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2020 Final Report:

- 1. <u>Project title and project number</u>: C1920-0201-Y2 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries
- 2. <u>Project leader and collaborators</u>: The project team consists of Ray Carmichael, MSc. Ag. and Andrew Systma, NBSCIA Club Agrologists. The collaborating farmers are Carpenter Farms Ltd., Charles McIntosh, and Sunset U-Pick.

ABSTRACT/RÉSUMÉ:

Root Lesion nematodes have an economic impact on potato production that could be in the range of 10% in Atlantic Canada. Root lesion nematodes and Verticillium sp. are associated with a major cause of potato yield reduction commonly referred to as Early Dying Complex (PED). Root Lesion nematodes and Verticillium sp. singularly and combined have similar negative impacts on a range of crops, including strawberries. The NBSCIA established mustard bio-fumigant cultivars in a field in 2019 prior to potatoes in 2020 to observe its potential as a fumigant to reduce nematodes and Verticillium populations. Similar treatments were established at two locations in 2020 preceding potatoes and strawberries in 2021. The effectiveness of chemical fumigation was observed in a single location in strawberries and random samples were collected in six fields in a potato rotation to establish typical levels of infestation in commercial potato rotations. The objective of this project was to evaluate soil sampling and analytical methodologies for nematodes and Verticillium sp. to demonstrate the management of bio fumigant control in potatoes and strawberries. On average, Root lesion nematode populations increased from spring to fall in 2019 and 2020 in the potato field, and the strawberry field in 2020 under the mustard bio-fumigant. Root lesion nematode populations were significantly reduced from the fall of 2019 to the spring of 2020 at the original location. Root lesion populations were reduced under oats and mustard in the Home 1 field in 2020, however there was little observed difference between the two crop species. Chemical fumigation with Vapam in the Sunset strawberry field clearly reduced Root lesion nematode populations in 2020. V. dahliae increased from an average 6644 cells per gram to 23,721 cells per gram of soil under potatoes at the field site in the summer of 2020. Mustard bio-fumigant was observed to reduce V. dahliae population in the potato and strawberry fields during the summer of 2020. The Caliente mustard crop decreased the average number of cells per gram of soil from 9040 to 6003 over the summer of 2020. Oats as a cover crop did not reduce V. dahliae. Over the summer of 2020 the oats cover crop brought V. dahliae from an average of 7701 to 9866 cells per gram of soil.

Les nématodes des racines ont un impact économique sur la production de pommes de terre évalué à environ 10 % au Canada atlantique. Les nématodes des racines et le verticillium sont associés à un phénomène majeur affectant le rendement de la pomme de terre, communément appelé le complexe de mort précoce (PED). Les nématodes des racines et le verticillium, seuls ou combinés, ont les mêmes effets négatifs sur une gamme de cultures, y compris les fraises. L'AASCNB a semé des cultivars de moutarde biofumigante dans un champ en 2019 préalablement à la culture de pommes de terre en 2020 afin d'en observer le potentiel comme fumigant de sol pour réduire les populations de nématodes et de verticillium. Des traitements du même type ont été appliqués à deux endroits en 2020 préalablement à la culture de pommes de terre et de fraises en 2021. La fumigation chimique s'est avérée efficace à un seul endroit sur les fraises et des échantillons aléatoires ont été prélevés dans six champs d'une rotation de pommes de terre afin d'établir les niveaux d'infestation typiques des rotations commerciales de pommes de terre. L'objectif de ce projet était d'évaluer les méthodes d'échantillonnage et d'analyse du sol relatives aux nématodes et au verticillium aux fins de démonstration de la gestion parasitaire par biofumigant dans les pommes de terre et les fraises. En moyenne, les populations de nématodes des racines ont augmenté du printemps à l'automne en 2019 et 2020 dans le champ de pommes de terre, et dans le champ de fraises en 2020 sous la moutarde biofumigante. Les populations de nématodes des racines ont connu une forte baisse entre l'automne 2019 et le printemps 2020 à l'endroit d'origine. Les populations de nématodes des racines ont connu une baisse sous l'avoine et la moutarde dans le champ Home 1 en 2020, mais peu de différences ont été observées entre les deux espèces de cultures. La fumigation chimique au Vapam dans le champ de fraises Sunset a clairement réduit les populations de nématodes des racines en 2020. La V. dahliae est passée d'une moyenne de 6644 cellules à 23 721 cellules par gramme de sol sous les pommes de terre du champ à l'été 2020. On a observé que la moutarde biofumigante réduisait l'incidence de la V. dahliae dans les champs de pommes de terre et de fraises au cours de l'été 2020. La culture de moutarde Caliente a entraîné une baisse du nombre moyen de cellules par gramme de sol (de 9040 à 6003) au cours de l'été 2020. L'avoine comme culture de couverture n'a pas réduit l'incidence de la V. dahliae. Au cours de l'été 2020, la culture de couverture d'avoine a fait passer le nombre moyen de cellules de V. dahliae de 7701 à 9866 par gramme de sol.

3. <u>Summary</u>: Root Lesion nematodes have an economic impact on potato production that could be in the range of 10% in Atlantic Canada. Root lesion nematodes and Verticillium sp. are associated with a major cause of potato yield reduction commonly referred to as Early Dying Complex (PED). Root Lesion nematodes and Verticillium sp. singularly and combined have similar negative impacts on a range of crops. These pests can cause significant economic losses in strawberries.

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NBSCIA established mustard bio-fumigant cultivars in a field in 2019 prior to potatoes in 2020 to observe the potential as a fumigant to reduce nematodes and Verticillium populations. Similar treatments were established at two locations in 2020 preceding potatoes and strawberries in 2021. The effectiveness of chemical fumigation was observed in a single location in strawberries and random samples were collected in six fields in a potato rotation to establish typical levels of infestation in commercial potato rotations.

The objective of this project was to evaluate soil sampling and analytical methodologies for nematodes and Verticillium sp. to demonstrate the management of bio fumigant control in potatoes and strawberries.

On average, Root lesion nematode populations increased from spring to fall in 2019 and 2020 in the HW potato field, and the CM strawberry field in 2020 under the mustard bio-fumigant. Root lesion nematode populations were significantly reduced from the fall of 2019 to the spring of 2020 at the original HW location. Root lesion populations were reduced under oats and mustard in the Home 1 field in 2020, however there was little observed difference between the two crop species. Chemical fumigation with Vapam in the Sunset strawberry field clearly reduced Root lesion nematode populations in 2020.

V. dahliae increased from an average 6644 cells per gram to 23,721 cells per gram of soil under potatoes at the HW field site in the summer of 2020. Mustard bio-fumigant was observed to reduce V. dahliae population in the potato and strawberry fields during the summer of 2020. The

Caliente mustard crop decreased the average number of cells per gram of soil from 9040 to 6003 over the summer of 2020. Oats as a cover crop did not reduce V. dahliae. Over the summer of 2020 the oats cover crop brought the V. dahliae from an average of 7701 to 9866 cells per gram of soil.

4. <u>Introduction:</u> There are no statistically validated estimates in Canada of yield losses in potatoes due to nematodes, however in the United States, it is estimated that 10% of the potato crop is lost annually because of nematodes, and it is reasonable to assume that this percentage is also applicable to Canada and the Atlantic region. The most important nematode parasite of potatoes in the northeastern United States and in eastern Canada is the root lesion nematode, Pratylenchus penetrans. Several other root lesion nematode species attack potatoes but are not important or have not been detected in the Atlantic region.

Since nematodes usually attack underground plant parts, there are no reliable foliar symptoms to signify that nematodes may be the major cause of poor growth and reduced tuber yields in potatoes. In roots, injury by nematodes may be detected by the presence of lesions, cysts or galls. After a few weeks, however, roots are attacked by other pathogens such as bacteria and fungi (Verticillium sp.), and the original damage by nematodes may not be obvious. Consequently, nematode damage has often been attributed to other factors. Root lesion nematodes invade and migrate in potato roots, though tubers are sometimes invaded when nematode populations are very high. Root lesion nematodes and Verticillium sp. are associated with a major cause of potato yield reduction commonly referred to as Early Dying Complex (PED).

Adult females lay eggs in roots or soil. Second stage juveniles hatch from the eggs, invade roots, and develop through the third and fourth stages to adults. Second, third, and fourth stage juveniles, as well as adult males and females are all capable of invading and migrating through roots. A life cycle takes 20 to 60 days to complete, and depends mostly on the condition of the host and on soil temperature. Maximum damage occurs in sandy soils, since these nematodes prefer this type of soil, and also because sandy soils are suited for potato culture. Above-ground symptoms caused by high populations of root lesion nematodes are sometimes falsely attributed to lack of water or nutrients.

Fumigant nematicides are an expedient way to control nematodes. The major disadvantage of chemical control is the cost of fumigants and the need for specialized equipment. Planting is delayed by three or four weeks if fumigants are applied in the spring. In addition, there is the possibility that residues may be left in tubers or may contaminate groundwater. Soil fumigants also are not discriminating in the organisms they control and may destroy beneficial soil life as well as the target pests.

Mustard is a well understood bio fumigant. Its bio fumigation properties have been studied for a number of years and scientists have developed a method to maximize these properties. Mustard has been shown to control a variety of soil borne pests. These include Verticillium spp., Rhizoctonia spp., Fusarium spp., Pythium spp., Sclerotinia spp., common scab and a range of nematodes. The use of mustard as a bio fumigant has also shown a decrease in damage caused by wireworm. However, using a bio fumigant non-cash crop results in lost income for the year and significant input costs which can approximate the cost of chemical fumigants.

Compounding the situation is finding crops that complement the potato crop in regards to soil health and disease cycles while providing an economic benefit. Pearl millet also has properties that control root lesion nematodes and can act as a green manure crop to improve soil health in

the break years of potato rotations. Pearl millet following a mustard crop could potentially further reduce Root lesion nematodes.

The bio fumigant (mustard) crop must be cut and incorporated into the soil not more than 60 days after planting and before the crop goes to seed. While there is time to establish a second crop the cutting and incorporation will conflict with potato harvest. The use of a millet crop following the mustard crop may eliminate the need to cut and incorporate a second mustard crop during potato harvest while still having an impact on the nematode population.

- 5. <u>Project Objective(s)</u>: To evaluate soil sampling and analytical methodologies for nematodes and Verticillium sp. to demonstrate the management of bio fumigant control in potatoes and strawberries.
- 6. <u>Project Deliverable(s):</u>
  - 1. The level of Root lesion nematode and Verticillium sp. in the field prior to establishing the bio fumigant crop and levels at seasons end.
  - 2. Management of the break crop by the producer to control the nematode and virus population in the field, as well as improving soil organic matter and acting as a nutrient sink.
  - 3. A cost benefit comparison between bio fumigation and chemical fumigation.
- 7. <u>Material and Methods</u>: The collaborating producer (Carpenter Farms) indicated that one of their potato fields exhibited poor growth in sections of the field in 2017. Soil samples from a Carpenter Farms' field (HW) in the fall of 2018 identified higher levels of Root Lesion Nematode in sections of the field where the crop production was reduced compared to the "newer" area. However, nematode levels did not exceed the critical threshold of 2000 per kg.

| Root Lesion Nematodes/kg of Dried Soil |            |  |  |  |  |  |  |
|----------------------------------------|------------|--|--|--|--|--|--|
| Plot                                   | 14-Sept-18 |  |  |  |  |  |  |
| HW1                                    | 1440       |  |  |  |  |  |  |
| HW2 (new area)                         | 420        |  |  |  |  |  |  |
| HW3                                    | 980        |  |  |  |  |  |  |
| HW4                                    | 980        |  |  |  |  |  |  |

Potato Early Die (PED) symptoms are not expressed uniformly throughout a field, therefore georeferenced soil sites approximating these locations (Appendix Illustration 1) were identified prior to the establishment of the mustard and annual cereal crops in the spring of 2019 to get a base level of root lesion nematode populations in the spring.

Replicated bio-fumigant treatments of Mighty, Attack and Centennial mustard and annual cereal crops were established and incorporated in the HW field as reported in C1920-0201 Demonstrate Bio-fumigants as a Control of Root Lesion Nematode. Samples from the geo-referenced locations were collected in the spring and fall of 2019 and assessed for nematodes and Verticillum sp. Samples from the geo-referenced locations were collected in the spring of 2020, prior to planting the potato crop and in the fall after potato harvest and assessed for nematodes (University of Guelph, Laboratory Services) and Verticillum sp. using qPCR techniques by Agricultural Certification Services Inc.

A second field location (Home-1) was identified and six geo-referenced sample sites were established in the fall of 2019 (Appendix Illustration 2). The field was split with Caliente mustard and oats and seeded June 5, 2020. Both crops were chopped, prior to mustard seed formation,

incorporated and reseeded as described in Appendix Illustrations 2 and 3 of the C1920-0201 Demonstrate Bio-fumigants as a Control of Root Lesion Nematode report on July 22, 2020. A second crop of each species was replanted on August 27, 2020. Soil samples from the georeferenced locations were collected in the fall of 2019, spring of 2020, prior to planting the mustard and oat crop and in the fall of 2020 and assessed for nematodes and Verticillum sp.

As shown in Appendix Illustration 3 the oat crop demonstrated greater biomass accumulation from the August 27 planting prior to final incorporation on October 30, 2020 than mustard.

Nematode counts provided by the Potato Quality Institute Inc. in 2019 were reported to be considerably higher than comparative samples submitted to University of Guelph, Laboratory Services by NBDAAF staff in other projects. Since the University of Guelph Laboratory Services is the preference for NBDAAF researchers to determine nematode species and populations, all 2020 samples were forward to Guelph for nematode assessments. All Verticillium assessments were undertaken using qPCR techniques by Agricultural Certification Services Inc.

To provide comparative values for the general nematode and Verticillium sp. status in production fields, geo-referenced samples were collected from four potato and two strawberry fields. As a comparison one of the strawberry farm cooperators has a history of chemical fumigation and one field site was treated with a chemical fumigant in 2020. Two of the Carpenter Farms fields will be planted to potatoes in 2021.

COVID-19 public safety restrictions inhibited the identification of additional demonstration sites and the preparation of detailed comparative cost benefit analysis of bio-fumigants and chemical fumigation.

#### 8. Results and Discussion:

Spring and fall-harvest nematode populations for the field sites are reported in Appendix Tables 1, 2 and 3. Observed variance is considered to be within the limits of random error due to timing and the absence of replication. Root lesion nematode populations increased from spring to fall each year (2019, 2020) in the HW field and the CM strawberry field in 2020.

Root lesion nematode populations were reduced under oats and mustard in the Home 1 field in 2020, however there was little difference between the species. Chemical fumigation clearly reduced Root lesion nematode populations in the strawberry field in 2020.

2020 spring and fall Verticillium sp. counts for each field sample site are reported in Appendix Tables 4 and 5.

Mustard bio-fumigant was observed to reduce V. dahliae population in the potato and strawberry fields during the summer of 2020. Oats as a cover crop did not reduce V. dahliae. The average cells per gram of soil for the three oat sites in the Home 1 field increased from 7701 to 9866 under oats but decreased from 9866 to 6003 under Caliente mustard.

V. dahliae populations increased in the HW field planted to potatoes in 2020. The number of cells per gram of soil were observed to be similar between the fall of 2019 and the spring of 2020 but exhibited a large increase during the 2020 growing season. This result suggests that V. dahliae populations may be capable of rebuilding quickly with a susceptible host such as potatoes.

With Verticillium qPCR testing, the level of Verticillium species in soil is quantified directly by the extractable DNA from soil samples. For practical purposes, this is converted into an estimate of C1920\_0201\_Demonstrate Biofumigant - 2020 Report - AGedits 5

the number of cells per gram of soil based upon the known DNA content of Verticillium dahliae. The quantity of Verticillium DNA as reported is not atypically very high or low compared to representative Verticillium-positive samples in New Brunswick potato rotations. How these DNA concentrations relate to infection risk, symptom expression and possible yield reduction in the field, however, likely depends on many factors.

A series of greenhouse experiments recently completed by Agricultural Certification Services determined that tuber yield of greenhouse-grown Russet Burbank potato plants was reduced by 50% at V. dahliae inoculum levels of 0.035 ng/g soil, compared to control plants not exposed to V. dahliae in the soil; this increased to 75% reduction in tuber yield at 0.123 ng/g soil. Given that the values recorded lay close to the conditions of the ACS greenhouse experiment, the potential for substantial infection and yield reduction exists.

The ACS experiment did not determine a safe lower limit of Verticillium DNA that does not cause tuber yield reduction compared to the control. Literature values of Verticillium DNA levels in soil corresponding to crop outcomes are rare for potatoes, but in other susceptible crops, DNA levels as low as 0.003 ng/g soil can cause detectable levels of plant infection.

Geo-referenced soil samples were collected from several other field locations to provide reference values for nematode and Verticillium infestation levels in commercial fields.

#### 9. Conclusions:

Chemical fumigation clearly reduced Root lesion nematode populations in 2020. However, the impact of mustard bio-fumigant crops on Root lesion nematode populations is less definitive, as populations were reduced under oats and mustard in 2020. The extremely dry weather experienced during the growing season of 2020 may have been a factor in reducing the chemical reaction of the bio-fumigant, or nematodes may have remained below the layer of incorporation of the mustard foliage. Mustard bio-fumigant appeared to be effective in reducing V. dahliae population in the potato and strawberry fields during the summer of 2020.

Utilizing the same laboratory for nematode and Verticillium sp. identification and geo-referenced sampling sites increased the confidence of treatment effects observed from spring to fall compared to previous years. However, given the range of variability observed from year to year and location to location, further work is required to validate the effectiveness of mustard bio-fumigant treatments. The data presented suggests that V. dahliae populations may be capable of rebuilding quickly with a susceptible host, such as potatoes.

#### 10. Required next steps:

Further testing at multiple sites is required to compare the efficacy and cost of mustard bio fumigant treatments with registered chemical treatment such as Chloropicrin, Veleum Prime and Elatus to control nematode and Verticillium species.

These data should also be supported with Soil Health Analysis provided by PEI Analytical Laboratories (PEIAL). This analysis includes the standard soil sample analysis: pH, OM, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, Ca, Mg, Cu, Zn, Fe, Mn, S, B, Na, AI, Lime Index, CEC, and % Base Saturation, in addition to Soil Respiration, Aggregate Stability, Active Carbon, Biological Nitrogen Availability, and Soil Texture. Of particular interest would be any correlation with OM, Soil Respiration and Biological Nitrogen Availability.

## 11. Communication:

The data generated by this project will be analyzed and reported in the NBSCIA annual report and used at producer meetings, and local association meetings when requested.

### 12. Intellectual Property:

There are no intellectual properties involved with this project.

## C1920-0201-Y2 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries

| Table 1: Carpenter Farms Number of Nematodes/kg of Dried Soil |                |            |            |                     |            |            |            |           |            |  |  |
|---------------------------------------------------------------|----------------|------------|------------|---------------------|------------|------------|------------|-----------|------------|--|--|
|                                                               |                |            | Root L     | Other (Pin, Spiral) |            |            |            |           |            |  |  |
| Plot                                                          | Treatment      | 25-Jun2019 | 26-Sep2019 | 5-Jun2020           | 30-Oct2020 | 25-Jun2019 | 26-Sep2019 | 5-Jun2020 | 30-Oct2020 |  |  |
| HW1                                                           | Mighty         | 1800       | 12341      | 40                  | 520        | 6301       | 32369      | 0         | 0          |  |  |
| HW2                                                           | Attack         | 1768       | 1549       | 260                 | 560        | 5894       | 19204      | 0         | 0          |  |  |
| HW3                                                           | Attack/Centen. | 3143       | 3990       | 200                 | 100        | 6601       | 19338      | 0         | 0          |  |  |
| HW4                                                           | Centennial     | 1261       | 4494       | 220                 | 180        | 4414       | 40147      | 0         | 0          |  |  |
|                                                               | Average        | 1993       | 5593       | 180                 | 340        |            |            |           |            |  |  |

| Table 2: Carpenter Farms Nematodes/kg of Dried Soil |             |         |     |               |        |             |     |             |        |     |  |
|-----------------------------------------------------|-------------|---------|-----|---------------|--------|-------------|-----|-------------|--------|-----|--|
|                                                     | Nov.2       | 26,2019 |     | 2020          |        | June 5,2020 |     | Oct 30,2020 |        |     |  |
|                                                     |             |         |     | Сгор          | Root-  |             |     |             |        |     |  |
| Plot                                                | Root-lesion | Spiral  | Pin |               | lesion | Spiral      | Pin | Root-lesion | Spiral | Pin |  |
| Home1-1                                             | 3680        | 260     | 0   | Oat           | 760    | 0           | 0   | 0           | 60     | 0   |  |
| Home1-2                                             | 1680        | 80      | 0   | Oat           | 1220   | 80          | 0   | 160         | 0      | 0   |  |
| Home1-3                                             | 1400        | 0       | 0   | Oat           | 200    | 20          | 0   | 140         | 0      | 0   |  |
| Average                                             | 2253        |         |     |               | 726    |             |     | 100         |        |     |  |
| Home1-4                                             | 500         | 0       | 0   | Caliente      | 1060   | 60          | 0   | 60          | 20     | 0   |  |
| Home1-5                                             | 1340        | 120     | 20  | Caliente      | 240    | 20          | 100 | 240         | 120    | 0   |  |
| Home1-6                                             | 440         |         | 120 | Caliente      | 520    | 40          |     | 80          | 0      | 140 |  |
| Average                                             | 760         |         |     |               | 607    |             |     | 127         |        |     |  |
|                                                     |             |         |     |               |        |             |     |             |        |     |  |
| Home6                                               |             |         |     | Caliente      |        |             |     | 200         | 0      | 0   |  |
| Paul 47                                             |             |         |     | Alfalfa/grass |        |             |     | 360         | 300    | 0   |  |
| Paul 48-1B                                          |             |         |     | Barley/under  |        |             |     | 20          | 0      | 0   |  |
| Paul 48-2B                                          |             |         |     | Barley/under  |        |             |     | 20          | 0      | 0   |  |
| KT-1                                                |             |         |     | Soybean       |        |             |     | 2180        | 0      | 0   |  |
| BP-1                                                |             |         |     | Potato        |        |             |     | 1760        | 0      | 20  |  |

## Appendix

| Table 3: Strawberry Field Nematodes/kg of Dried Soil |              |                    |        |     |                    |      |      |  |  |
|------------------------------------------------------|--------------|--------------------|--------|-----|--------------------|------|------|--|--|
|                                                      |              | June               | 5,2020 |     | Oct 30,2020        |      |      |  |  |
| Field                                                | Crop         | <b>Root-lesion</b> | Spiral | Pin | <b>Root-lesion</b> | Pin  | Knot |  |  |
| CM-1                                                 | Caliente     | 300                | 0      | 0   | 380                | 0    | 0    |  |  |
| CM-2                                                 | Pacific Gold | 60                 | 0      | 0   | 900                | 0    | 0    |  |  |
| CM-3 Timothy-Clover                                  |              | na                 | na     | na  | 280                | 4380 | 0    |  |  |
|                                                      |              |                    |        |     |                    |      |      |  |  |
| Jordie Strawberry                                    |              | na                 | na     | na  | 600                | 0    | 520  |  |  |
| Blueberry field Strawberry                           |              | na                 | na     | na  | 80                 | 0    | 0    |  |  |
| Wrong Way Strawberry                                 |              | na                 | na     | na  | 20                 | 0    | 0    |  |  |
| Hill Strawberry                                      |              | na                 | na     | na  | 640                | 0    | 60   |  |  |
| Blueberry pre-fumigant Strawberry                    |              | na                 | na     | na  | 140                | 0    | 60   |  |  |
| Blueberry after fumigant                             | Strawberry   | na                 | na     | na  | 20                 | 0    | 0    |  |  |

| Table 4: 2020 Strawberry Field qPCR Results for Verticillium dahliae, and Verticillium albo- |                    |             |                                            |                                          |                             |                  |                                             |                   |   |  |
|----------------------------------------------------------------------------------------------|--------------------|-------------|--------------------------------------------|------------------------------------------|-----------------------------|------------------|---------------------------------------------|-------------------|---|--|
| Field                                                                                        | Crop               | DNA<br>ng/g | <b>V. da</b> l<br>Standard<br>Error (ng/g) | hliae<br>cells per<br>gram soil*<br>5-Ju | Standard<br>Error<br>n-2020 | DNA<br>ng/g soil | <b>V. albo-</b><br>Standard<br>Error (nq/q) | Standard<br>Error |   |  |
| CM-1                                                                                         | Caliente           | 0.035       | 0.016                                      | 958                                      | 451                         | 0.000            | 0.000                                       | 0                 | 0 |  |
| CM-2                                                                                         | Pacific Gold       | 0.010       | 0.007                                      | 261                                      | 184                         | 0.000            | 0.000                                       | 0                 | 0 |  |
|                                                                                              |                    |             |                                            | 28-0                                     | ct-2020                     |                  | -                                           | -                 |   |  |
| СМ-1                                                                                         | Caliente           | 0.000       | 0.000                                      | 0                                        | 0                           | 0.000            | 0.000                                       | 0                 | 0 |  |
| СМ-2                                                                                         | Pacific Gold       | 0.000       | 0.000                                      | 0                                        | 0                           | 0.000            | 0.000                                       | 0                 | 0 |  |
| CM-3                                                                                         | Timothy-<br>Clover | 0.093       | 0.038                                      | 2555                                     | 1041                        | 0.000            | 0.000                                       | 0                 | 0 |  |

|         | Table 5a:   | 2020 Potato      | o Field qPCR             | Results for             | Verticillium (              | dahliae, and     | d Verticillium           | n albo-atrur            | n                           |  |  |  |
|---------|-------------|------------------|--------------------------|-------------------------|-----------------------------|------------------|--------------------------|-------------------------|-----------------------------|--|--|--|
|         |             |                  | V. di                    | ahliae                  |                             |                  | V. albo                  | o-atrum                 |                             |  |  |  |
| Crop    | Client ID   | DNA<br>ng/g soil | Standard<br>Error (ng/g) | cells per<br>gram soil* | Standard Error<br>(cells/g) | DNA<br>ng/g soil | Standard<br>Error (ng/g) | cells per<br>gram soil* | Standard Error<br>(cells/g) |  |  |  |
|         |             |                  |                          | 16-Oct-201              |                             |                  |                          |                         |                             |  |  |  |
| Austard | 16-Oct-2019 |                  |                          |                         |                             |                  |                          |                         |                             |  |  |  |
| Mustard | HW-2        | 0.16             | 0.09                     | 4434                    | 2355                        | 0.00             | 0.00                     | 0                       | 0                           |  |  |  |
| Mustard | HW/-3       | 0.19             | 0.02                     | 5201                    | 556                         | 0.32             | 0.08                     | 8778                    | 2202                        |  |  |  |
| Mustard | HW/-4       | 0.10             | 0.02                     | 2842                    | 608                         | 0.09             | 0.02                     | 2519                    | 471                         |  |  |  |
| Wastara | 1100-4      |                  | Average                  | 5376                    |                             |                  |                          | 2315                    |                             |  |  |  |
|         |             |                  |                          | 5-Jun-2020              | , <mark></mark>             | <u></u>          |                          |                         |                             |  |  |  |
| Potato  | HW 1        | 0.263            | 0.049                    | 7199                    | 1353                        | 0.000            | 0.000                    | 0                       | 0                           |  |  |  |
| Potato  | HW 2        | 0.084            | 0.026                    | 2310                    | 706                         | 0.000            | 0.000                    | 0                       | 0                           |  |  |  |
| Potato  | HW 3        | 0.260            | 0.030                    | 7121                    | 815                         | 0.001            | 0.001                    | 18                      | 18                          |  |  |  |
| Potato  | HW 4        | 0.363            | 0.033                    | 9948                    | 899                         | 0.000            | 0.000                    | 0                       | 0                           |  |  |  |
|         |             | Τ                | Average                  | 6644                    |                             |                  | 1                        |                         |                             |  |  |  |
|         |             |                  |                          | 28-0                    | Dct-2020                    |                  |                          |                         |                             |  |  |  |
|         | HW-1        | 0.612            | 0.097                    | 16767                   | 2664                        | 0.014            | 0.009                    | 381                     | 243                         |  |  |  |
|         | HW-2        | 0.191            | 0.057                    | 5226                    | 1559                        | 0.031            | 0.021                    | 845                     | 564                         |  |  |  |
|         | HW-3        | 1.920            | 0.187                    | 52599                   | 5136                        | 0.090            | 0.016                    | 2470                    | 429                         |  |  |  |
|         | HW-4        | 0.741            | 0.095                    | 20293                   | 2601                        | 0.078            | 0.026                    | 2142                    | 721                         |  |  |  |
|         |             |                  | Average                  | 23721                   |                             |                  |                          |                         |                             |  |  |  |

| Table 5b: 2020 Potato Field qPCR Results for Verticillium dahliae, and Verticillium albo-atrum |                                |                            |                              |                              |                                 |                                      |                                   |                              |                             |  |
|------------------------------------------------------------------------------------------------|--------------------------------|----------------------------|------------------------------|------------------------------|---------------------------------|--------------------------------------|-----------------------------------|------------------------------|-----------------------------|--|
|                                                                                                |                                |                            | V. da                        | ahliae                       |                                 | V. albo-atrum                        |                                   |                              |                             |  |
| Crop                                                                                           | Client ID                      | DNA<br>ng/g soil           | Standard<br>Error (ng/g)     | cells per<br>gram soil*      | Standard<br>Error (ng/g)        | DNA<br>ng/g soil                     | Standard<br>Error (ng/g)          | cells per<br>gram soil*      | Standard Error<br>(cells/g) |  |
|                                                                                                |                                |                            |                              | 26-N                         | ov-2019                         |                                      |                                   |                              |                             |  |
| Oat                                                                                            | Home 1-1                       | 0.32                       | 0.03                         | 8776                         | 941                             | 0.00                                 | 0.00                              | 0                            | 0                           |  |
| Oat                                                                                            | Home 1-2                       | 0.93                       | 0.18                         | 25494                        | 4871                            | 0.08                                 | 0.04                              | 2113                         | 1118                        |  |
| Oat                                                                                            | Home 1-3                       | 0.35                       | 0.04                         | 9636                         | 1004                            | 0.00                                 | 0.00                              | 0                            | 0                           |  |
| Mustard                                                                                        | Home 1-4                       | 0.42                       | 0.08                         | 11624                        | 2326                            | 0.06                                 | 0.04                              | 1690                         | 1082                        |  |
| Mustard                                                                                        | Home 1-5                       | 0.47                       | 0.10                         | 12937                        | 2802                            | 0.00                                 | 0.00                              | 0                            | 0                           |  |
| Mustard                                                                                        | Home 1-6                       | 0.40                       | 0.08                         | 10841                        | 2139                            | 0.00                                 | 0.00                              | 0                            | 0                           |  |
|                                                                                                |                                |                            |                              | 5-Ju                         | in-2020                         |                                      |                                   |                              |                             |  |
| Oat                                                                                            | Home 1-1                       | 0.230                      | 0.048                        | 6293                         | 1320                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Oat                                                                                            | Home 1-2                       | 0.242                      | 0.062                        | 6621                         | 1700                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Oat                                                                                            | Home 1-3                       | 0.372                      | 0.061                        | 10190                        | 1682                            | 0.001                                | 0.001                             | 40                           | 40                          |  |
| Mustard                                                                                        | Home 1-4                       | 0.288                      | 0.060                        | 7890                         | 1639                            | 0.004                                | 0.004                             | 103                          | 103                         |  |
| Mustard                                                                                        | Home 1-5                       | 0.294                      | 0.032                        | 8067                         | 878                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Mustard                                                                                        | Home 1-6                       | 0.408                      | 0.094                        | 11165                        | 2578                            | 0.004                                | 0.004                             | 111                          | 111                         |  |
|                                                                                                |                                | -                          | _                            | 28-0                         | ct-2020                         |                                      |                                   |                              |                             |  |
| Oat                                                                                            | Home1-1                        | 0.424                      | 0.022                        | 11616                        | 600                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Oat                                                                                            | Home1-2                        | 0.283                      | 0.037                        | 7746                         | 1004                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Oat                                                                                            | Home1-3                        | 0.374                      | 0.027                        | 10237                        | 727                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Mustard                                                                                        | Home1-4                        | 0.214                      | 0.066                        | 5868                         | 1821                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Mustard                                                                                        | Home1-5                        | 0.257                      | 0.041                        | 7029                         | 1113                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Mustard                                                                                        | Home1-6                        | 0.187                      | 0.043                        | 5112                         | 1170                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Mustard                                                                                        | Field6                         | 0.414                      | 0.064                        | 11336                        | 1760                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Covercrop                                                                                      | Paul47                         | 0.370                      | 0.075                        | 10137                        | 2045                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Barley/un<br>derseeded                                                                         | Paul48-1A                      | 0.207                      | 0.024                        | 5662                         | 644                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
|                                                                                                | Paul48-2A                      | 0.439                      | 0.025                        | 12034                        | 672                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Sovbean                                                                                        | KT-1                           | 0.615                      | 0.112                        | 16837                        | 3076                            | 0.000                                | 0.000                             | 0                            | 0                           |  |
| Potato                                                                                         | BP-1                           | 0.267                      | 0.028                        | 7319                         | 754                             | 0.000                                | 0.000                             | 0                            | 0                           |  |
| *cells per<br>organism                                                                         | gram soil est<br>-specific DNA | imate based<br>was near ze | on known Di<br>ro, with only | NA size of V.<br>1 of 6 qPCR | dahliae genon<br>reactions show | ne = 36.5 <i>fg</i><br>ving positive | /cell. † "trace<br>e reaction nea | " here indica<br>r detection | ates<br>limit               |  |

## C1920-0201-Y2 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries Appendix



Illustration 1: Carpenter Farms HW field sample location.



Illustration 2: Carpenter Farms Home 1 and Home 6 field sample locations.



Illustration 3: Carpenter Farms Home 1-oat and mustard October 3, 2020