MANAGEMENT STRATEGIES FOR POTATO EARLY DYING COMPLEX IN SOUTHERN ALBERTA

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Dedication

This thesis is dedicated to Isabelle Dowd, who, along with my potato-loving ancestors, I would have loved sharing my research with.

Abstract

Alberta potato production expanded in acres and processing recently. Production faces a yield barrier identified as potato early dying disease complex, involving soil-borne *Verticillium dahliae* and *Pratylenchus penetrans* pathogens. To address this barrier, soil treatments of Elatus® fungicide, Velum Prime® nematicide, and Pic Plus® fumigant were evaluated in commercial potato fields growing cultivar Russet Burbank. Reduced soil disturbance using a bed freshener was also investigated. *Verticillium* spp. and *Pratylenchus* spp. were morphologically identified and quantified. In-field tuber samples were assessed for yield and quality according to industry standards. Soil microbial communities were characterized using metagenomic techniques. Three site-years of results showed soil pathogen levels declined and recovered in the same years. Soil applied chemicals significantly increased potato yield with responses dependent on soil pathogen levels. The most efficacious fumigation included bed freshening. Soil microbial communities were not significantly affected by soil treatments in the short-term but longer study is recommended.

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List of Abbreviations

$ - Canadian dollars
ac - acre
AU - amplification units
CFU - colony forming units
cwt - hundredweight (*100 pounds*)
FRAC - Fungicide Resistance Action Committee
IRDA - Research and Development Institute for the Agri-Environment
MeBr - methyl bromide
NE - Northeast
NW - Northwest
PED - Potato Early Dying
SE - Southeast
QOI - quinone outside inhibitors
SHDI - succinate dehydrogenase inhibitors

1. Introduction

1.1. Thesis arrangement

The thesis is arranged into five chapters. Chapter 1 provides a literature review of current knowledge and gaps regarding the topic. The chapter also details recent progress on the topic and outlines the objectives of the project and hypotheses. Chapter 2 describes the research approach and methods both in-field and in-laboratory along with challenges that were encountered. Chapter 3 provides results of the field experimentation and laboratory analyses through words, tables, and figures. Interpretation of results, a discussion of adopting innovation, and recommended future investigation constitutes Chapter 4. Chapter 5 synthesizes the aforementioned chapters into a conclusion. Appendix A is a summarized cost analysis calculation.

1.2. Literature review

1.2.1. Potato importance

The potato (*Solanum tuberosum* L.) is a staple in diets globally, with over 4000 cultivars constituting the diverse food item (Wijesinha-Bettoni and Mouille, 2019). The inclusion of potatoes within diets of performance athletes also places the tuberous vegetable within a category of high nutritional value (Kanter and Elkin, 2019). Related to nutrition, starch derived from potato is an option for prebiotic supplementation, thus being applicable to gastrointestinal improvement (Fuentes-Zaragoza et al., 2011). Pharmaceutical applications of potato starch are also a component of potato's widespread utilization, whereby encapsulation methods are developed using the starch (Bae et al., 2008). Looking beyond consumption, starch of potato can also be used in the textile industry; whereby, wastewater is treated with the powdered form (Zafar et al., 2015).

Beyond the broader importance of the potato, there exists an economic component directly relevant to potato production.

Potato production globally ranks 5th in total volume among all agricultural crops as of 2017, with a total production of approximately 388 million tonnes (Food and Agricultural Organization of the United Nations, 2019a). As of 2013, China leads the world in the volume of potatoes and potato products supplied to its people, reaching over 57 million tonnes, while Canada places 21st with about 2.5 million tonnes (Food and Agricultural Organization of the United Nations, 2019b). Potato production in Alberta experienced positive trends in recent years. Yields increased by 31% between the years 2000 and 2018, seeded acres increased 16% between the years 2017 and 2019, and commodity values increased 46% between the years 2000 and 2012 (Statistics Canada, 2020). Statistics Canada further details that Alberta led Canada with the highest provincial average potato yield and was the province producing the greatest volume of potatoes in 2018. Potato production in Alberta results in several distinct commodities and foodstuffs. These include seed potatoes, frozen potato products, chipped potatoes, fresh market table potatoes, and dehydrated potatoes. Recent industrial expansion announcements by major processors signals an opportunity for continued growth of Alberta potato production. A main barrier to this growth, identified by growers and the industry, is Potato Early Dying Complex (PED) or a similar disease complex, which involves fungi and nematodes as pathogens.

1.2.2. Interaction of host and pathogen

The disease triangle is a model which describes host-pathogen interaction and is generally applied across disciplines which include the study of pathogens (Scholthof,

2007). This model includes the three main components of the disease triangle; the pathogen, the host, and environmental or abiotic factors (Figure 1).

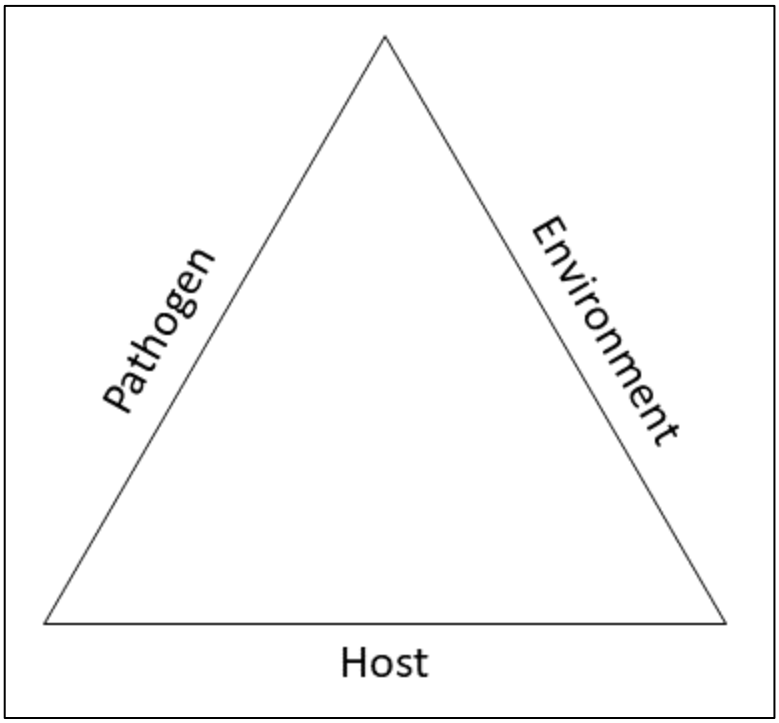

Figure 1 The disease triangle and its three components. Adapted from Scholthof (2007).

If any one of the three components is missing or significantly lacking, pathogenesis proceeds slowly, if at all, demonstrating the equal importance of each component. The pathogen can be of various pathotypes or strains, and subsequently can vary in virulence on the host. Rare strains of pathogens can become predominant when selection acts on populations. The host can vary in susceptibility and tolerance to a pathogen. The susceptibility or resistance of a host to a pathogen is a determining factor in how easily pathogenesis can proceed and complete a life cycle. The tolerance of a host to a pathogen is slightly different in that a tolerant host will allow pathogenesis to proceed without appreciably reducing fitness of the host or its yield if it is a crop. The third component – the environment – is arguably the component with the most sub-

components and influences. With regards to the soil-borne pathogens *Verticillium dahliae* and *Pratylenchus penetrans*, soil characteristics constitute a major portion of the environmental component. Soil moisture content, temperature, structure, and soil organic matter levels are a few characteristics which contribute to the environmental component that soil represents.

1.2.3. Fungi *Verticillium* spp. as pathogens of potato

The *Verticillium* genus includes several fungal species. The species *V. albo-atrum*, *V. nonalfalfae*, and *V. tricorpus* are less virulent on potato than the species *V. dahliae* (Inderbitzin et al., 2011). *Verticillium* spp. colonize roots of potato, eventually entering xylem tissue and vessel elements (Schnathorst, 1981) (Figure 2). Growth of the pathogen proceeds optimally between 20°C and 28°C and follows the stem direction acropetally (Agrios, 2005). The plant will respond with attempts to occlude the pathogen's advance. This physiological response leads to blockages in xylem and subsequent wilting of the canopy. Upon tissue necrosis, the formation of fungal resting structures begins. The type of resting structures formed is dependent on the species. *V. dahliae* forms resilient microsclerotia while other species form less resilient melanised hyphae (Fradin and Thomma, 2006).

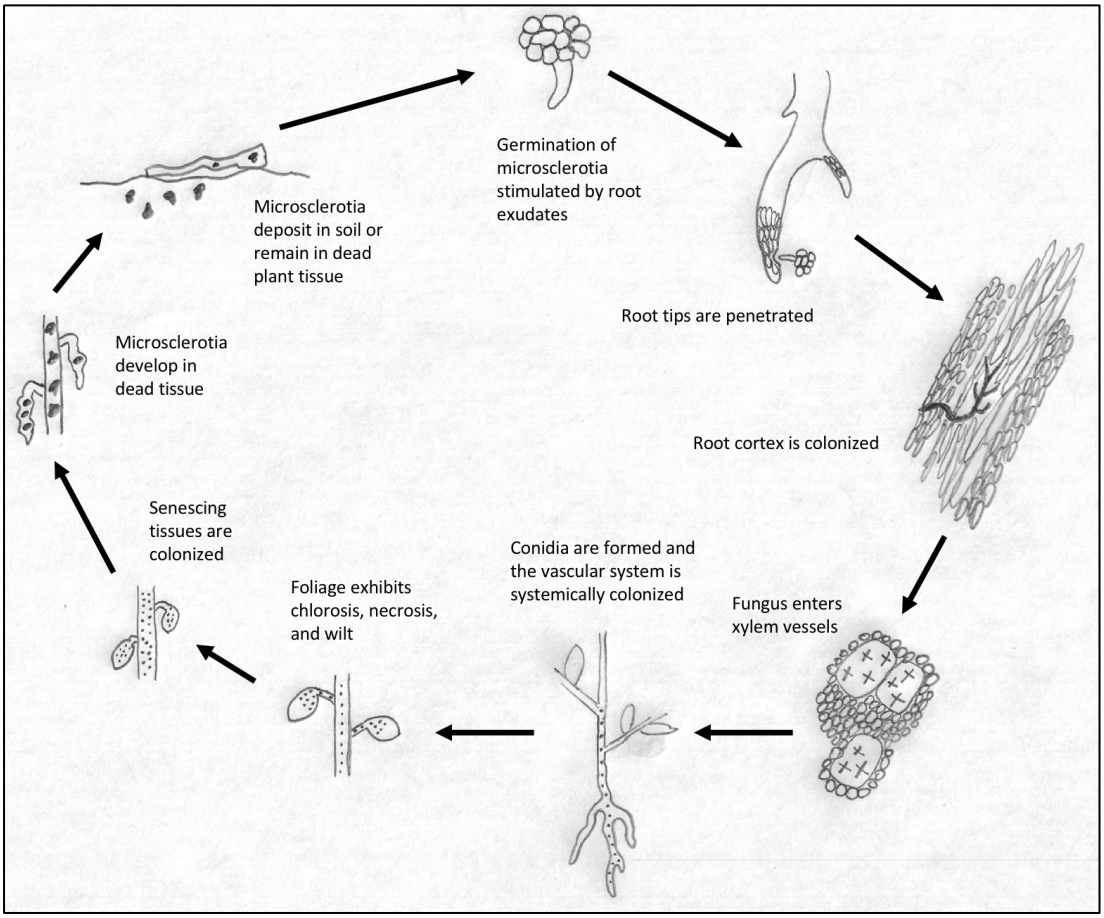

Figure 2 Disease life cycle of vascular wilt fungus *Verticillium dahliae*. Illustration adapted from Rowe and Powelson (2002), drawn by Colby Robertson.

All fungal structures which enter the soil can be quantified in some way, but resting microsclerotia are the best structure to quantify to gauge future long-term pathogen pressure. *Verticillium dahliae* microsclerotia can persist beyond a decade (Rowe and Powelson, 2002), with early reports of up to 14 years regardless of the presence of a suitable host (Wilhelm, 1955). This persistence is in part why *V. dahliae* is the *Verticillium* wilt fungus of concern in potato. Soil is regarded as an inoculum source more important than seed tubers displaying symptoms of vascular infection (Dung et al., 2012). The economic threshold of *V. dahliae* is established at a range of 5 to 30 microsclerotia propagules per g of soil (Powelson and Rowe, 1993) with more recent

work suggesting a narrower range of 8 to 12 (Gudmestad et al., 2007). This threshold can be lowered when the nematode *Pratylenchus penetrans* is present (Johnson and Dung, 2010).

1.2.4. Nematodes *Pratylenchus* spp. as pathogens of potato

Pratylenchus spp. have been reported as pathogens of potato (Koenning et al., 1999; Kotcon and Loria, 1986). As an individual pathogen, *Pratylenchus penetrans* is considered a highly pathogenic nematode of potatoes, with an economic yield threshold established at a range of 1000 to 2000 nematodes counted per kg of soil (Olthof, 1987). *Pratylenchus* spp. undergo a total of six life stages; egg, juvenile stages 1-4, and adult (Figure 3).

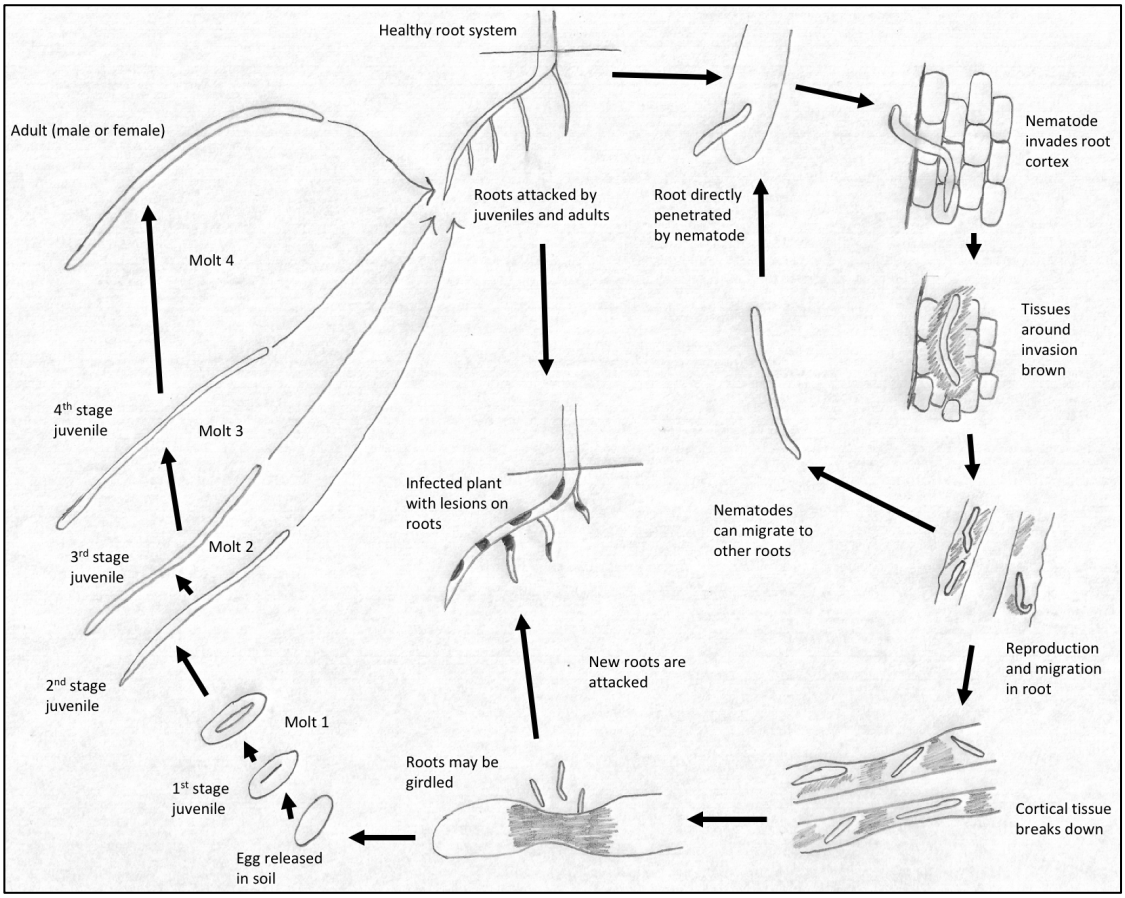

Figure 3 Disease life cycle of the root-lesion nematode *Pratylenchus* spp.. Illustration adapted from Agrios (2005), drawn by Colby Robertson.

The endoparasites can complete a lifecycle in 45 to 65 days, depending on environmental and host factors (Taylor et al., 2000). From the second juvenile stage up to and including the adult stage, the nematode is capable of parasitizing and re-parasitizing root tissue. Eggs can be oviposited in both host roots and soil by females (Pudasaini et al., 2008). *Pratylenchus* spp. is a genus of nematodes commonly referred to as root-lesion nematodes. Root-lesion nematodes create lesions on plant roots during infection. *Pratylenchus penetrans* is the species of greatest concern within the PED complex. It will run its lip region on the surface of the root in search of an area to penetrate, touching the surface root cells with its stylet gently (Zunke, 1990). After penetrating the host, lesion development can influence susceptibility of the plant to further infection by the *Verticillium* fungi. When identifying and quantifying *Pratylenchus* spp. Mokrini et al. (2019) discussed that morphological characteristics can be unreliable, but that species-specific and quantitative real-time polymerase chain reaction methods are in development to aid in identification and quantification of *Pratylenchus* species.

1.2.5. Potato Early Dying Complex

PED complex is defined as the synergistic pathogenesis involving both *V. dahliae* and *P. penetrans* in potato (MacGuidwin and Rouse, 1990). PED has been identified across North America, with presence in midwest and Pacific northwest areas (Rowe and Powelson, 2002) and the northeast (Borza et al., 2018) regions of the United States and Canada. Total impact on potato yields is as much as 50% reduction in marketable yield (Powelson and Rowe, 1993). A lower yield impact is observed when the pathogens act on potato independently. It has been demonstrated that various pathotypes of *V. dahliae* will result in synergies with *P. penetrans* of varying significance (Botseas and Rowe, 1994).

This synergy is in part due to root exudates that can be produced in response to nematode attack on plant roots, whereby the exudates stimulate the germination of *Verticillium* microsclerotia in the soil (Johnson and Dung, 2010; Mol, 1995). The synergy of root-lesion nematodes and *Verticillium* fungi is also suggested to be due to the wounds the nematodes cause in root tissues. Damaged root tissue can provide an opportunity for fungi, such as *Verticillium* spp., to colonize the root (Agrios, 2005). In contrast, several studies conclude that *Verticillium* spp. are proficient in colonizing roots without added entry points via wounds on root tissues by recording similar infection regardless of artificially created lesions (Bowers et al., 1996; Eynck et al., 2007; Perry and Evert, 1983), suggesting that the role root exudates play in the Complex is paramount.

The PED Complex can be complicated further by other pathogens. The fungal pathogen known commonly as black dot, or formally *Colletotrichum coccodes*, is one such example. Davis and Howard (1976) first observed black dot and *Verticillium dahliae* showing an additive effect by reducing potato yield in tandem more so than when separate. Barkdoll and Davis (1992) mention that symptoms of *C. coccodes* appear similar to those typical of *Verticillium dahliae* infection. They also summarize that the two pathogens have demonstrated an interaction effect further reducing tuber yield of potatoes. Tsror and Hazanovsky (2001) studied dual inoculation of the fungal pathogens on four potato cultivars of various susceptibility. Similar to what was suggested by Barkdoll and Davis, they concluded that depending on the cultivar, dual infection by the two pathogens can lead to substantial yield decreases. Lees and Hilton (2003) suggests that *C. coccodes* is increasing in importance throughout potato production systems, further complicating future research on PED.

1.2.6. Managing the PED complex

1.2.6.1. Genetic resistance

Full resistance to either of the PED causal agents, *P. penetrans* or *V. dahliae*, is not a common characteristic of potato cultivars commercially grown in North America, but various levels of resistance exist in cultivars, such as the moderate resistance of Ranger Russet described by Pavek et al. (1992). Resistance is favored over tolerance in terms of cultivar development and selection. This is due to the persistent nature of *Verticillium* spp. propagules in soil, regardless of survivability of the root-lesion nematodes. A plant which tolerates infection by *Verticillium* spp. will allow for growth and reproduction of the pathogen to proceed. Upon plant death, *Verticillium* spp. is reintroduced to the soil via resting structures, building up total pathogen load in the soil. Over many crop cycles this leads to a pathogen load in the soil which can have a significant impact on a potato crop if a susceptible cultivar is planted, even when practicing rotational breaks longer than three years. When selecting tolerant cultivars as a method of managing PED, tolerant or resistant cultivars must continue to be used across rotations to avoid a significant infection event. In terms of managing yield loss due to *Pratylenchus* spp. infestation, Mokrini et al. (2019) concluded that selecting a resistant cultivar is the most economical solution in cropping systems. Russet Burbank is the most prevalent commercially grown cultivar in North America, in part due to customer demands, and is moderately susceptible to Verticillium wilt (Arbogast et al., 1999; Johnson and Dung, 2010). The dependence on a cultivar moderately susceptible to Verticillium wilt further emphasizes the need for a management options other than utilizing cultivars with genetic resistance.

1.2.6.2. Chemical control

1.2.6.2.1. Soil fumigation

The standard soil fumigant used in agricultural production for decades was methyl bromide (MeBr) (Gamliel et al., 1997). Due to ozone depleting properties of the product and practice, the Montreal Protocol has required that MeBr be phased out of agricultural production systems (Velders et al., 2007). As an alternative to MeBr, metam sodium-based fumigants have been successful in reducing fungal pathogen levels while increasing potato yield (Tsror et al., 2005; Yellareddygari and Gudmestad, 2018). Thiophanate-methyl, another alternative to MeBr, has been effective in reducing Verticillium wilt incidence while increasing plant canopy size in greenhouse experimentation, but has been unsuccessful in increasing potato crop yield when applied in the field (Bubici et al., 2019). Chloropicrin fumigation has demonstrated to be effective at reducing soil levels of the fungal *Verticillium* pathogen (Frederick et al., 2018; Gullino et al., 2002; Tsror, Erlich, Peretz-Alon, et al., 2000). As an active ingredient, chloropicrin degrades faster in soil than traditional methyl bromide, with the rate dependent on microbial activity (Gan et al., 2000). Degradation and metabolization rates can be influenced by mixing active ingredients, such as a combination of 1,3-D and chloropicrin (Zheng et al., 2003). Chloropicrin is ultimately metabolized by microbes via dechlorination to dichloronitromethane, then chloronitromethane, and finally nitromethane (Castro et al., 1983).

1.2.6.2.2. In-furrow fungicides and nematicides

There are other soil applied chemicals beyond fumigants which can be used to manage PED. There are two specific groups of chemicals employed within the scope of

this project that were used in managing PED; Group 7 and Group 11 fungicides. Succinate dehydrogenase inhibitors (SDHI) are categorized by the Fungicide Resistance Action Committee (FRAC) as Group 7 fungicides. Succinate dehydrogenase is a critical enzyme in electron transport for cellular respiration and important in the citric acid cycle. SDHI fungicides date back to the 1960's, when they were initially explored as a treatment on cotton to protect seedlings from *Rhizoctonia solani* (Borum and Sinclair, 1968). An SDHI fungicide of interest in this project was Velum Prime (fluopyram 500 g/L, Bayer CropScience Inc.). Velum Prime is registered for suppression of three genera of soil-borne nematodes, including *Pratylenchus* spp., and imparts systemic suppression of early blight (*Alternaria solani*) and black dot (*Colletotrichum coccodes*) in the potato plant. Velum Prime is currently the only non-fumigant nematicide registered for use on potatoes in Canada. Quinone outside inhibitors (QOI) are categorized in the FRAC Group 11. QOI fungicides inhibit the activity of cytochrome bc1 complex and disrupt electron transport and respiration. Commercial use of QOI fungicides began in 1996 (Gisi et al., 2002). A QOI fungicide of interest in this project is Elatus (azoxystrobin 250 g/L and benzovindiflupyr 100 g/L, Syngenta Canada Inc.). Elatus is comprised of two parts, Elatus A and Elatus B, with the latter of the two containing the active ingredient benzovindiflupyr, which is a Group 7 fungicide, and exhibiting activity on the *Verticillium* pathogen of interest in this project.

1.2.6.3. Cultural and biological control

Composts have demonstrated pathogen control and yield improvement in potato (Molina et al., 2014). Aside from improving nutrient availability in the soil, composts have been shown to contribute to pathogen control and yield improvement by stimulating

microbial activity antagonistic of *V. dahaliae* (Termorshuizen et al., 2006). Pathogen control and yield improvement associated with compost use was observed alongside improved availability of the nutrients phosphorus and sulfate in the soil. Composts with appropriate certification can be sourced from a farm or city programs, to name a few. The use of green manures consisting of plants from the *Brassicaceae* family have also been reported to be effective biofumigation options to reduce root lesion nematode levels in potato growing regions across the globe (Fourie et al., 2016). Green manures require the preceding year's crop to be terminated at the flowering stage to maximize available plant biomass returned to the soil and to contribute the desired level of vegetation and/or phytochemical to the soil. In this case, the grower must forego a cash crop with the goal of increasing the upcoming potato crop's profitability. The classical agricultural practice of crop rotation is often regarded as a method of reducing pest pressure. Challenging this dogma is research from the Columbia Basin of Washington showing that regardless of total crop rotations and time between potato rotations, *Verticillium dahliae* continues to pervade production areas (Johnson and Cummings, 2015). This is logical given the persistence of *Verticillium* microsclerotia within soil and across the pathogen's diverse host range (Wheeler and Johnson, 2016). Mokrini et al. (2019) discussed how crop rotation can help with reducing *Pratylenchus* spp. soil levels, but that a thorough understanding of alternative host crops and interactions is necessary for effective execution. Cover crops are another form of cultural control, with the goal of improving the soil environment to foster improved soil microbial communities. Root lesion nematode and *Verticillium* soil levels have decreased with the use of multi-species mixes of cover crops, including marigold flowers (Kimpinski et al., 2000; Korthals et al., 2014).

The alternative cultural control method of soil solarisation sees little use in commercial scale processing potato production in Southern Alberta. The method involves placing a clear tarp over the intended seeding area and allowing the heat of the sun and subsequent steam to sterilize the soil. Despite its lack of use, soil solarisation has been observed as an effective method for controlling PED pathogens in Central Ontario (Lazarovits et al., 1991). Therefore, in theory it may be a successful but impractical method of reducing pathogen pressure, chiefly due to the large size of agricultural fields in Southern Alberta.

Biological control involves the use of beneficial organisms that are antagonistic towards non-beneficial organisms. In the case of Verticillium wilt, biological control of the pathogen's propagules has been explored. Recent study has shown that lipopeptides originating from *Bacillus subtilis* strain C232 are responsible for inhibiting the formation of fungal microsclerotia (Yu et al., 2019). *Trichoderma* spp. in general have been studied as biocontrol agents of multiple agricultural pathogens (Harman, 2006). A recently discovered species *Trichoderma cyanodichotomus* was shown to inhibit *Verticillium dahliae* during *in vitro* assay (Li et al., 2018). Although not applied to a host-pathogen environment, this *Trichoderma* species may become a practical biocontrol if further study shows *in vivo* success.

1.2.7. Soil microbial communities and PED management

As stewards of the land, growers take into consideration the effects their food production practices have on the environment. When producers choose a novel management option for their production system, they consider how the novel product or practice affects the soil it is applied to. How the products and practices in this project effect soil microbial communities is not well understood. With the potential to change

microbial structure and function there is a need to better understand the interactions which take place at the biological level within the soil. These interactions are what drive a soil's complexity beyond its physical and chemical properties. For example, microbial communities play important roles in nutrient cycles and meeting plant growth needs (Hayat et al., 2010). The nitrogen cycle is one such important nutrient cycle and can be affected by chloropicrin fumigation, demonstrated through greenhouse experimentation (Li et al., 2017).

Fumigation with chloropicrin based products has demonstrated increased N_2O soil emissions resulting from increased denitrification (Fang et al., 2018; Spokas et al., 2006; Yan et al., 2015) due to changes in soil microbial communities. The return of nitrification rates to pre-fumigation rates has been observed and may be dependent on soil texture, which is determined by the proportion of soil particle size fractions (Yan et al., 2017). This observation can be connected to research conducted by Hemkemeyer et al. (2015) that demonstrated microbes have preferred soil particle size fractions, whereby various bacteria are found in association with small particle sizes instead of large particle sizes and vice versa. The soil texture influences nitrification rates post-fumigation as well as the types of bacteria present due to the soil texture.

The return of microbial abundance to normal soil levels within experimental timelines following application of chloropicrin has been observed in both broad microbial communities (Fang et al., 2018) and specifically bacteria (Ibekwe et al., 2001). In contrast, chloropicrin has shown to be effective at reducing fungi levels and restricting their return to previous levels for up to four months (Tanaka et al., 2003). Studies have shown that soil fumigants without chloropicrin as their active ingredient allow bacterial

levels to rebound (Fang et al., 2019; Ibekwe et al., 2001), thus allowing nitrification rates to recover (Stromberger et al., 2005). One analysis has shown gram-positive bacteria dominating microbial communities following various fumigant applications, including chloropicrin (Ibekwe et al., 2001).

The aforementioned studies do not all follow the same methods of quantification. Non-standardized methods make it difficult to compare results across all experiments. Methods of quantifying soil microbial activity range from practices involving the measurement of phospholipid fatty acids, respiration rates, and enzyme assays (Ibekwe et al., 2001; Stromberger et al., 2005; Zelles et al., 1997), to more recent methods involving molecular tools and assessing the abundance of genes coding for functional enzymes (Fang et al., 2019; Fang et al., 2018; Fierer et al., 2012; Frederick et al., 2018).

1.2.8. Effects of hail on potato

The performance of the cultivar Russet Burbank has been evaluated in presence of simulated hail. Wille and Kleinkopf (1992) discovered that an increase in defoliation correlated inversely with crop yield and tuber quality. They further observed that when the crop was reaching physiological maturity, a late season hail event could detrimentally impact the specific gravity of tuber, which is directly related to the proportion of the tuber which is dry matter. This was due to broken stems preventing translocation of carbohydrates from the canopy into the tubers. A depression in carbohydrate translocation, evident by a change in specific gravity, has a broader impact on tuber quality attributes, such as texture and consistency. Pavek et al. (2018) found similar yield results with cultivars Russet Norkotah and Ranger Russet. They observed that defoliation was inversely correlated with crop yield. Furthermore, they discovered that the timing of

defoliation also affected the yield. Nearly complete defoliation at the early bulking growth stage halved yield, whereas the same level of defoliation at tuber initiation and late bulking stages did not have as large of an effect. In studying the Jaerla, Red-Pontiac, and Baraka cultivars, Irigoyen et al. (2011) found that cultivar characteristics can also influence a potato crop's ability to recover. They observed that an earlier maturing cultivar, such as Jaerla, recovered less so than Red-Pontiac and Baraka, when hail was simulated at an early phenological growth stage. Jalali (2013) studied simulated hail damage on the Agria cultivar and concluded that while intensity was important, the more important factor was the timing of the defoliation, similar to Wille and Kleinkopf (1992) and Pavek et al. (2018). Jalali also determined that the most critical defoliation time was 5-11 weeks post-emergence of the crop while defoliation during early development led to the best recovery of the potato crop.

1.2.9. Innovation adoption in agricultural production

Agricultural production requires the coordination of many resources, such as natural, human, and financial. A major factor in the agricultural decision-making process is profit margin. A farm is a business with a goal of maximizing profits while conducting sustainable food, feed, fibre, and/or fuel production. With this in mind, an important step in the adoption of a new product or practice into an agricultural production system is the determination of its economic viability. A grower is less likely to implement a new practice in their business if it will detrimentally affect their financial status. A basic comparison of cost and returned benefit is a minimum requirement for any agronomic validation experiment. The process of a grower adopting an innovation as proposed by Pannell (1999) includes four conditions that must be met; the grower must be aware of

the innovation, they must perceive that the product or practice is practical to trial, they must perceive that the innovation is worth trialing, and they must perceive that the change helps them achieve their objectives. Marketing departments of agrochemical companies generally achieve the first condition. The second condition can be achieved through communication with trusted professionals who have worked in relevant aspects of the industry. The third condition can be satisfied through partnership with innovation providers, leading to donated chemical product or a demonstration of a piece of equipment. The final condition is satisfied dependent on the growers' objectives, with Pannell (1999) concluding the greatest challenge is developing an innovation that is more profitable than the standard practice.

1.3. Recent progress

Previously published research involving PED in Alberta is limited, with the only published literature representing a portion of this thesis (Neilson et al., 2020). *Verticillium* spp. have been studied in Alberta as pathogens of other host crops, such as alfalfa (Calpas and Rahe, 1995; Howard, 1985; Howard et al., 1991) and canola (Hwang et al., 2017). *Pratylenchus neglectus*, a species which is less virulent on potato, has been identified in agricultural fields of Alberta (Forge et al., 2015; Yu, 2008). Recent soil analyses have demonstrated the presence of both *Verticillium* spp., with *V. dahliae* identified, and *Pratylenchus* spp. at various levels, some above economic thresholds described by Powelson and Rowe (1993). Fumigation with chloropicrin as an active ingredient is novel to the growing region. Little research has focused on the soil microbiome in Alberta soils. Zaheer et al. (2019) surveyed soil collected from agricultural fields that had experienced cattle manure in search of microbes resistant to

certain antibiotics. These fields were in Southern Alberta, but not part of Alberta's potato growing regions.

In terms of land reclamation, Mitter et al. (2017); Mitter et al. (2018) studied soil of previous oil sands, where barley was grown on reclaimed soil. These fields were located in northern Alberta and outside of potato growing regions. There is limited research conducted regarding how novel agricultural practices affect the microbiome structure of soils in commercial potato fields. PED research exists in other growing regions of North America, the framework of which can be adapted for conducting region-specific research to investigate the unique PED problem experienced in Southern Alberta.

1.4. Objectives and hypotheses

1.4.1. Objectives

Four short-term objectives were achieved through this project:

1) Evaluation of the effect(s) of new and commercially available soil treatments on pathogenic *Verticillium* spp. soil levels through pre- and post-treatment soil analyses;
2) Evaluation of the effect(s) of the treatments on pathogenic *Pratylenchus* spp. soil levels through pre- and post-treatment soil analyses;
3) Evaluation of the effect(s) of the soil treatments on potato crop yield in agricultural fields of Alberta;
4) Analysis of differences in soil microbial communities affected by the soil treatments using metagenomic techniques.

Each of these objectives culminate in developing a recommended solution to mitigate PED in Alberta. The long-term objective of this project was to contribute to the

development of efficient methods to control PED in Alberta, thereby enhancing sustainable food production.

1.4.2. Hypotheses

The following three hypotheses were tested in this study:

1) The outlined soil treatments reduce pathogen soil levels below economic thresholds and keep them below established thresholds throughout the growing season;
2) The soil treatments significantly increase the yield of the potato crop in comparison to areas without the treatments;
3) The soil treatments affect soil microbial communities.

2. Methods

2.1. Experimental design and setup

To complete all objectives, this project was executed through a commercial scale field trial with treated strips. This was chosen in place of growth chamber experimentation, which has been successfully used to study Verticillium wilt management options in the past (Nagtzaam et al., 1998). This approach was used because it approximates on-farm methods well, increases applicability of results to local growers, and aids in demonstrating to the growers how the applied products and practices could be adopted. A commercial scale approach also increases the collaboration between this author and growers. The increased collaboration facilitated learning opportunities at the farm level. The treatment areas were defined as a minimum of 48 potato rows wide (44 metres) and variable lengths dependent on the shape of the overall field (400-800 metres). A list of field locations and names are provided (Table 1). One field was prepared and ready for study on short notice upon commencement of the project during the first field season. Two fields were selected in the second field season as a means of increasing available data and is an experimental design improvement from the first field season. It is recognized that small plot trials, randomized complete block design, or other assays established in highly regulated environments offer the benefit of experimental control and robust statistics, but an applied approach was desirable for extension to potato producers and agronomic training. Conducting a commercial scale field trial was the only available field trial approach available due to the lack of equipment and dedicated land at the University of Lethbridge required for small plot trials. Electing for a commercial scale

approach reduced the level of control over the experiments in terms of weather, soil variability, and environmental conditions.

Table 1 Experimental field locations and names. Precise land locations are reserved for collaborating grower privacy.

Field season	Municipal District	Common name
2018	Municipal District of Taber	SE 5
2019	Municipal District of Taber	NE 8
2019	Municipal District of Taber	NW 13

There were two factors in the design of the experiment. The first factor was the application of a chemical control product. The first factor contained five levels; Velum Prime (fluopyram 500 g/L, Bayer CropScience Inc.) and Elatus (azoxystrobin 250 g/L and benzovindiflupyr 100 g/L, Syngenta Canada Inc.) applied in combination in the first field season and separately in the second field season, Pic Plus fumigant (chloropicrin 85.1% active, TriEst Ag Group Inc.) applied alone, and an untreated control. Elatus and Velum Prime were applied separately in the second field season on recommendation of product representatives. The in-furrow chemicals were also considered separately with the goal of addressing a single component of the disease complex, rather than two components. Velum Prime had a cost of $57.20 per acre (Lorenz, 2020), Elatus had a cost of about $32.00 per acre (Heal, 2020), and the Pic Plus fumigant had a cost of $450.00 per acre applied (Douglas, 2019). All products were applied according to Health Canada label rates. Elatus is marketed as a co-pack, meaning that components A and B are mixed at the farm level. Only the latter of the two has activity on *Verticillium dahliae*. Given that it is unrealistic for a grower to apply only component B and not component A, both

components were applied. Velum Prime is classified as both a nematicide and systemic fungicide, for suppression of *Pratylenchus* spp. in soil and the early blight pathogen *Alternaria solani* in the plant. This nematicide can be combined with Verticillium wilt suppression products, such as Elatus, to theoretically provide better PED suppression than when applied separately. The second factor was the mixing of the soil bed in the spring at planting. This factor contained two levels. One level was the grower's standard planting practice of using a hilling implement driven by a tractor's power take-off to prepare the bed before planting into it. This practice mixes soil from outside of zones treated with fumigant with soil from inside zones treated with fumigant. The second level was an alternative planting practice of using a reduced soil disturbance implement not driven by a power take-off. This implement is referred to as a bed freshener (Figure 4) and loosens soil without moving it between treated and untreated zones of the formed bed, thus loosening the soil for planting without compromising the integrity of the fumigated soil zone. The bed freshener is an uncommercialized piece of equipment, originating from tobacco production, but at the time of this study could not be readily purchased by potato growers on the open market. The equipment can be fabricated through custom request. Throughout the project the bed freshener level is applied to only some levels of the chemical factor. Velum Prime and Elatus in SE 5 was not paired with the bed freshener level because at the time of experimental design the need to address bed freshener use alongside in-furrow fungicides had not been identified. During the second field season field NW 13 was designated for full bed freshener inclusion while field NE 8 was designated for bed freshener exclusion as a means to simplify design and determine if the fumigant was effective without said implement. Separation of the bed freshener

level between fields reduces comparability between fields while easing comparisons between adjacent strips within fields. In the autumn leading up to the second field season, field NW 13 had a corn straw manure applied to a portion of it. Although not part of the experimental design, the manure was a requirement to mitigate soil erosion. The manure was recorded and is marked appropriately on the field's map. All other production aspects were equal across the production fields and matched the growers' standard practices. A list of treatments applied to each field is provided (Table 2, Table 3, and Table 4). Experimental layouts for each field and season are shown in Table 5, Table 6, and Table 7.

Fields were selected through consultation with local growers, agronomists, and industry representatives. Field SE 5 from the first field season was planted on May 3, 2018. In the second field season, field NE 8 was planted on April 19, 2019 and field NW 13 was planted on May 3, 2019. A hail event occurred on both fields during the second field season on August 6th, 2019. Field NE 8 experienced 100% defoliation while field NW 13 experienced 70%. Defoliation was consistent across fields and treatment areas.

Figure 4 Bed freshener implement being used in potato field NW 13. (photo by Colby Robertson)

Table 2 A numbered list of treatment combinations for field SE 5 in 2018.

Strip number	Chemical	Soil disturbance
1	Control	No freshener
2	Chloropicrin	No freshener
3	Velum Prime + Elatus	No freshener
5	Velum Prime + Elatus	No freshener
6	Chloropicrin	Bed freshener
7	Control	Bed freshener
8	Chloropicrin	Bed freshener
9	Control	Bed freshener

Table 3 A numbered list of treatment combinations for field NE 8 in 2019.

Strip number	Chemical	Soil disturbance
1	Velum Prime	No freshener
2	Control	No freshener
3	Chloropicrin	No freshener
4	Control	No freshener
5	Chloropicrin	No freshener
6	Control	No freshener
7	Elatus	No freshener

Table 4 A numbered list of treatment combinations for field NW 13 in 2019. Strip numbering begins at 2.

Strip number	Chemical	Soil disturbance	Manure
2	Chloropicrin	Bed freshener	No
3	Velum Prime	Bed freshener	Yes
4	Control	Bed freshener	Yes
5	Elatus	Bed freshener	Yes
6	Chloropicrin	Bed freshener	Yes

Table 5 Experimental layout of field SE 5. Numbered boxes define the location of treatment strips relative to each other. Strips are oriented North.

1	2	3	Not applicable	5	6	7	8	9

Table 6 Experimental layout of field NE 8. Numbered boxes define the location of treatment strips relative to each other. Strips are oriented North.

1	2	3	4	5	6	7

Table 7 Experimental layout of field NW 13. Numbered boxes define the location of treatment strips relative to each other. Strips are oriented North. Strip numbering begins at 2.

2	3	4	5	6

2.2. Soil sampling

To complete objectives 1, 2, and 3, soil sampling for pathogenic fungi and nematodes was conducted in the fall prior to the trial year to determine fields which were ideal candidates. The pre-treatment sampling dates before treatments were not recorded. Soil samples were collected in the spring, shortly after planting of the crop, to determine a baseline of pathogen levels after treatment but before growth of the host crop. A second round of in-season soil samples were taken immediately prior to harvest to capture soil pathogen levels after the completion of a growing season. Table 8 details sampling dates of each field. Soil was collected from the middle of each row to a depth of 30 centimetres with a 5-centimetre diameter Dutch auger. There were 20 cores bulked from a repeating "W" pattern per treatment area and subsampled into 3.8 l zipper sealed bags. The auger was brushed free of dirt and sanitized with 70% ethanol solution between treatment areas. Bagged soil was placed on ice in a cooler for transport to short term storage at 4°C before shipping. All soil was shipped to the respective laboratories for analysis in insulated boxes and accompanied by ice packs. All soil was received for processing no later than 72 hours after shipping. Soil sampling in the second field season was conducted earlier in the growing season compared to the first field season to accommodate an earlier tuber harvest due to canopy defoliation from hail.

Table 8 Soil sampling dates of experimental fields.

Sample timing	SE 5	NE 8	NW 13
Pre-treatment	October, 2017	October, 2018	October, 2018
After treatments	May 22, 2018	May 6, 2019	May 9, 2019
End of growing season	September 11, 2018	August 19, 2019	September 5, 2019

2.3. Soil pathogen quantification

2.3.1. *Verticillium* spp. quantification

To complete objective 1, the number of *Verticillium* microsclerotia propagules per g of soil, also known as colony forming units (CFU), were quantified for each sampling date. *Verticillium* quantification took place at the Agricultural Certification Services Inc. lab in Fredericton, NB and followed previously defined plating procedures with modifications (Molina et al., 2014). This method involved extracting 5 g of air-dried soil from the overall sample. The air-dried soil was then passed through a 2 mm mesh sieve. Solution preparation for soil dilution first required mixing of 100 ml of Type One (ultrapure) water and 0.1 g of agar in a 250 ml Erlenmeyer flask. The flask was covered with aluminum foil and gently shaken before autoclaving. The flask and mixture were allowed to cool overnight. The 5 g of air dried and sieved soil was added to the water agar mix the following day and agitated on an orbital shaker at a speed of 50 to 60 revolutions per minute. While continuing to agitate the flask in a laminar flow hood, 1 ml of the solution was pipetted onto each of 10 petri dishes with NP-10 semi-selective medium (Kabir et al., 2004). The NP-10 medium provided an environment conducive for microsclerotia formation while retarding bacterial growth, therefore reducing competition for nutrients from the medium. The total soil pipetted onto plates was 0.5 g, with 0.05 g on each plate. Solution was spread across the plates with a spreader. Plates were

permitted time to dry with lids partially covering them. The plates were placed inverted in a Ziploc bag in the dark for an incubation period of 3 weeks, which allowed formation of microsclerotia. After incubation, plates were gently washed free of debris under cool running water. A gridline background was drawn onto an additional petri dish lid to aid in counting. Identification and counting of *Verticillium* microsclerotia colony forming units via morphology was carried out with a stereoscope. The number of CFU per plate were summed, providing a count per 0.5 g of soil, and multiplied by 2 to determine provide a total CFU count per 1 g of soil.

A potential limitation in utilizing this quantification technique was misidentification of organisms. Past research has shown that *V. tricorpus* was distinguishable from *V. dahliae* during plate counting (Goud and Termorshuizen, 2003), but inter-laboratory comparisons have demonstrated that visually identifying and separating species within the *Verticillium* genus can be a challenge (Termorshuizen and Davis, 1998). It is acknowledged that species level confirmation of *Verticillium* requires more than visual identification. The cooperating laboratory, Agricultural Certification Services Inc., is confident in their *Verticillium dahliae* quantification but conservatively report the counts only to the *Verticillium* genus. Although molecular techniques are established for identifying individual species, this level of analysis is beyond the scope of the project. Since samples were available for conducting the molecular analyses, some were retained for species identification outside of this project and will still contribute to a broader research topic in Alberta soils.

2.3.2. *Pratylenchus* spp. quantification

To complete objective 2, *Pratylenchus* spp. quantification took place at the University of Guelph Agri-Food Laboratory and utilized the Baermann pan method to extract the nematodes from 50 g of fresh, undried soil per sample (Forge and Kimpinksi, 2007; Townshend, 1963). Although 50 g of soil is proportionally small compared to the overall sample, increasing extraction samples above 50 g reduces the efficiency of quantification and recovers fewer nematodes (Bell and Watson, 2001). The Baremann pan method relies on nematode mobility. Soil was placed on 3-ply paper tissue, which was placed on a non-metallic mesh screen and subsequently placed in a pan filled with water. The pan held enough water to saturate the soil without fully immersing it. Pans were stacked for efficient use of space, then covered by plastic to limit evaporation. Incubation proceeded at room temperature for 3 to 14 days with water added to the edges of the screens to maintain a consistent water level (Barker, 1985). Screens were removed from pans and had their bottoms rinsed into their respective pan. Contents of pans were collected into large test tubes and left undisturbed for 1 hour minimum to allow nematodes to settle to the bottom. Supernatant was siphoned off to leave between 5 and 10 ml remaining. The remaining contents were placed in a counting dish. Visual identification was conducted via stereoscope at 10 to 70x magnification after the extraction settled in the dish for a few minutes. Nematode identifications were performed using a dichotomous key (Tarjan et al., 1977). The described methods provided the best estimate of the number of root-lesion nematodes per kg of soil. Other methods, such as species specific and/or quantitate polymerase chain reaction can capture DNA content of the target organism(s) following death. Given that the Baremann pan extraction method

relies on mobility of live organisms, it reduces the chance of quantifying terminated nematodes. The mixed-species populations, which existed in these soil samples, prevented accurate species-level identification (Mokrini et al., 2016). Therefore, it is acknowledged that molecular identification is required to go beyond genus level identification but was not conducted within the scope of this project.

2.4. Visual wilt symptom quantification

To complete objective 3, the incidence of wilt symptoms on potato plants were quantified visually. This involved the selection of 15 consecutive plants within a single row per treatment and categorization based on the level of symptoms within the canopy (MacGuidwin and Rouse, 1990). The categories followed a scale of 0 to 4, with 0 = no symptoms, 1 = 1-32% of foliage with wilting, necrosis, or chlorosis, 2 = 33-65%, 3 = 66-99%, and 4 = dead plant. Examples of each rating were recorded in Figure 5. Three replicated observations were conducted near the end of the growing season for each treatment area. Field season one had scores recorded on August 29, 2018. No plant tissue was excised for diagnostic confirmation of the presence of pathogens. The second field season did not have scores recorded due to a hail event prior to the planned scoring date.

Figure 5 Examples of visual wilt ratings of potato plants taken from experimental field SE 5 on August 29, 2018. **A**) no symptoms of wilt, **B**) category 1 showing 1-32% of foliage with wilting, necrosis, or chlorosis, **C**) 33-65%, **D**) 66-99%, **E**) dead plant, scale bar = 28 cm (photos by Colby Robertson).

2.5. Yield quantification and cost analysis

Tuber samples were collected prior to commercial harvest and yield, size profile, and quality of the crop were determined. Each sample consisted of all tubers collected from an approximately three metre (ten feet) section of a row. There were eight samples taken per treatment strip. The total number of plants and stems in the sample areas was recorded before tuber harvest. Tubers were lifted by a single row chain digger pulled by tractor and collected by hand (Figure 6). Gross yield quantification was conducted based

on a ten foot sample row. Gross yield is a direct estimate of total production in the field but does not relate directly to the harvested yield due to potential harvest losses. A gross yield value is the simplest way of determining what, if any, added value is provided by treatments in the field. Size profile grading and weighing were carried out by a third party according to local potato production contract specifications. “Smalls” are tubers which are less than 4.45 centimetres (1.75 inches) in diameter and less than 7.62 centimetres (3 inches) in length. “Smalls” are a size category defined by processors to help them understand which tubers are appropriate for specific finished products. The proportion of tuber weight over ten ounces was recorded. Recording this metric in conjunction with the smalls metric allows one to determine how large of a shift happens within the tuber size profile. Specific gravity was determined through the weight in air and weight in water method (Sharma et al., 1958). The specific gravity of a tuber is a ratio involving the weight of the tuber in the air and the weight of the tuber suspended in water. Tubers are first weighed in a tared bucket on a scale while another tared bucket is suspended in water hanging from the underside of the scale. The second measurement includes the same buckets but with the tubers weighed in the bucket that is suspended in water. The ratio can be used to determine the percent dry matter of the tuber, which has a direct influence on the texture of the internal flesh and therefore affects processing of the tuber into a finished product (i.e. fries). The ideal specific gravity of a tuber depends on its intended end utilization. The total stem and tuber number were recorded so that stems per plant and tubers per plant could be calculated.

Figure 6 One row chain digger pulled by tractor. Used in all experimental fields, featured here in field NE 8. (photo by Colby Robertson)

Collection of crop data in the described manner is a common method used by growers and agronomists for tracking yield and quality data. Field SE 5 from the first field season was yield sampled on September 10, 2018. From the second field season, the majority of field NE 8 was yield sampled on August 27, 2019 while field NW 13 was yield sampled on September 3, 2019. In response to a hail event and potential labour constraints, field NE 8 had 14 of the 56 tuber samples collected by hand 1 and 2 weeks ahead of the samples collected by the chain digger.

A basic cost analysis of the applied treatments compared to control strips and associated treatment pricing provided a suggested break-even payment per hundredweight of tubers. In results showing a negative yield response due to treatment

there was a calculation of dollars lost per cwt of crop produced in the relevant treatment areas. The cost of the product per acre was compared to the added yield per acre due to treatment effect, with the quotient of the two determining the additional break-even payment required to cover the cost of the added treatment.

2.6. Statistical analyses

To complete objectives 1, 2, and 3, statistical analyses of tuber yields and visual wilt observations were conducted, with basic comparisons of mean pathogen levels in soil samples. Design limitations do not allow separate field locations and seasons to be combined for analyses. Statistical comparisons were performed among adjacent treatments at single sampling time points. Regarding pathogen levels, there was a single observation made per treatment area, which was derived from an aggregate soil sample. Aggregating 20 cores in each treatment area across the commercial potato field aims to mitigate fluctuations of pathogen levels along the landscape and capture a sample representative of the treated area. Since a single mean was produced per treatment strip, statistical analysis of soil pathogen levels could not be conducted. Repeated measures of the same soil sample are an aspect of the quantification methodology and as such a standard error is available for each pathogen level. The standard error is from repeated measures and not experimental replication. Regarding visual wilt observations, each mean of 15 plants was considered a single observation within its respective treatment area. Aggregating 15 plants into a single observation mitigated extremes and provided a more representative sample. Variability within each treatment strip was captured through replicated observations. Each tuber sample was considered a single observation of its respective treatment. Similar to previous sampling methods, the multiple samples across

any given treatment area aims to provide an appropriate representation of the area while capturing variability across the area. Treatment strip tuber and visual wilt metrics were statistically analyzed via paired comparisons with a Tukey adjustment for multiple comparisons. The standard errors of the means were derived from a sample size of eight samples per treatment strip and were not representing repeated measures. Only adjacent strips were compared due to their close proximity. Assumptions of residuals include a constant variance, mean zero, and normal distribution. In all cases, the program R (version 3.6.0) was utilized for statistical analysis.

2.7. Soil microbiome analyses

To complete objective 4, a structural analysis of organism communities in the soil was conducted. Soil collected from the final sampling date in each experimental field was sent to the Research and Development Institute for the Agri-Environment (IRDA), Quebec City. Metagenomic techniques were used in the microbiome analysis. The first field season included soil samples from each treatment strip pseudo-replicated by subsampling three times from each aggregate sample. Pseudo-replication was conducted to gauge the uniformity within the aggregated samples. As a cost-saving measure, the second field season had no pseudo-replication and instead each treatment strip was represented by a single sample. Therefore, means and standard errors were calculated for the first field season while only a single sample value was reported for attributes quantified in each treatment strip during the second field season. Prokaryotes were identified and characterized based on 16S ribosomal RNA gene sequences, focusing on the V3-V4 regions, according to previous research (Brassard et al., 2018). Fungi were identified and characterized based on internal transcribed spacer sequences, focusing on

the ITS1, according to previous research (McGuire et al., 2013). Eukarya were identified and characterized based on 18S ribosomal RNA gene sequences according to previous research (Comeau et al., 2017; Comeau et al., 2011). Eukarya quantification was completed for related research outside the scope of this project. Bioinformatics were completed with the Qiime 2 platform and involved a DADA2 filter step described in a past publication (Callahan et al., 2016). Three reference databases were used for taxonomic assignment and were Greengenes 13.8 (DeSantis et al., 2006), SILVA132 (Quast et al., 2013), and UNITE7 (Kõljalg et al., 2013). The bioinformatics data was visualized using R. Resulting visualizations showed relationships in microorganism community structures among soil treatments.

2.8. Challenges

A challenge encountered during soil sampling was maintaining sample integrity during transport to analyses locations. This was addressed by using an insulated cooler during sampling and Styrofoam boxes during shipping, both with ice packs for chilling. Adequate interior packaging was included to separate samples from ice packs and reduce the risk of damaging samples via freezing.

A random act of nature affected the integrity of the second field season. During the evening of August 6, 2019 a hailstorm completely defoliated field NE 8 and partially defoliated field NW 13. Given the nature of commercial scale field trials, this event was unavoidable. Data was still collected as per described methods. Due to uncertainty regarding the fate of field NE 8 following the hail event and limited labour, two small rounds of tuber harvesting were conducted in a one week interval prior to a complete sampling campaign.

3. Results

3.1. Levels of *Verticillium* spp. in soil

3.1.1. Field SE 5 of 2018

After field SE 5 was selected for the 2018 field season it was recorded to have 142 *Verticillium* spp. propagules per g of soil (Table 9). A count of 142 propagules per g of dry soil is four times the highest economic threshold of 30 propagules per g of dry soil. Between the pre-treatment sampling time and the May 22nd post-treatment sampling time, there was a decline in pathogen levels across all treatment strips. Strips Fumigation6 and Control9 resulted in an undetectable soil level of *Verticillium* spp. propagules. Strip Fumigation2, which lacked the use of a bed freshener, had a smaller reduction in *Verticillium* spp. propagules when compared to those seen in strips Fumigation6 and Fumigation8. There was an increase in the pathogen's soil levels in most of the treatment strips after the growing season. The exception was in VPE3, which experienced a decline in *Verticillium* spp. propagules between May 22nd and September 11th.

Table 9 Quantity of *Verticillium* spp. microsclerotia detected in soil of field SE 5 as count/g of dried soil with standard errors. Standard errors originate from multiple measurements on the same soil sample.

Treatment	Microsclerotia count/g of soil		
	Pre-treatment	Post-treatment, May 22	Post-treatment, September 11
Control1	142	6 ± 2	20 ± 3
Fumigation2		10 ± 3	36 ± 4
VPE3		10 ± 2	8 ± 2
VPE5		4 ± 2	10 ± 2
Fumigation6		0 ± 0	4 ± 1
Control7		8 ± 2	28 ± 3
Fumigation8		2 ± 1	22 ± 4
Control9		0 ± 0	18 ± 2

3.1.2. Field NE 8 of 2019

After field NE 8 was selected for the 2019 field season it was recorded to have 40 *Verticillium* spp. propagules per g of soil (Table 10). A count of 40 propagules per g of dry soil is 1.3 times that of highest economic threshold of 30 propagules per g of dry soil. Between the pre-treatment sampling time and post-treatment sampling time of May 6th, there were increases of propagules in some treatment strips and decreases in others. The control strips trended with an increase, with one of three strips decreasing in propagules. The fumigation strips varied, with Fumigation3 decreasing and Fumigation5 increasing compared to pre-treatment levels. Velum Prime1 on May 6th did not change appreciably from the pre-treatment levels while Elatus7 became half of the pre-treatment levels. On August 19th it was observed that Fumigation5 and Control6 strips declined compared to the May 6th sampling date. All other strips experienced an increase in *Verticillium* spp. propagules to above pre-treatment levels. Beyond the typical colonies one would expect from *Verticillium* spp., there were other atypical colonies observed on August 19th and recorded separately. The colonies were "heavy" in appearance compared to the typical microsclerotia one would expect *V. dahliae* to form (Figure 7). The atypical microsclerotia were present in soil from field NE 8 in quantities sometimes higher and sometimes lower than the typical colonies.

Table 10 Quantity of *Verticillium* spp. microsclerotia and atypical colonies detected in soil of field NE 8 as count/g of dried soil with standard errors. Standard errors originate from multiple measurements on the same soil sample.

Treatment	Microsclerotia count/g of soil			
	Pre-treatment	Post-treatment, May 6	Post-treatment, August 19 (typical colonies)	Post-treatment, August 19 (atypical colonies)
Velum Prime1		38 ± 7	94 ± 17	132 ± 14
Control2		2 ± 2	80 ± 17	74 ± 19
Fumigation3		26 ± 7	74 ± 17	24 ± 7
Control4	40 ± 7	58 ± 9	98 ± 14	47 ± 7
Fumigation5		88 ± 11	43 ± 6	78 ± 10
Control6		56 ± 12	42 ± 10	79 ± 27
Elatus7		18 ± 6	62 ± 16	30 ± 5

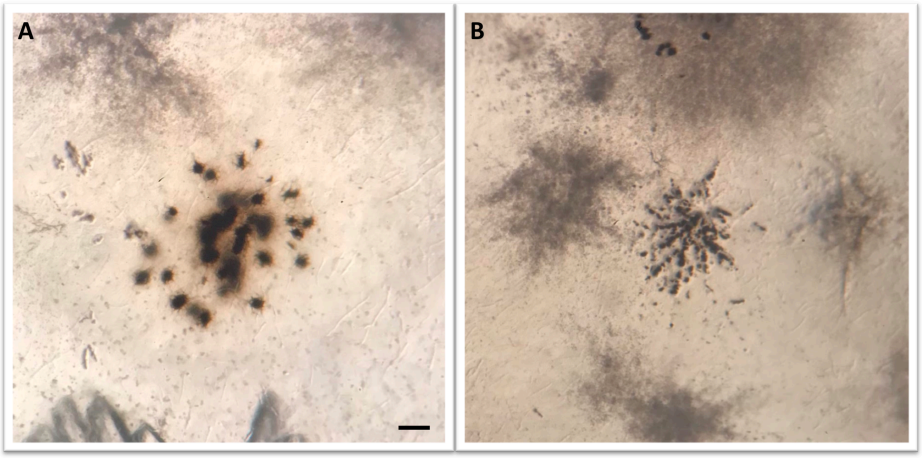

Figure 7 Colonies of microsclerotia originating from soil of field NE 8. **A**) Atypical colonies of unidentified organism on NP-10 media appearing heavy in nature, scale bar = 80 µm (Dr. Tyler MacKenzie, ACS Inc., used with permission), **B**) Typical *Verticillium* spp. colonies on NP-10 media appearing in a starburst shape (Dr. Tyler MacKenzie, ACS Inc., used with permission).

3.1.3. Field NW 13 of 2019

Field NW 13 was sampled for *Verticillium* spp. levels before experimental field selection and was observed to contain 40 propagules per g of soil (Table 11). A count of 40 propagules per g of dry soil is above the previously mentioned highest economic

threshold of 30 by a factor of 1.3. Soil levels recorded on May 9th showed that a decline occurred in all treatment strips, with the greatest declines to single digits in strips Elatus5 and Fumigation6. Declines in *Verticillium* spp. levels from the pre-treatment time to May 9th was not consistent between fumigation strips, evident with a difference of 24 propagules per g of soil. An increase in *Verticillium* spp. levels was observed across all treatment strips between May 9th and September 5th. Velum Prime3, Control4, and Fumigation6 had *Verticillium* spp. levels rise above levels initially determined before treatment. By September 5th, Velum Prime3 had the highest levels of *Verticillium* spp. propagules. Unlike field NE 8, there were very few atypical colonies observed in field NW 13 on the final soil sampling date of September 5th. The quantity of atypical colonies ranged from 0 to 4 propagules per g of soil.

Table 11 Quantity of *Verticillium* spp. microsclerotia and atypical colonies detected in soil of field NW 13 as count/g of dried soil with standard errors. Standard errors originate from multiple measurements on the same soil sample.

Treatment	Microsclerotia count/g of soil			
	Pre-treatment	Post-treatment, May 9	Post-treatment, September 5 (typical colonies)	Post-treatment, September 5 (atypical colonies)
Fumigation2		26 ± 8	30 ± 13	2 ± 2
Velum Prime3		16 ± 7	124 ± 14	4 ± 2
Control4	40 ± 4	20 ± 11	68 ± 8	0 ± 0
Elatus5		6 ± 2	22 ± 4	0 ± 0
Fumigation6		2 ± 2	104 ± 9	2 ± 2

3.2. Levels of *Pratylenchus* spp. in soil

3.2.1. Field SE 5 of 2018

After field SE 5 was selected for the 2018 field season it was sampled and recorded to contain 120 *Pratylenchus* spp. per kg of fresh, undried soil (Table 12). A

count of 120 nematodes per kg of fresh soil is below the previously mentioned lowest economic threshold by a factor of 9. Between the pre-treatment sampling time and the spring sampling time of May 22nd, there was a decline in *Pratylenchus* spp. levels across all treatment strips. There was an increase in *Pratylenchus* spp. soil levels in all the treatment strips between May 22nd and September 11th. There were no apparent trends in differences between treatment strips which received the bed freshener (strips 6, 7, 8, and 9) compared to those which did not receive the bed freshener (strips 1, 2, 3, and 5). There were no trends apparent in the choice of chemical treatment as results varied among the strips of the same soil treatment. Fumigation strips differed by up to 1220 counts on September 11th and VPE strips differed between each other by the same amount. Disregarding the level of bed freshener treatment, the control strips exhibited the least variable differences across the field on September 11th. By September 11th the Fumigation8 strip was exhibiting the highest soil levels of *Pratylenchus* spp., above the upper level of established economic thresholds for *P. penetrans* (Olthof, 1987).

Table 12 Quantity of *Pratylenchus* spp. detected in soil of field SE 5 as count/kg of fresh, undried soil.

Treatment	Nematode count/kg of fresh, undried soil		
	Pre-treatment	Post-treatment, May 22	Post-treatment, September 11
Control1	120	20	1020
Fumigation2		40	1080
VPE3		0	100
VPE5		20	1320
Fumigation6		0	1400
Control7		40	1160
Fumigation8		60	2300
Control9		0	1060

3.2.2. Field NE 8 2019

After field NE 8 was selected for the 2019 field season it was sampled and recorded to contain 900 *Pratylenchus* spp. counted per kg of fresh, undried soil (Table 13). A count of 900 nematodes per kg of fresh soil is barely below the previously mentioned lowest economic threshold by a count of 100. Between the pre-treatment sampling time and the spring sampling time of May 6th, there was a decline in *Pratylenchus* spp. levels across all treatment strips. The greatest decline was in the Fumigation3 strip, which had a reported quantity of zero. The Fumigation5 strip had a higher value than the aforementioned one, but still below economic threshold for *P. penetrans*. There were variable increases and decreases in *Pratylenchus* spp. soil levels in all the treatment strips between May 6th and August 19th. Two of the three control strips showed increases in the soil levels between May 6th and August 19th while one fumigation strip increased and the other decreased. By August 19th, Elatus7 had six times the quantity of root lesion nematodes compared to the Velum Prime1 strip. Furthermore, the highest reported *Pratylenchus* spp. soil levels were in the three control strips. All strips were below the economic threshold for *P. penetrans* throughout the field experiment.

Table 13 Quantity of *Pratylenchus* spp. detected in soil of field NE 8 as count/kg of fresh, undried soil.

Treatment	Nematode count/kg of fresh, undried soil		
	Pre-treatment	Post-treatment, May 6	Post-treatment, August 19
Velum Prime1		140	20
Control2		260	360
Fumigation3		0	60
Control4	900	80	360
Fumigation5		220	60
Control6		660	240
Elatus7		20	120

3.2.3. Field NW 13 of 2019

After field NW 13 was selected for the 2019 field season it was sampled and recorded to contain 440 *Pratylenchus* spp. counted per kg of fresh, undried soil (Table 14). A count of 440 nematodes per kg of fresh soil is below the previously mentioned lowest economic threshold by a factor of 2.3. There was a decline in root-lesion nematode levels across the experimental field between pre-treatment sampling and May 9th. Very few root-lesion nematodes remained in strips Fumigation2 and Elatus5, with zero reported for all other strips. By September 5th, the experimental field soil had zero root-lesion nematodes in it, regardless of treatment strip. No trends were observed for the applied soil treatments due to the overall low levels of *Pratylenchus* nematodes in this field.

Table 14 Quantity of *Pratylenchus* spp. detected in soil of field NW 13 as count/kg of fresh, undried soil.

Treatment	Nematode count/kg of fresh, undried soil		
	Pre-treatment	Post-treatment, May 9	Post-treatment, September 5
Fumigation2		20	0
Velum Prime3		0	0
Control4	440	0	0
Elatus5		40	0
Fumigation6		0	0

3.3. Soil microbial analyses

3.3.1. Abundance, ratios, and relative compositions of field SE 5 of 2018

The relative abundance of prokaryotes and fungi and ratios of the two in each treatment strip are available in Table 15. The units of measure are amplification units resulting from molecular analyses. Control strips consistently resulted in more abundant prokaryotes compared to Fumigation strips, regardless of the presence of a bed freshener (Figure 8). Velum Prime and Elatus strips resulted in different abundances of prokaryotes but still had a high quantity compared to other strips. When there was no bed freshener used, Fumigation2 resulted in a higher abundance of fungi compared to the adjacent Control1 strip (Figure 9). This trend did not continue when a bed freshener was used, as Fumigation6 resulted in less fungi than the adjacent Control7 strip while Fumigation8 resulted in more fungi than the adjacent Control9 strip. Both Control7 and Control9 strips were very similar in fungal abundance. Velum Prime and Elatus strips, when established without a bed freshener, produced a similar fungal abundance that was also close to Control7 and Control 9 strips. When the abundance of prokaryotes is compared to fungi, a resulting ratio provides insight on microbial community structure. Without the use of a bed freshener, the Control1 strip resulted in a ratio favoring prokaryotes over fungi more

so than the Fumigation2 strip (Figure 10). With the use of a bed freshener, the Fumigaiton6 strip resulted in a ratio favoring prokaryotes over fungi more so than the Control7 strip. The opposite is true of the Fumigation8 and Control9 strips.

Data detailing relative abundances were visualized by the IRDA. The composition of prokaryotic phyla varied little among treatment strips (Figure 11). No trends were observed alongside minor shifts in microbial community structure. The composition of fungal orders varied among treatment strips and included differences with some trends (Figure 12). Control1 and Fumigation2 strips appear similar to each other. Strips VPE3 and VPE5 showed differences from strips Control1 and Fumigation2 through a higher relative composition of organisms in the *Cystofilobasidiales* order. Among the strips that included a bed freshener, Fumigation8 resulted in the greatest proportion of *Cystofilobasidiales*. Fumigation6 and Control7 strips appear more similar to each other than Fumigation8 and Control9, of which the latter two show marked differences in the relative composition of *Cystofilobasidiales* and *Glomerellales*. The Control9 strip produced a microbial structure very similar to that of Control1, despite the two strips differing in the use of a bed freshener and being located on opposite sides of the experimental field. Relative abundances of eukaryotic organisms were observed to be similar across treatment strips, with fungi dominating the composition of all strips (Figure 13).

Table 15 Means of amplification units, ratios for prokaryotes and fungi, and standard errors in each treatment strip of field SE 5 of 2018. Standard errors originate from repeated measurements on subsamples from one aggregated soil sample, where n = 3 through subsampling.

Treatment	Prokaryote AU	Fungal AU	Ratio of prokaryote to fungal AU
Control1	3.94E+09 ± 1.48E+08	9.68E+07 ± 1.09E+07	42 ± 4
Fumigation2	3.86E+09 ± 2.47E+07	1.29E+08 ± 1.10E+07	30 ± 3
VPE3	5.61E+09 ± 8.73E+07	1.59E+08 ± 1.35E+07	36 ± 3
VPE5	4.79E+09 ± 2.67E+08	1.63E+08 ± 1.40E+07	30 ± 4
Fumigation6	4.37E+09 ± 1.85E+08	1.10E+08 ± 3.62E+06	40 ± 3
Control7	5.30E+09 ± 1.35E+08	1.64E+08 ± 1.20E+07	33 ± 2
Fumigation8	3.84E+09 ± 2.80E+08	1.89E+08 ± 2.94E+07	21 ± 3
Control9	4.45E+09 ± 3.33E+08	1.67E+08 ± 1.28E+07	27 ± 1

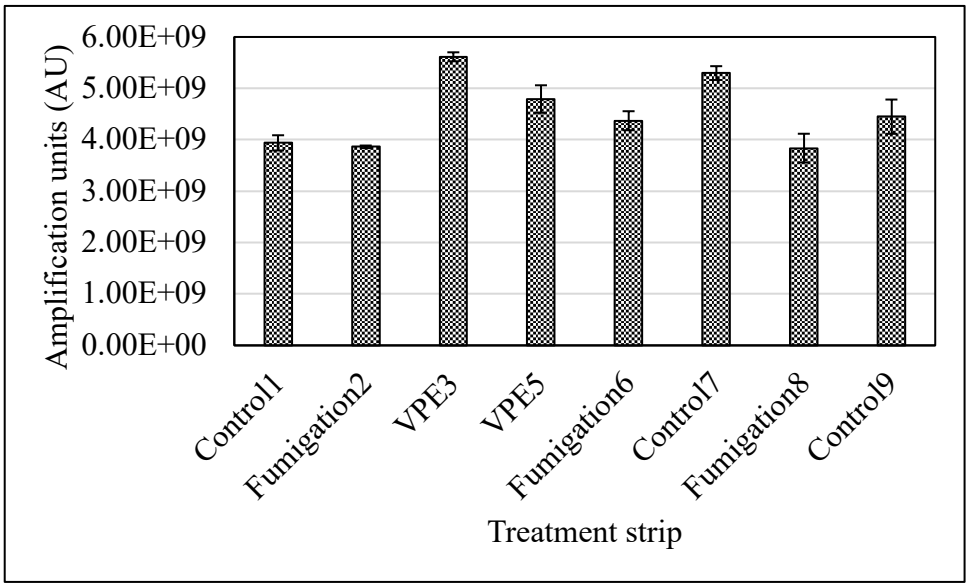

Figure 8 Amplification units providing a measure of abundance of prokaryotes in each treatment strip of field SE 5 of 2018. Standard errors originate from repeated measurements on subsamples from one aggregated soil sample, where n = 3 through subsampling.

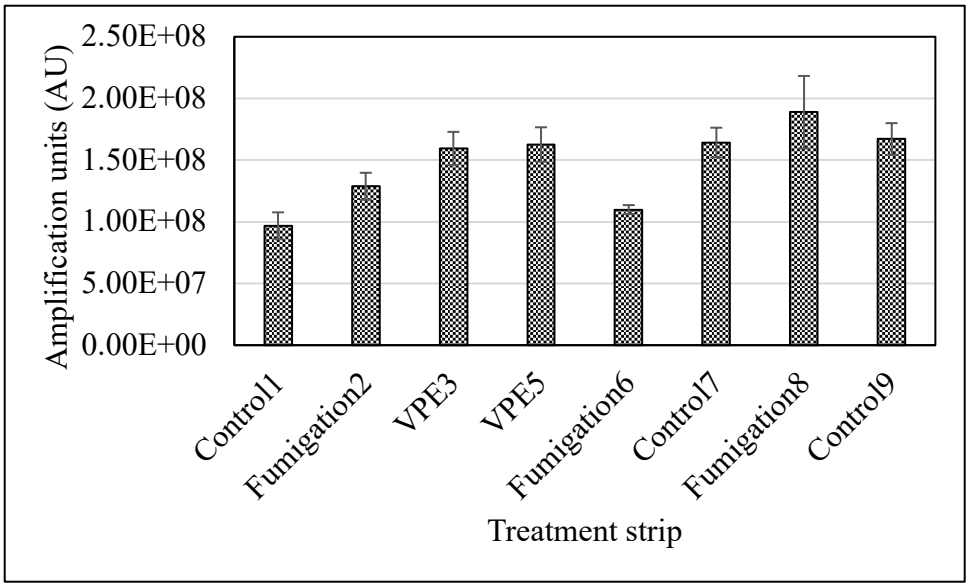

Figure 9 Amplification units providing a measure of abundance of fungi in each treatment strip of field SE 5 of 2018. Standard errors originate from repeated measurements on subsamples from one aggregated soil sample, where n = 3 through subsampling.

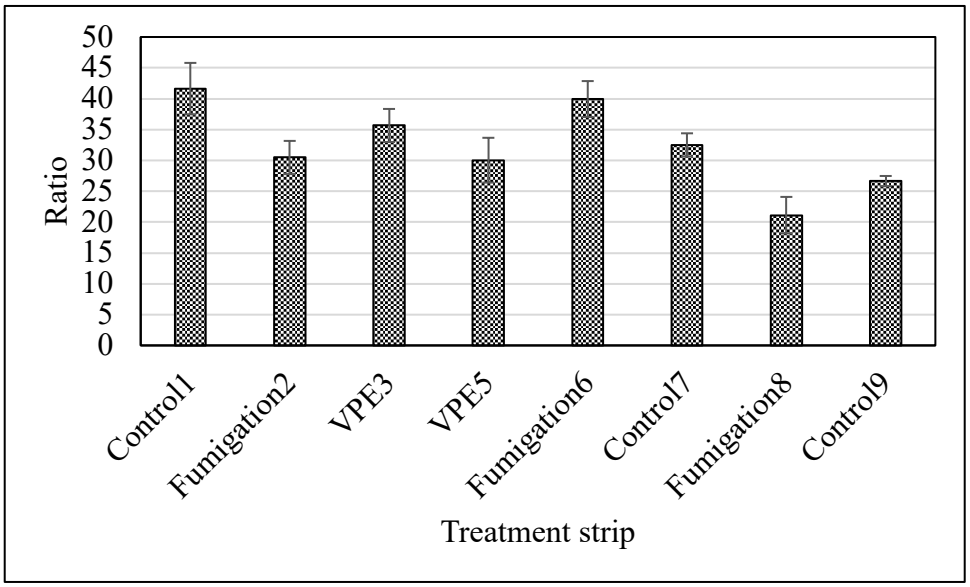

Figure 10 Ratios providing a measure of balance between prokaryotes and fungi in each treatment strip of field SE 5 of 2018. Standard errors originate from repeated measurements on subsamples from one aggregated soil sample, where n = 3 through subsampling.

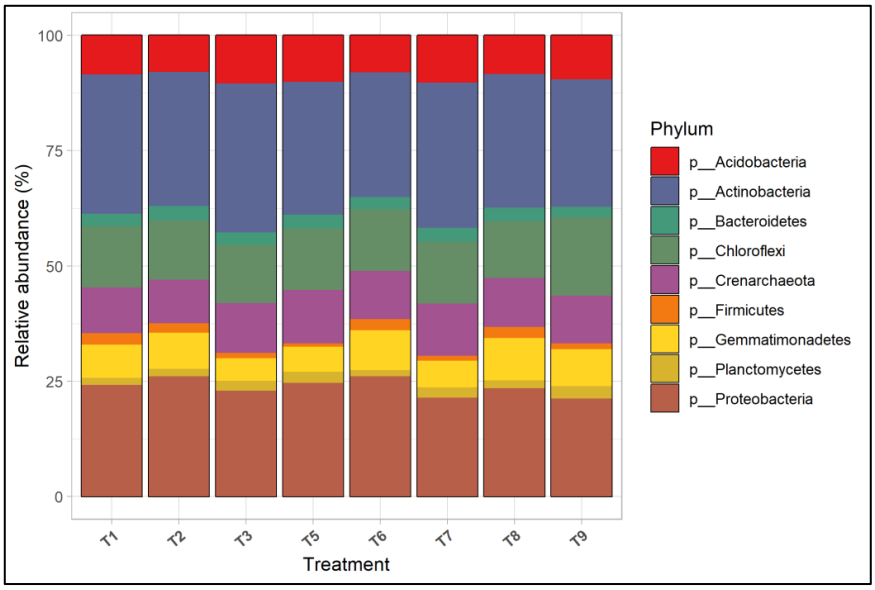

Figure 11 Composition of prokaryote phyla in field SE 5 of 2018. Treatment numbers correspond to equivalent numbers of treatment strips previously defined. Visualisation provided by the IRDA.

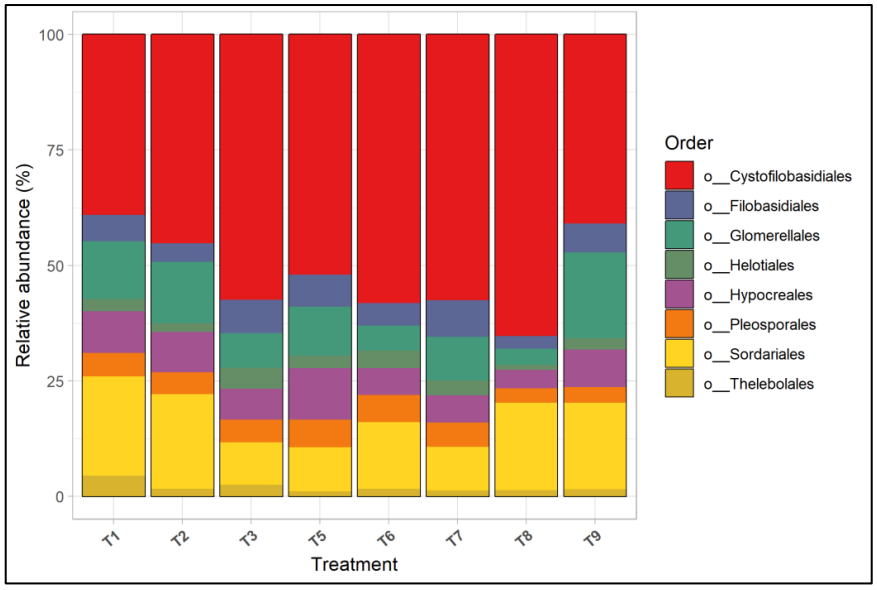

Figure 12 Composition of fungal orders in field SE 5 of 2018. Treatment numbers correspond to equivalent numbers of treatment strips previously defined. Visualisation provided by the IRDA.

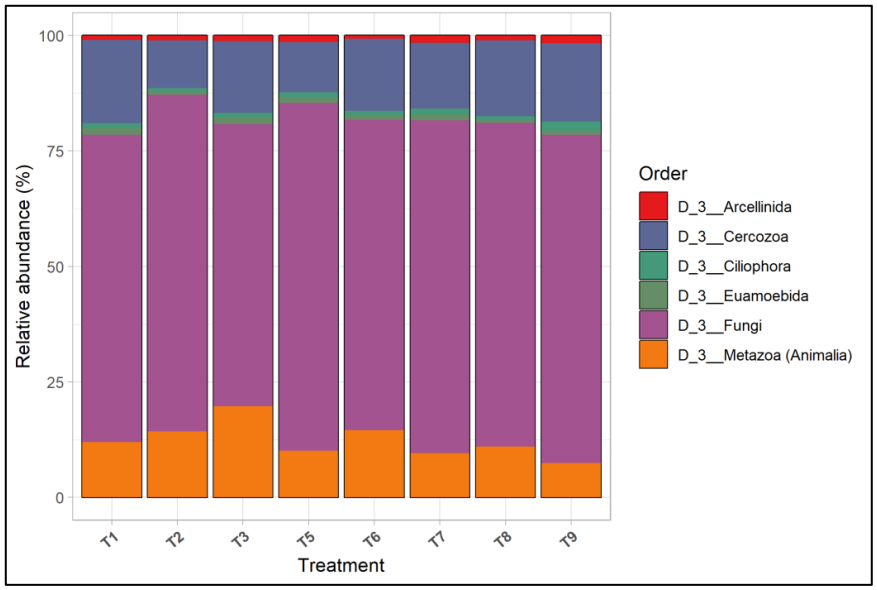

Figure 13 Composition of eukaryotic orders in field SE 5 of 2018. Treatment numbers correspond to equivalent numbers of treatment strips previously defined. Visualisation provided by the IRDA.

3.3.2. Abundance and ratios of field NE 8 of 2019

The relative abundance of prokaryotes and fungi and ratios of the two in each treatment strip are available in Table 16. The units of measure are amplification units resulting from molecular analyses. Control strips varied in their abundances of prokaryotes, with Control6 resulting in the lowest abundance and Control4 the highest abundance in the experimental field (Figure 14). Fumigation strips also varied, with Fumigation5 resulting in an abundance of prokaryotes above Control6 and Fumigation3 having abundances below Control4. Velum Prime1 and Elatus7 strips resulted in similar prokaryotic abundances on opposite sides of the field while being within the range of control strip abundances. Control strips varied in their abundances of fungi, with Control4 resulting in the lowest abundance and Control2 the third highest abundance in the experimental field (Figure 15). The Control2 strip and Velum Prime1 strip showed similar fungal abundances. There was no obvious trend observed in terms of fungal

abundances and the effect of fumigation. The Fumigation3 strip showed a fungal abundance between that observed in strips Control2 and Control4, while Fumigation5 showed a fungal abundance higher than adjacent Control4 and Control6 strips. When the abundance of prokaryotes is compared to fungi, a resulting ratio provides insight on microbial community structure. Fumigation3 and Control4 strips stand out among the treatment strips, with Control4 resulting in the highest ratio (Figure 16). The ratio of Control4 was over twice that of the lowest observed ratio observed in strip Control2. The ratios show similarities among each treatment strip aside from the notable Fumigation3 and Control4 strips.

Table 16 Amplification units and ratios for prokaryote and fungi for field NE 8 of 2019.

Treatment	Prokaryote AU	Fungal AU	Ratio of prokaryote to fungal AU
Velum Prime1	8.60E+09	1.94E+08	44
Control2	7.46E+09	1.90E+08	39
Fumigation3	8.04E+09	1.32E+08	61
Control4	9.34E+09	1.15E+08	81
Fumigation5	7.35E+09	1.52E+08	48
Control6	6.68E+09	1.35E+08	50
Elatus7	8.18E+09	2.11E+08	39

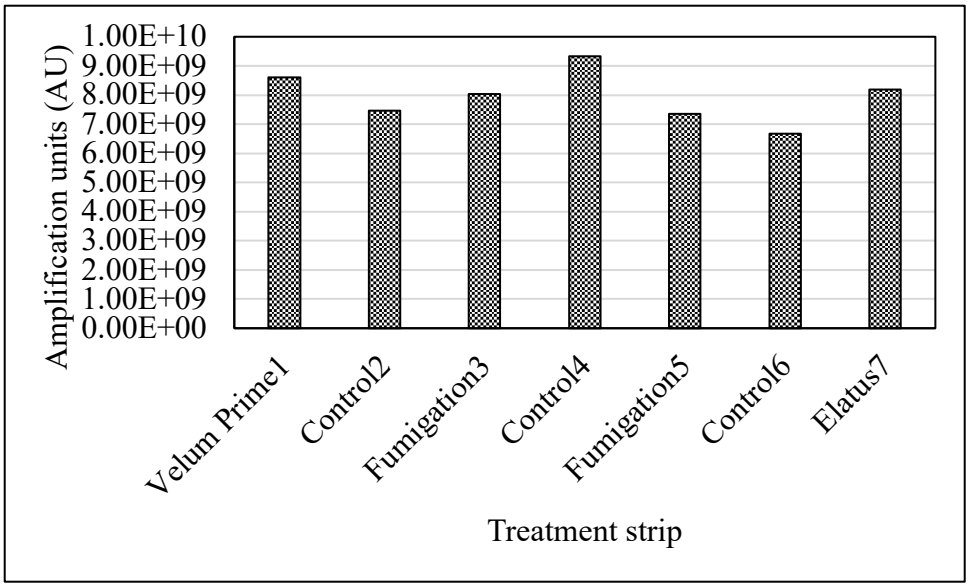

Figure 14 Amplification units providing a measure of abundance of prokaryote in each treatment strip of field NE 8 of 2019.

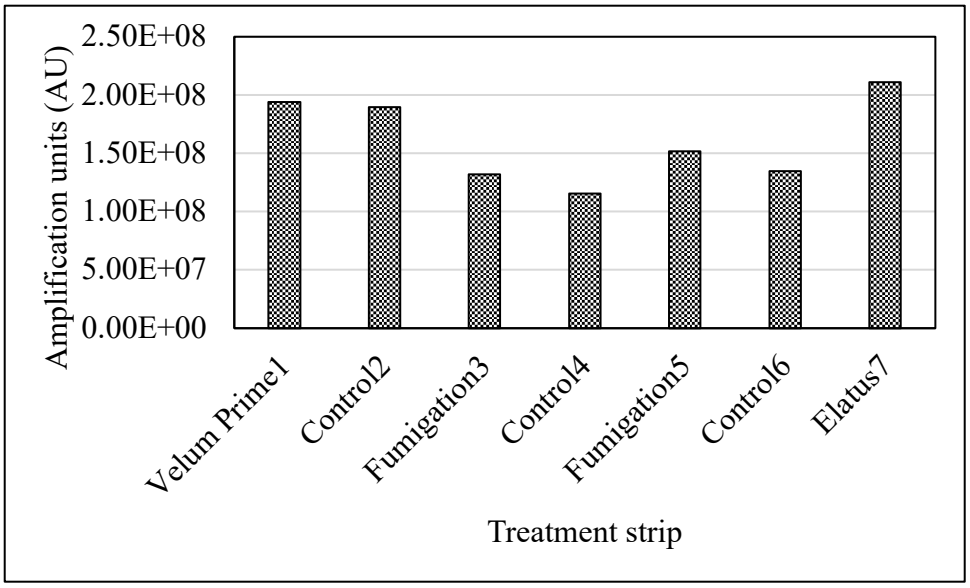

Figure 15 Amplification units providing a measure of abundance of fungi in each treatment strip of field NE 8 of 2019.

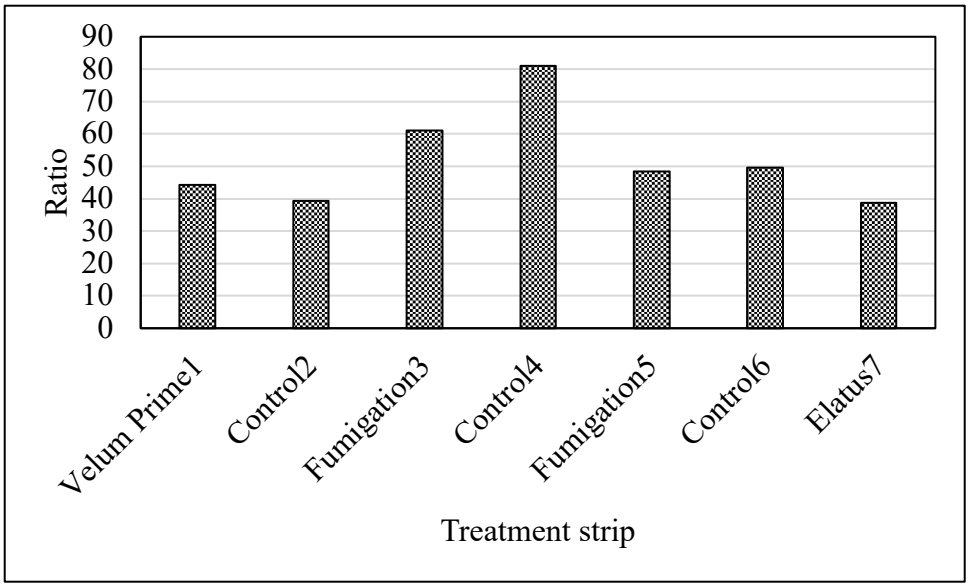

Figure 16 Ratios providing a measure of balance between prokaryote and fungi in each treatment strip of field NE 8 of 2019.

3.3.3. Abundance and ratios of field NW 13 of 2019

The relative abundance of prokaryotes and fungi and ratios of the two in each treatment strip are available in Table 17. The abundance of prokaryotes in field NW 13 ranged from lowest to highest starting on the western edge of the field and moving to the east (Figure 17). The Fumigation2 strip resulted in the lowest abundance of prokaryotes while the Fumigation6 strip resulted in the highest abundance. Velum Prime3 resulted in a prokaryotic abundance between those seen in Fumigation2 and Control4 strips, while Elatus5 resulted in a prokaryotic abundance between those seen in Control4 and Fumigation6 strips. Fungal abundances did not follow the same trend in spatial distribution as prokaryotic abundances did. Fungal abundances were similar among treatment strips with the exception of Control4 (Figure 18). The Control4 strip resulted in an appreciably higher fungal abundance than all other strips in the experimental field. When the abundance of prokaryotes is compared to fungi, a resulting ratio provides

insight on microbial community structure. The lowest ratio of prokaryotes to fungi was observed in the Control4 strip, coinciding with a noticeably higher fungal abundance compared to other treatment strips (Figure 19). Fumigation treatment strips ranged from a ratio of approximately 61 to approximately 86. Velum Prime3 and Elatus5 strips resulted in ratios similar to each other and between those observed in fumigation strips.

Table 17 Amplification units and ratios for prokaryote and fungi for field NW 13 of 2019.

Treatment	Prokaryote AU	Fungal AU	Ratio of prokaryote to fungal AU
Fumigation2	6.67E+09	1.09E+08	61
Velum Prime3	8.03E+09	1.09E+08	74
Control4	8.85E+09	1.76E+08	50
Elatus5	9.13E+09	1.21E+08	76
Fumigation6	9.18E+09	1.06E+08	86

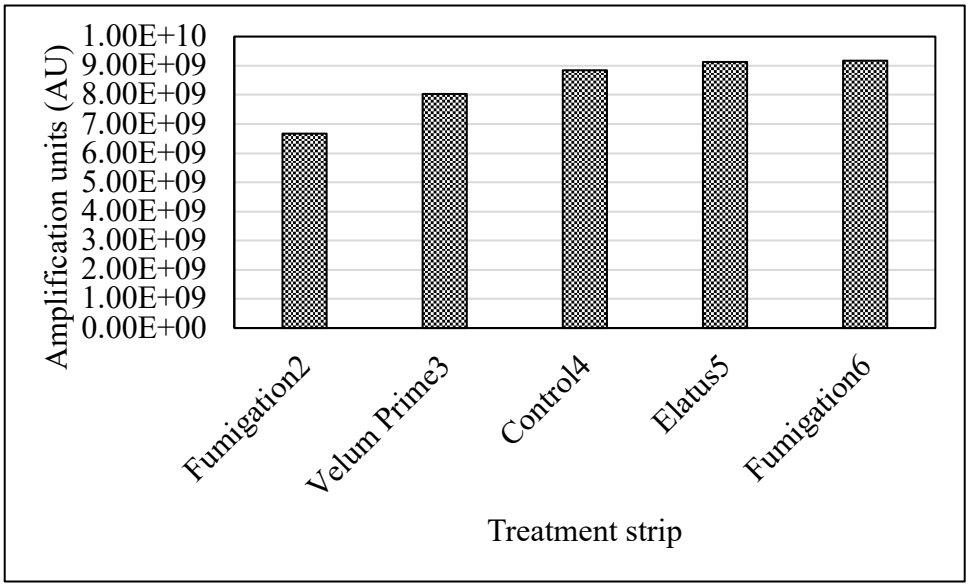

Figure 17 Amplification units providing a measure of abundance of prokaryote in each treatment strip of field NW 13 of 2019.

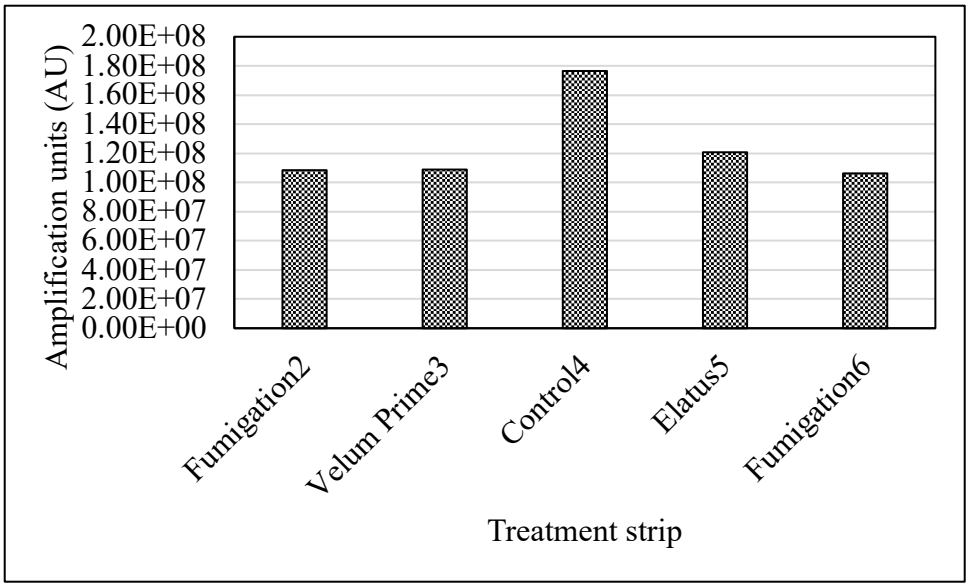

Figure 18 Amplification units providing a measure of abundance of fungi in each treatment strip of field NW 13 of 2019.

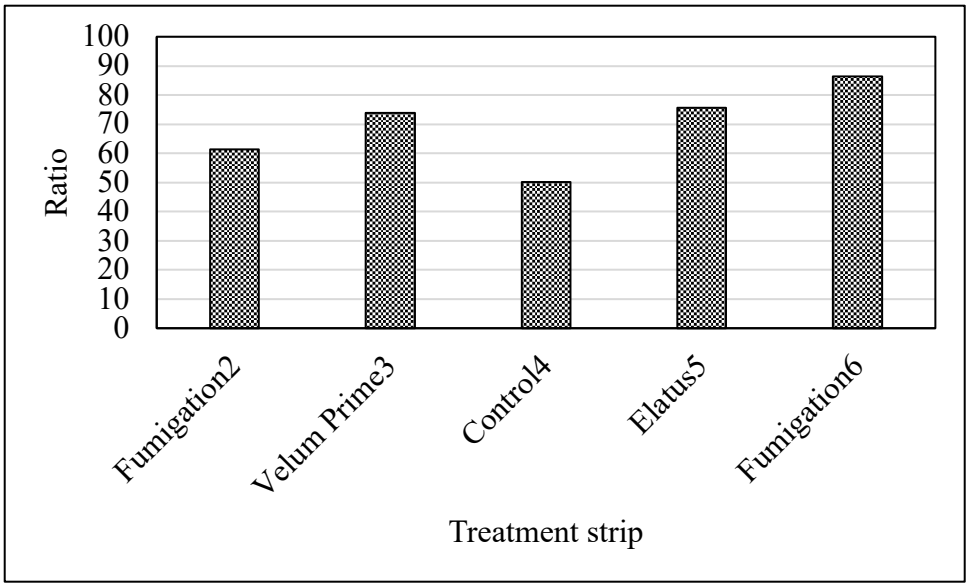

Figure 19 Ratios providing a measure of balance between prokaryote and fungi in each treatment strip of field NW 13 of 2019.

3.3.4. Relative compositions of fields NE 8 and NW 13 of 2019

Table 18 and Table 19 provide details of each treatment strip coded for analyses with the IRDA, showing chemical applied, level of soil disturbance, and presence of

manure. Parallel to the first field season, the second field season's soil microbial structures were visualized by the IRDA and show relative compositions of phyla for prokaryotes (Figure 20), orders for fungi (Figure 21), and orders for eukarya (Figure 22).

Table 18 Coded treatments for relative microbial composition in field NE 8.

Code	Strip number	Chemical	Soil disturbance
CC1	1	Velum Prime	No freshener
CC2	2	Control	No freshener
CC3	3	Chloropicrin	No freshener
CC4	4	Control	No freshener
CC5	5	Chloropicrin	No freshener
CC6	6	Control	No freshener
CC7	7	Elatus	No freshener

Table 19 Coded treatments for relative microbial composition in field NW 13.

Code	Strip number	Chemical	Soil disturbance	Manure
CP2	2	Chloropicrin	Bed freshener	No
CP3	3	Velum Prime	Bed freshener	Yes
CP4	4	Control	Bed freshener	Yes
CP5	5	Elatus	Bed freshener	Yes
CP6	6	Chloropicrin	Bed freshener	Yes

Attention should be directed to Figure 20 for graphical representation of prokaryotic phylum relative abundances. CC3 and CC5 fumigation strips differed from the adjacent control strips by resulting in larger relative quantities of *Gemmatimonadetes* and *Bacteroidetes*, while resulting in lower relative quantities of *Acidobacteria*. CC1 and CC7 in-furrow chemical strips appeared very similar in relative quantities of prokaryotes, despite being on opposite sides of experimental field NE 8. Control strips CC6, CC4, and CC2 located across the experimental field appeared similar to each other in prokaryote relative composition. In experimental field NW 13, CP2 and CP6 fumigation strips resulted in lower *Acidobacteria* quantities compared to control and in-furrow treatment

strips. CP2 and CP6 fumigation strips also resulted in larger quantities of *Bacteroidetes* compared to control and in-furrow treatment strips. The CP5 Elatus strip comparatively resulted in a larger relative quantity of *Firmicutes* than CP3 Velum Prime and CP4 Control strips while remaining similar in other prokaryotic phyla. The phyla *Bacteroidetes* trended higher while *Acidobacteria* trended lower in relative quantities in fumigation portions of both experimental fields, regardless of the use of a bed freshener. Small differences among treatment strips were noted within experimental fields but no major differences in relative quantities of prokaryotic phyla were visualized between the experimental fields NE 8 and NW 13.

Attention should be directed to Figure 21 for graphical representation of fungal order relative abundances. The relative composition of fungal orders across both experimental fields varied more than that recorded for prokaryotic phyla. There was less consistency within fields and between fields. The CC3 fumigation strip of experimental field NE 8 resulted in a larger relative quantity of *Agaricales* fungi compared to all other treatment strips, many of which exhibited zero relative quantities of the fungal order. Treatment strip CC3 also resulted in the largest relative quantity of unidentified fungal orders among all treatment strips. The CC3 and CC5 fumigation strips resulted in the largest relative quantities of *Thelebolales* of all treatment strips in experimental field NE 8. The fungal order *Helotiales* were relatively absent in the CC5 fumigation strip but were present in the CC3 fumigation strip in relative quantities similar to adjacent control strips CC2 and CC4. The fungal order *Pezizales* was found in a relatively absent quantity in fumigation strip CC3 compared to all other treatment strips in experimental field NE 8, which resulted in similar relative quantities of the order. The fungal order *Microascales*

were found in the highest relative quantity on the eastern side of experimental NE 8 and gradually lessened in relative quantity in a westerly direction across the experimental field. Fumigation strips CP2 and CP6 in experimental field NW 13 resulted in the highest relative quantities of the fungal order *Sordariales* among all treatment strips. The fungal order *Mortierellales* was in the largest relative composition in the CP4 control strip, while all other treatment strips in field NW 13 resulted in relative quantities similar to each other. The CP3 Velum Prime strip of field NW 13 resulted in a low relative quantity of *Eurotiales* but exhibited the largest relative quantity of *Auriculariales* and *Cystofilobasidiales* of all treatment strips in the experimental field. The *Microascales* fungal order was relatively absent in all treatment strips of experimental field NW 13 except in CP5 Elatus and CP6 fumigation strips. Overall, experimental field NW 13 resulted in noticeably different relative quantities of fungal orders compared to NE 8. The former of the two utilized a bed freshener prior to application of soil chemicals and planting in place of a standard power hiller. The *Cystofilobasidiales* fungal order was in relatively greater quantities in field NW 13 than NE 8. Meanwhile, the *Sordariales* order was in relatively greater quantities in field NE 8 than in NW 13.

The following results include eukaryotic order relative abundances and attention should be directed to Figure 22 for graphical representation. The CC7 fumigation strip of experimental field NE 8 resulted in the highest relative quantity of *Didymium* while all other treatment strips in the field were relatively absent of the order. Fumigation strips CC3 and CC5 of field NE 8 resulted in relatively absent quantities of WIM 1 lineage organisms. This was a trend of fumigation strips in the field but was also observed in control strip CC6 and Elatus strip CC7. The CC1 Velum Prime treatment strip resulted in

a relatively higher quantity of *Euamoebida* compared to all other treatments of experimental fields NE 8 and NW 13. This result was not similarly observed in the CP3 Velum Prime strip of NW 13, showing a difference coinciding with differing bed freshener use. The CP6 fumigation strip of experimental field NW 13 resulted in the highest relative quantity of *Apicomplexa* while all other treatment strips of both experimental fields resulted in relatively zero quantities of the order. Experimental field NW 13 resulted in higher relative quantities of the eukaryotic order *Cercozoa* than field NE 8. Technically considered a multi-cellular organism, the results of relative quantities of fungi were included in the relative compositions of eukaryotic organisms in experimental fields NW 13 and NE 8. Field NW 13 resulted in a relative quantity of fungi lower than field NE 8. The relative quantities of fungi compared among strips in either of the two fields appear similar. Therefore, while there was a noticeable difference in relative quantities of fungi between fields, within each field there were similar relative quantities of fungi among treatment strips.

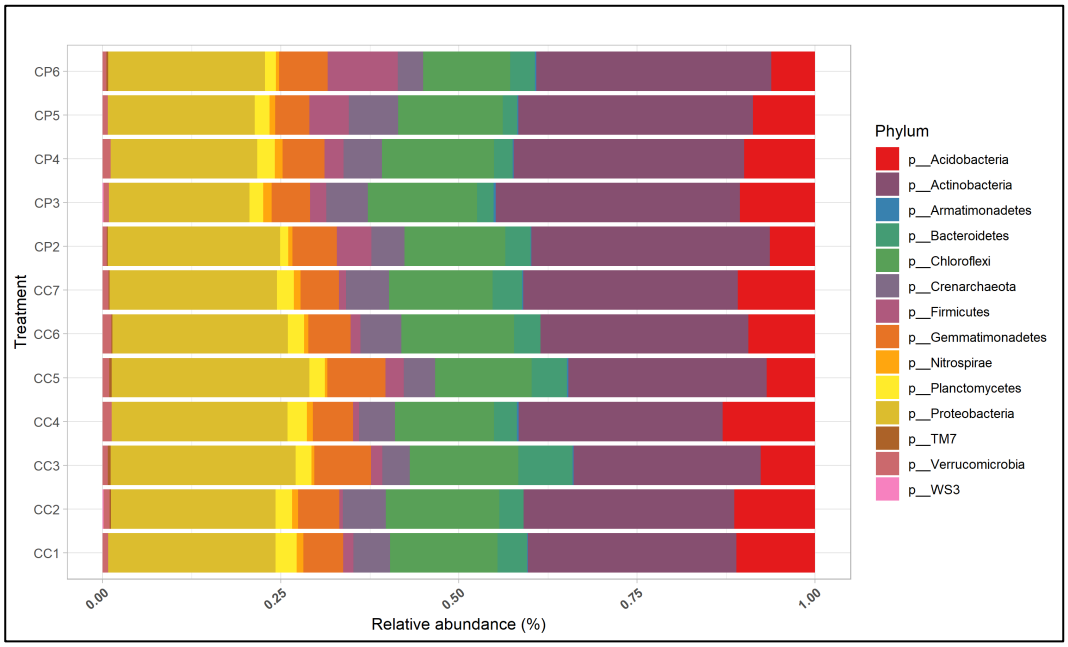

Figure 20 Composition of prokaryote phyla in fields NE 8 and NW 13 of 2019. Visualisation provided by the IRDA.

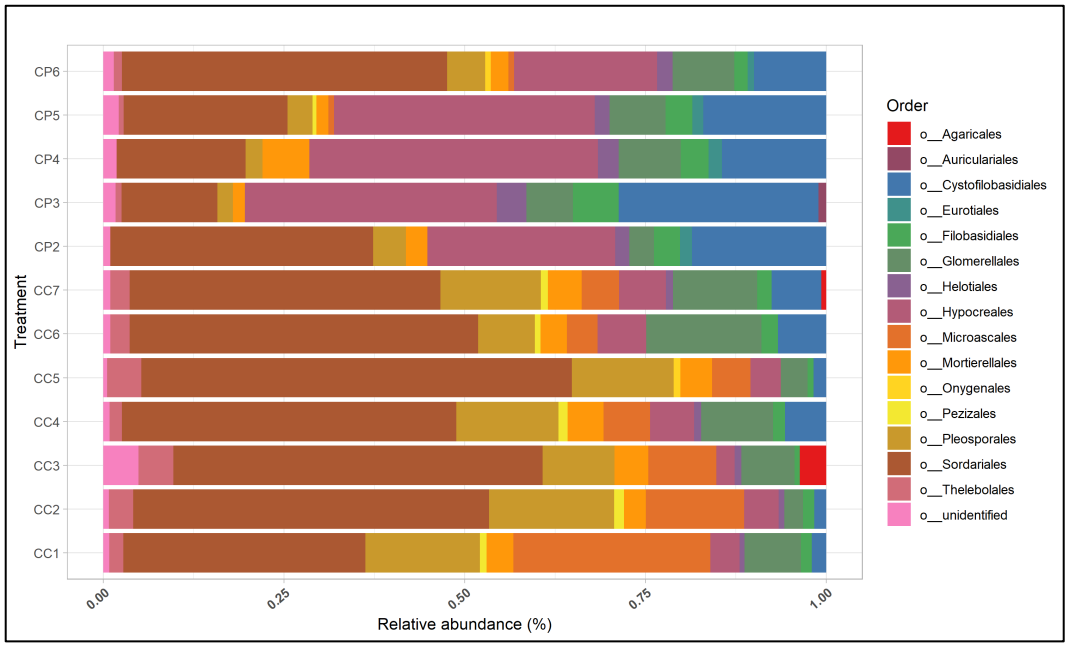

Figure 21 Composition of fungal orders in fields NE 8 and NW 13 of 2019. Visualisation provided by the IRDA.

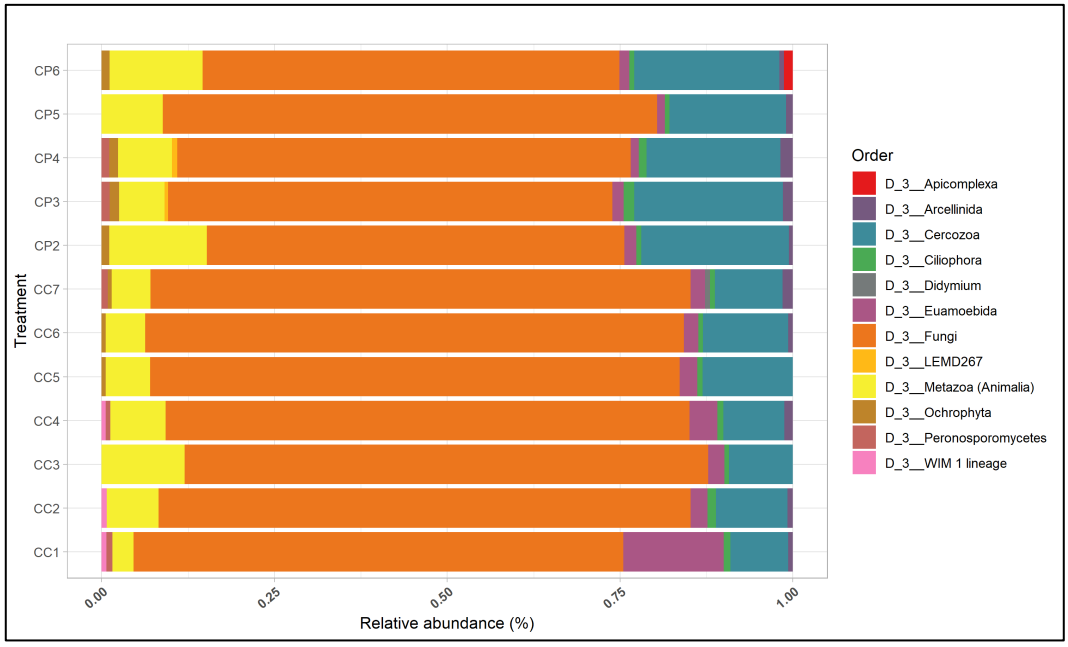

Figure 22 Composition of eukaryotic orders in fields NE 8 and NW 13 of 2019. Visualisation provided by the IRDA.

3.4. Visual wilt ratings of the canopy

Statistically significant results were observed when comparing strips Control1 and Fumigation2 and strips Fumigation2 and VPE3 (Table 20). These strips lacked the use of a bed freshener in their execution. Fumigation2 scored a statistically significantly lower wilt rating than both the Control1 and VPE3 strips by a full visual rating category (Table 21). While this trend was observed with other fumigation and control strip comparisons, these other comparisons were not statistically significant. A general trend towards lower wilt was observed in the fumigated strips of the field compared to other strips regardless of bed freshener use. Wilt scores were similar in control and Velum Prime plus Elatus strips. Control strips including the use of a bed freshener, Control7 and Control9, scored lower in their visual wilt compared to the control strip without the use of a bed freshener, Control1.

Data from the 2019 field season was unavailable due to hail.

Table 20 Visual wilt rating statistically significant differences between pairs of importance in field SE 5 of 2018. ** is significant at the 0.01 level and NS is not significant at the 0.05 level, and n = 3. Statistical significance determined through Tukey adjusted multiple comparisons with relevant comparisons displayed.

Treatment 1	Treatment 2	Statistical significance of visual wilt rating
Control1	Fumigation2	**
Fumigation2	VPE3	**
Fumigation6	Control7	NS
Control7	Fumigation8	NS
Fumigation8	Control9	NS

Table 21 Visual wilt rating mean values and standard errors in field SE 5 of 2018. Through replication, n = 3.

Treatment	Visual wilt rating
Control1	2.7 ± 0.22
Fumigation2	1.6 ± 0.19
VPE3	2.7 ± 0.14
VPE5	2.4 ± 0.12
Fumigation6	1.7 ± 0.10
Control7	2.3 ± 0.19
Fumigation8	2.0 ± 0.13
Control9	2.4 ± 0.06

3.5. Potato yield and tuber quality attributes

3.5.1. Field SE 5 of 2018

There were trends apparent from the first field season but statistical significance was lacking (Table 22). The fumigation treatment trended higher in mean gross yield compared to the adjacent control strips regardless of the bed freshener treatment (Table 23). Fumigation also trended higher in the greater than 10 ounce metric compared to the control while the smalls metric trended lower in 2 of the 3 paired comparisons. The stems per plant trended higher in fumigation strips compared to the control, but only in strips which included the use a bed freshener. Velum Prime plus Elatus treatment consistently

resulted in fewer tubers per plant than other treatment strips, excluding one control strip. VPE3 produced gross yield similar to Control1, while VPE5 was similar to Fumigation2, with an overall difference between the two VPE strips of 30.1 cwt/ac. Specific gravity remained consistent across the field. The only statistically significant result in paired comparisons was that Fumigation8 produce significantly higher tubers per plant than Control9 (Table 22 and Table 23). This trend was not observed in other paired comparisons involving control and fumigation strips.

Table 22 Tuber related attribute statistically significant differences in paired comparisons of interest in field SE 5. * is statistically significant at 0.05, NS is not statistically significant at 0.05, and n = 8. Statistical significance determined through Tukey adjusted multiple comparisons with relevant comparisons displayed.

Treatment 1	Treatment 2	Gross Yield	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
Control1	Fumigation2	NS	NS	NS	NS	NS	NS
Fumigation2	VPE3	NS	NS	NS	NS	NS	NS
Fumigation6	Control7	NS	NS	NS	NS	NS	NS
Control7	Fumigation8	NS	NS	NS	NS	NS	NS
Fumigation8	Control9	NS	*	NS	NS	NS	NS

Table 23 Tuber related attribute mean values and standard errors in field SE 5. Sample size of n = 8.

Treatment	Gross Yield (cwt/ac)	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
Control1	463.4 ± 26.3	13.0 ± 0.4	4.6 ± 0.3	6.2 ± 0.9	8.1 ± 2.7	1.091 ± 0.0
Fumigation2	496.1 ± 25.1	12.5 ± 0.5	4.2 ± 0.3	6.1 ± 0.5	8.3 ± 1.4	1.091 ± 0.0
VPE3	467.4 ± 15.7	11.5 ± 0.6	3.6 ± 0.2	4.5 ± 1.0	11.8 ± 3.7	1.094 ± 0.0
VPE5	497.5 ± 13.3	11.4 ± 0.4	3.5 ± 0.2	5.2 ± 1.0	7.2 ± 1.9	1.094 ± 0.0
Fumigation6	545.1 ± 28.5	12.4 ± 0.4	3.7 ± 0.1	4.6 ± 0.4	16.6 ± 2.4	1.093 ± 0.0
Control7	476.8 ± 18.2	12.6 ± 0.4	3.4 ± 0.1	5.4 ± 0.8	13.1 ± 4.8	1.094 ± 0.0
Fumigation8	512.8 ± 27.7	13.1 ± 0.3	3.8 ± 0.2	5.1 ± 0.5	23.8 ± 4.7	1.093 ± 0.0
Control9	479.0 ± 42.1	10.8 ± 0.7	3.5 ± 0.1	3.9 ± 0.9	21.6 ± 4.1	1.096 ± 0.0

3.5.2. Field NE 8 2019

There were significant differences in paired comparisons of field NE 8 in all metrics except tubers per plant and percent smalls. Fumigation5 was statistically significantly lower in gross yield compared to both the Control4 and Control6 strips (Table 24). This trend was also seen when comparing the Fumigation3 strip to Control2 and Control4, although it was not statistically significant (Table 25). VP1 showed a higher mean gross yield compared to its nearest control strip while Elatus7 showed a lower mean gross yield compared to its nearest control strip. No trends were apparent in the tubers per plant metric because strips varied in their results. A statistically significant result in stems per plant entailed the Control2 strip counting higher than Fumigation3. This trend was observed in all other comparisons between control and fumigation strips, although only the single comparison was statistically significant. Elatus7 showed stem

counts per plant lower than the controls while VP1 resulted in stem counts on par with controls. No trends were apparent from the percent smalls metric because strips varied in their results. Although there were no statistically significant results in the percent smalls, results showed statistical significance in the percent greater than 10 ounces metric. Control4 significantly outperformed strips Fumigation3 and Fumigation5 in percent greater than 10 ounces, without the same statistically significant difference appearing in the percent smalls. All other treatment strips were relatively similar in greater than 10 ounce metric without further statistical significance between paired comparisons of interest. The specific gravity of Fumigation5 was statistically significantly lower than Control4, but no other statistical significance was found in other paired comparisons.

Hail occurred on August 6, 2019 in field NE 8. The result was 100% defoliation of the canopy. Effect of the hail on the canopy was subjectively scored as a percentage. The effect of hail on yield and tuber metrics was not formally quantified. The date of the hail was at least 30 days prior to the intended commercial harvest date. This represents premature termination of the crop and likely affected the quantified tuber metrics and measured yield.

Table 24 Tuber related attribute statistically significant differences in paired comparisons of interest in field NE 8. * is statistically significant at 0.05, ** at 0.01, *** at 0.001, NS is not statistically significant at 0.05, and n = 8. Statistical significance determined through Tukey adjusted multiple comparisons with relevant comparisons displayed.

Treatment 1	Treatment 2	Gross Yield	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
VP1	Control2	NS	NS	NS	NS	NS	NS
Control2	Fumigation3	NS	NS	*	NS	NS	NS
Fumigation3	Control4	NS	NS	NS	NS	***	NS
Control4	Fumigation5	**	NS	NS	NS	**	*
Fumigation5	Control6	*	NS	NS	NS	NS	NS
Control6	Elatus7	NS	NS	NS	NS	NS	NS

Table 25 Tuber related attribute mean values and standard errors in field NE 8. Sample size of n = 8.

Treatment	Gross Yield (cwt/ac)	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
VP1	362.1 ± 15.0	9.3 ± 0.4	3.0 ± 0.1	16.07 ± 1.2	3.63 ± 1.1	1.077 ± 0.0
Control2	330.3 ± 9.8	8.7 ± 0.3	3.1 ± 0.1	20.76 ± 4.1	3.52 ± 1.3	1.076 ± 0.0
Fumigation3	308.8 ± 19.6	7.8 ± 0.5	2.6 ± 0.1	17.51 ± 1.8	2.18 ± 0.7	1.074 ± 0.0
Control4	361.6 ± 12.7	7.7 ± 0.4	2.8 ± 0.1	13.60 ± 1.4	11.00 ± 1.8	1.076 ± 0.0
Fumigation5	269.5 ± 23.4	7.2 ± 0.6	2.5 ± 0.1	23.10 ± 2.0	3.61 ± 1.3	1.069 ± 0.0
Control6	349.8 ± 22.3	9.2 ± 0.6	3.0 ± 0.2	19.48 ± 4.5	4.03 ± 1.6	1.074 ± 0.0
Elatus7	332.5 ± 14.1	8.4 ± 0.5	2.7 ± 0.1	19.03 ± 1.3	2.83 ± 1.4	1.074 ± 0.0

3.5.3. Field NW 13 of 2019

There were significant differences in paired comparisons of field NW 13 in the metrics of gross yield, tubers per plant, and percent smalls (Table 26). The gross yield of Fumigation2 was statistically higher than the adjacent VP3 strip. The gross yield of Elatus5 was statistically higher than the adjacent Control4 strip, with a significant

difference of approximately 143 cwt/ac (Table 27). Elatus5 also outperformed the control by statistically differing in the tubers per plant, resulting in an additional 3.4 tubers per plant compared to the Control4 strip. No statistically significant results were observed in the stems per plant metric, with both fumigation strips providing the highest and lowest mean values across the experimental field. The Elatus5 strip statistically differed from the Control4 strip in the percent smalls metric, with Elatus5 producing about 18% less small tubers than the adjacent control strip. This difference in the smalls metric was not paralleled in the percent greater than ten ounces metric, as all treatment strips had a near zero reading for the greater than ten ounces metric. No statistically significant results were observed in the specific gravity metric, with both fumigation strips providing the highest and lowest mean values across the experimental field.

Hail occurred on August 6, 2019 in field NW 13. The result was 70% defoliation of the canopy. Effect of the hail on the canopy was subjectively scored as a percentage. The effect of hail on yield and tuber metrics was not formally quantified. The date of the hail was at least 30 days prior to the intended commercial harvest date. This represents premature termination of the crop and likely affected the quantified tuber metrics and measured yield.

Table 26 Tuber related attribute statistically significant differences in paired comparisons of interest in field NW 13. * is statistically significant at 0.05, ** at 0.01, **** at 0.0001, NS is not statistically significant at 0.05, and n = 8. Statistical significance determined through Tukey adjusted multiple comparisons with relevant comparisons displayed.

Treatment 1	Treatment 2	Gross Yield	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
Fumigation2	VP3	*	NS	NS	NS	NS	NS
VP3	Control4	NS	NS	NS	NS	NS	NS
Control4	Elatus5	****	**	NS	*	NS	NS
Elatus5	Fumigation6	NS	NS	NS	NS	NS	NS

Table 27 Tuber related attribute mean values and standard errors in field NW 13. Sample size of n = 8.

Treatment	Gross Yield (cwt/ac)	Tubers per plant	Stems per plant	Smalls (%)	Greater than 10 ounces (%)	Specific gravity
Fumigation2	251.9 ± 14.1	9.4 ± 0.4	3.0 ± 0.1	34.3 ± 2.5	0.0 ± 0.0	1.080 ± 0.0
VP3	169.6 ± 23.1	7.5 ± 0.9	2.8 ± 0.1	47.4 ± 4.1	0.0 ± 0.0	1.077 ± 0.0
Control4	133.9 ± 5.9	6.6 ± 0.3	2.8 ± 0.2	56.7 ± 6.9	0.0 ± 0.0	1.077 ± 0.0
Elatus5	277.2 ± 21.5	10.0 ± 0.5	2.9 ± 0.1	38.4 ± 2.9	0.4 ± 0.4	1.075 ± 0.0
Fumigation6	295.2 ± 15.0	10.0 ± 0.4	2.7 ± 0.1	28.5 ± 2.3	0.0 ± 0.0	1.075 ± 0.0

3.6. Cost analysis

A basic comparison among treatment means alongside per acre treatment cost without accounting for experimental design or statistical assumptions provided a general cost analysis. Across all site-years, two unfavourable treatment combinations were observed in a single field, one treatment combination was conditionally favorable, and all other treatment combinations were financially favorable (Table 28). To cover the cost of fumigating without using a bed freshener in field SE 5, the grower would need to be paid

nearly $14.00 for each added cwt of tubers produced by the fumigation treatment strip. This payment compares to the latest recorded average farm price of potatoes of $11.84 per cwt in 2012 (Statistics Canada, 2020). The use of fumigation alongside the recommended bed freshener showed a break-even payment within expected industry market value. The use of Velum Prime and Elatus without a bed freshener resulted in the lowest required break-even payment of all treatment combinations in field SE 5 of the 2018 season.

In field NE 8 there was one financially favorable treatment. Velum Prime applied without the use of a bed freshener showed a break-even payment of under $4.00 per cwt. Application of fumigation or Elatus without the use of a bed freshener resulted in a financial loss compared to the control strips, with fumigation resulting in the greatest loss. Field NW 13 resulted in the most financially favorable outcomes. Elatus alongside a bed freshener resulted in the most favorable break-even payment, with a required payment of under $1.00 per cwt of added yield to cover the cost of treatment. Fumigation with the use of a bed freshener resulted in a required break-even payment of about $3.00 per cwt to cover the cost of treatment. Velum Prime and the use of a bed freshener resulted in a break-even payment between the aforementioned treatments, requiring about $1.50 per cwt to cover the cost of treatment.

Table 28 Basic economic analysis of soil treatment combinations in all experimental potato fields. Treatments with low positive break-even payments per cwt, denoted by green, require very little payment from a potential buyer of the added yield to cover the cost of the treatment. The treatment with a high positive break-even payment, denoted by yellow, requires a payment on the upper limit of market value. The treatments with a negative break-even payment, denoted by red, represent the value lost per cwt due to treatment and would need to be recuperated through other acres of untreated potato crop.

Experimental Field	Treatment combination	Break-even payment per cwt relative to control
SE 5	Fumigation without bed freshener	13.76
	Velum Prime and Elatus without bed freshener	4.68
	Fumigation with bed freshener	8.81
NE 8	Velum Prime without bed freshener	3.85
	Fumigation without bed freshener	-7.75
	Elatus without bed freshener	-2.17
NW 13	Fumigation with bed freshener	3.22
	Velum Prime with bed freshener	1.60
	Elatus with bed freshener	0.22

4. Discussion

4.1. Levels of *Verticillium* spp. in soil

As with all methods and techniques, there are inherent strengths and weaknesses to each of them. Morphological identification was a limitation of the method used to identify *Verticillium* spp. microsclerotia. Morphological identification included an interpretation whereby the exact species of *Verticillium* could not be determined without further analysis with molecular tools. Therefore, results provided an approximation of *Verticillium dahliae* pressure in the soil, while recognizing it may include species which are less pathogenic on potato and still form microsclerotia, such as *V. tricorpus* and *V. longisporum* (Inderbitzin et al., 2011). The quantifying laboratory, Agricultural Certification Services Inc., is confident in the method they use to quantify *V. dahliae*, but they can only conservatively report genus level quantification without further analysis (MacKenzie, 2019).

The atypical microsclerotial colonies observed from soil sampled in field NE 8, and to a lesser extent field NW 13, is perplexing. The “heavy” colonies were not observed in the previous field season. Environmental *in vitro* stress during the plating and incubating procedure is unlikely because the atypical colonies were present alongside typical colonies. Although *V. tricorpus*, *V. longisporum*, and *V. albo-atrum* are known to form microsclerotia (Inderbitzin et al., 2011), the quantifying laboratory did not identify the “heavy” colonies as belonging to those species. The quantifying laboratory speculated that the sclerotia may be the result of the *Rhizoctonia solani* pathogen of potato. This cannot be confirmed without molecular analyses but is possible given that *R. solani* produces sclerotia of varying size (Haque et al., 2019; Takashi and Tadao, 1978). The

pathogen *Colletotrichum coccodes*, commonly known as Black Dot, may have also produced the sclerotia given that it is known to produce sclerotia in the soil (Dillard and Cobb, 1998) and has been discussed in the potato production region as a present concern. The undesired presence of non-target organisms in the quantification process emphasises the need for improved quantification methods.

All experimental fields exhibited pre-treatment *Verticillium* spp. levels above economic thresholds established for *V. dahliae*, which range from 5 to 30 microsclerotial propagules per g of air-dried soil (Powelson and Rowe, 1993). All experimental fields showed that a rebound of *Verticillium* spp. levels, although not consistent in magnitude, is possible within a single growing season. This indicates that treatment with chemical intervention like fumigation and in-furrow fungicides is most effective for the year of its use without multi-year efficacy. A similar recovery of *Verticillium* spp. levels in the soil nearing the end of the growing season has been observed in Manitoba during PED management with a metam sodium fumigant (Tenuta, 2017). Aside from observing rebounds in *Verticillium* spp. soil levels in each experimental field, specific attention should be directed to Velum Prime strips, which showed a rebound to levels higher than or near equal to control strips. While any decreased *Verticillium* spp. levels in the spring cannot be explained, it is logical for strips treated with Velum Prime to eventually result in such high *Verticillium* spp. soil levels. This is because Velum Prime does not have activity on *Verticillium* spp.

The cause of reduced *Verticillium* spp. propagules in some control strips of each experimental field between autumn and spring sampling dates cannot be determined with absolute certainty. Although there is limited literature regarding microsclerotial survival

in adverse field conditions, one can speculate the unique climate of Southern Alberta may have affected *Verticillium* spp. survival structures during the winter. Chinooks in Alberta have been observed to influence soil in fields by blowing snow off, driving evaporation, and affecting freezing and thawing during winter months (MacDonald et al., 2018). Chinook effects may have interacted with landscape and soil features inherent of the experimental fields, variably affecting *Verticillium* spp. survivability over the winter. A means of tracking survivability of *Verticillium* spp. propagules during the winter months could include periodic soil sampling beginning in November and leading up to planting of the potato crop, with greater intensity during chinook events. Another factor which may have influenced the significant drop in *Verticillium* spp. pressure in control strips without chemical intervention is natural field variability. The pre-treatment soil sampling method involved a broad assessment of the whole experimental field while the post-treatment spring season soil sampling focused on individual treatment strips. Knowing that *Verticillium* spp. are inherently not uniformly distributed across a landscape (Wei et al., 2015), it is likely that soil sampling encountered pockets of high and low pressure variably across sampling areas. This would explain an apparent drop in control strips compared to a pre-treatment baseline assessed across the experimental field.

Similar to the results in this project's potato fields, chloropicrin, as well as other fumigants, have been observed to be effective in reducing *Verticillium* spp. levels in several different cropping systems. Mpofu and Hall (2002) observed that the application of the fumigant metam sodium, both alone and in conjunction with burning of residue, reduced *V. dahliae* soil levels. Gullino et al. (2002) observed that chloropicrin applied via irrigation system can effectively reduce *V. dahliae* levels in soils used for tomato

production. Ślusarski and Spotti (2016) observed that chloropicrin applied via irrigation is also effective in reducing *V. dahliae* soil levels in a greenhouse pepper production system. Short et al. (2015) observed that chloropicrin applied with methyl bromide reduced the quantity of *V. dahliae* microsclerotia in fields of a lettuce production system. Tsror, Erlich, Cahlon, et al. (2000) observed that Telopic, which contains chloropicrin in combination with another soil fumigant, was effective in reducing the quantity of *Verticillium* spp. microsclerotia in stems of potato crops. The effectiveness of chloropicrin soil treatment in reducing *Verticillium* spp. levels in agricultural systems has been demonstrated globally, which this project's results do not fully support but indicate further investigation is required for confirmation in Southern Alberta.

4.2. Levels of *Pratylenchus* spp. in soil

The method of quantifying *Pratylenchus* spp. involved counting nematodes based on morphological identification following extraction from soil and was inclusive of the *Pratylenchus* genus. Therefore, the method was not specifically tailored for *P. penetrans*. It is likely that the actual levels of *P. penetrans* are lower than those reported for *Pratylenchus* spp. An estimate of *P. penetrans* via *Pratylenchus* spp. quantification was the best option available due to the unreliable nature of current molecular techniques quantifying DNA from terminated nematodes.

Pre-treatment soil levels of *Pratylenchus* spp. were below the established economic threshold of 1000-2000 counts per kg of soil for *P. penetrans* in all fields (Olthof, 1987). One would not use control methods targeting the determined soil level of *Pratylenchus* spp. nematodes because they were below the previously mentioned economic threshold. The additive effect of the two pathogens needs to be addressed. How

to properly account for the interaction of *P. penetrans* and *V. dahliae* has been addressed with one model assessing the nematode interaction based on a check for presence or absence of the nematodes (Wheeler et al., 1992). Other research assessed the nematode and fungus interaction quantitatively (Francl et al., 1987; Martin et al., 1982). Powelson and Rowe (1993) summarizes that the economic threshold of *V. dahliae* can drop from the range of 5-30 colony forming units to a range of 2-12 colony forming units per g of dried soil in the presence of 10-20 *P. penetrans* per 100 cm^3 of soil. The experimental fields in this project were selected in consultation with local agronomic expertise and collaborating growers. They were selected due to symptoms in past potato crops being characteristic of the PED complex but without confirmation of pathogen presence until initiation of this project. Applying a nematode targeting control option, such as Velum Prime, allows one to determine if it will elicit a yield response in absence of the targeted nematode. Beyond this, the application of Velum Prime in suspected PED affected fields is congruent with similar projects across Canada feeding into a Canada-wide network.

The only recorded level of *Pratylenchus* spp. above the economic threshold of *P. penetrans* was in a fumigated strip of field SE 5 nearing harvest of the crop. This treatment strip, as well as many others in field SE 5, experienced a rebound in the pathogen level to above the pre-treatment levels. This suggests that the applied treatments are only effective in the year of application. A single treatment strip of Elatus plus Velum Prime in field SE 5 is the only exception to the trend of soil pathogen levels rebounding.

The reduction of *Pratylenchus* spp. levels in the soil of field NE 8, including control strips, and the drop of levels in NW 13 to nearly zero across all treatments, suggests there is a confounding factor influencing the survival of the nematodes between

the first two soil sampling dates. Potential interaction between landscape and soil features with adverse environmental and weather events may have killed nematodes. This is similar in speculation to the previous section discussing unexplained changes in *Verticillium* spp. soil levels over winter months. The speculation of environmental and weather effects raises the question about whether certain agroecosystems in Alberta are less suitable for pathogen survival than others. *Pratylenchus* spp. have a migratory nature within the soil profile and are susceptible to drying (Agrios, 2005). A dry summer during the 2019 field season, marked by reduced availability of irrigation water, is considered a contributing factor to the observed nematode levels. Soil sampling reached a depth of 30 centimetres, while *Pratylenchus* spp. nematodes could have rested below that depth during dry periods in order to survive.

Other research has shown fumigation and fluopyram containing nematicides to be variably effective methods of reducing *Pratylenchus* spp. levels, increasing crop yield, or a combination of both in agricultural production systems. Olthof (1987) demonstrated that various fumigants other than chloropicrin can reduce *P. penetrans* soil levels while increasing total potato crop yield. Rudolph et al. (2019) observed reduced *P. penetrans* soil levels in a raspberry production system when chloropicrin fumigation was applied. Watson and Desaeger (2019) observed that Velum Prime did not reduce *Pratylenchus* spp. levels or increase a strawberry crop's yield in a statistically significant magnitude. Shank injection of chloropicrin alone and in combination with other fumigants, has been effective in controlling other nematodes of agricultural concern, such as *Meloidogyne* spp. and *Tylenchulus semipenetrans*, in a strawberry production system (Schneider et al., 2008). These results of other experiments cannot be supported nor challenged by my own

experimental results because a decline in *Pratylenchus* spp. soil levels was observed in control strips in addition to chemical treatment strips. The effectiveness of chloropicrin and Velum Prime soil treatments in reducing *Pratylenchus* spp. levels in agricultural systems is not fully understood, which parallels the results of this project.

4.3. Soil microbial community

4.3.1. Field SE 5 of 2018

By generally considering the abundance of prokaryotes and fungi in treatment strips one can determine superficial treatment effects on the soil microbiome before considering more detailed relative abundances of phyla and orders. The result of fumigated strips consistently having less abundant prokaryotes than control strips is a trend regardless of the use of a bed freshener, suggesting a main treatment effect. It was observed that fumigation can reduce the abundance of prokaryotes in soil. Li et al. (2017) did not consider total prokaryotic abundance but observed significant changes in the abundance of bacterial species constituting the microbiome after chloropicrin fumigation. Prokaryotic abundances of field SE 5 and the mentioned research by Li et al. (2017) indicate that there may be a treatment effect due to chloropicrin fumigation. This indication was further explored in the second field season.

When considering strips without the use of a bed freshener, Velum Prime plus Elatus strips showed higher relative abundances of the *Cystofilobasidiales* order compared to fumigation and control strips, indicating a treatment effect due to Velum Prime plus Elatus. The *Cystofilobasidiales* order is described as yeasts (Fell et al., 1999) and thus influence fermentation in soil. Zhang et al. (2019) observed chloropicrin fumigation in a strawberry production environment resulting in a higher mean proportion

of *Basidiomycota* compared to unfumigated controls. This phylum contains the order of interest – *Cystofilobasidiales*. Unlike Zhang et al. (2019), field SE 5 did not show differences between fumigation and control strips, but rather between Velum Prime plus Elatus and control strips. Treatment strips using a bed freshener were inconsistent and minor in their changes of relative abundances of *Cystofilobasidiales*.

Mowlick et al. (2013) observed a slight decrease in relative abundance of *Acidobacteria* with the application of a chloropicrin fumigant. This contrasts results of field SE 5 showing no appreciable difference in *Acidobacteria* relative abundances among treatment strips. *Acidobacteria* have been studied as organisms important to carbon breakdown in agricultural production (de Chaves et al., 2019; Kielak et al., 2016). Mowlick et al. (2013) and Fang et al. (2020) observed a major increase in *Firmicutes* following chloropicrin fumigation compared to controls, contrasting results of field SE 5 showing no appreciable difference in *Firmicutes* relative abundances among treatment strips. *Firmicutes* decompose organic matter and are important in carbon cycling (Sykes and Skinner, 1973). Zhang et al. (2019) observed no significant differences in mean proportions of *Actinobacteria* when comparing chloropicrin fumigation to an unfumigated control in strawberry production, paralleling results of field SE 5. Similar to *Firmicutes*, *Actinobacteria* are involved in the carbon cycle and decompose organic material (Sykes and Skinner, 1973). Although the difference notably occurred in only the paired comparison between Fumigation8 and Control9, the *Glomerellales* order was lower in the fumigated strip than the control. This could be the result of a treatment effect, but is unlikely since it was only observed in a single paired comparison. The

Glomerellales order includes some organisms pathogenic on potato, such as the *Verticillium* genus and the black dot causal agent *Colletotrichum coccodes*.

In terms of fungal abundance, comparing fumigation with control strips provides no insightful trend regardless of bed freshener use, suggesting no treatment effect in field SE 5. The same is true when considering the ratio of prokaryotes to fungi, presenting inconsistencies and suggesting no treatment effect. A similar microbial community composition in strips Control1 and Control9 is peculiar given their spatial separation and difference in bed freshener use. Overall, these irregularities across treatment strips resulted in few discernable trends, suggesting there is overall no major treatment effects beyond those previously outlined. To simplify comparisons of soil microbial community structures, bed freshener use in the second field season was modified, such that one experimental field was fully bed freshened and the second excluded the bed freshener.

4.3.2. Field NE 8 and field NW 13 of 2019

Fumigated strips did not consistently have a lower abundance of prokaryotes than control strips in fields NE 8 and NW 13, unlike the results of SE 5. Prokaryotic abundances in fumigation strips when compared to control strips do not show trended differences in fields NE 8 and NW 13, despite differing in bed freshener use. Assuming the method was sensitive enough to detect differences at these levels, it indicates that fumigation with chloropicrin does not affect the abundance of prokaryotes in soil. This indication is contrasted by research from Dangi et al. (2014). They observed bacterial and fungal groups were in greatest abundance in areas which had not been fumigated, albeit with methyl bromide.

The abundance of fungi in field NE 8 follows a spatial trend across the field, increasing from one side to the other. The lack of treatment effect on fungal abundance is indicated by the spatial trend in addition to fumigation strips resulting in the upper and lower bounds of fungal abundance. Considering the control strip in field NW 13 produced a noticeably higher fungal abundance than all other treatment strips in the field, it indicates that any form of chemical intervention in this field produced a lower fungal abundance. This result is logical due to the antagonistic activities of chloropicrin, Elatus, and Velum Prime on fungal organisms. Although Velum Prime does not have a soil-borne fungus as a target on its registration label, as a Group 7 fungicide it will have activity on non-target fungi in the soil.

The variability in the ratio of prokaryotes to fungi across control strips of field NE 8 suggests there was an inherent variability in the experimental field. This inherent variability presents a confounding factor when considering treatment effects. The ratio of prokaryotes to fungi in field NW 13 was lowest in the control strip while all chemical interventions increased the ratio. This indicates that any form of chemical intervention in this field produced a higher ratio of prokaryotes to fungi, which can be related back to the lower abundance of fungi.

There were no appreciable differences to the relative abundances of prokaryotes at the phylum level when chloropicrin was applied in field NE 8. This result is similar to results from Li et al. (2017), who observed no significant differences when comparing the community structure of bacteria to a control. Although trended differences were not observed, the gradually increasing relative abundance of *Microascales* from one end of the field to the other suggested that this order of fungi was unaffected by the applied

chemical soil treatments. It also indicated the experimental field may have an inherent microbial structure varying from one edge to the other. Field NW 13 exhibited a similar trend, whereby *Microascales* were relatively absent in fumigation on one side of the field and relatively higher in the same treatment on the opposite side. In terms of how *Microascales* has responded to chloropicrin fumigation in the published literature, little information is available. A genus within *Microascales* – *Ceratocystis* – was controlled in the soil after chloropicrin application as a means of managing a canker disease in Japanese fig production (Hirota et al., 1984). *Microascales* are by majority saprobic organisms contributing to organic matter breakdown in the soil, although one genus in the order has been recorded to infect humans (Lake et al., 1990). More investigation is required to understand the role of *Microascales* in fumigated production systems.

Both fumigation strips in field NE 8, Fumigation3 and Fumigation5, showed a relative abundance of WIM 1 lineage eukarya that were similar to Control6 and Elatus7 strips, indicating that the low relative quantities present in fumigated areas were not due to treatment effect. Differences in the relative abundance of WIM 1 lineage eukarya could not be used as an indicator of treatment effect in field NW 13 as the order was relatively absent compared to other orders.

Fumigation strips in experimental fields NW 13 and NE 8 resulted in the highest relative quantities of the fungal order *Sordariales* among all treatment strips, suggesting a major trend. This trend was less prominent in field SE 5. This indicated that fumigation has a treatment effect that increased the relative abundance of the order *Sordariales*. Lu et al. (2013) identified that *Sordariales* were a major phylogenetic group of fungi more prevalent in soils exhibiting a healthy network as opposed to a diseased network in fields

continuously cultivated with potato. Based on work by Lu et al., the *Sordariales* order could be used as an indication that the fumigated soils of both experimental fields have a healthy microbial network. Liu et al. (2019) also identified the *Sordariales* order as a disease suppression agent.

Another major trend that was apparent in all fumigated portions of fields NE 8 and NW 13 was that the phyla *Bacteroidetes* trended higher and *Acidobacteria* trended lower in relative quantities compared to control strips, regardless of the use of a bed freshener. This contrasts results of field SE 5, which showed no appreciable differences in *Acidobacteria* and *Bacteroidetes* relative quantities among any treatment strips. Wei et al. (2019) observed that a higher abundance of *Bacteroidetes* was associated with diseased microbiomes and a higher abundance of *Acidobacteria* was associated with healthy microbiome. The relative abundance of *Actinobacteria* existing in similar amounts across all treatments of fields NE 8 and NW 13 indicate there is no treatment effect on the phyla. This result is complemented by research from Zhang et al. (2017) showing a gradual increase in the relative abundance of the phyla with additional years of chloropicrin fumigation. This result is also contrasted by research from Fang et al. (2020) showing that *Actinobacteria* were in a higher quantity after chloropicrin fumigation compared to a control. While there was no treatment effect observed in the first year of application, a baseline has been established for the growers to compare to if they decide to monitor in the long-term and use soil fumigation again.

The relative abundance of the *Cercozoa* order appeared to be higher in field NW 13 than NE 8, with the only treatment difference being the use of a bed freshener. This marked difference could in part be due to the treatment effect of bed freshening. Due to

soil differences between fields introducing site variability, it is currently uncertain if the bed freshener had a true treatment effect. *Cercozoa* are an order of eukarya recognized as predators, exhibiting various feeding preferences; bacteria, other eukarya, a mixture of both, plant parasitism, and oomycete parasitism (Fiore-Donno et al., 2019). Powdery scab is an important disease of potato and its causal agent, *Spongospora subterranea*, is classified in the *Cercozoa* order (Bittara et al., 2017). The eukaryotic order *Euamoebida* was observed in different relative quantities in the Velum Prime treatment strips of fields NW 13 and NE 8. While this may indicate an effect from the bed freshener treatment, the inherent differences in the fields again present a confounding factor. The *Euamoebida* order is recognized as organisms key to reducing bacterial biomass and cycling nutrients in the soil (Vaerewijck et al., 2014).

Rudolph et al. (2019) observed differences in the soil bacterial community when a seed meal amendment was used instead of metam sodium fumigation in a raspberry production system. This difference was only apparent early in the experiment and dissipated at later soil sampling dates, paralleling a general lack of notable differences in this project's results. They also attributed many of the changes in the bacterial and fungal communities to seasonal variation rather than treatment effect.

No major differences in soil microbial structures elucidated from my soil sampling and analyses indicated that the soil treatments do not have an immediate detrimental effect on soil microbial community structures. As a proactive measure, one may consider alternating management options, such as the practices investigated by Zhang et al. (2019). They alternated chloropicrin fumigation with biofumigation as a

means of improving a strawberry production system. This would help mitigate potential long-term effects of chloropicrin fumigation that have yet to be quantified.

While it is tempting to speculate how differing abundances and relative compositions could affect soil processes, it would be spurious to do so without additional data. Information such as the presence of genes coding for enzymes of interest, similar to work by Li et al. (2017) and Fang et al. (2020), would permit further discussion on topics such as nutrient cycling. This project's results showed that the broad soil microbial structures were not consistently altered by treatment with fumigant or in-furrow chemicals, indicating growers can use these products and practices while continuing to be stewards of the land. The compiled results only consider a single field season at each site, without regard for long-term effects. Long-term effects need to be investigated before growers are likely to fully adopt the practice of fumigation and novel in-furrow chemical applications into a multi-decadal production system involving crop rotation.

4.4. Visual wilt ratings of the canopy

Visual wilt ratings were conducted solely on field SE 5 because an unexpected hail event preceded the scheduled visual wilt ratings in fields NE 8 and NW 13. Visual wilt ratings were scheduled for fields NE 8 and NW 13 to take place later in August, when canopy symptoms would have been more pronounced. If visual wilt ratings had been conducted following the hail event, they would have resulted in data that did not accurately reflect wilt differences due to treatment effect.

Basic visual wilt ratings were conducted whereby wilt, chlorosis, and necrosis were scored without differentiating among natural senescence, pathogenic influences, or nutrient deficiencies. The wilt ratings did not include a confirmation of pathogens present

via tissue preparation and pathogen isolation, as has been done in past research (Ashworth, 1983). Without confirming the presence of pathogens *in planta*, one cannot determine if significant differences in the canopy are wholly the result of treatment effects on pathogen levels. For example, changes in soil nutrient levels due to treatment intervention on microbial processes may make yield-limiting nutrients more or less plant available (Fang et al., 2019; Fang et al., 2018). A visual canopy wilt rating scale has been used by Ashworth (1983) on cotton plants, but was less robust than what was used in this project. Ashworth's scale relied on the rater to subjectively decide what is "little", "moderate", "severe", and "pronounced". Isaac and Rogers (1974) used a scale to rate wilt of peas by segmenting the canopy into four regions, determining the progress of wilt acropetally, and indexing the data. A detailed wilt rating scale requiring laboratory analyses focuses on vascular discoloration *in planta*, such as the one developed by Uppal et al. (2008). Embracing technology, Yellareddygari and Gudmestad (2017) explored the use of a smartphone app named Canopeo as a means of assessing wilt, but it was not in agreement with standard visual assessments. Even in the absence of canopy symptoms, potato plants infected by *V. dahliae* have been observed to have reduced photosynthesis (Bowden et al., 1990). This presents a limitation of methods relying solely on visual assessment of wilt. While the utilized method in this project had limitations, it provided an efficient rating system *in agro* and was consistent with similar projects across Canada.

The addition of a bed freshener to fumigation treatment applications coincided with the elimination of statistical significance when comparing adjacent fumigation and control strips. The addition of a bed freshener also improved the control strips and reduced the improvement observed in fumigation strips. This suggests that the practice of

bed freshening in place of power hilling has a moderating effect with regards to visual wilt symptoms. The Velum Prime plus Elatus treatment strips resulting in visual wilt ratings indifferent from control strips suggests that their activity on soil and plant characteristics does not directly correlate with visible canopy differences. This further emphasizes the value of quantifying pathogen levels in plant tissue. Fumigating with chloropicrin appears to lower the severity of visual wilt symptoms, statistically significantly without the use of a bed freshener and insignificantly with the use of a bed freshener. The application of in-furrow fungicide Elatus and nematicide Velum Prime in combination does not affect the incidence of visual wilt symptoms.

While there was a statistically significant decrease of wilt symptoms when comparing fumigation and no bed freshening to the control and no bed freshening, the same was not true of yield responses in field SE 5. This suggests that treatment effects on canopy wilt are not equal in the tubers belowground. This result also demonstrates that measurements of aboveground canopy are not an appropriate metric to estimate tuber metrics.

There is limited peer reviewed literature on the efficacy of the in-furrow treatments on reducing visual wilt symptoms in potato. The Northwest Potato Research Consortium has released preliminary results detailing that Velum Prime and Elatus treatments statistically significantly reduced wilt disease progression when applied independently (Johnson et al., 2018). This contrasts my results, but is not wholly comparable since products were applied in combination in my experiment and my project included fewer rating timepoints. Johnson et al. (2018) also determined there was no difference between the wilt of potato acres treated with Velum Prime or Elatus, implying

that the use of a single product will help manage the disease complex. Their findings support the application of Velum Prime and Elatus separately in the second field season of my project.

Peer reviewed literature is more readily available for research conducted on the efficacy of chloropicrin and other fumigants in reducing visual wilt symptoms. Similar to results of field SE 5, Tsror et al. (2005) observed significant reductions in canopy symptoms of Verticillium wilt when a soil fumigant was applied. Their research focused on the application of metam sodium, not chloropicrin. Again focusing on metam sodium, Mpofu and Hall (2002) observed reductions in canopy wilt symptoms coinciding with fumigation. They also observed a reduction in wilt symptoms when fumigation was paired with burning of residual vines in a sequential potato rotation. Yellareddygari and Gudmestad (2018) also observed that fumigating with metam sodium as the active ingredient significantly reduced symptoms of wilt in Russet Burbank compared to non-fumigated controls.

While there is no literature available on wilt symptom responses to chloropicrin fumigation in potato, my results show that chloropicrin effectively reduces visual wilt symptoms in potato canopy. This is in agreement with the aforementioned metam sodium fumigation successfully reducing wilt symptoms in potato. Results show that a combination of in-furrow treatments affords no additional decrease in wilt symptoms. Further investigation is required to address the potential to apply in-furrow treatments independently.

4.5. Potato yield and tuber qualities

4.5.1. Field SE 5 of 2018

Considering yield as the sole metric of concern would be flawed as it is important to consider other crop metrics, such as tuber size profile, specific gravity, and stems per plant, to name a few. Fumigation in combination with the use of a bed freshener trended higher in stems per plant compared to bed freshened control strips, but the same was not true when a bed freshener was not used. This suggests that the use of a bed freshener and fumigation in some way improved the fitness of sprouts on seed tubers, permitting more to survive compared to when a bed freshener was not used. The effect of chloropicrin on non-target pathogens may improve the environment for early season growth of the potato crop, explaining improved sprout survival. By bed freshening, the fumigated zone of treatment is not compromised, thus maintaining the improved growth environment.

Although the effect was not statistically significant, the use of fumigation with a bed freshener trended higher in the greater than 10 ounces category than the bed freshened control strips. This occurred without a change in the smalls category of equal magnitude. The unbalanced change in smalls, along with inconsistent change in tubers per plant, suggests that the added yield realized in bed freshened fumigated strips may be the result of bulkier tubers, as opposed to a greater number of tubers. While a greater yield is preferential from the grower's perspective, it can become problematic if the added yield is solely from enlarged tubers. Some processors and packers consider oversized tubers unfavorable due to customer requirements for specific sizes. Contrasting results of the first field season, Hutchinson (2005) observed no change in tuber size profile while realizing a significant increase in yield when comparing tubers grown in

chloropicrin fumigated soil to unfumigated soil. Bittara et al. (2017) observed significant yield increases in multiple cultivars, including Russet Burbank, when chloropicrin fumigation was applied, while my results showed some significant yield increase but not consistently across site-years. The concern of how much of the added yield is due to larger tubers was to be further investigated in the second field season. Hail ended the growing season prematurely, preventing results representative of a standard growing season.

There was no expectation for specific gravity of the tubers to change when comparing fumigated strips to control strips. Results showing no significant change in specific gravity parallels results of other research (Hutchinson, 2005; Kunkel et al., 1965; Molina et al., 2014).

Despite minimal statistical significance, all fumigation strips trended higher in yield than control strips regardless of the use of a bed freshener. The application of in-furrow treatments averaged yield above control strips but was inconsistent between adjacent strips. Results suggested that fumigation with chloropicrin can consistently increase yield regardless of bed freshening. This was further explored in the second field season by bed freshening a field in its entirety and omitting it in a second field.

4.5.2. Field NE 8 of 2019

It was unexpected for a control strip to result in a statistically significantly higher portion of larger tubers than the adjacent fumigation strips. The single control strip which resulted in this also had the highest yield of all control strips. Consistently, and at times significantly, control strips yielded higher than fumigated strips. This contrasts research discussed when evaluating field SE 5, where authors observed significant yield increases

compared to control areas when chloropicrin and other fumigants were utilized. Other published research has not considered the use of a bed freshener implement, which is recommended by the manufacturer and distributor (Godbehere, 2018). Given that Field NE 8 did not include the use a bed freshener, it is suggested that this reduced the efficacy of fumigation, perhaps to the extent of detrimentally affecting the potato crop yield.

Contrasting to results of field SE 5, results of field NE 8 show that stems per plant were depressed by the application of fumigation. The effect of fumigation in doing this was only statistically significant in one paired comparison, but the trend was consistent throughout the field. As to why this is so, this author suggests it relates to the absence of a bed freshener during planting. Results of field SE 5 only contrast with field NE 8 when the use of a bed freshener differs in the presence of fumigation. When using a standard power hiller, the zone of fumigation treatment in the bed is mixed with untreated areas from between beds. With limited literature available, one may speculate that this introduces pathogens from untreated zones into preferential areas of growth that are lacking competition. Therefore, various pathogenic organisms rebound and negatively affect early season growth, impacting sprout survival and subsequently stems per plant.

Lower than expected specific gravities in field NE 8 are due to the growth stage of the plants at time of termination by hail. Termination of the crop prior to completed translocation of carbohydrates from canopy to tuber would cause this result. While other aspects of production can influence specific gravity of the tubers, such as irrigation and plant nutrition, the premature growth termination produced this result. The single statistically significant difference in the specific gravity metric between a fumigated strip and control strip is unexpected. The magnitude of difference between the two strips, a

measure of 0.007, is enough to deem a tuber unfit for processing into specific recipes. This magnitude of difference did not occur in any other paired comparisons. While it is possible this result is due to treatment effect, the confounding effect of hail likely affected this result. It is uncertain how confounded the analysis is by hail, but it is certain the timing and severity of hail damage affected carbohydrate translocation from canopy to tubers and therefore tuber bulking. Specific gravity calculations from the 2019 field season should be interpreted with caution because of confounding effects from hail.

4.5.3. Field NW 13 of 2019

It was observed that *Verticillium* spp. could be a prominent pathogen of the PED complex in field NW 13. This is evident by the statistically significant yield improvement in the Elatus fungicide strip compared to the control strip. Other wilt pathogens, such as *Fusarium* and *Colletotrichum*, were not quantified but may have also been reduced by the Elatus fungicide. The lack of an equal yield response in the Velum Prime nematicide strip complements this understanding. Yield responses demonstrating a prominent pathogen are accompanied by quantification of soil pathogen levels, showing appreciable *Verticillium* spp. levels in-season while *Pratylenchus* spp. are nearly absent across the field. Consistently and significantly across the experimental field there was an increase of yield in fumigated strips compared to control strips. Although Mpofu and Hall (2002) used a fumigant containing metam sodium as the active ingredient, they did not observe a significant yield increase in fumigated potato acres, contrasting results of field NW 13. Drawing from previous discussion regarding field SE 5, both Bittara et al. (2017) and Hutchinson (2005) observed significant potato yield increases with the application of a chloropicrin fumigant. These results parallel the statistically significant yield increases

observed in a fumigation strip of field NW 13. Johnson et al. (2018) observed an increase in potato yield when Elatus was applied in-furrow, albeit statistically insignificant. This is similar to this project's results, differing only in statistical significance. They also observed an insignificant yield increase when Velum Prime was applied in-furrow, contrasting the results of this project. While these results differ, the level of target pathogens *Pratylenchus* spp. also undoubtedly differed between their experimental location and field NW 13.

Tuber grading showed that the greater than 10 ounces category was nearly zero across all samples. While this would be unexpected in a regular growing season, it is not unusual due to the timing of the hail event. If the crop was able to proceed to full maturity uninterrupted by hail, then differences within the percent greater than 10 ounces metric may have been discernable. Similar to field NE 8, tubers from field NW 13 showed a lower than expected specific gravity for the region. Given that specific gravity was consistently depressed across the field, it is determined that the lower than expected specific gravity is the result of hail prematurely slowing or halting translocation of carbohydrates from the canopy to the tuber. If the crop had reached full maturity, a difference in specific gravity of the Elatus, Velum Prime, and control tubers may have been discernable, similar to what Johnson et al. (2018) observed.

Recall that field SE 5 trended higher stems per plant in fumigated portions while NE 8 trended lower stems per plant. Results of field NW 13 show no trend in stems per plants with regards to fumigation. The inconsistencies across sites and years suggests that fumigation, regardless of use of a bed freshener, does not consistently affect stems per plant. Previous discussion suggested that improved sprout survival permitted higher

stems per plant, but this result from one the first field season cannot be confirmed with results from the second field season. In terms of Velum Prime and Elatus treatments, the number of stems per plant did not trend higher or lower than the control strips across site-years.

It is acknowledged that manure applied to the surface of field NW 13 during the autumn may have contributed an effect not accounted for in analyses of this project. This is especially important with respect to the soil microbiology. The application was not planned, but was required by the grower as an emergency soil conservation measure. Manure applications and their effect on soil nutrients, microbiome activity, and other pertinent soil characteristics was outside the scope of this project.

Overall, chloropicrin fumigation in tandem with a bed freshener, or the use of Elatus in-furrow fungicide, provided a yield increase to potatoes without negatively affecting the tuber size profile, specific gravity of the tubers, or stems and tubers per plant. The use of an in-furrow nematicide such as Velum Prime was not appropriate for PED control in this field, based on yield results and soil pathogen levels. This further emphasizes the importance of making PED management decisions with adequate soil testing completed in a timely manner.

4.6. Hail event

Emergence dates of the potato crop were not recorded for experimental fields, making an estimate of the foregone yield difficult. Days after recorded planting dates are the next best alternative. With a planting date of April 19th, field NE 8 was able to grow uninterrupted for 15 weeks. Field NW 13 was planted on May 3rd, providing it with 13 weeks of uninterrupted growth. According to Jalali (2013), severe defoliation results in

the greatest yield loss in the 5-11 week post-emergence period. Given that NE 8 experienced severe defoliation 15 weeks post-planting, the experimental field likely experienced some yield loss, but not the worst possible amount. Jalali (2013) also outlines that the key yield loss period for moderate defoliation, such as that experienced in field NW 13, is 8-11 weeks post-emergence. Given that NW 13 grew for 13 weeks post-planting before the hail event, it is likely that the field experienced an appreciable yield loss due to hail.

Wille and Kleinkopf (1992) provide a figure in an article outlining the location of carbohydrates during various growth stages of Russet Burbank. In this figure, it is around 60 days after planting that the tubers begin to receive dry matter translocated from the canopy, with the process slowing at around 110 days after planting. Knowing that field NE 8 experienced hail 109 days after planting, and field NW 13 experienced the same hail 95 days after planting, it is reasonable to suggest that the hail event adversely impacted the translocation of carbohydrates to the tubers and subsequently depressed the specific gravity of harvested samples. This explains why specific gravities of tubers samples from both fields were below what one would expect of a standard growing season.

The hail event undoubtedly affected the tuber size profile of fields in the second field season. Most prominently, none of the tubers sampled from field NW 13 weighed in at over 10 ounces, while one treatment strip showed the majority of its size profile was comprised of small tubers. This contrasts to field SE 5 of the previous field season, which was cultivated by the same grower and produced tuber samples ranging in the greater than 10 ounces category from 7 to 24 %. A similar result was seen in research by Pavek

et al. (2018). They observed the largest shift in size profile of cultivars Russet Norkotah and Ranger Russet when the crop experienced nearly complete defoliation at the early bulking stage of growth. Regardless of yield loss, specific gravity changes, or tuber size profile shifts across fields, the hail event was visually consistent within individual fields. Consistent defoliation in individual fields introduces a confounding effect when comparing between experimental fields and a confounding effect of lesser magnitude when comparing between treatments in individual fields.

4.7. Farm level adoption of innovation

It was assumed that the only yield limiting factor in the experimental fields was that of a disease complex involving the previously described fungal and nematological pathogens. Therefore, whichever treatment combination provided the lowest required break-even payment per cwt is perceived to be the most profitable while providing the best mitigation of the yield limiting factor.

In experimental field SE 5, fumigation appeared to be the most profitable when applied following use of a bed freshener. Break-even payment for using fumigation without a bed freshener neared $14.00. Drawing on personal industry experience, this is nearing the upper limit of potential payout to the grower. This was balanced out with a lower break-even payment when a bed freshener was used. Across all fumigated strips, the cooperating grower reported that the added yield value was about equal to the cost of fumigation. The results of the same field season showed that combining both Velum Prime and Elatus was more profitable for the grower than applying fumigation. In search of a cheaper treatment option, the two in-furrow chemicals were applied separately in the second field season.

The profitability of using fumigation alongside a bed freshener is further emphasized in comparing fields NE 8 and NW 13, where fumigation was only profitable when a bed freshener was used. Fumigation treatment without a bed freshener showed to be detrimental, requiring the grower to recuperate lost value via other potato acres or crops. When considering all site-years the most profitable practice for fumigating appears to be using a bed freshener. The bed freshener implement is not readily available for purchase from local agricultural equipment dealers. The added cost of the custom piece of equipment is not considered in the cost analysis because a fair market price has not been established. The use of a bed freshener in a potato production system introduces a cost variable that is of yet not quantified but will be important if a grower considers adopting the practice of fumigating.

The profitability of applying Velum Prime in field NW 13 is interesting because of the combination of a yield increase and low break-even cost in the midst of low target pathogen levels in the soil. This suggests that the issue the treatment may have helped resolve was not the targeted nematode. Velum Prime is labelled for early blight (*Alternaria solani*) suppression in addition to the target nematodes (Bayer, 2017). The suppression of early blight in the potato crop may have confounded yield results. As a Group 7 fungicide, Velum Prime may have also suppressed other off-label pathogens. One is limited in determining this because early blight and other pathogens were not quantified in the field. If the yield increase over the control was due to reasons other than nematode suppression, increasing profits may be achievable without specifically using Velum Prime. A cheaper or alternative intervention may provide the same added value.

If a grower continues to face a profit barrier while adopting fumigation into their production system, there are further options one can explore. King and Taberna Jr (2013) demonstrated that variable rate application of fumigants can be integrated into a Washington potato production system for control of root-knot nematode (*Meloidogyne chitwoodi*). The overall cost savings were up to $85 an acre. The most successful alternative practices involved a mixed use of fumigant and cheaper non-fumigant nematicide. Considering one of the major concerns of growers in Alberta is the cost of production, it may be beneficial to explore the opportunity for variable rate fumigant application. Hansen et al. (2018) has also looked at alternative fumigant application practices, comparing in-row application to broadcasted application in Idaho. The former of the two was cheaper in cost by about $50 per acre, but the latter of the two resulted in better yield and tuber quality of cultivar Russet Burbank. Broadcast application has not been explored in Alberta and may be a topic for further investigation.

4.8. Future investigation

There was no quantification of soil abiotic factors over time, such as how plant available nutrients or soil structure changed after treatments. Korthals et al. (2014) conducted a study which quantified nutrients within the soil to discern if soil treatments influence nutrient levels over time and space. Given that changes in soil fertility impact overall plant development, it is important that future experiments involving soil treatments for managing PED include an aspect of quantifying abiotic soil characteristics. Additional soil quantification should also include a functional analysis of the treatment effects on the soil microbial community. Larkin (2003) quantified the effect of varying crop rotations on soil microbes using multiple techniques; substrate utilization,

population dynamics, and fatty acid profile analyses. Zelles et al. (1997) also analyzed fatty acid profiles to determine the effects of chloroform fumigation on soil microbial properties. Li et al. (2017) used molecular techniques to determine the effects of fumigating with chloropicrin on enzymes important in the nitrogen cycle. They achieved this by determining the prevalence of genes which coded for the important enzymes. There are several methodology options for quantifying functional differences in soil microbial properties beyond a structural survey, such as quantifying the abundance of genes coding for enzymes integral to soil processes.

There is minimal literature to support the use of a bed freshener as recommended by manufacturers and distributors, with the only mention appearing in a tobacco conference presentation delivered by Godbehere (2018) that was not peer reviewed. Further investigation is required to understand this practice. Understanding if soil pathogens' distributions differ when a bed freshener is used is an important step. This could be achieved by soil sampling specific areas in and between beds before and after power hilling or bed freshening. It would be prudent to conduct this on multiple soils in the growing region to determine if soil texture interacts with the practice.

Longer term management options, such as green manures and modifications to rotations, need to be considered in future PED research in Southern Alberta. Multiple experiments have demonstrated the effectiveness of green manures and modified rotations in either reducing pathogen soil levels, increasing crop yield, or both (Davis et al., 2010; Larkin and Halloran, 2014; Larkin et al., 2017; Larkin et al., 2011; Ochiai et al., 2008; Ochiai et al., 2007). In conjunction with this, Li et al. (2017) observed long-

term changes in bacterial community composition following chloropicrin fumigation, further emphasising the need for longer term investigation.

Commercial scale field trials are valuable in that they directly involve the intended beneficiary of the research; growers. A small scale replicated field trial would be a complement to larger scale experiments. Small scale replicated field experimentation would provide the opportunity for more rigorous statistical analysis while lending itself well to publication in agricultural science journals.

5. Conclusion

After three site-years of experimental results, it is concluded that the addition of chloropicrin fumigant, soil fungicide Elatus, and soil nematicide Velum Prime can reduce *Verticillium* spp. and/or *Pratylenchus* spp. soil levels in Southern Alberta potato fields. The levels of pathogens in the soil can be reduced to levels below economic thresholds established in other regions but is followed by a recovery of soil pathogen levels later in the growing season. Results also showed that the addition of these soil treatments can significantly increase the yield of the potato crop but is conditional to the soil levels of the target pathogen(s). To select the appropriate management strategy, one should soil sample their field to first ascertain what pathogens are present and in what quantity. In conjunction with improving yield and decreasing pathogen levels in the soil, the use of a bed freshener is the best practice for the most efficacious treatment with chloropicrin fumigant. Microbial community structures were largely unaffected by soil treatment over the short-term duration of this project, with minimal or inconsistent differences from the control. The long-term effects of soil treatment have not yet been investigated and need to be considered in future projects while working towards the goal of enhancing sustainable potato production in Southern Alberta.

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Appendix A: Cost analysis calculation

The following is a worded example of how cost analysis was conducted.

I know that the cost of fumigant product and application is $450.00 per acre.

Results of fumigating without a bed freshener in field SE 5 showed a yield of 496.1 cwt/ac.

Results of not fumigating without a bed freshener in field SE 5, constituting the control or grower's standard practice, showed a yield of 463.4 cwt/ac.

The net difference is 32.7 cwt/ac, which is attributed to treatment effect.

Therefore, fumigation provided an additional yield of 32.7 cwt/ac at an additional cost of $450.00/ac.

To recuperate the additional cost with the additional yield, the grower would need to be paid $13.76/cwt for the additional yield. This is the quotient of $450/ac divided by $32.7/cwt.

$13.76/cwt is considered the break-even payment and does not represent a profit for the grower.