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Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries

Objective

To evaluate soil sampling and analytical methodologies for nematodes and *Verticillium* sp to demonstrate the management of bio fumigant control in potatoes and strawberries.

Summary

Root lesion nematodes have an economic impact on potato production that could be in the range of 10 per cent 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. When present, these pests can also cause significant economic losses in strawberries.

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 in 2020 and 2021. Canadian forage pearl millet was added as a potential biofumigant crop in 2021.

On average, root lesion nematode populations increased from spring to fall in each of 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. Treatment effects were less conclusive in 2021, possibly impacted by the record setting temperature and rainfall throughout the growing season. Chemical fumigation with Vapam in the Sunset strawberry field clearly reduced root lesion nematode populations in 2020 and 2021.

V. dahlia 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. dahlia* population in the potato and strawberry fields during the summer of 2020. Oats as a cover crop did not reduce. The average cells per gram of soil for the three oat sites increased from 7701 to 9866 but decreased from 9866 to 6003 under Caliente mustard. Overall *V. dahlia* populations increased slightly throughout the 2021 season.

Conclusion

Chemical fumigation clearly reduced root lesion nematode populations in 2020 and 2021. 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 and none were reported after potatoes in the fall of 2021. Nematode populations were reduced under both mustard and CFPM in 2021.

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. The extremely wet weather experienced during the growing season of 2021 may have impacted results, as nematodes may have moved below the sample depth. Nematode populations on average appear to be rebuilding in the HW field. Mustard bio-fumigant appeared to be effective in reducing *V. dahlia* population in the potato and strawberry fields during the summer of 2020.

Treatment effects were inconclusive in 2021.

Démontrer la présence de biofumigants pour lutter contre le nématode et le Verticillium dans les pommes de terre et les fraises

Objectif

Évaluer les méthodes d'échantillonnage et d'analyse du sol pour les nématodes et Verticillium sp afin de démontrer la gestion du contrôle des biofumigants dans les pommes de terre et les fraises.

Sommaire

Les nématodes à lésions radiculaires ont un impact économique sur la production de pommes de terre qui pourrait être de l'ordre de 10% au Canada atlantique. Les nématodes à lésions racinaires et Verticillium sp sont associés à une cause majeure de réduction du rendement de la pomme de terre communément appelée Complexe de mort précoce (DEP). Les nématodes à lésions racinaires et Verticillium sp, seuls et combinés, ont des impacts négatifs similaires sur une gamme de cultures. Lorsqu'ils sont présents, ces ravageurs peuvent également entraîner des pertes économiques importantes pour les fraises.

L'AASCNB a établi des cultivars de biofumigants de moutarde dans un champ en 2019 avant les pommes de terre en 2020 pour observer le potentiel en tant que fumigeant de réduire les populations de nématodes et de Verticillium. Des traitements similaires ont été mis en place à deux endroits en 2020 avant les pommes de terre et les fraises en 2021. L'efficacité de la fumigation chimique a été observée en un seul endroit dans les fraises en 2020 et 2021. Le millet perlé fourrager canadien a été ajouté comme culture biofumigants potentielle en 2021.

En moyenne, les populations de nématodes à lésions racinaires ont augmenté du printemps à l'automne en 2019 et 2020 dans le champ de pommes de terre HW et le champ de fraises CM en 2020 sous le biofumigant moutarde. Les populations de nématodes à lésions radiculaires ont été considérablement réduites de l'automne 2019 au printemps 2020 à l'emplacement d'origine de HW. Les populations de lésions racinaires ont été réduites sous l'avoine et la moutarde dans le champ de la Maison 1 en 2020, mais il y avait peu de différence observée entre les deux espèces de cultures. Les effets du traitement ont été moins concluants en 2021, peut-être affectés par la température record et les précipitations tout au long de la saison de croissance. La fumigation chimique au Vapam dans le champ de fraises Sunset a clairement réduit les populations de nématodes à lésions racinaires en 2020 et 2021.

V. dahlia est passé d'une moyenne de 6644 cellules par gramme à 23 721 cellules par gramme de terre sous les pommes de terre sur le site du champ HW à l'été 2020. Un biofumigant à la moutarde a été observé pour réduire la population de V. dahlia dans les champs de pommes de terre et de fraises au cours de l'été 2020. L'avoine en tant que culture de couverture n'a pas diminué. La moyenne des cellules par gramme de sol pour les trois sites d'avoine est passée de 7701 à 9866, mais a diminué de 9866 à 6003 sous la moutarde Caliente. Les populations globales de V. dahlia ont légèrement augmenté tout au long de la saison 2021.

Conclusion

La fumigation chimique a clairement réduit les populations de nématodes à lésions racinaires en 2020 et 2021. Cependant, l'impact des cultures biofumigants à la moutarde sur les populations de nématodes à lésions racinaires est moins définitif, car les populations ont été réduites sous l'avoine et la moutarde en 2020 et aucune n'a été signalée après la pomme de terre à l'automne 2021. Les populations de nématodes ont été réduites sous la moutarde et le CFPM en 2021. Le temps extrêmement sec connu pendant la saison de croissance de 2020 peut avoir été un facteur de réduction de la réaction chimique du biofumigant ou les nématodes peuvent être restés sous la couche d'incorporation du feuillage de moutarde. Le temps extrêmement humide connu pendant la saison de croissance de 2021 peut avoir eu un impact sur les résultats, car les nématodes peuvent s'être déplacés sous la profondeur de l'échantillon. Les populations de nématodes semblent en moyenne se reconstituer dans le champ HW. Le biofumigant moutarde semblait efficace pour réduire le V. population de dahlias dans les champs de pommes de terre et de fraises pendant l'été 2020. Les effets du traitement n'étaient pas concluants en 2021.

2021 Interim Report:

1. Project title and project number: C1920-0201-Y3 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries
2. Project leader and collaborators: The project team consists Ray Carmichael, MSc. Ag. and Andrew Systma, NBSCIA Club Agrologists. The collaborating farmers are Carpenter Farms Ltd Charles McIntosh and Sunset U-Pick.
3. Summary: 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. When present these pests can also cause significant economic losses in strawberries.

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 in 2020 and 2021. Canadian Forage Pearl Millet was added as a potential biofumigant crop in 2021.

The objective of this project is 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 each of 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. Treatment effects were less conclusive in 2021, possibly impacted by the record setting temperature and rainfall throughout the growing season. Chemical fumigation with Vapam in the Sunset strawberry field clearly reduced Root lesion nematode populations in 2020 and 2021.

V. dahlia 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. dahlia population in the potato and strawberry fields during the summer of 2020. Oats as a cover crop did not reduce. The average cells per gram of soil for the three oat sites increased from 7701 to 9866 but decreased from 9866 to 6003 under Caliente mustard. Overall V. dahlia populations increased slightly throughout the 2021 season.

Initial soil health sampling data established benchmark procedures for future work.

4. Introduction: 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.

A soil health analysis including parameters that measure microbial or biological activity could provide some comparison of the impact of chemical and biofumigant treatments. The soil health analysis conducted by PEI Analytical Laboratories provided a measure of soil respiration and biological nitrogen availability.

Microbes, including bacteria and fungi, play a critical role in regulating the carbon cycle and mineralizing nutrients, turning them into plant-available forms. Soil microbes also influence tilth (soil structure) and help protect crops against pests and disease. As the name implies, the soil respiration test assesses microbial activity by measuring the release of carbon dioxide (CO₂) from the soil. CO₂ respiration is a by-product of microbial metabolism, which includes mineralizing nutrients and breaking down residues. This test is a good indicator of overall microbial activity. The value reported for the soil respiration test is in milligrams of CO₂ per gram of dry soil. The higher the value - the better.

Nitrogen is stored in the soil in two forms – one is immediately plant available (inorganic), and the other (organic) is tied-up in a variety forms (i.e. in organic matter, microbial organisms, plant and root residues, etc.). Nitrogen becomes plant available when it is broken down (also known as mineralized) into an “inorganic” form, and can then be actively taken up by plant roots. This breakdown process occurs by microbes metabolizing these compounds and releasing nitrogen into a plant available form. This process is driven by microbes and is dependent on soil temperatures and moisture levels.

To measure how well your soil can provide plant-available nitrogen during the growing season, the biological nitrogen availability is tested by taking a dry, relatively inactive soil and exposing it to optimum moisture and temperature conditions over two weeks. This allows microbial activity to resume and the amount of nitrogen that gets mineralized into plant-available forms can be measured. The amount of plant-available (inorganic) nitrogen that is mineralized during this period is reported and the higher the value – the better. This test was adapted for use specifically for PEI producers by the Atlantic Soil Health Lab in Truro, NS.

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 fully use 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. Project Objective(s): 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. Project Deliverable(s):
 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. Material and Methods: 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. (Appendix Table 1). Because Potato Early Die (PED) symptoms are not expressed uniformly throughout a field. All soil sample sites were geo-referenced.

Replicated bio-fumigant treatments of Mighty, Attack and Centennial mustard were established in 2019 in the HW field as reported in C1920-0201 Demonstrate Bio-fumigants as a Control of Root Lesion Nematode. Pearl Millet, Sorghum Sudan grass and oats were planted as cover crops following incorporation of the mustard bio fumigant species. Samples from the geo-referenced

locations were collected in the spring and fall of 2019, 2020 and fall of 2021 (Appendix Tables 1 & 2).

A second field location (Home-8) was identified and six geo-referenced sample sites were established in the fall of 2019. The field was split with Caliente mustard and oats and seeded June 5, 2020. Both crops were chopped, prior to mustard seed formation and incorporated on July 22, 2020. A second crop of each species was replanted on August 27, 2020. Soil samples from the geo-referenced locations were collected in the fall of 2019, spring of 2020, prior to planting the mustard and oat crop, spring of 2021, prior to planting the potato crop and in the fall of 2020 and 2021 and assessed for nematodes and *Verticillium* sp. (Appendix Tables 3 & 4).

A third site was established in Home 8 in the spring of 2021 and split to compare Canadian Forage Pearl Millet (CFPM) with Caliente mustard. Three geo-referenced sample sites were located in each crop species as illustrated in Appendix Figures 1. A similar side by side comparison with geo-referenced sample sites was established in a second field site CampMid. Samples were collected from each field location in the spring and fall of 2021 and assessed for nematodes and *Verticillium* sp. (Appendix Tables 3 & 4).

Biomass imagery from Climate Fieldview was monitored throughout the 2021 season to identify potential visual symptoms of PED for Carpenter Home 8 and HW fields (Appendix Figures 2).

Spring and fall samples for nematode and *Verticillium* sp. identification were collected from two strawberry farm sites identified in 2020. 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 and 2021. (Appendix Tables 5 & 6)

A number of samples for soil health analysis were collected from selected geo-referenced sites from across all locations for observation of potential correlation between soil health parameters and nematode and *Verticillium* sp. occurrence (Appendix Tables 7 & 8)

Experience from the initial work in 2019 identified variance between laboratory methodologies and interpretation of results regarding critical levels for both nematodes and *Verticillium* spp.. To eliminate this variance all nematode evaluations were conducted by the University of Guelph, Agriculture and Food Laboratory and soil scans using qPCR technology for DNA identification of *Verticillium* sp. were undertaken at Agricultural Certification Service.

Basic soil health analysis conducted by PEI Analytical Laboratories (<https://www.princeedwardisland.ca/en/information/agriculture-and-land/pei-soil-health-test-how-to-interpret-your-results>) was initiated this year.

8. Results and Discussion:

Fall sample results for 2021 for nematode and *Verticillium* sp. for the Carpenter HW field with all historical values are presented in Appendix Tables 1 and 2, attached. Root-lesion population as reported were depressed in the spring of 2020 prior to the potato crop and subsequent to the biofumigant treatments of 2019. Populations increased during 2020 with potatoes and again in 2021 under the oat crop. However considerably lower than the levels reported throughout the 2019 crop season.

Verticillium dahliae cells per gram of soil increased during the same three crop seasons. *V dahlia* 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 and increased again in 2021 under oats. Suggesting that *V. dahlia* populations may be capable of rebuilding quickly with a susceptible host such as potatoes.

Spring and fall nematode populations for 2019, 2020 and 2021 for all Carpenter field sites and others are presented in Appendix Table 3, attached. Observed variance is considered to be within the limits of random error due to timing and the absence of replication. In the fall of 2021 Root-lesion nematode counts were 0 for the three sample sites with oats in 2020 compared to Caliente mustard with 20 nematodes per kg of soil in the fall of 2021. On average the three sample sites treated with Canadian Forage Pearl Millet demonstrated a greater reduction in Root-lesion nematode counts per kg of soil than the three sites with Caliente mustard.

The exact sample locations and biomass ratings from Climate Fieldview are illustrated in Appendix Figures 2.

Spring and fall *Verticillium* sp cells per gram of soil for 2019, 2020 and 2021 for all Carpenter field sites and others are presented in Appendix Table 4, attached

Mustard bio-fumigant was observed to reduce *V. dahlia* population in the potato and strawberry fields during the summer of 2020. Oats as a cover crop did not reduce *V. dahlia*. The average cells per gram of soil for the three oat sites in the field Home 8-1, 2, and 3 increased from 7701 to 9866 under oats but decreased from 9866 to 6003 under Caliente mustard(Sites 4,5,and6). *Verticillium dahliae* concentrations increased in each treatment under potatoes in 2021, somewhat greater in the Caliente mustard treatment.

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 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. The DNA levels (ng/g of soil) reported for all samples are in excess of the suggested low limit of 0.003ng/g.

Soil samples were collected from several other field locations to provide reference values for nematode and *Verticillium* infestation levels in commercial fields.

Nematode populations for 2020 and 2021 in strawberry field sites are reported in Appendix Table 5, attached. The Root-lesion populations for the CM samples sites are inclusive and variable,

demonstrating real trend. Chemical fumigation clearly reduced Root lesion nematode populations in the strawberry fields in 2020 and 2021.

Verticillium sp concentrations for 2020 and 2021 in strawberry field sites are reported in Appendix Table 6, attached. A very low level of Verticillium dahliae was reported in only one sample site in the fall 2020 and 2021 but not the spring of 2021.

Soil health Verticillium sp and Root-lesion nematodes results are illustrated in Appendix Figures 7 & 8, attached. This is a very preliminary look at potential relationships between soil health parameters and the occurrence of Verticillium and Root-lesion nematodes, as such is impossible to draw any conclusions. It is worth noting that two parameters related to soil biological activity Respiration and Biological Nitrogen Activity should slight increase during the growing season.

9. Conclusions:

Chemical fumigation clearly reduced Root lesion nematode populations in 2020 and 2021. 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 and none were reported after potatoes in the fall of 2021. Nematode populations were reduced under both mustard and CFPM in 2021. 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. The extremely wet weather experienced during the growing season of 2021 may have impacted results, as nematodes may have moved below the sample depth. Nematode populations on average appear to be rebuilding in the HW field. Mustard bio-fumigant appeared to be effective in reducing V. dahlia population in the potato and strawberry fields during the summer of 2020. Treatment effects were inconclusive in 2021.

Utilizing the same laboratory for nematode and Verticillium sp identification and geo-referenced sampling sites increases the confidence of treatment effects observed over time. 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 and CFPM as bio-fumigant treatments. The data presented suggests that V dahlia 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 crop bio fumigant treatments with registered chemical treatment such as Chloropicrin, Veleum Prime and Elatus to control nematode and Verticillium species.

These data collected should also be supported with Soil Health Analysis provided by PEI Analytical Laboratories (PEIAL, of particular interest would be any correlation with OM, Soil Respiration, Biological Nitrogen Availability and soil texture.

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.

Appendix Tables: C1920-0201-V3 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries

Appendix Table 1: Carpenter Farms Number of Nematodes/kg of Dried Soil														
Plot	Treatment Crop	Fall 2018	25-Jun-19		26-Sep-19		2020 Crop	5-Jun-20		30-Oct-20		2021 Crop	29-Sep-21	
		Root-lesion	Root-lesion	Other	Root-lesion	Other		Root-lesion	Other	Root-lesion	Other		Root-lesion	Other
HW-1	Mighty	1440	1800	6301	12341	32369	Potatoes	40	0	520	0	Oats	340	0
HW-2(new)	Attack	420	1768	5894	1549	19204	Potatoes	260	0	560	0	Oats	1300	0
HW-3	Attack/Cent	980	3143	6601	3990	19338	Potatoes	200	0	100	0	Oats	300	0
HW-4	Centennial	980	1261	4414	4494	40147	Potatoes	220	0	180	0	Oats	360	0
	Average	955	1993	5803	5594	27165		180	0	340	0		575	0

Appendix Table 2: Carpenter Farms Number of Verticillium Cells per gram of Soil*																			
Plot	2019 Crop	16-Oct-19				2020 Crop	5-Jun-20		16-Jul-20		28-Oct-20		9-Dec-20		5-Oct-21				
		<i>V. dahliae</i>		<i>V. albo-atrum</i>			<i>V. dahliae</i>		<i>V. albo-atrum</i>		<i>V. dahliae</i>		<i>V. albo-atrum</i>		<i>V. dahliae</i>		<i>V. albo-atrum</i>		
		DNA	cells per	DNA	cells per		DNA	cells per	DNA	cells per	DNA	cells per	DNA	cells per	DNA	cells per	DNA	cells per	
		ng/g soil	gram soil*	ng/g soil	gram soil*		ng/g soil	gram soil*	ng/g soil	gram soil*	ng/g soil	gram soil*	ng/g soil	gram soil*	ng/g soil	gram soil*	ng/g soil	gram soil*	
HW-1	Mighty	0.33	9030	0	0	Potatoes	0.263	7199	0	0	0.612	16767	0.014	381	Oats	0.667	18283	0.000	0
HW-2(new)	Attack	0.16	4434	0	0	Potatoes	0.084	2310	0	0	0.191	5226	0.031	845	Oats	1.059	29022	0.000	0
HW-3	Attack/Cent	0.19	5201	0.32	8778	Potatoes	0.26	7121	0.001	18	1.92	52599	0.09	2470	Oats	0.967	26496	0.000	0
HW-4	Centennial	0.1	2842	0.09	2519	Potatoes	0.363	9948	0	0	0.741	20293	0.078	2142	Oats	0.980	26852	0.095	2608
	Average	0.20	5377	0.10	2824		0.243	6645	na	na	0.866	23721	0.053	1460		0.918	75164	0.024	na

*cells per gram soil estimate based on known DNA size of *V. dahliae* genome = 36.5 fg/cell

Appendix Table 3: 2019-2021 Nematodes/kg of Dried Soil

Plot	Oct.16,2019			2020	June 5,2020			Oct 30,2020			2021	May 17, 2021			Sept. 29, 2021		
	Root-lesion	Spiral	Pin	Crop	Root-lesion	Spiral	Pin	Root-lesion	Spiral	Pin	Crop	Root-lesion	Spiral	Pin	Root-lesion	Spiral	Pin
Home8-1	3680	260	0	Oat	760	0	0	0	60	0	Potato	80	0	0	0	40	0
Home8-2	1680	80	0	Oat	1220	80	0	160	0	0	Potato	40	0	0	0	0	0
Home8-3	1400	0	0	Oat	200	20	0	140	0	0	Potato	60	0	0	0	0	20
Average	2253	113	0		727	33	0	100	20		Average	60	0	0	0	13	7
Home8-4	500	0	0	Caliente	1060	60	0	60	20	0	Potato	20	0	0	0	20	0
Home8-5	1340	120	20	Caliente	240	20	100	240	120	0	Potato	20	0	20	0	0	60
Home8-6	440	0	120	Caliente	520	40	0	80	0	140	Potato	20	0	60	60	0	20
Average	760	40	47		607	40	33,	127	47			20	0	27	20	7	27
Home8-7	na	na	na		na	na	na	no	na	no	CFPM	4360	60	0	80	0	0
Home8-8	na	na	na		na	na	na	na	no	na	CFPM	5120	300	0	280	0	0
Home8-9	na	na	na		na	na	na	na	no	na	CFPM	2400	20	0	140	0	0
											Average,	3960	127	0	167	0	0
Home8-10	na	na	na		na	na	na	na	na	na	Caliente	10400	40	0	2480	20	0
Home8-11	na	na	na		na	na	na	na	na	na	Caliente	2680	60	0	960	0	0
Home8-12	na	na	no		na	na	na	na	na	na	Caliente	16000	60	0	1060	0	0
											Average	9693	53	0	1500	7	0
CampMid1	na	na	na		na	na	na	na	na	na	Caliente	2560	0	0	20	0	0
CampMid2	na	na	na		na	na	na	na	na	na	CFPM	560	0	0	40		
											Average	1560	0	0	30	0	0
Max-1	no	no	na		no	na	na	na	na	na	Oats	na	na	na	620	0	0
Max-2	na	na	nor		no	no	na	na	na	na	Oats	na	na	na	120	0	0

								Oct 30,2020			Client ID			Dec 8,2021		
Home6	na	na	na	Caliente	na	na	na	200	0	0	101 10 FOF-103V			3200	40	20
Paul 47	na	na	na	Alfalfa/grass	na	na	na	360	300	0	D1 10 FOF-104V Nov 2nd			4280		40
Paul 48-1B	na	na	na	Barley/under	na	na	na	20	0	0	A2 10 FOF-102V Nov 1st			4360		
Paul 48-2B	na	na	na	Barley/under	na	na	na	20	0	0	A2 10 FOF-106V Nov 3rd			3480	20	860
KT-1	na	na	na	Soybean	na	na	na	2180	0	0	A3 10 FOF-105V Nov 3rd			1100		80
BP-1	na	na	na	Potato	na	na	na	1760	0	20	A3 10 FOF-101V Nov ?1st			980		100
											A3 10 FOF-109V Nov 10th			280		20
											A4 10 FOF-107V Nov 3rd			2400	40	20
											A4 10 FOF-108V Nov 3rd			1940		20

Appendix Table 4: Verticillium sp. qPCR Results Summary All Years All Sites Carpenter

Organism(s): Verticillium dahliae, Verticillium albo-atrum

'cells per gram soil estimate based on known DNA size of V. dahliae genome - 36.5 fg/cel

Client ID	<i>V. dahliae</i>		<i>V. albo-atrum</i>		2020 Crop	<i>V. dahliae</i>		<i>V. albo-atrum</i>		2021 Crop	<i>V. dahliae</i>		<i>V. albo-atrum</i>		<i>V. dahliae</i>		<i>V. albo-atrum</i>			
	DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil		DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil		DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil
	16-Oct-19		5-Jan-20			28-Oct-20		18-May-21			5-Oct-21									
Home8.1	0.32	8776	0.00	0	Oat	0.23	6293	0	0	Potato	0.113	3097	0.000	0	0.181	4958	0.138	3792		
Home8.2	0.93	2594	0.08	2113	Oat	0.242	6621	0	0	Potato	0.089	2429	0.000	0	0.147	4027	0.250	6847		
Home8.3	0.35	9636	0.00	0	Oat	0.372	10190	0.001	40	Potato	0.178	4884	0.000	0	0.106	2917	0.131	3582		
Average	0.53	7002	0.03	704		0.28	7701	0.00	13	Average	0.13	3470	0.00	0	0.14	3967	0.17	4740		
Home8.4	0.42	11324	0.06	1690	Caliente	0.288	7890	0.004	103	Potato	0.121	3326	0.000	0	0.679	18610	0.290	7951		
Home8-5	0.47	12937	0.00	0	Caliente	0.294	8067	0	0	Potato	0.184	5050	0.000	0	0.426	11682	0.184	5036		
Home8.6	0.40	10841	0.00	0	Caliente	0.408	11165	0.004	111	Potato	0.138	3780	0.000	0	0.309	8460	0.140	3847		
Average	0.43	11701	0.02	563		0.33	9041	0.00	71	Average	0.15	4052	0.0	0	0.47	12918	0.20	6511		
Home8-7	na	na	na	na		na	na	na	na	CFPM	0.113	3105	0.000	0	0.067	1848	0.000	0		
Home8-8	na	na	na	na		na	na	na	na	CFPM	0.052	1424	0.000	0	0.103	2834	0.000	0		
Home8-9	na	na	na	na		na	na	na	na	CFPM	0.069	1885	0.000	0	0.078	2129	0.000	0		
										Average	0.08	2138	0.00	0	0.08	2270	0.00	0		
Home8 10	na	na	na	na		na	na	na	na	113 Caliente	0.113	3089	0.000	0	0.046	1249	0.000	0		
Home8-11	na	na	na	na		na	na	na	na	Caliente	0.036	981	0.000	0	0.097	2671	0.000	0		
Home8-12	na	na	na	na		na	na	na	na	Caliente	0.028	764	0.000	0	0.117	3212	0.000	0		
										Average	0.06	1612	0.00	0	0.09	2377	0.00	0		
CampMidi	na	na	na	na		na	na	na	na	Caliente	0.164	4497	0.000	0	0.162	4436	0.000	0		
campmid2	na	na	na	na		na	na	na	na	CFPM	0.275	7524	0.000	0	0.206	5654	0.000	0		
										Average	0.22	6010	0.00	0.00	0.18	5045	0.00	0		
Max 1	na	na	na	na		na	na	na	na	Oats	na	na	na	na	0.496	40988	0.025	693		
Max 2	na	na	na	na		na	na	na	na	Oats	na	na	na	na	0.455	12454	0.000	0		
										Average	na	na	na	na	0.98.....	26721	0.01	346.63		

Farm of the Future	<i>V. dahliae</i>		<i>V. albo-atrum</i>	
	DNA ng/g soil	cells per gram soil	DNA ng/g soil	cells per gram soil
Client ID	Sample Date: 12-Nov-21			
D1 10 FOF-103V	0.081	2225	0.060	1637
D1 10 101-104V Nov 2nd	0.193	5300	0.000	0
A2 10 FOF-102V Nov 1st	0.074	2029	0.000	0
A2 10 FOF-106V Nov 3rd	0.112	3062	0.000	0
A3 10 FOF-105V Nov 3rd	0.186	5099	0.000	0
A3 10 FOF-101V Nov 21st	0.144	3949	0.000	0
A3 10 FOF-109V Nov 10th	0.248	6802	0.000	0
A4 10 FOF-107V Nov 3rd	0.103	2820	0.021	587
A4 10 FOF-108V Nov 3rd	0.111	3046	0.117	3206

Source: Agricultural Certification Service Inc.

Appendix Table 5: Strawberry Fields Nematodes/kg of Dried Soil

		June 5,2020			Oct 30,2020							May 17,2021			Oct 4,2021		
Field	Crop	Root-lesion	Spiral	Pin	Root-lesion	Pin	Knot	Field	Crop	Root-lesion	Pin	Knot	Root-lesion	Pin	Knot		
CM-1	Caliente	300	0	0	380	0	0	CM-1	Oats/clover	200	0		180	160			
CM-2	Pacific Gold	60	0	0	900	0	0	CM-2	Oats/clover	940	0		900	20			
CM-3	Timothy-Clover	na	na	na	280	4380	0	CM-3	Strawberry	1660	1460		400	1280			
CM-4								CM-4	CFPM	280	0		140	80			
CM-5								CM-5	Mustard/oats	700	0		520	0			
CM-6								CM-6	Mustard/oats	1600	20		800	140			
Jordie	Strawberry	na	na	na	600	0	520	Field	Crop	Oct 26,2021 -PreFumigant			Nov 15,2021-PostFumigant				
Blueberry field	Strawberry	na	na	na	80	0	0	Beside Hill		1200	20	60(Dagger)	40		20		
Wrong Way	Strawberry	na	na	na	20	0	0	By SprayTank		120		180(Knot)	20	0	0		
Hill	Strawberry	na	na	na	640	0	60	Raspberry		180			220	0	0		
		PreFumigant			PostFumigant												
Blueberry	Strawberry	140	0	60	20	0	0										

Appendix Table 6: Verticillium sp. qPCR Results Summary All Years All Berry Sites

Verticillium qPCR results

Organism(s): Verticillium dahliae, Verticillium albo-atrum

*cells per gram soil estimate based on known DNA size of V. dahliae

Client ID	<i>V. dahliae</i>		<i>V. albo-atrum</i>		<i>V. dahliae</i>		<i>V. albo-atrum</i>		2021 Crop	<i>V. dahliae</i>		<i>V. albo-atrum</i>		<i>V. dahliae</i>		<i>V. albo-atrum</i>		
	DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*		DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*	DNA ng/g soil	cells per gram soil*	
Sample Date:	5-Jun-20				28-Oct-20					18-May-21				5-Oct 21				
CM1	0.035	958	0	0	0	0	0	0	Oats/clover	0.000	0	0.000	0	0.011	291	0.000	0	
CM2	0.01	261	0	0	0	0	0	0	Oats/clover	0.000	0	0.000	0	0.020	548	0.000	0	
CM-3					0.093	2555	0	0	Strawberry	0.000	0	0.000	0	0.071	1935	0.000	0	
CM-4									CFPM	0.000	0	0.000	0	0.017	455	0.000	0	
CM-5									Mustard/oats	0.000	0	0.000	0	0.000	0	0.000	0	
CM-6									Mustard/oats	0.000	0	0.000	0	0.129	3547	0.000	0	
														Oct 26,2021 -PreFumigant				
														By Spray Tanks	0.000	0	0.000	0
														Beside Hill	0.000	0	0.000	0
														Raspberry	0.000	0	0.000	0
														Nov 15,2021-PostFumigant				
														Beside Hill	0.000	0	0.000	0
														Sprayer	0.000	0	0.000	0
														Raspberry	0.000	0	0.000	0

Appendix Table 7: 2021 Soil Health, Verticillium dahliae and Root-lesion Nematode Summary

DATE_REC'D	FIELD_ID	Date	%SAND	%SILT	%CLAY	TEXTURE	OM	ACTIVE_C	RESPIRATION	AG_STABILITY	BNA	pH	P_INDEX	C:NRATIO	%C	%N					
		May 17 & 18, 2021																V. dahliae			
		Sept. 29 & Oct 5, 2021																cells/gram soil*		Root-lesion/kg of soil	
																		spring	fall	spring	fall
5-May-21	Cbell Mid 1 Caliente	Spring	22.6	59.9	17.5	Silt Loam	3	349	0.36	27.1	19.7	6.4	14.62	9.16	1.74	0.19	4,497		2560		
4-Oct-21	CbellMid1 Caliente	Fall	25.8	55.7	18.5	Silt Loam	3	403	0.47	34.6	25.5	6.2	15.51	8.29	1.74	0.21		4,436		20	
5-May-21	Cbell Mid 2-CFPM	Spring	23.5	60.1	16.4	Silt Loam	2.6	467	0.32	26.9	18.1	6.4	18.17	8.39	1.51	0.18	7,524		560		
4-Oct-21	CbellMid2-CFPM	Fall	21.6	59.3	19.1	Silt Loam	2.6	358	0.42	25	23.4	6	15.6	7.95	1.51	0.19		5,654		40	
5-May-21	Home 8 8- CFPM	Spring	18.1	63.4	18.5	Silt Loam	4.8	585	0.41	28.6	23.7	6	8.06	11.12	2.78	0.25	1,424		5120		
4-Oct-21	Home 8 8- CFPM	Fall	20.7	59.2	20.2	Silt Loam	4.7	591	0.47	26.4	44.2	6.1	6.53	9.75	2.73	0.28		2,834		280	
5-May-21	Home 8 11 Caliente	Spring	17.6	63.1	19.2	Silt Loam	4.7	569	0.4	27.9	29.4	6.1	10.02	10.5	2.73	0.26	981		2680		
4-Oct-21	Home 8 11 Caliente	Fall	18.7	61.3	20	Silt Loam	4.4	601	0.51	26.6	34	6.1	11.39	9.11	2.55	0.28		2,671		960	
4-Oct-21	Max 1	Fall	25.4	57.7	16.9	Silt Loam	4.7	489	0.54	47.2	19.5	6.2	6.87	9.41	2.73	0.29		40,988	na	620	
4-Oct-21	Max 2	Fall	21.9	58.6	19.5	Silt Loam	4.9	548	0.43	49.1	29.2	6.5	6.29	9.16	2.84	0.31		12,454	na	120	
4-Oct-21	HW 1	Fall	26.4	53.7	19.9	Silt Loam	4.3	460	0.4	26.4	6.4	6.4	8.52	9.58	2.49	0.26		18,283		340	
4-Oct-21	HW 2	Fall	25.8	55.3	18.9	Silt Loam	4.8	503	0.67	35.9	31.9	7.1	5.18	10.69	2.78	0.26		29,022		1300	
4-Oct-21	HW 3	Fall	23.7	55.4	20.9	Silt Loam	4.9	471	0.62	39.6	24.9	6.1	7.45	10.14	2.84	0.28		26,496		300	
4-Oct-21	HW 4	Fall	24.3	56	19.7	Silt Loam	5.3	508	0.49	46.5	33.5	6.5	6.82	10.96	3.07	0.28		26,852		360	

Appendix Table 8: 2021 Soil Health, Verticillium dahliae and Root-lesion Nematode Summary

FIELD_ID	%SAND	%SILT	%CLAY	TEXTURE	OM	ACTIVE_C	RESPIRATION	AG_STABILITY	BNA	pH	P_INDEX	C:NRATIO	%C	%N	Sample Date: Oct 5, 2021				Soil Health Date	
																Root-lesion /kg of Dried Soil	Root-lesion/kg of Dried Soil	V. dahliae cells pergram soil*		
CM 4	25.7	57	17.3	Silt Loam	4.8	715	0.52	50.8	28.7	6.2	15.6	10.3	2.78	0.27	CM-4	280		0		May 17,20
CM-4	36.2	46.6	17.2	Loam	4.9	569	0.5	52.9	49.4	5.9	15	11.36	2.84	0.25			140	455		Oct 4, 2021
CM 5	27.2	55.1	17.7	Silt Loam	4.6	678	0.51	61	29.3	6.1	15.7	9.89	2.67	0.27	CM-5	700		0		May 17,20
CM-5	35.7	50.3	14	Silt Loam	5.4	612	0.7	65.5	45.2	6	16.9	11.59	3.13	0.27			520	0		Oct 4, 2021
Average:	31.2	52.25	16.55		4.925	643.5	0.5575	57.55	38.15	6.05	15.8	10.785	2.855	0.265		Oct 26, 2021 - PreFumigant	Nov 15, 2021- PostFumigant	Oct 26, 2021 - PreFumigant	Nov 15, 2021- PostFumigant	
Beside Hill	70.9	19.1	10	Sandy Loam	3.6	541	0.57	69.5	21.8	6.5	6.4	13.06	2.09	0.16	Beside Hill	1200	40	0	0	Oct 28, 202
Spray Tank	68.8	21.7	9.6	Sandy Loam	2.8	369	0.45	76.8	27.3	5.6	1.89	10.12	1.62	0.16	By Spray Tanks	120	20	0	0	Oct 7, 2021
Raspberry	73.7	18.2	8	Sandy Loam	4.2	498	0.81	88.3	29.9	4.9	15.9	11.62	2.44	0.21	Raspberry	180	220	0	0	Oct 28, 202

Appendix Figures 1: C1920-0201-Y3 Demonstrate Bio-fumigants as a Control of Nematode and Verticillium in Potatoes and Strawberries

Carpenter Home 8



Carpenter HW & Maxine



Carpenter Campbell



McIntosh Strawberry



Sunset U-pick Strawberry



Appendix Figures 2;  Image Summary
Geo-referenced Sample Locations



May 14, 2021



July 13, 2021



July 23, 2021



Aug 14, 2021



Sept. 8, 2021



Sept.21, 2021



Oct.11, 2021



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