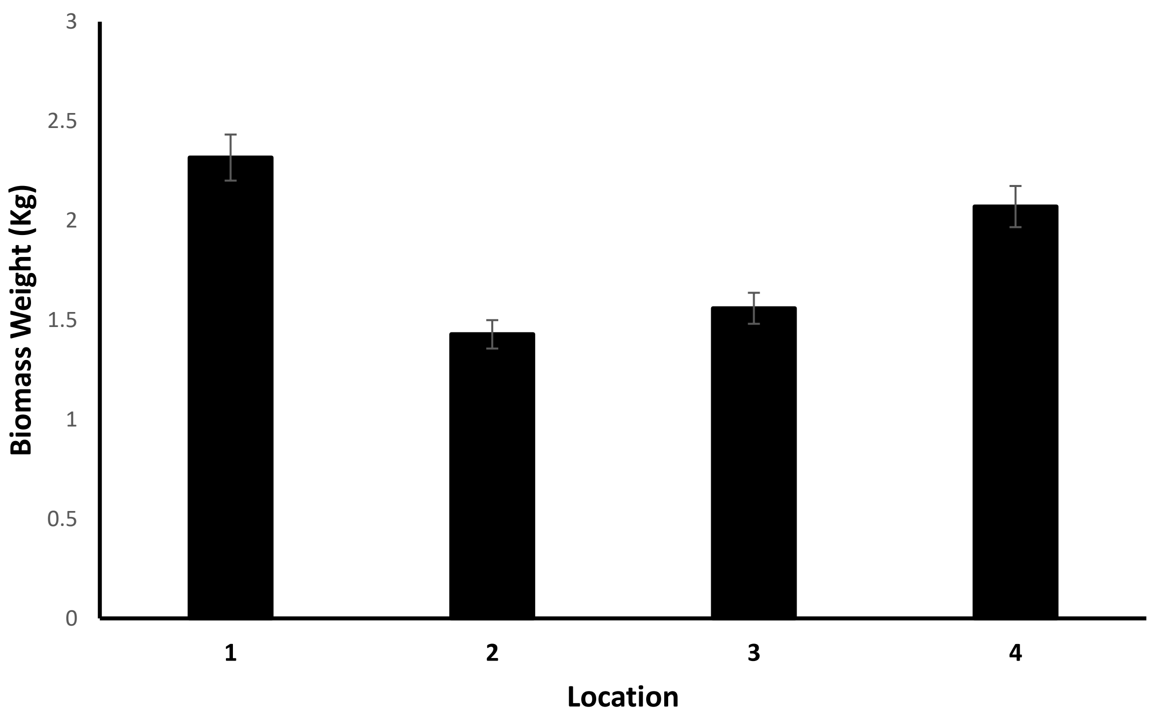


Fig. 30 Biomass weight at MB-10

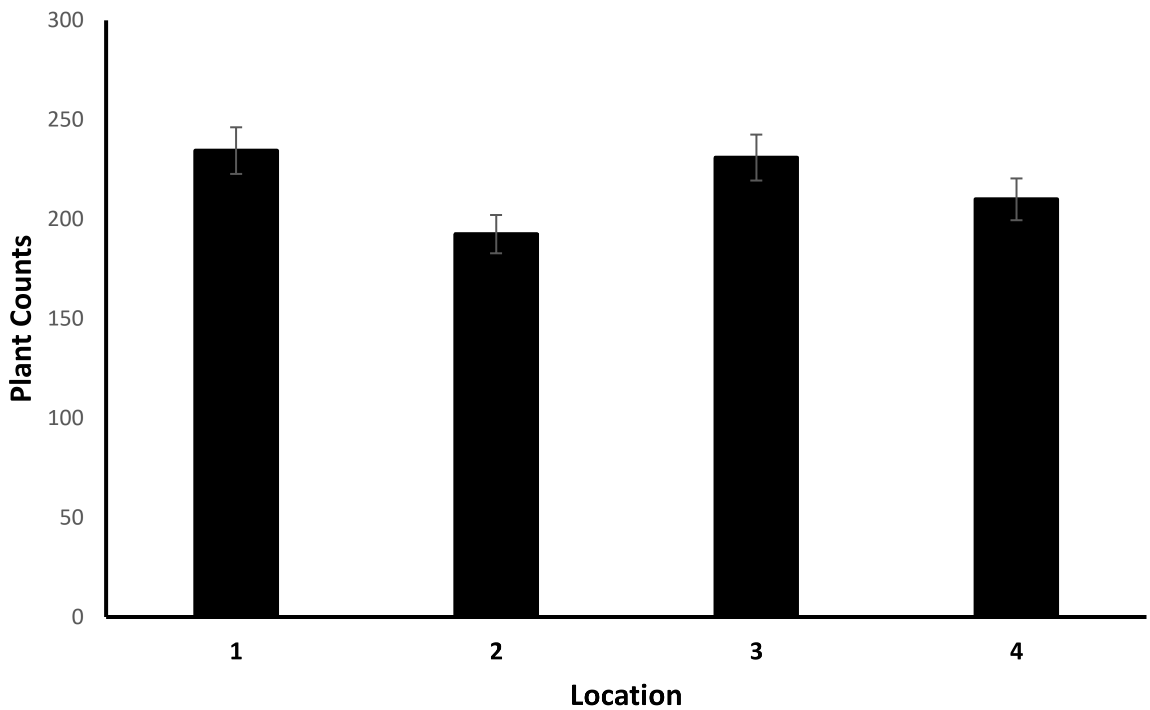


Fig. 31 Plant Counts at MB-10

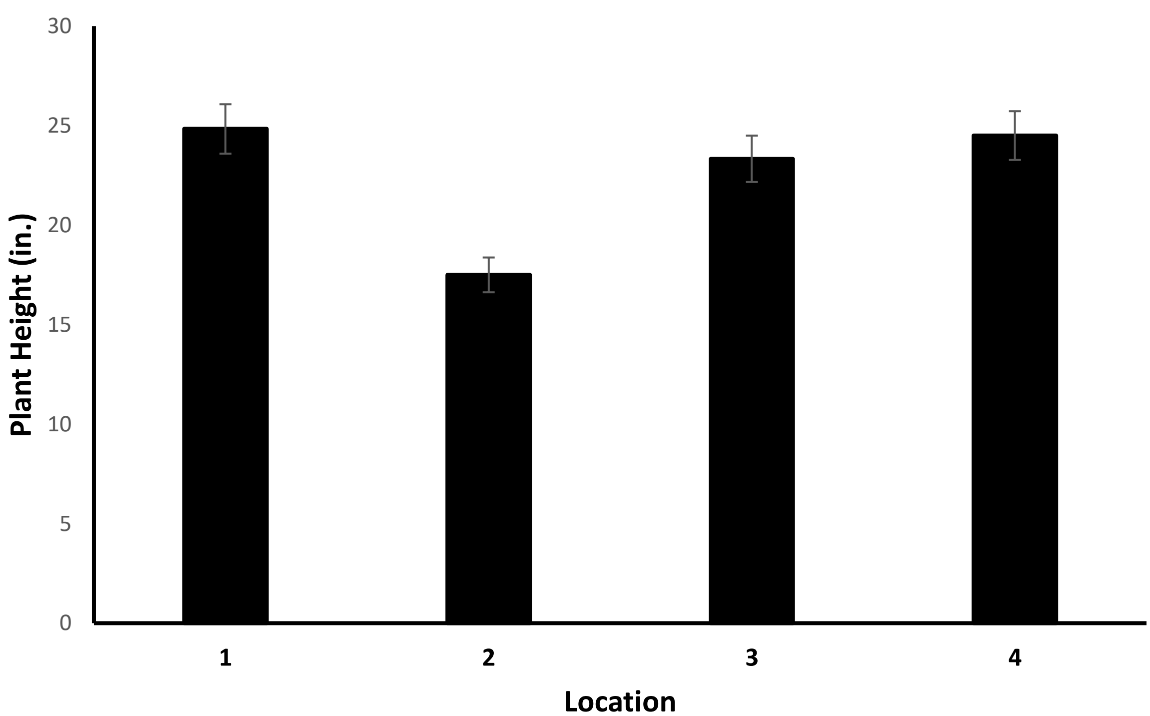


Fig. 31 Plant Heights at MB-10

Discussion:

This study demonstrates the necessity for planting the mustard crop in the early growing season if the goal is to maximize biomass production prior to late fall incorporation. Biofumigant mustard varieties planted early in the season (date 1) produced substantially more biomass than mustard planted late in the growing season (date 2). Moreover, mustard seed treated with Gaucho seed treatment produced more biomass and had higher percent cover in terms of plant counts and plant height in all mustard varieties. When using mustard as a biofumigant tool in the potato production systems, mustard should be planted as soon as the soil can be worked to maximize biomass production.

Biomass production is important, even when a cover crop is selected for a specific function. A mustard cover crop grown for its bio-fumigation properties or a legume cover crop grown for its nitrogen contribution is more likely to perform its intended function if it produces maximum amounts of biomass. Biomass production can be optimized by selecting the ideal cultivar and planting date.

Objective 2

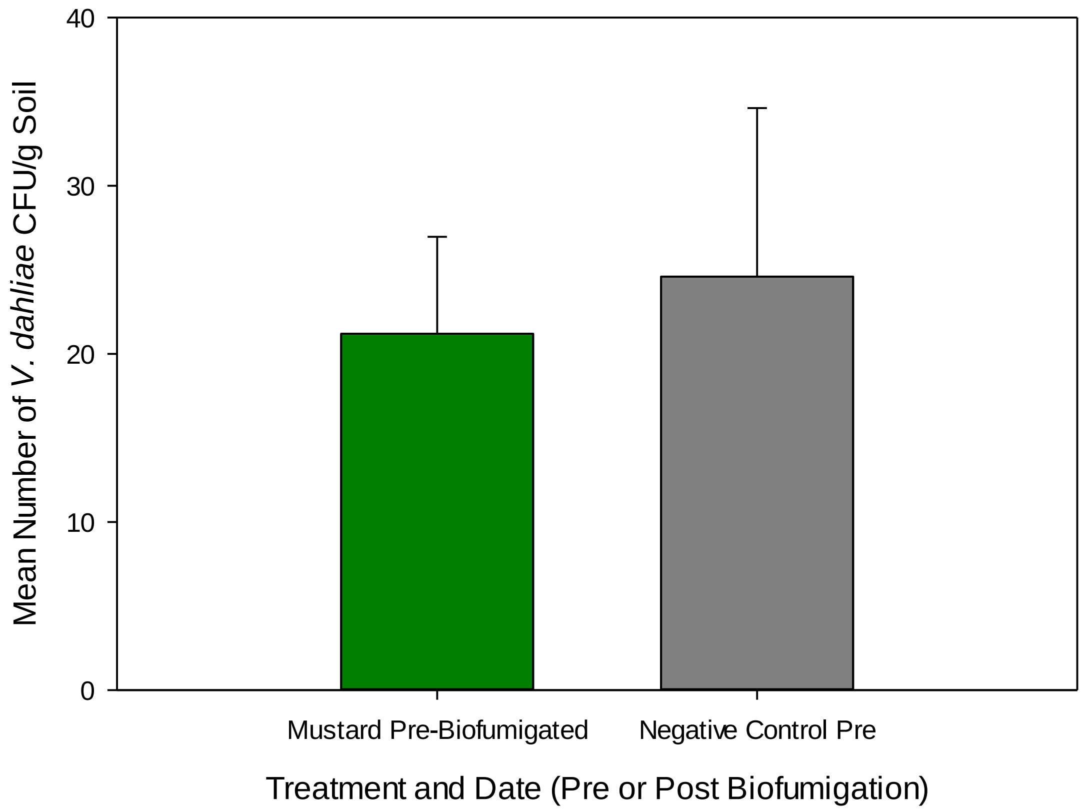
Six field sites were established in 2020 with generally one field site per grower cooperator (Table 1, below). 'Caliente Rojo' seed was treated with 16 cwt/acre of Gaucho in 2020, which when coupled with generally lower flea beetle pressure in 2020 than 2019, led to all mustard fields surviving until 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

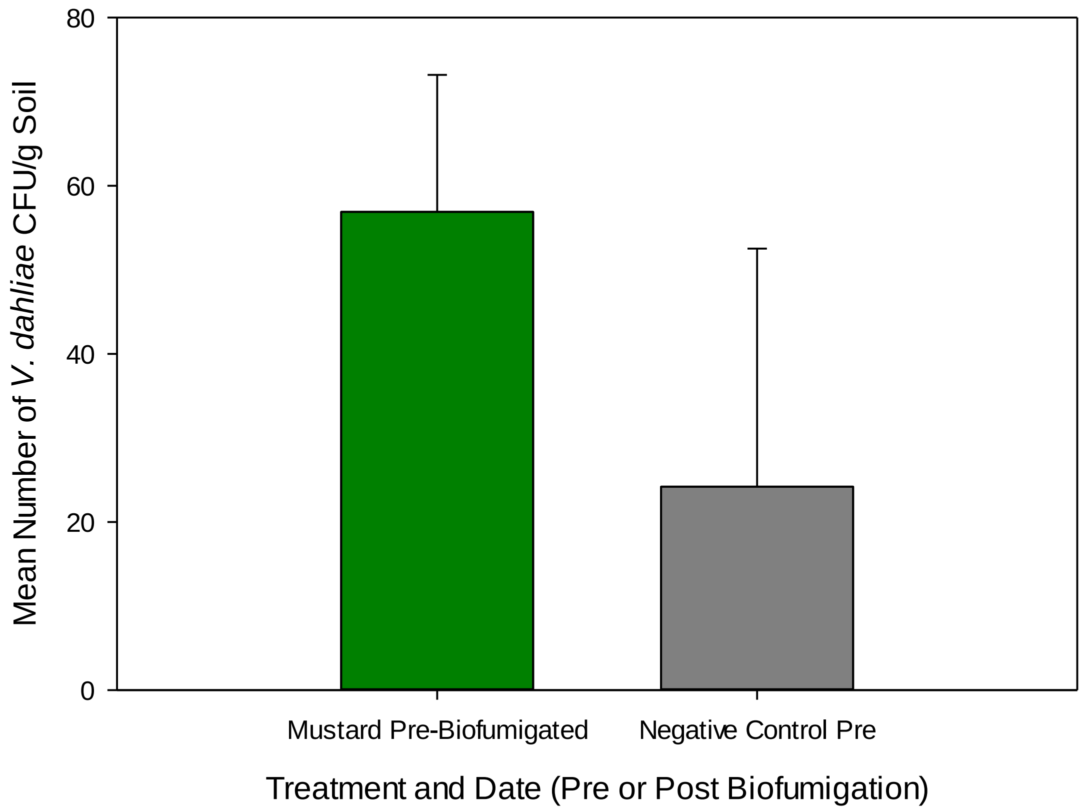
Table 1 – All relevant planting, biofumigation, irrigation status, flea beetle damage, and cold damage information relevant to raising the mustard crops in each field that participated in the study

Field MB-5



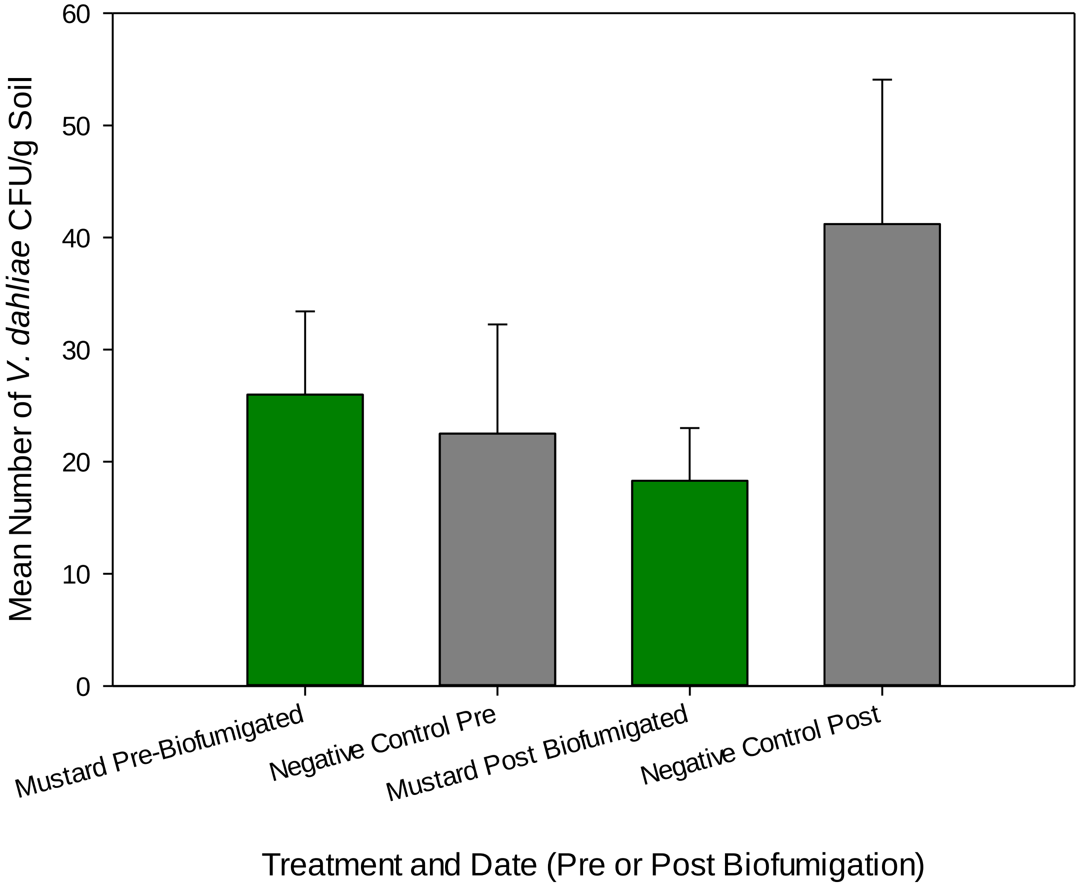
This field did not have sufficient biomass (at least 5 feet) for biofumigation at a rate that is expected to kill *V. dahliae* microsclerotia, meaning no post biofumigation testing was completed. However, this information from the pre-biofumigation does confirm that *V. dahliae* levels are over the 10 CFU threshold needed for disease in both plots, and both plots have very similar loads of *V. dahliae* in the soil. From the grower perspective, the levels of *V. dahliae* are not extreme with no sample exceeding 40 CFU, and in severe fields upwards of 600 CFU can be found in Manitoba.

Field MB-6



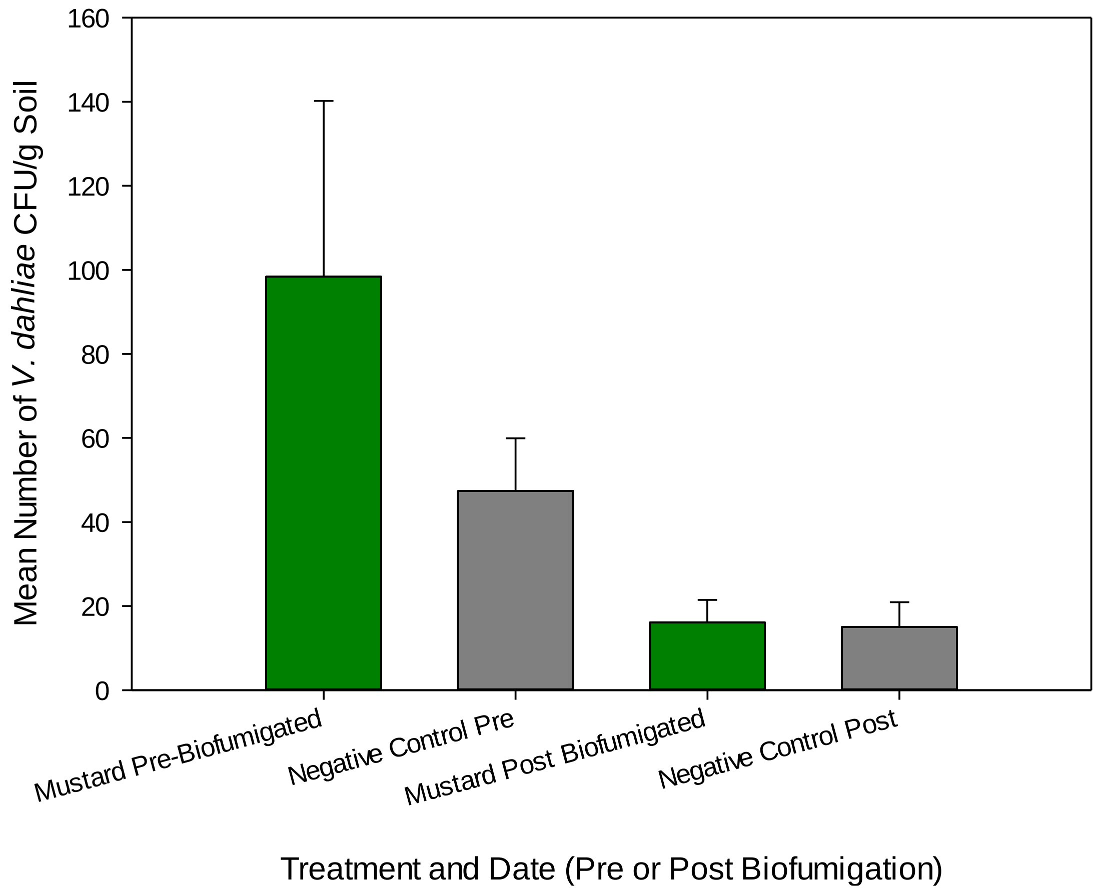
This field did not have sufficient biomass (at least 5 feet) for biofumigation at a rate that is expected to kill *V. dahliae* microsclerotia, meaning no post biofumigation testing was completed. However, this information from the pre-biofumigation does confirm that *V. dahliae* levels are over the 10 CFU threshold needed for disease in both plots, and both plots have very similar loads of *V. dahliae* in the soil. From the grower perspective, the levels of *V. dahliae* are not extreme with no sample exceeding 80 CFU, and in severe fields upwards of 600 CFU can be found in Manitoba. However, 80 CFU is eight times the minimum threshold, meaning that treatment should be considered very soon for this field.

Field MB-7



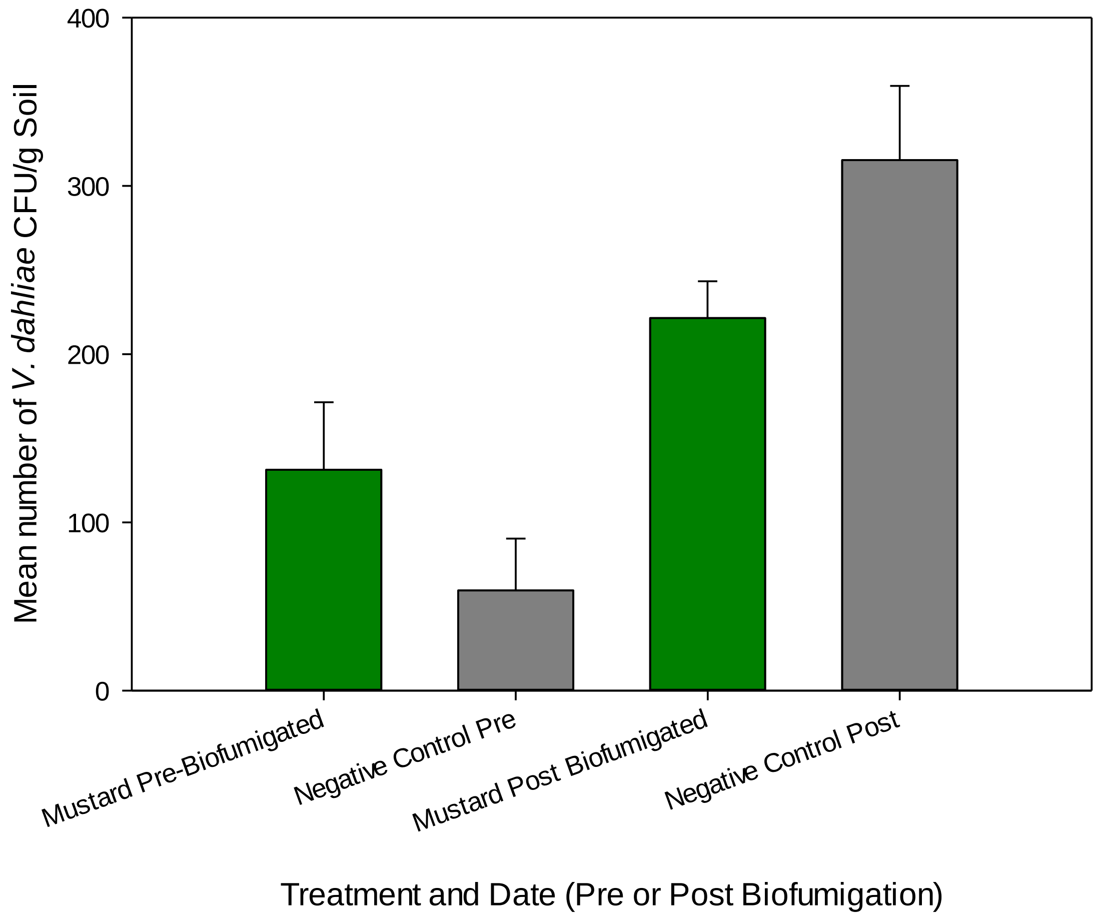
This field did have sufficient biomass (at least 5 feet) of mustard in order to biofumigate and possibly kill *V. dahliae* CFU in the soil. The pre-biofumigation plots had nearly identical amounts of *V.* dahliae CFU in the soil, as indicated by similar means and overlapping error bars, which is a positive attribute as the experiment started in the same place and the main difference in the post-biofumigation experimental means between treatments suggests that mustard biofumigation reduced *V. dahliae* CFU in the soil compared to the negative control.

Field MB-8



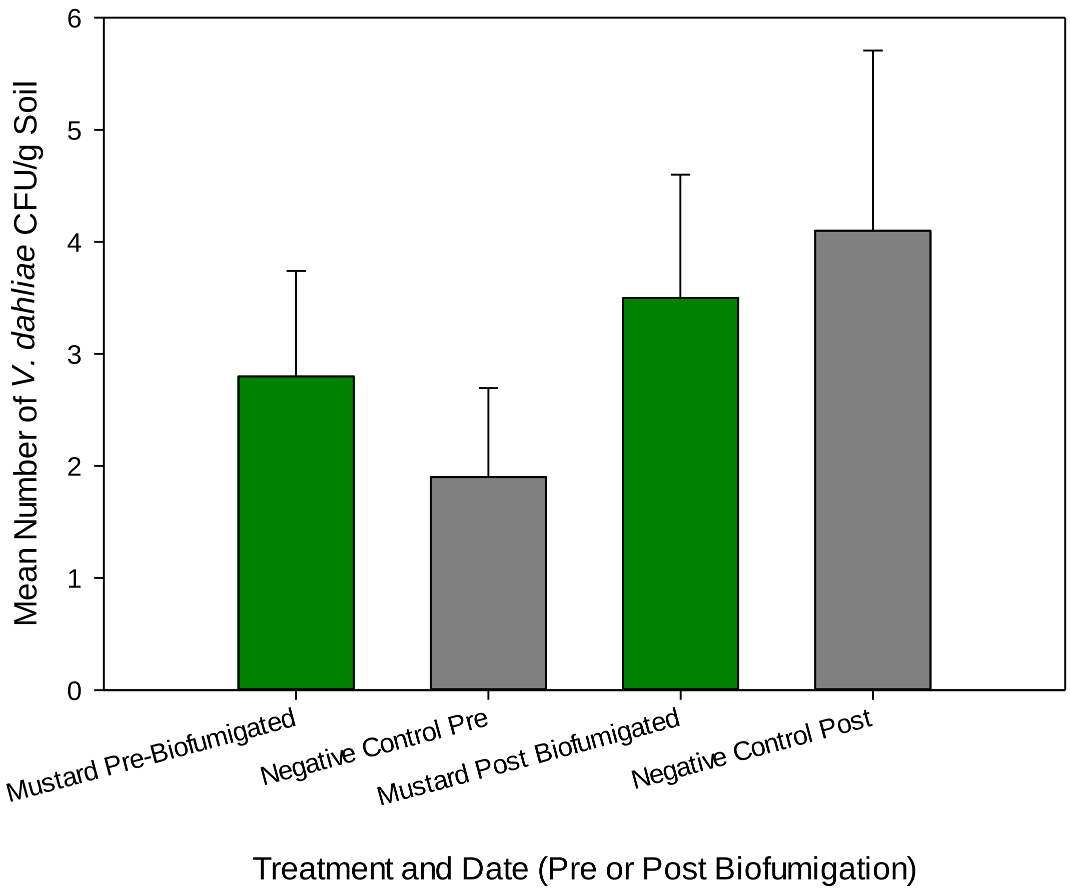
This field did have sufficient biomass (at least 5 feet) of mustard in order to biofumigate and possibly kill *V. dahliae* CFU in the soil. The pre-biofumigation plots did have similar amounts of *V.* dahliae CFU in the soil, as indicated by dissimilar means <u>but overlapping error bars</u>, which is a positive attribute as the experiment started in nearly the same place. The fact that pre and post biofumigation mustard plots had different levels of *V. dahliae* CFU suggests that mustard biofumigation reduced *V. dahliae* CFU. However, the negative control also went down over time, indicating that the negative controls were either contaminated with mustard or the sample sites had different levels of *V. dahliae*. Whatever happened in the negative control plots, it was very consistent across all 16 sample sites because the error bar is very small, indicating the load of Verticillium was virtually the same.

Field MB-9



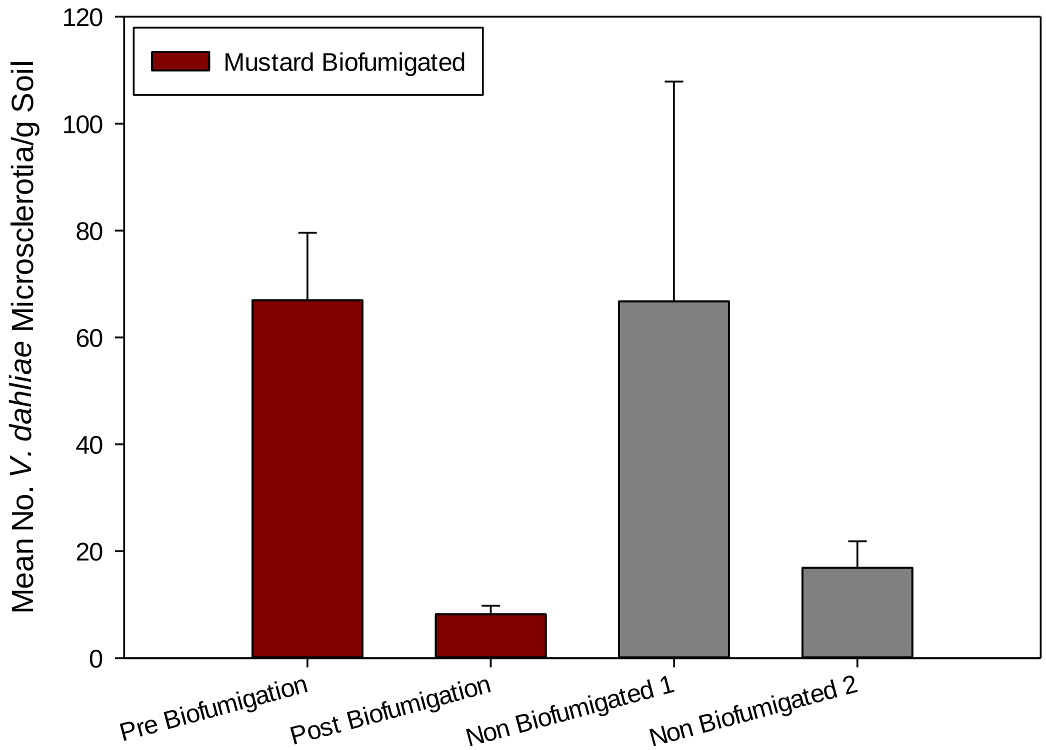
This field did not have sufficient biomass (at least 5 feet) for biofumigation at a rate that is expected to kill *V. dahliae* microsclerotia. Post biofumigation samples were completed because the load of *V. dahliae* CFU in the soil was so high (10 times minimum threshold of 10 CFU on average). The pre-biofumigation plots did have similar amounts of V. dahliae CFU in the soil, as indicated by dissimilar means but overlapping error bars, which is a positive attribute as the experiment started in nearly the same place. The fact htat the error between the 16 sample sites in this field exceeds the total *V. dahliae* CFU found in some Manitoba fields means that the levels of Verticillium in this field, while high, also vary by large gradients in the field in an area that is only 2 inches apart. In this field, the mustard biofumigation could have reduced *V. dahliae* CFU, as the negative control generally has more CFU in it than the biofumigation plot after biofumigation.

Field MB-10



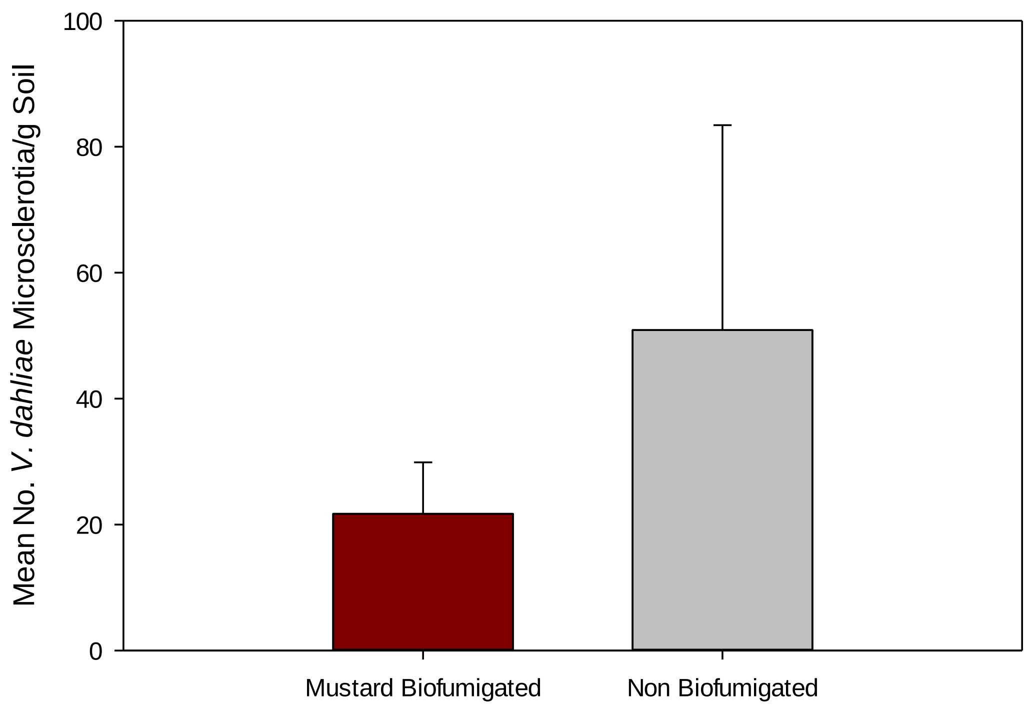
This field did have sufficient biomass (at least 5 feet) of mustard in order to biofumigate and possibly kill *V. dahliae* CFU in the soil. Any differences you can see between the treatments isn't biologically meaningful. If you look on left axis, the highest number of *V. dahliae* CFU ever observed in this field was 6, and the minimum needed for potato infection is 10. Biofumigation wasn't really successful in this field because no plot had enough *V. dahliae* to cause disease.

Field MB-2 Mustard Year 1 (2019) Results averaged amongst all four plots – year of mustard biofumigation



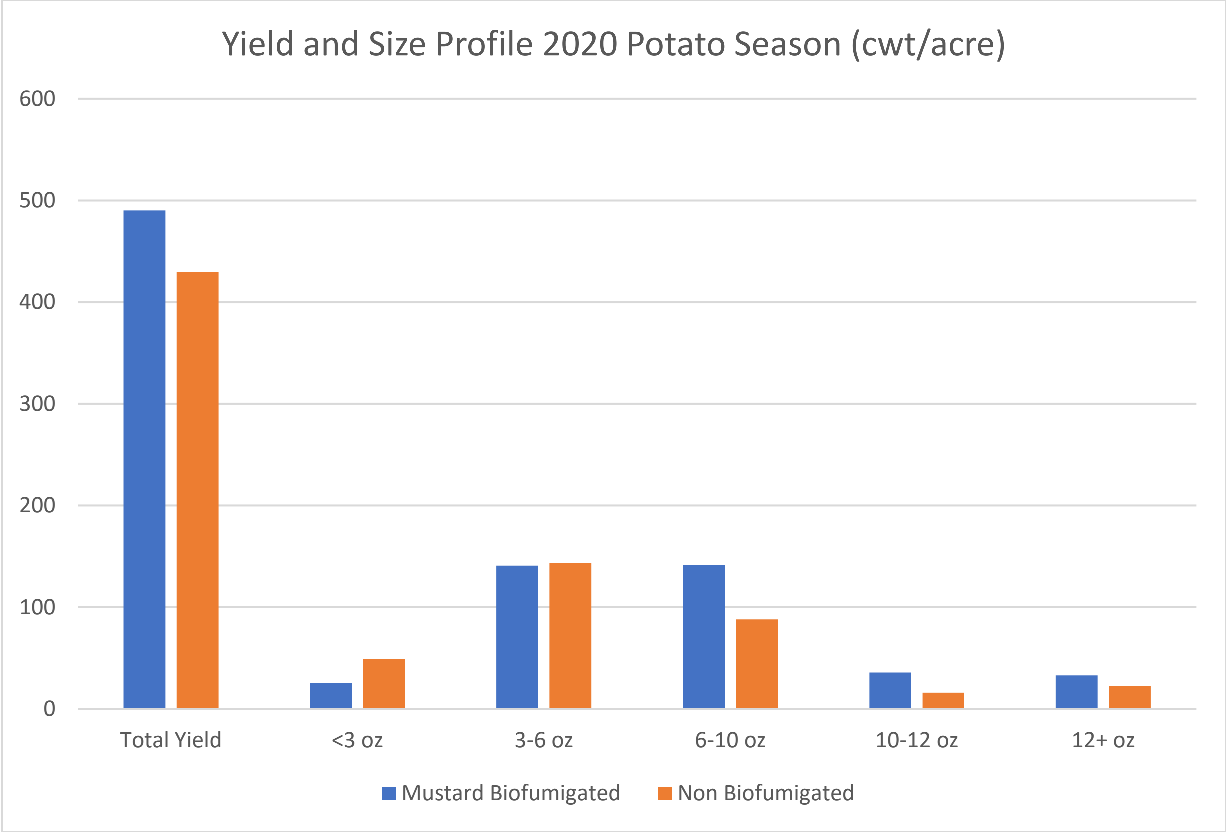
MB-2 was selected for more detailed analysis in 2019 and 2020 because the field survived in 2019 (Table 1), even though the relative amount of mustard was not at the maximum thought possible for Manitoba because the field was grown dryland instead of irrigated. The initial results are promising despite the limitations of the dryland method. The red columns appear to show that less *Verticillium* was found after biofumigating than before. However, the second set of samples after biofumigating had less *Verticillium* on average because the nonbiofumigated areas were also lower on the second sampling date. It is hard to say whether the *Verticillium* reduction was caused by sampling differences or by mustard biofumigation. The mustard crop was 3 feet tall at the most, meaning the biofumigant dose wasn't at full strength. More study is needed for definitive answer on whether mustard is working or not.

Field MB-2 First year after mustard (2020) Results averaged amongst all four plots – potato year



The initial results still appear promising but are not definitive. The error lines above the bars extend both up and down, and when they overlap as they do here then there is no statistical difference. That being said, the overall numbers in all plots decreased between 2018 and 2020 by hundreds of microsclerotia and many plots are just at the threshold where Verticillium wilt can occur (30 CFU/g) rather than 10 times over threshold as they were in 2018 (300+ CFU/g). The are three probable reasons why complete success remains elusive: mustard biofumigation is known to take several attempts to bring a *Verticillium* epidemic of this scale under control, the mustard biofumigation that was done in 2019 was with a crop that did not pack as much biomass as possible, and the nightshade problem remains at large every year. The jump in *Verticillium* counts from 2017-2018 was attributed to nightshade presence, and it is possible that continued nightshade growth will muddy the water because Verticillium counts will increase annually as long as there is this much nightshade around.

Field MB-2 Yield Data from potato year (2020, averaged between four plots in each treatment, harvest date Sept 3)



The largest impact on total yield came from a difference in 6-10 oz tubers between the plots subjected to biofumigation and those that were not. Offhand it appears that mustard biofumigation increased the number of 6-10 oz tubers and decreased the number of tubers under 3 ozs. These results need to be viewed with some caution as Verticillium wilt wasn't the only problem observed in the field. Nightshade weed density was still extremely high in the northeast part of the field, although the plants were only half as tall as 2017. Black dot was also present on the dead potato vines in addition to Verticillium wilt. Water erosion also washed away the hills in most plots. Regardless, a 60-cwt increase in yield in mustard plots over plots without biofumigation is a promising early lead. It is possible that further improvements to the 10-12 oz and 12+ oz categories can be made if other issues can be addressed so that potato vines are not dead by Sep 3. Many of these plots would not have experienced any additional bulking in the month of September in 2020.