APHANOMYCES EUTEICHES SPATIAL DISTRIBUTION, HOST STUDIES, AND CHARACTERIZATION IN MONTANA

by

Carmen Yvette Murphy

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Plant Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2022

ACKNOWLEDGEMENTS

This dissertation and the work herein could not have been completed without the guidance and patience of my primary advisor, Dr. Mary Burrows. Mary's expertise in the field of plant pathology and her direction in all matters research, science communication, leadership, and empowerment, were essential for my success. Many thanks to Mary and my doctoral committee: Dr. Kevin McPhee, Dr. Jessica Rupp, and Dr. Tim Seipel who provided guidance, support, knowledge, and wisdom. To Monica Brelsford, thank you for your assistance digging and collecting soil samples, for managing the lab, and for our lengthy research discussions. I'm grateful for Dr. Uta McKelvy's friendship and her guidance in molecular techniques. To Dr. Collins Bugingo, Everet Owen, and Syd Atencio, for being my lab buddies. Thank you to Dr. Julie Pasche and Dr. Kim Zitnick-Anderson from North Dakota State University for teaching me isolation and maintenance techniques for Aphanomyces root rot, and to Lulu McMahon for her assistance with my greenhouse trials. Thanks to Jeannie Rude and Luke Dighans for facilitating sampling in Northeast Montana. This research came to fruition thanks to pulse crop producers who allowed me to sample their fields and dig up their soil and those who sent pulses with root rot symptoms to the Montana State Schutter Diagnostic Clinic. Thank you to my family and husband for your love and support. This project was made possible thanks to funding from the Montana Department of Agriculture Specialty Crop Block Grant, USDA.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

ABSTRACT

Growing pulse crops in Montana has been inhibited by biotic constraints to production, including a complex of pathogens causing root rot. Aphanomyces root rot, caused by the soilborne oomycete, *Aphanomyces euteiches*, causes plant stunting and yellowing, root browning and constriction, and reduces yield in dry pea and lentil in the state. Twelve fields with a history of pulse root rot were sampled in northeast Montana with three 100 m entrance transects and one 50 m transect at a low spot or problem area. Soil from each 10 m quadrat within transects was assessed for root rot using a greenhouse bioassay with a susceptible dry pea variety, and with PCR. Samples were also analyzed for soil properties and nutrients. Distribution of the pathogen was sporadic in most fields, except for fields that had been growing pulses in a consistent rotation, where root rot severity was high and consistent. Soil pH, organic matter, potassium, and sulfur concentration were correlated with Aphanomyces root rot, and isolates varied in their response to acidic pH *in vitro*. Using a highly virulent *A. euteiches* isolate, greenhouse trials were conducted to assess the pathogen load of inoculated soil after growing host and non-host plant species, measured with a bioassay. Greenhouse pots were inoculated with 500 oospores per gram prior to planting plant treatments. Growing host plants resulted in higher root rot severity on dry pea bait plants compared to non-host plant treatments. When five cycles of plants were grown in greenhouse pots inoculated with *A. euteiches*, using five 'rotation' treatments, one treatment with three consecutive rounds of non-host plants reduced the disease severity score in one trial repetition compared to treatments with less than three successive rounds growing a non-host. This research indicates that sampling strategies for Aphanomyces root rot requires multiple sampling locations within a field to enhance the probability of detection, and that crop rotation is an important tool for management of pathogen load in the soil.

CHAPTER ONE

INTRODUCTION

Pulse Crop Production

In the United States, pulse crops increase sustainability of farms in Washington, Idaho, North Dakota, and Montana through crop diversity, breaking pest cycles, fixing atmospheric nitrogen, efficiently utilizing soil moisture, and reducing economic uncertainty for growers (Miller et al. 2015; Zhou et al. 2017). The term 'pulse crop' refers to plants in the Fabaceae family that are produced as dry seed for humans and animal food, distinguishing them from legumes produced for the fresh market. Pulse crops are an excellent source of fiber and protein and are often incorporated into vegetarian diets as a replacement for meat protein. Some of the main pulse crops produced in the United States are dry pea (*Pisum sativum*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*). Dry pea, lentil, and chickpea are the pulse crops grown on a large scale in Montana (NASS 2019b). The popularity of these crops in Montana has risen in the last twenty years due to their versatility and inclusion in crop rotations, with a shift away from fallow in the traditional wheat-fallow rotation (Long et al. 2014).

Dry pea and lentil are herbaceous, dicotyledonous, cool-season annual grains, and grow well under semi-arid conditions. They are excellent additions to human diets and animal feed for their nutritional benefits, and cover crop mixes for soil health benefits (Smýkal et al. 2012; Kumar et al. 2013). Dry pea and lentil varieties can have over 20%

protein in the grain, and they are a good source of vitamins, minerals, and fiber (Joshi et al. 2017; Kumar et al. 2013; Shekib et al. 1986; Saharan and Khetarpaul 1994). There are important biotic constraints to producing pulse crops, including weeds, insects, and diseases. Here we focus on soilborne plant diseases and the root rot complex.

Root Rot Complex of Pulse Crops

Limitations to pulse crop production include a highly damaging swath of root rot pathogens, including fungi, oomycetes, and nematodes – collectively called a root rot complex. These include members of the *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani* Kühn, *Aphanomyces euteiches* Drechs., and a few nematode genera including *Paratylenchus*, *Tylenchorhynchus*, and *Ditylenchus* (Hajihassani et al. 2016; Upadhaya et al. 2019; Gossen et al. 2016; Ogoshi 1987). These are known to be important biotic stressors impacting pulse production across the world (Kraft and Pfleger 2001).

Soilborne pathogens on pulses can cause other issues in conjunction with root rot. These include seed rot, seedling blight, damping off, reduced stand, reduced nodulation leading to decreased nitrogen fixation, and wilt-like symptoms from loss of vasculature, all of which can have significant impacts on yield (Xue 2003b; Gossen et al. 2016). Although seed rot and decay is possible with all of the root rot pathogens, it is most often caused by *Pythium* spp. (Kraft and Pfleger 2001). When conditions are conducive to soilborne pathogens, yield loss can be severe, with up to 70% yield loss reported in dry pea fields (Tu 1987; Hwang et al. 1991). One of the main issues with estimating yield

loss is that the primary symptoms occur belowground, and distribution is often patchy, therefore, root rot symptoms can easily be missed or mistaken for abiotic issues (Navarro et al. 2008; Moussart et al. 2009).

One of the members of the pulse root rot complex, *Aphanomyces euteiches* f. sp. *Pisi*, was recently discovered in Montana dry pea fields in 2016. This pathogen is of concern due to its long-lived oospore and limited management options. The focus of this work is on this highly damaging oomycete pathogen, which causes Aphanomyces root rot.

Aphanomyces Root Rot of Pulse Crops

Background

Aphanomyces euteiches Drechs. is the causal agent of Aphanomyces root rot on dry pea, lentil, vetch, common bean, alfalfa, and red clover (Gaulin et al. 2007; Malvick et al. 1998; Tofte et al. 1992; Wu et al. 2018; Pfender and Hagedorn 1982; Sherwood and Hagedorn 1962). *A. euteiches* is distinguished from fungal pathogens as it is an oomycete, or 'water mold' from the order Saprolegniales. It has been detected in North America, Australia, New Zealand, Japan, and multiple European countries (Wicker et al. 2003). It was first found in Wisconsin in the 1920s, although it wasn't considered a threat to the production of pulse crops until recent years (Gossen et al. 2016; Kraft and Pfleger 2001). Some *A. euteiches* isolates are specific to host species, and do not infect a wide host range of legumes (Moussart et al. 2008; Malvick and Percich 1999; Levenfors et al.

2003). For example, isolates of *A. euteiches* collected from alfalfa fields do not always have cross-pathogenicity on dry pea and vice versa (Holub and Grau 1990).

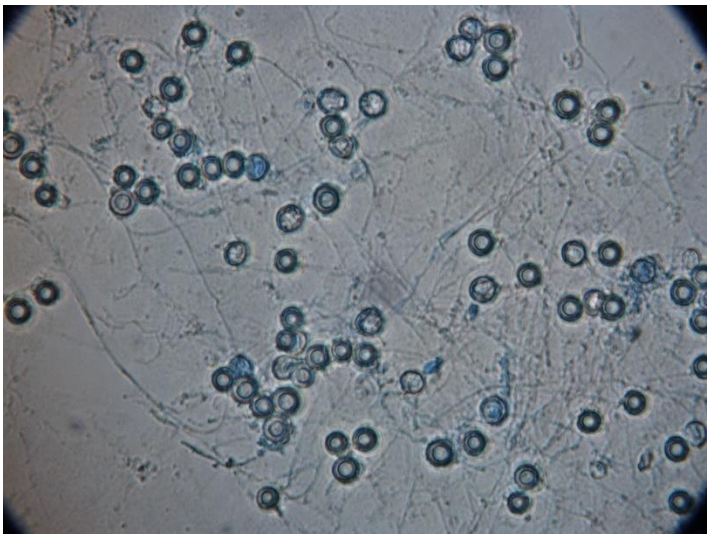
In the Northern Great Plains regions, Pacific Northwest, and Canadian Prairies of North America, where pulses are frequently grown, Aphanomyces root rot can infect at any time in the growing season. However, certain climactic conditions are ideal for pathogen infection and reproduction on host plants. *A. euteiches* thrives in warmer ambient temperatures, up to 28°C, so cold spring conditions can postpone infection on dry pea and lentil (Lewis and Papavizas 1971). Due to the cooler ambient temperature in winter, Austrian winter pea can typically escape severe root rot issues caused by *A. euteiches* (McGee et al. 2017). Water is important in the lifecycle of this pathogen. Other conducive conditions are high clay content soils between 35 to 40%, damaged root systems from soil compaction, and numerous soil nutrients (Papavizas and Ayers 1974; Zitnick-Anderson et al. 2020; Karppinen et al. 2020).

The Lifecycle of *Aphanomyces euteiches*

Both a sexual and vegetative stage occur in the lifecycle of Aphanomyces root rot, in a complex cycle that occurs entirely below ground. The vegetative stage consists of hyphae, zoospores, and zoosporangia and the sexual stage consists of oogonia, antheridia, and oospores (Scott 1961). The lifecycle of the pathogen typically commences in response to plant root exudates prompting germination of oospores living in soil or rotted root tissue (Papavizas and Ayers 1974). Oospores have a thick wall and serve as the survival and overwintering structure of the oomycete (Fig. 1.01). They have a large apleurotic gap, the gap between the spore and the oogonium wall, filled with a lipid sac

(Shang et al. 2000). This protective layer allows oospores to persist in soils for up to ten years when conditions are conducive by avoiding freezing, thawing, and drying out (Gaulin et al. 2007; Pfender and Hagedorn 1983; Sherwood and Hagedorn 1962).

Fig. 1.01 Multiple oospores of *A. euteiches* in solution, photo: C. Murphy

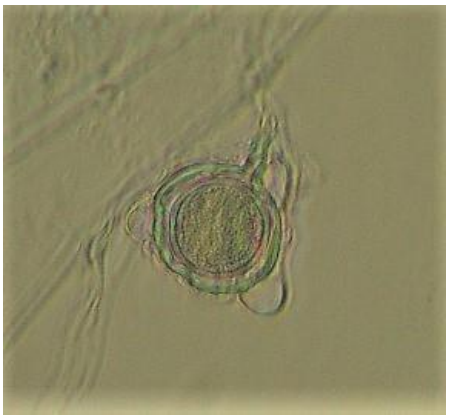


Direct germination of oospores produces a germ tube that enters plant roots and produces hyphae and oospores inside the root tissue, while indirect germination is when the oospore produces a sporangium (Scott 1961). Sporangia can release biflagellate zoospores, with an anterior flagellum and posterior flagellum, that are able to swim to neighboring plants when soil conditions are favorable. Zoospores use chemotaxis to locate nearby plant root elongation zones and may then encyst on susceptible roots to commence the secondary infection cycle (Cannesan et al. 2011; Scott 1961; Sekizaki and Yokosawa 1988). The initial zoospores to emerge from the sporangia are the primary zoospores. These are typically slower and weaker than the secondary zoospores that follow (Walker and van West 2007). Zoospores create cysts and are able to germinate a

tube via repeated discharge and assimilation of calcium ions on the surface of roots (Deacon and Saxena 1998). The pathogen then invades the root system in a similar fashion to the colonization by direct oospore germination, initially with hyphae, and then oospores are formed (Kjøller and Rosendahl 1998).

The hyphae of *A. euteiches* can move intercellularly and intracellularly in susceptible root tissue, however, most movement is intercellular (Gaulin et al. 2007). Hyphae are coenocytic and have a diameter of approximately 4 to 10 µm in diameter (Scott 1961). Aphanomyces hypha are homothallic, requiring only one thallus for sexual reproduction. Sexual reproduction occurs when male nuclei from the antheridia are transported to the oogonium using a fertilization hypha, producing diploid oospores (Scott 1961). For *A. euteiches*, multiple antheridia can often be seen around the oogonium wall during this phase (Fig. 1.02).

Fig. 1.02 Oogonium of *A. euteiches* with multiple antheridia attaching to the oogonium wall, photo: C. Murphy



Symptoms of Aphanomyces Root Rot on Dry Pea and Lentil

Characteristic features of Aphanomyces root rot on susceptible hosts are caramel brown roots that may initially appear water soaked. Later in the disease cycle root browning becomes uniform, with stripping of lateral roots leading to a loss of functionality and impacting pod fill (Fig. 1.03) (Gangneux et al. 2014). When symptoms are severe, the entire root system may slough off when plants are uprooted. When symptomatic plants are viewed under a dissecting microscope, oospores can be seen in infected root, epicotyl, and hypocotyl tissue.

Fig. 1.03 Symptoms of *A. euteiches* on dry pea plants 21 days (left) after greenhouse oospore inoculation in comparison to healthy dry pea plants (right), photo: C. Murphy

Above ground symptoms of Aphanomyces root rot on pulses includes yellowing that typically starts at the soil line, and dead plants may be visible (Fig. 1.04). Diseased plants are often stunted and appear desiccated.

Fig. 1.04 Montana field sown to dry pea where *A. euteiches* was detected using a soil bioassay and PCR in 2019. Dry pea plants show characteristic above-ground yellowing in a sporadic distribution across the field, photo: L. Dighans

Aphanomyces Root Rot Detection

Numerous techniques to detect *A. euteiches* and determine its inoculum density in field soil have been used, with varying levels of success and difficulty. Field soil samples can be wet-sieved to suspend oospores in water for counting under a microscope (Chan and Close 1987; Boosalis and Scharen 1959). The number of oospores in the soil system can give a rough idea of disease severity potential on host plants (Lockwood 1960). These direct oospore counting techniques are labor intensive, and do not consider the virulence of the spores counted (Mitchell et al. 1969; Pfender et al. 1981). More recently, researchers attempt to measure the inoculum potential of field soil. Inoculum potential typically measures propagule infectivity, propagule density, and soil factors impacting infection (Malvick et al. 1994). Propagules are the disease-causing structures, and for *A. euteiches* this can be oospores, zoospores, and hyphae.

To get an indirect measure of propagule infectivity and density, soil bioassays are a popular technique and can be used in conjunction with soil testing for chemical and structural factors that may be influencing disease. Bioassays measure the disease potential of field samples without directly counting propagules. A technique called the most probable number (MPN) was an early bioassay used for *A. euteiches*, whereby serial dilutions of clean and infested soils were used to rate root rot on fresh pea, and a statistical algorithm determined likely oospore values (Pfender et al. 1981). Rolled towel assays are a bioassay where fresh pea seedlings are placed in wet paper towels with a soil sample of interest and inoculum potential is calculated from the proportion of plants with root rot symptoms (Kraft et al. 1990; Malvick et al. 1994).

Greenhouse bioassays are a technique where susceptible plants are grown in field soil samples and disease severity on plant roots is visually scored. Although growing plants in the greenhouse takes time, it is less labor intensive than counting propagules directly, and does not overestimate the virulence of spores in a sample. A greenhouse soil bioassay is also useful for collecting isolates from field samples, as fresh plant material can be harvested and plated immediately onto selective media. A semi-selective medium for isolating *A. euteiches* typically uses metalaxyl, vancomycin, benomyl, rifampicin, and amphotericin B to eliminate competing fungi, oomycetes, and bacteria (Pfender et al. 1984; Zitnick-Anderson et al. 2021b). After evidence of *A. euteiches* is seen on selective media, isolates can be stored on corn meal agar (on which most isolates produce sexual structures) or potato dextrose agar (on which most isolates produce asexual structures)

(Yokosawa et al. 1995). On media, *A. euteiches* is a white color with dense hyphal growth with an indiscriminate arrangement (Zitnick-Anderson et al. 2021b).

Isolating *Aphanomyces* spp. is difficult, and often requires attempts to isolate from newly infected plants, as seen with *A. cochlioides* causing root rot on sugar beet (Windels 1996). *A. euteiches* research has shown that pathogen DNA is highest 7 days after infection and then starts to decline (Vandemark and Ariss 2007). It is almost impossible to recover isolates from field samples due to degradation of the plant tissue. In a field survey over multiple years on dry pea and lentil crops in the Canadian prairies, 37 fields were positive for *A. euteiches* in 2014, and 45 fields in 2015 based on PCR results, however, no isolates were obtained, while *Fusarium* isolates were more readily collected (Chatterton et al. 2019). This difficulty of isolation contributed to the underestimation of the importance of *A. euteiches* in the pulse root rot complex until the use of molecular techniques made detection more straightforward (Banniza et al. 2013; Gossen et al. 2016).

Since the year 2000, molecular primers for detection of *A. euteiches* have been available for PCR, effective at distinguishing it from the closely related root rot pathogen of sugar beet, *A. cochlioides* (Vandemark et al. 2000). Since then, real-time primers and probes have been designed to quantify DNA amounts of *A. euteiches* from roots and soil, although some issues can occur (Vandemark and Ariss 2007; Willsey et al. 2018). The difficulties with detection of Aphanomyces in soil include issues with extracting enough pathogen DNA for analysis, the need to disrupt thick-walled oospores without degrading pathogen DNA, and inhibitors in the environment such as tannins and humic acid

(Vandemark et al. 2000). Soil type can also influence the ability to detect *A. euteiches*, with evidence suggesting that high clay content soils are more difficult to extract from than sandy soils (Almquist et al. 2016). Soil samples should be from the rhizosphere and rooting zone, as oospore concentration is highest in the top 20 cm of the soil profile (Moussart et al. 2009).

Detection of pathogens may require modification for different pathogen races and pathotypes. On alfalfa, two races have been classified of *A. euteiches* (Malvick et al. 2009; Malvick and Grau 2001). Known pathotypes are recognized from *A. euteiches* from dry pea based on disease responses on a differential set of legume species (McGee et al. 2012; Sivachandra Kumar et al. 2021). In one study, cross-pathogenicity in isolates was observed with 20% of *A. euteiches* isolates obtained from alfalfa causing root rot on dry pea, and 80-100% of isolates from dry pea roots virulent on alfalfa (Holub and Grau 1990).

Aphanomyces Root Rot Management

Current management methods for Aphanomyces root rot focus on widening crop rotations out of susceptible legumes and avoiding infested fields (Gaulin et al. 2007; Wu et al. 2019). This is because susceptible species and cultivars are very favorable to pathogen multiplication, and continuous cultivation of these crops increases the inoculum potential of *A. euteiches* in soil (Moussart et al. 2013). In Canada, current recommendations for farmers with existing Aphanomyces problems are to rotate out of dry pea and lentil for at least six years, which is not sustainable for the industry. *A. euteiches* is particularly problematic as there are limited crop variety and fungicide

management options available, and currently no resistant dry pea and lentil varieties are on the market.

Releasing plant varieties with Aphanomyces root rot resistance is impeded by the complex polygenic nature of resistance, with minimal heritability, and large environmental impacts (Pilet-Nayel et al. 2002). Studies on the genomic regions associated with Aphanomyces root rot resistance have been conducted for lentil, *Medicago* spp., and dry pea, and have revealed partial resistance in germplasm materials. For *Medicago truncatula*, commonly called barrel clover, resistance to *A. euteiches* occurs in the root stele due to a major quantitative trait loci near the top of chromosome 3 (Djébali et al. 2009). From genome-wide association mapping, 52 loci have been identified in relation to dry pea resistance to Aphanomyces root rot (Desgroux et al. 2016). In lentil, QTL mapping showed 19 QTL and association mapping 38 QTL in association with resistance from a recombinant inbred line (Ma et al. 2020). In a study in France, traits unrelated to geographic origin were found in a few clusters of *A. euteiches* isolates overcoming plants with QTL resistance, indicating that more work is required to determine the mode of action of plant resistance to *A. euteiches*, and QTL pyramiding may be necessary to avoid resistance breakdown in future breeding programs (Quillévéré-Hamard et al. 2021).

Biological control has been investigated for its effectiveness at managing Aphanomyces root rot. Bacterial suppression of *A. euteiches* was achieved in greenhouse trials with strains of *Bacillus* and *Paenibacillus* collected from New Zealand soils, namely *B. pumilus*, *B. subtilis*, *B. cereus*, *B. mycoides*, and *P. polymyxa* by reducing

oospore production in dry pea roots, although field applications did not show a reduction in root rot (Wakelin et al. 2002). A field study in Wisconsin on fresh pea showed reduction of Aphanomyces root rot using select bacterial strains of *Pseudomonas cepacian* and *P. fluorescens* by up to 19% at harvest compared to controls (Bowers and Parke 1993). Greenhouse trials testing arbuscular mycorrhizal fungi *Glomus intraradices* and *Glomus claroideum* for suppression of Aphanomyces root rot showed a slight reduction in rot on dry pea variety Bodil compared to controls (Thygesen et al. 2004). Field trials with a strain of the Ascomycete fungus *Clonostachys rosea* had variable efficacy in field trials, with evidence of the fungus increasing emergence and reducing root rot severity in dry pea in one year of the trial (Xue 2003a). These biocontrol treatments, while promising, incur challenges when applying to larger field scales.

Synthetic fungicides useful on other members of the dry pea root rot complex have not shown efficacy in managing Aphanomyces root rot (Xue 2003a). Newer chemistries, though, have proven useful in greenhouse trials, although yield benefits have not been seen under field conditions. In Canada, registered fungicides for *A. euteiches* include INTEGO™ Solo (ethaboxam) and Vibrance® Maxx RFC (sedaxane, metalaxyl, and fludioxonil), although these products are only effective for a few weeks after planting and yield increases are not consistently associated with product use (Wu et al. 2019). Further work is required in this area and may need to be tailored for specific races and isolates of *A. euteiches* and crop sensitivities.

References

Almquist, C., Persson, L., Olsson, Å., Sundström, J. & Jonsson, A. 2016. Disease risk assessment of sugar beet root rot using quantitative real-time PCR analysis of *Aphanomyces cochlioides*. European Journal of Plant Pathology. 145: 731-742.

Banniza, S., Bhadauria, V., Peluola, C., Armstrong-Cho, C. & Morrall, R. 2013. First report of *Aphanomyces euteiches* in Saskatchewan. Canadian Plant Disease Survey. 93: 163-164.

Boosalis, M. & Scharen, A. L. 1959. Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. Phytopathology. 49: 192-198.

Bowers, J. & Parke, J. 1993. Epidemiology of Pythium damping-off and Aphanomyces root rot of peas after seed treatment with bacterial agents for biological control. Phytopathology. 83: 1466-1473.

Cannesan, M. A., Gangneux, C., Lanoue, A., Giron, D., Laval, K., Hawes, M., Driouich, A. & Vicré-Gibouin, M. 2011. Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. Annals of Botany. 108: 459-469.

Chan, M. & Close, R. 1987. Aphanomyces root rot of peas 1. Evaluation of methods for assessing inoculum density of *Aphanomyces euteiches* in soil. New Zealand Journal of Agricultural Research. 30: 213-217.

Chatterton, S., Harding, M. W., Bowness, R., Mclaren, D. L., Banniza, S. & Gossen, B. D. 2019. Importance and causal agents of root rot on field pea and lentil on the Canadian prairies, 2014–2017. Canadian Journal of Plant Pathology. 41: 98-114.

Deacon, J. & Saxena, G. 1998. Germination triggers of zoospore cysts of *Aphanomyces euteiches* and *Phytophthora parasitica*. Mycological Research. 102: 33-41.

Desgroux, A., L'anthoëne, V., Roux-Duparque, M., Rivière, J.-P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P. & Piriou, C. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics. 17: 1-21.

Djébali, N., Jauneau, A., Ameline-Torregrosa, C., Chardon, F., Jaulneau, V., Mathé, C., Bottin, A., Cazaux, M., Pilet-Nayel, M.-L. & Baranger, A. 2009. Partial resistance of *Medicago truncatula* to *Aphanomyces euteiches* is associated with protection of the root stele and is controlled by a major QTL rich in proteasome-related genes. Molecular Plant-Microbe Interactions. 22: 1043-1055.

Gangneux, C., Cannesan, M.-A., Bressan, M., Castel, L., Moussart, A., Vicré-Gibouin, M., Driouich, A., Trinsoutrot-Gattin, I. & Laval, K. 2014. A sensitive assay for rapid detection and quantification of *Aphanomyces euteiches* in soil. Phytopathology. 104: 1138-1147.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Molecular Plant Pathology. 8: 539-548.

Gossen, B. D., Conner, R. L., Chang, K.-F., Pasche, J. S., Mclaren, D. L., Henriquez, M. A., Chatterton, S. & Hwang, S.-F. 2016. Identifying and managing root rot of pulses on the Northern Great Plains. Plant Disease. 100: 1965-1978.

Hajihassani, A., Tenuta, M. & Gulden, R. H. 2016. Host preference and seedborne transmission of *Ditylenchus weischeri* and *D. dipsaci* on select pulse and non-pulse crops grown in the Canadian Prairies. Plant Disease. 100: 1087-1092.

Holub, E. & Grau, C. 1990. Specificity of resistance to *Aphanomyces euteiches* in seedling alfalfa. Plant Disease. 74: 164-168.

Hwang, S., Lopetinskyz, K. & Evans, L. 1991. Effects of seed infection by *Ascochyta* spp., fungicide seed treatment, and cultivar on yield parameters of field pea under field conditions. Canadian Plant Disease Survey. 71: 169-172.

Joshi, M., Timilsena, Y. & Adhikari, B. 2017. Global production, processing and utilization of lentil: a review. Journal of Integrative Agriculture. 16: 2898-2913.

Karppinen, E. M., Payment, J., Chatterton, S., Bainard, J. D., Hubbard, M., Gan, Y. & Bainard, L. D. 2020. Distribution and abundance of *Aphanomyces euteiches* in agricultural soils: effect of land use type, soil properties, and crop management practices. Applied Soil Ecology. 150: 103470.

Kjøller, R. & Rosendahl, S. 1998. Enzymatic activity of the mycelium compared with oospore development during infection of pea roots by *Aphanomyces euteiches*. Phytopathology. 88: 992-996.

Kraft, J., Marcinkowska, J. & Muehlbauer, F. 1990. Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet-sieving/baiting technique. Plant Disease. 74: 716-718.

Kraft, J. M. & Pfleger, F. L. 2001. Compendium of pea diseases and pests, American Phytopathological Society (APS Press).

Kumar, S., Barpete, S., Kumar, J., Gupta, P. & Sarker, A. 2013. Global lentil production: constraints and strategies. SATSA Mukhapatra-Annual Technical. 17: 1-13.

Levenfors, J. P., Wikström, M., Persson, L. & Gerhardson, B. 2003. Pathogenicity of *Aphanomyces* spp. from different leguminous crops in Sweden. European Journal of Plant Pathology. 109: 535-543.

Lewis, J. & Papavizas, G. 1971. Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. Phytopathology. 61: 208-214.

Lockwood, J. L. 1960. Pea introductions with partial resistance to Aphanomyces root rot. Phytopathology. 50: 621-4.

Long, J. A., Lawrence, R. L., Miller, P. R. & Marshall, L. A. 2014. Changes in field-level cropping sequences: Indicators of shifting agricultural practices. Agriculture, Ecosystems & Environment. 189: 11-20.

Ma, Y., Marzougui, A., Coyne, C. J., Sankaran, S., Main, D., Porter, L. D., Mugabe, D., Smitchger, J. A., Zhang, C. & Amin, M. 2020. Dissecting the genetic architecture of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. International Journal of Molecular Sciences. 21: 2129.

Malvick, D. & Grau, C. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Disease. 85: 740-744.

Malvick, D., Grau, C. & Percich, J. 1998. Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. Mycological Research. 102: 465-475.

Malvick, D. & Percich, J. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Disease. 83: 51-54.

Malvick, D., Percich, J., Pfleger, F., Givens, J. & Williams, J. 1994. Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Disease. 78: 361-365.

Malvick, D. K., Grünwald, N. J. & Dyer, A. T. 2009. Population structure, races, and host range of *Aphanomyces euteiches* from alfalfa production fields in the central USA. European Journal of Plant Pathology. 123: 171.

Mcgee, R. J., Coyne, C. J., Pilet-Nayel, M.-L., Moussart, A., Tivoli, B., Baranger, A., Hamon, C., Vandemark, G. & Mcphee, K. 2012. Registration of pea germplasm lines partially resistant to *Aphanomyces* root rot for breeding fresh or freezer pea and dry pea types. Journal of Plant Registrations. 6: 203-207.

Mcgee, R. J., Eigenbrode, S., Nelson, H. & Schillinger, W. 2017. Re-inventing Austrian winter pea towards developing food quality winter peas. Crops & Soils. 50: 4-46.

Miller, P. R., Bekkerman, A., Jones, C. A., Burgess, M. H., Holmes, J. A. & Engel, R. E. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. Agronomy Journal. 107: 541-550.

Mitchell, J. E., Balla, H. S. & Yang, G. 1969. An approach to the study of the population dynamics of *Aphanomyces euteiches* in soil. Phytopathology. 59: 206-212.

Moussart, A., Even, M.-N., Lesne, A. & Tivoli, B. 2013. Successive legumes tested in a greenhouse crop rotation experiment modify the inoculum potential of soils naturally infested by *Aphanomyces euteiches*. Plant Pathology. 62: 545-551.

Moussart, A., Even, M.-N. & Tivoli, B. 2008. Reaction of genotypes from several species of grain and forage legumes to infection with a French pea isolate of the oomycete *Aphanomyces euteiches*. European Journal of Plant Pathology. 122: 321-333.

Moussart, A., Wicker, E., Le Delliou, B., Abelard, J.-M., Esnault, R., Lemarchand, E., Rouault, F., Le Guennou, F., Pilet-Nayel, M.-L. & Baranger, A. 2009. Spatial distribution of *Aphanomyces euteiches* inoculum in a naturally infested pea field. European Journal of Plant Pathology. 123: 153-158.

Nass 2019b. National Agricultural Statistics Service, Commodity Statistics. In: AGRICULTURE, U. S. D. O. (ed.).

Navarro, F., Sass, M. E. & Nienhuis, J. 2008. Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Science. 48: 962-972.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annual Review of Phytopathology. 25: 125-143.

Papavizas, G. C. & Ayers, W. A. 1974. Aphanomyces species and their root diseases in pea and sugarbeet. United States Department of Agriculture, Economic Research Service.

Pfender, W., Delwiche, P., Grau, C. & Hagedorn, D. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Disease. 68: 845-847.

Pfender, W. & Hagedorn, D. 1982. *Aphanomyces euteiches* f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology. 72: 306-310.

Pfender, W. & Hagedorn, D. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology. 73: 1109-1113.

Pfender, W., Rouse, D. & Hagedorn, D. 1981. A "most probable number" method for estimating inoculum density of *Aphanomyces euteiches* in naturally infested soil. Phytopathology. 71: 1169-1172.

Pilet-Nayel, M., Muehlbauer, F., Mcgee, R., Kraft, J., Baranger, A. & Coyne, C. 2002. Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theoretical and Applied Genetics. 106: 28-39.

Quillévéré-Hamard, A., Le Roy, G., Lesné, A., Le May, C. & Pilet-Nayel, M.-L. 2021. Aggressiveness of diverse French *Aphanomyces euteiches* isolates on pea near isogenic lines differing in resistance quantitative trait loci. Phytopathology. 111: 695-702.

Saharan, K. & Khetarpaul, N. 1994. Protein quality traits of vegetable and field peas: varietal differences. Plant Foods for Human Nutrition. 45: 11-22.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bulletin. Virginia Agricultural Experiment Station. 151.

Sekizaki, H. & Yokosawa, R. 1988. Studies on zoospore-attracting activity. I. Synthesis of isoflavones and their attracting activity to *Aphanomyces euteiches* zoospore. Chemical and Pharmaceutical Bulletin. 36: 4876-4880.

Shang, H., Grau, C. & Peters, R. 2000. Oospore germination of *Aphanomyces euteiches* in root exudates and on the rhizoplanes of crop plants. Plant Disease. 84: 994-998.

Shekib, L. A., Zoueil, M., Youssef, M. & Mohamed, M. S. 1986. Amino acid composition and *in vitro* digestibility of lentil and rice proteins and their mixture (*Koshary*). Food Chemistry. 20: 61-67.

Sherwood, R. & Hagedorn, D. 1962. Studies on biology of *Aphanomyces euteiches*. Phytopathology. 52: 150-154.

Sivachandra Kumar, N. T., Caudillo-Ruiz, K. B., Chatterton, S. & Banniza, S. 2021. Characterization of *Aphanomyces euteiches* pathotypes infecting peas in Western Canada. Plant Disease. PDIS-04-21-0874-RE.

Smýkal, P., Aubert, G., Burstin, J., Coyne, C. J., Ellis, N. T., Flavell, A. J., Ford, R., Hýbl, M., Macas, J. & Neumann, P. 2012. Pea (*Pisum sativum* L.) in the genomic era. Agronomy. 2: 74-115.

Thygesen, K., Larsen, J. & Bødker, L. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. European Journal of Plant Pathology. 110: 411-419.

Tofte, J., Smith, R. & Grau, C. 1992. Reaction of red clover to *Aphanomyces euteiches*. Plant Disease.

Tu, J. 1987. Integrated control of the pea root rot disease complex in Ontario. Plant Disease (USA). 71: 9-13.

Upadhaya, A., Yan, G., Pasche, J. & Kalil, A. 2019. Occurrence and distribution of vermiform plant-parasitic nematodes and the relationship with soil factors in field pea (*Pisum sativum*) in North Dakota, USA. Nematology. 21: 445-457.

Vandemark, G. & Ariss, J. 2007. Examining interactions between legumes and *Aphanomyces euteiches* with real-time PCR. Australasian Plant Pathology. 36: 102-108.

Vandemark, G., Kraft, J., Larsen, R., Gritsenko, M. & Boge, W. 2000. A PCR-based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. Phytopathology. 90: 1137-1144.

Wakelin, S. A., Walter, M., Jaspers, M. & Stewart, A. 2002. Biological control of *Aphanomyces euteiches* root-rot of pea with spore-forming bacteria. Australasian Plant Pathology. 31: 401-407.

Walker, C. A. & Van West, P. 2007. Zoospore development in the oomycetes. Fungal Biology Reviews. 21: 10-18.

Wicker, E., Moussart, A., Duparque, M. & Rouxel, F. 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. European Journal of Plant Pathology. 109: 47-60.

Willsey, T., Chatterton, S., Heynen, M. & Erickson, A. 2018. Detection of interactions between the pea root rot pathogens *Aphanomyces euteiches* and *Fusarium* spp. using a multiplex qPCR assay. Plant Pathology. 67: 1912-1923.

Windels, C. 1996. Limitations of a greenhouse assay for determining potential of Aphanomyces root rot in sugarbeet fields. Journal of Sugar Beet Research. 33: 1-13.

Wu, L., Chang, K.-F., Conner, R. L., Strelkov, S., Fredua-Agyeman, R., Hwang, S.-F. & Feindel, D. 2018. *Aphanomyces euteiches*: a threat to Canadian field pea production. Engineering. 4: 542-551.

Wu, L., Chang, K.-F., Hwang, S.-F., Conner, R., Fredua-Agyeman, R., Feindel, D. & Strelkov, S. E. 2019. Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. The Crop Journal. 7: 38-48.

Xue, A. 2003a. Efficacy of *Clonostachys rosea* strain ACM941 and fungicide seed treatments for controlling the root rot complex of field pea. Canadian Journal of Plant Science. 83: 519-524.

Xue, A. G. 2003b. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. Phytopathology. 93: 329-335.

Yokosawa, R., Kuninaga, S., Sakushima, A. & Sekizaki, H. 1995. Induction of oospore formation of *Aphanomyces euteiches* Drechsler by calcium ion. Japanese Journal of Phytopathology. 61: 434-438.

Zhou, Y., Zhu, H., Fu, S. & Yao, Q. 2017. Variation in soil microbial community structure associated with different legume species is greater than that associated with different grass species. Frontiers in Microbiology. 8: 1007.

Zitnick-Anderson, K., Luis, E., Forster, S. & Pasche, J. S. 2020. Associations among the communities of soil-borne pathogens, soil edaphic properties and disease incidence in the field pea root rot complex. Plant and Soil. 457: 339-354.

Zitnick-Anderson, K., Porter, L. D., Hanson, L. E. & Pasche, J. S. 2021b. Identification, laboratory, greenhouse, and field handling of *Aphanomyces euteiches* on pea (*Pisum sativum*). Plant Health Progress. PHP-01-21-0011-FI.

CHAPTER TWO

SPATIAL DISTRIBUTION AND IMPACT OF SOIL PROPERTIES ON *APHANOMYCES EUTEICHES* IN MONTANA DRY PEA AND LENTIL FIELDS

Abstract

Dry pea and lentil in Montana have been experiencing losses due to root rot caused by the soilborne oomycete, *Aphanomyces euteiches*. Due to reports in neighboring states and provinces, a survey was conducted, and the pathogen identified in 2016. Detection can be difficult due to the patchy nature of root rot pathogens. To ascertain the distribution of *A. euteiches* in Montana fields, twelve fields were strategically sampled using entrance and low spot transects. Soil from transects was assessed for root rot using a greenhouse bioassay with a susceptible dry pea variety (cv. Majoret). Soil edaphic properties were compared to root rot as measured by a disease severity index to analyze trends in four of the fields sampled. Aphanomyces root rot was highly variable across field transects, except for two fields where root rot severity was high across all transects. Multiple fields had only one transect with root rot hot spots, and sporadic distribution of the pathogen was typical. Soil pH was positively associated with root rot in one field when soil was acidic and was negatively associated in a field when soil was more alkaline. Organic matter, potassium, and sulfur concentration were correlated with root rot, although correlations were not consistent between fields. This research indicates that sampling entryways alone is often not adequate to detect the presence of disease, and sampling at multiple field locations may be necessary for *A. euteiches* detection.

Introduction

Production of pulses in the Northern Great Plains of the United States has increased since they were first grown in the 1990s. Pulses have assisted producers filling fallow acres, moving away from a traditional cereal-fallow rotation (Long et al. 2014). This movement was enhanced by price supports in the 2014 Farm Bill, fitting well with the cropping system, favorable economics, and secondary benefits including reduced nitrogen fertility needs for the succeeding crop, and soil health benefits (Miller et al. 2015; Burgess et al. 2012). The good fit of pulses with climate smart practices, human health nutrition benefits, as key ingredients in the growing alternative meat market, and the status of pulses as a 'clean' crop, with no genetically modified varieties, and increasing domestic consumption is likely to sustain the industry long term.

Constraints to pulse crop production include root rot pathogens that limit yield and can persist in the soil system for multiple years. Aphanomyces root rot, caused by the soilborne oomycete *Aphanomyces euteiches*, was identified in Montana in 2016 after it was reported in neighboring Provinces and states (Vandemark and Porter 2010; Chatterton et al. 2016; Zitnick-Anderson and Pasche 2016). Aphanomyces is highly virulent on the pulse crops dry pea (*Pisum sativum*) and lentil (*Lens culinaris*). In Canada and France as well as western North Dakota, Aphanomyces root rot has forced dry pea production out of wetter regions (Wu et al. 2018; Zitnick-Anderson and Pasche 2016). Other legumes susceptible to *A. euteiches* include alfalfa, faba bean, common bean, vetch, cowpea, and red clover (Sherwood and Hagedorn 1962; Pfender and Hagedorn 1982; Tofte et al. 1992; Malvick et al. 1998; Gaulin et al. 2007; Wu et al. 2018).

Aphanomyces root rot is difficult to manage due to a long-lived oospore, shown to be able to live for ten years in the absence of a host, and limited management options (Gaulin et al. 2007). Currently no dry pea and lentil varieties are on the market with resistance to *A. euteiches*, however, quantitative trait loci (QTLs) for resistance have been identified in dry pea and lentil germplasm (Hamon et al. 2013; Ma et al. 2020; Pilet-Nayel et al. 2005). Seed treatments available for *A. euteiches* suppression, including the active ingredient ethaboxam, are not effective for the entire growing season and yield benefits have not been seen (Willsey et al. 2021; Wu et al. 2019).

Pathogen avoidance is currently the best management strategy when root rot is severe, particularly rotating to non-host crops. Chickpea is a pulse crop option for growers in this predicament, as it is not a host to *A. euteiches*. Evidence also suggests that winter pea can escape root rot stress due to cold temperatures that are unfavorable for Aphanomyces root rot development (McGee et al. 2017). Recent studies have indicated that other factors will also impact disease risk. Soil moisture, nitrogen, and organic carbon were positively correlated with *A. euteiches* abundance when surveys of dry pea and lentil fields were conducted in Saskatchewan (Karppinen et al. 2020). In addition, data from surveys of dry pea fields in North Dakota supported the finding that potassium in soil had a negative association with *A. euteiches* disease incidence (Zitnick-Anderson et al. 2020).

Detection of *A. euteiches* requires either analysis of root samples or soil samples, due to the primary stages of the lifecycle occurring in soil and host plant root tissue. Targeted sampling is useful if susceptible host plants are growing with visible

aboveground symptoms of disease. However, this is not always the case, and for disease prediction purposes, sampling needs to be undertaken prior to planting of a susceptible crop. *A. euteiches* is difficult to detect without molecular tools, as the pathogen is very difficult to isolate and easily missed in samples, and the pathogen is known to occur in a root rot complex consisting of multiple oomycete and fungal species (Zitnick-Anderson et al. 2020). Due to the primary symptoms occurring belowground, Aphanomyces root rot can easily be missed or confused with abiotic issues on dry pea and lentil. This is also due to the sporadic and patchy spatial distribution of *A. euteiches*, with problem areas or foci of disease in fields (Moussart et al. 2009). Sampling strategies are, therefore, difficult to design.

Field entrances are often a site with compaction issues due to the high frequency of vehicle entry and exit in a constrained area. Soil compaction is known to exacerbate root rot, as roots are typically weaker and more vulnerable to invasion (Allmaras et al. 2003). Pea roots are quite susceptible to compaction and poor water drainage, and are more sensitive to it than other plant species (Allmaras et al. 1988). Due to motility of zoospores in water, *A. euteiches* detection may be enhanced by looking at waterlogged soil patches, or elevation depressions where moisture may gather (Zitnick-Anderson et al. 2021b). It was, therefore, hypothesized that sampling dry pea and lentil fields in Montana would reveal the pathogen at the entrance of fields, when present, and in low spot or ‘problem areas’. Of interest was if any soil factors were correlated with disease in field transects.

Materials and Methods

Field Sampling

From 2017 to 2021, fields with a history of dry pea and lentil were systematically sampled from fields in northeast Montana where root rot was reported. With assistance from County agents, MSU extension agents, pulse producers, soil surveys, and root rot samples submitted to the MSU Schutter Diagnostic Laboratory, fields were selected for sampling based on presence of root rot on soil bioassays with dry pea test plants and positive PCR tests (described below). Two fields sampled in 2017 did not have detectable root rot in the soil bioassay, even with initial positive PCR tests, and have been excluded (data not shown). Data tables of eight fields are included in Appendix A (Table A.01 through A.12).

Due to the large volume of soil required, and the disruptive nature of sampling to a crop in the ground, soil sampling was conducted in September and October after dry pea or lentil crop harvest. Field entrances were flagged in 3 x 100 m transects, at 45^{o} angles, with one sampling line parallel to the outer edge of the field, another on the other edge, and the other transect in the center (Fig 2.01). When fields had obstructions such as waterways, the angle of the transect was adjusted.

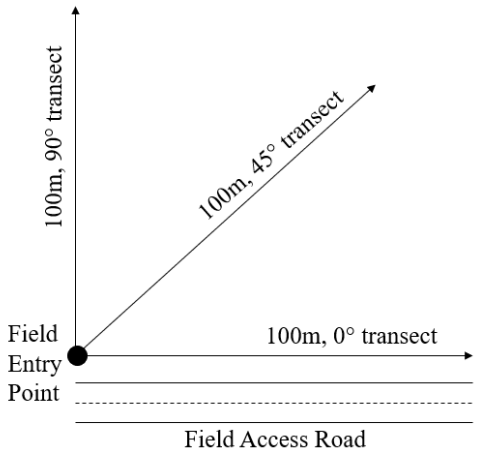


Fig. 2.01. Visual representation of field entrance transects.

In addition to the three entrance transects, a 50 m sampling transect within the field perimeter at a low, wet spot was selected. Each transect line was divided into 10 m x 10 m quadrats and approximately 250 g soil was collected from four randomly assigned subsampling points within each transect, for a total of 1 kg of soil. A shovel (blade 21 x 25 cm, AmesTrue Temper, Inc, Camp Hill, PA) was used to take samples from the top six inches of the soil profile. Soil samples were stored in gallon Ziploc® bags and held in cold (4ºC) storage until processing.

Prior to conducting a greenhouse bioassay, the four random sub-samples from each quadrat were combined and mixed into a composite sample and sieved using a 6.3 mm (0.25 in) U.S.A standard test sieve (Fisherbrand™, Ottawa, ON) to remove rocks and larger debris. Due to extremely dry soil conditions impeding sample collection, and restrictions on travel, fields sampled in 2020 had only one 50 m entrance transect, and one 50 m low spot transect (Supplemental Tables A.11. and A.12.).

Soil Bioassay

Soil bioassays are an effective tool for identifying the presence of soilborne plant pathogens in field samples. A bioassay for the detection of *Aphanomyces euteiches* in field soil was designed and tested based on existing protocols for alfalfa and fresh pea (Holub and Grau 1990; Persson et al. 1999). Sieved soil samples from each quadrat were divided between four pots with a diameter of 12.7 cm and 9.2 cm height, with a Kimwipe™ (Kimtech, Roswell, GA) at the bottom of each pot. A susceptible green dry pea variety, cultivar Majoret, was used in bioassays and seed was treated with Apron Maxx RTA® (Syngenta, Greensboro, NC) at the labelled rate (11.75 g / a.i. / ha of mefonoxam and 9.43 g / a.i. / ha fludioxonil) to reduce effects of soilborne *Pythium* spp. and fungi. Six seeds were planted and thinned to five plants after germination. Each pot was placed in a 15 cm diameter water saucer (Vigoro, Northbrook, IL) and watered daily. Greenhouse conditions were 22/18°C, and a 16/8 h day/night.

Pots were arranged on the greenhouse bench in a completely randomized design, with four pot replicates per quadrat. Four control pots using MSU mix soil (1:1:1 Canadian sphagnum peat moss, mineral soil mix, and Aquagro 2000G [Aquatrols, NJ]) were included to check for cross-contamination in pots. Approximately four inches of buffer room was left between pots. Dry pea plants were grown for 21 days then plants were carefully removed from pots and the roots were washed in water and evaluated for root rot using a disease severity scale, with disease severity scores (DSS) from 0-5 (Fig. 2.02). Root tissue was frozen at -20°C for molecular analysis.

Fig. 2.02. Disease severity scale for *A. euteiches* on dry pea, where 0=healthy root, 1=slight root discoloration, 2=slight to moderate browning with detectable root restriction, 3=moderate caramel browning and root constriction, 4=moderate to severe root browning and constriction, 5=severe root browning and root tissue sloughed off from seed attachment, photo: C. Murphy

Isolation and Maintenance of *Aphanomyces euteiches*

Four root samples from each field quadrat were plated onto semi-selective media for recovering isolates of *A. euteiches*. The selective medium 'MBV' containing metalaxyl, benomyl, and vancomycin (Pfender et al. 1984; Zitnick-Anderson et al. 2021b) was prepared in petri plates (100 mm x 15 mm, FisherBrand, Ottawa, ON). In addition to the three ingredients listed above, rifampicin (100 mg / L) and amphotericin B (0.5 mg /L) were incorporated, and the 'under-the-block' technique was used for pathogen isolation as described in Zitnick-Anderson et al. (2021). This is the procedure where a small square is cut into the agar and the sterilized root tissue is tucked underneath the agar square, to reduce bacterial contamination.

After 2-5 days at 20°C, petri plates were checked for growth indicative of *A. euteiches*. Typical growth consisted of transparent radial growth in a mat, visible only under high lighting. Microscope slides were prepared to check for the presence of oospores. Samples were sub-cultured using the hyphal tip method when suspected to be *A. euteiches* and plated on corn meal agar (CMA, PhytoTech Labs, Lenexa, KS) and potato dextrose agar (PDA, Difco™, BD, Franklin Lakes, NJ). CMA is known to induce sporulation for many *A. euteiches* isolates, while PDA is a useful storage medium for *A. euteiches* (Yokosawa et al. 1995). When isolates were successfully collected from plant roots, fresh petri plates were prepared with agar squares every 14 days. Inoculum of each isolate was pipetted onto dry pea roots in greenhouse pots and re-isolated approximately every six months to maintain virulence.

Molecular Detection of *Aphanomyces euteiches*

Frozen root tissue from greenhouse bioassays was used for molecular analysis. DNA was extracted from four samples of plant roots (~50 mg wet weight) per field quadrat using DNeasy plant pro kits (Qiagen, Germantown, MD). When isolates collected from semi-selective media were suspected to be *A. euteiches* based on morphological characteristics, DNA was extracted from hyphae (~10 mg) using the PrepMan™ ultra sample preparation reagent (Applied Biosystems™). Polymerase chain reaction (PCR) confirmation of *A. euteiches* presence was completed using published primers (Vandemark et al. 2000). PCR cycling conditions were 95°C for 9 min, 40 cycles of 94°C for 1 min, 70°C for 1 min, 72°C for 1 min, then an extension at 72°C for 7 min, and maintenance at 4°C. A relatively thick agarose gel (1.5 to 2%) was used to visualize

amplicons. When more than a binary presence / absence was desired, techniques were used for multiplex quantification of root and pathogen DNA using a CFX Opus 96 Real-Time PCR system (Bio-Rad Laboratories, Hercules, CA) using dry pea and *A. euteiches* primers (Psat_TUB1_3F, Psat_TUB1_3R, Psat_Tub1_3Pr, Ae1.2_ITS1F, Ae1.2_ITS1R, Ae1.2_ITS1Pr) and cycling conditions described in Willsey et al., 2018.

Soil Properties

Soil samples from four fields with significant and variable Aphanomyces root rot distribution between quadrats were sent to Agvise Laboratories (Northwood, ND) for soil testing. Samples were analyzed for the macronutrients nitrate-nitrogen, phosphorous, potassium, and sulfate-sulfur. Base cations included were calcium, magnesium, and sodium. Micronutrients measured were boron, chloride, copper, iron, manganese, and zinc. Soil properties analyzed were soil pH, soluble salts by electrical conductivity (EC), organic matter, carbonate, cation-exchange capacity (CEC), and base saturation.

Statistical Analysis

All statistical analysis was conducted in R version 4.1.2 (R Core Team 2018). Standard error of the mean was calculated from raw greenhouse bioassay DSS, for inclusion in data tables. DSS from greenhouse bioassays were converted to a disease severity index (DSI) for each field quadrat, using the formula DSI (%) = $\{[(a * 0) + (b * 1) + (c * 2) + (d * 3) + (e * 4) + (f * 5)]/[(a + b + c + d + e + f) * g]\}$ x 100, where *a*, *b*, *c*, *d*, *e*, and *f* indicate how many plants were rated at each disease severity score and *g* represents the maximal disease score, in this case, 5 (Li et al. 2014; McKinney 1923).

Data from control pots using greenhouse soil were removed prior to statistical analysis and each field was analyzed separately. DSI was used as the response variable with the independent factors of elevation, transect line, and soil properties, for analysis using the 'lm' linear model functions in the 'lme4' package in R (Bates et al. 2015). All soil factors were included in an initial full linear model, and terms were reduced first by the least relevant term, with a cut-off point using the 'anova' function of $P < 0.1$ (Fox and Weisberg 2011). The Akaike information criterion (AIC) was used to test model variations for fit to assist in model selection, using the 'AICcmodavg' package (Mazerolle 2020).

Based on information from model selection, a matrix of correlation coefficients with DSI and relevant soil factors was computed using the R package Hmisc, which uses a Pearson's rank correlation coefficient (Harrell Jr and Dupont 2019). The cutoff for evidence of a correlation between DSI and soil properties was $P < 0.05$.

Results

Two fields (Field 1 and Field 2) were managed by the same pulse producer in Roosevelt County, Montana. These fields had high Aphanomyces root rot severity in multiple quadrats in entryway transects and low spot transects. Field 1 had experienced dry pea crop failure in the year of sampling, 2018, with severe root rot reported by the producer as the issue. Root rot severity varied between transects of Field 1 ($P < 0.001$), with 90% of quadrats with a DSS of 4, severe root rot, in entrance line 1 and 2, while line 3 scores ranged from a DSS of 2-4 for Aphanomyces root rot (Table 2.01)

Table 2.01. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 1, sampled in 2018 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	3	0.2	4	0.1	3	0.3
20	4	0.2	2	0.3	2	0.4
30	4	0.1	4	0.1	2	0.3
40	4	0.2	4	0.1	3	0.2
50	4	0.1	4	0.1	3	0.2
60	4	0.1	4	0.1	2	0.2
70	4	0.2	4	0.1	3	0.2
80	4	0.1	4	0.1	3	0.2
90	4	0.1	4	0.1	4	0.3
100	4	0.2	4	0.2	2	0.2

In the low spot transect of Field 1, severe root rot was present in all five quadrats sampled in the 50 m line (Table 2.02). Elevation in the field did not influence root rot in quadrats ($P = 0.67$). Organic matter in the soil samples ranged from 2.6 to 4.7%, with an average of 3.9% and was inversely correlated (-0.43, $P = 0.01$) with disease severity across quadrats. Potassium in soil samples had an average of 612 ppm and was negatively correlated with root rot (-0.39. $P = 0.02$). The same relationship was seen with sulfur, with an average of 33 kg/ha in samples (-0.53, $P = 0.001$). Soil pH in samples ranged from 4.3 to 5.2, with an average of 4.6, and was positively correlated with root rot in the soil bioassay (0.5, $P = 0.002$).

Table 2.02. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 1, sampled in 2018 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	5	0.2
2	10	5	0.4
3	0	4	0.6
4	10	4	0.5
5	20	5	0.2

Field 2 was managed differently and had fewer years of pulses in the rotation. The three transects starting at the entryway had highly sporadic disease, with focal points in the field where root rot was severe, and no to moderate root rot symptoms in other sections (Table 2.03), with differences between transects ($P = 0.004$). Elevation of quadrats did not impact disease ($P = 0.93$). In the low spot transect of Field 2 there was a focal point of root rot disease for two transects (Table 2.04). Organic matter of soil samples ranged from 2.8 to 4%, averaging 3.5% and was positively correlated with root rot (0.46, $P = 0.01$). The range of pH values of soil samples collected from this field was 4.4 to 5.7, and no evidence of a correlation between Aphanomyces root rot and pH was found ($P = 0.2$). There were no other correlations found between root rot and soil properties in this field.

Table 2.03. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 2 sampled in 2018 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	5	0.3	5	0.2	5	0.3
20	4	0.4	2	0.5	4	0.5
30	0	0.4	1	0.4	3	0.3
40	1	0.3	3	0.3	0	0.3
50	1	0.3	4	0.4	4	0.5
60	0	0.4	5	0.3	2	0.4
70	0	0.2	5	0.5	2	0.4
80	0	0.1	5	0.1	3	0.4
90	1	0.2	5	0.3	5	0.7
100	0	0.1	4	0.4	5	0.3

Table 2.04. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 2 sampled in 2018 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	5	0.3
2	10	5	0.4
3	0	0	0.7
4	10	1	0.4
5	20	1	0.5

In Field 3, sampling lines differed in root rot severity ($P < 0.001$). The first entrance line ran alongside a road and had significant vehicle tracks within the field margin and moderate to severe Aphanomyces root rot was detected in eight quadrats of the ten quadrats (Table 2.05). Root rot was barely detectable in the other transect lines starting at the entryway and elevation of quadrats had an impact on root rot severity ($P < 0.001$). There was one quadrat with moderate to severe root rot in the low spot transect, while the other quadrats had predominantly healthy dry pea roots in the bioassay (Table

2.06). In Field 3, soil pH varied from 6.2 to 8.2, with an average of 7.5, and was negatively correlated with root rot (-0.6, $P < 0.001$).

Table 2.05. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 3 sampled in 2019 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	0	0.1	0	0.1	1	0.2
20	2	0.2	1	0.2	1	0.3
30	3	0.2	1	0.1	1	0.2
40	3	0.1	1	0.1	0	0.1
50	3	0.1	1	0.1	1	0.2
60	3	0.1	1	0.2	1	0.2
70	4	0.1	0	0.1	0	0.2
80	4	0.2	0	0.1	1	0.1
90	4	0.1	0	0.1	1	0.2
100	3	0.2	0	0.1	1	0.1

Table 2.06. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 3 sampled in 2019 in Roosevelt County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	4	0.1
2	10	1	0.2
3	0	0	0.1
4	10	0	0.1
5	20	0	0.2

Fairly consistent root rot pressure was discovered in Field 4 entrance transects, with a few ‘clean’ quadrat exceptions, where *A. euteiches* was not detected in the greenhouse bioassay or with PCR (Table 2.07). Differences were not detected between sampling transects ($P = 0.42$). Elevation in field quadrats also did not have an impact on root rot ($P = 0.64$). Lines 2 and 3 in the entrance were 30° apart, instead of 45°, due to a

pond that was situated on the edge of the field. The low spot transect was not selected near the pond, due to the proximity to entrance lines. The low spot quadrat selected was located at a dip in the middle of the field and had moderate to severe root rot pressure (Table 2.08). Soil properties were not correlated with disease in Field 4.

Table 2.07. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 4 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	3	0.2	3	0.2	3	0.1
20	3	0.2	3	0.2	3	0.2
30	2	0.3	0	0.2	4	0.2
40	2	0.3	3	0.1	4	0.2
50	3	0.2	3	0.2	3	0.1
60	0	0.0	3	0.2	3	0.3
70	4	0.2	4	0.3	4	0.2
80	2	0.1	4	0.2	3	0.2
90	0	0.2	3	0.2	3	0.3
100	0	0.2	3	0.2	3	0.1

Table 2.08. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 4 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	3	0.2
2	10	4	0.1
3	0	4	0.2
4	10	4	0.2
5	20	3	0.2

Discussion

The aim of this study was to get a better understanding of *A. euteiches* in-field horizontal distribution to assist in determining sampling strategies for optimal detection of *A. euteiches* in Montana dry pea and lentil fields. When dry pea and lentil are growing, symptoms of the disease can be used for targeted sampling, such as yellow patches in the field, typically following water run-off or localized to waterlogged areas (Gangneux et al. 2014; Scott 1961). On closer inspection, Aphanomyces root rot can be distinguished from abiotic stressors by digging up roots and looking for caramel brown roots, often with sloughing of lateral roots leaving an exposed stele. For root rot risk assessments prior to planting dry pea and lentil, however, a more targeted location to sample is required. It was hypothesized that sampling beginning at the field entrance would be optimal, as it is often an area of high vehicle movement, leading to soil compaction. Soil compaction causes root rot issues to be more pronounced, as roots are weaker and more vulnerable to pathogens and soils are more likely to be waterlogged due to issues with drainage (Allmaras et al. 2003; Tu 1994). Also, it was hypothesized that it could be where Aphanomyces root rot, a soilborne pathogen, is first introduced to a field by movement of soil on vehicles.

While Aphanomyces root rot was found at the quadrats close to the field entrance for most of the fields sampled, it is apparent that sampling at the field entrance alone is not always adequate. For example, some fields sampled had moderate to severe root rot that started twenty meters from the entrance of the field, which would have been missed if only the first quadrat at the entrance had been sampled. Root rot was also not always

consistently detected in low or water-logged regions in the fields. In two fields, moderate root rot was found only at one end of the low spot transect, but not in the middle of the quadrat, the 'hot spot'. Sporadic dispersal of the pathogen in fields with low to moderate disease means that the best sampling strategy for soil sampling is composite samples from multiple sampling locations in the field. Similar to the results found here, a study of the distribution of *A. euteiches* in one field with a history of dry pea production in France found that root rot was sporadic in fields, in disease foci (Moussart et al. 2009). In a field with *Aphanomyces cochlioides* presence, disease foci were present mid-season based on greenhouse bioassays, and by harvest the sugar beet root rot was uniform in the field sampled due to conducive moisture conditions (Beale et al. 2002).

Uniformity of *A. euteiches* in a field indicates that conditions have been conducive for pathogen spread. In some fields sampled in this study, root rot severity was uniform, for example, Field 1 had moderate root rot found in all quadrats. Unfortunately, detection of *A. euteiches* in this field was not timely, as sampling occurred after dry pea crop failure in the year of sampling. Detection is necessary prior to this point, so that producers can avoid planting host crops and make risk assessment decisions to mitigate yield losses in dry pea and lentil. To make such risk assessments, a disease modelling tool may be necessary that considers crop history, environmental conditions, and soil properties for prediction of root disease, with considerations for other root rot pathogens in the region.

Certain soil edaphic properties were correlated with Aphanomyces root rot with individual fields, however, these properties differed between fields sampled. In a field

with acidic soil conditions, root rot was positively correlated with pH, and when soil was neutral to alkaline, an inverse correlation was seen. This could indicate that soils with acidity issues are suppressive to Aphanomyces root rot in the region, and neutral to slightly alkaline pH conditions are favorable for the disease cycle. Similarly, surveys of dry pea in North Dakota revealed a positive association with pH and *A. euteiches* presence (Zitnick-Anderson et al. 2020). In contrast, recent surveys in the Canadian prairies did not detect correlations in pulse fields between pH and *A. euteiches* abundance, however a negative correlation with pH and root rot was seen in non-cropped regions, including roadsides (Karppinen et al. 2020). The closely related sugar beet root pathogen, *A. cochlioides*, was shown to have a negative correlation with disease presence and pH, and liming fields proved effective for improving yields when the pathogen was present (Olsson et al. 2011; Olsson et al. 2019). Further work is required to elucidate if amending soil pH would be beneficial for suppression of *A. euteiches* in pulse fields, and whether differences in pH preference for pathotypes or isolates occur between geographic regions.

Correlations between Aphanomyces root rot and organic matter were discovered in two fields sampled, with a positive correlation in one field and a negative correlation in the other. In the literature, the relationship with organic matter and soilborne pathogens has been shown to be positive for pathogens in some cases, and negative in others. For root rot caused by *Pythium* spp., organic matter in the form of humus in soil typically assists the pathogen by providing nutrients in an accessible form (Stone et al. 2001). In other instances, organic matter promotes beneficial biodiversity in soils, increasing

competition for pathogens, as seen with the addition of composts suppressing *Phytophthora* spp. (Broadbent and Baker 1974; Hoitink et al. 1996).

In one field, disease severity of *A. euteiches* was inversely correlated with potassium measured in the soil sample. Studies in the literature of oomycetes pathogenic to plants have shown that potassium salts can reduce the ability of zoospores to swim to neighboring plants (Appiah et al. 2005). It has also been shown that potassium salts can inhibit the growth of oomycete hyphae, as witnessed with *Phytophthora* root rot on lettuce and tomato and *Pythium* spp. on tomato (El-Mohamedy et al. 2013; Jee et al. 2002). In the same field, the same inverse relationship was seen with sulfur and Aphanomyces root rot. Sulfur has been shown in the literature to reduce infection with oomycete and fungal pathogens by destroying disease propagules (Williams and Cooper 2004; Tweedy 1981). These elements may therefore be playing a role in disease suppression of *A. euteiches* in the sampled field.

In conclusion, composite sampling at multiple points in a field will increase the chance of detecting *A. euteiches* for disease risk assessment in dry pea and lentil fields. Soil testing for edaphic soil properties, including soil pH, organic matter, potassium, and sulfur, may be useful for determining risk factors or disease suppressive attributes of fields for Aphanomyces root rot disease, however, further work is required to test these outcomes and elucidate other factors important for disease risk modelling.

References

Allmaras, R., Fritz, V., Pfleger, F. & Copeland, S. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil and Tillage Research. 70: 41-52.

Allmaras, R., Kraft, J. & Miller, D. 1988. Effects of soil compaction and incorporated crop residue on root health. Annual Review of Phytopathology. 26: 219-243.

Appiah, A. A., Van West, P., Osborne, M. C. & Gow, N. A. 2005. Potassium homeostasis influences the locomotion and encystment of zoospores of plant pathogenic oomycetes. Fungal Genetics and Biology. 42: 213-223.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software.

Beale, J. W., Windels, C. E. & Kinkel, L. L. 2002. Spatial distribution of *Aphanomyces cochlioides* and root rot in sugar beet fields. Plant Disease. 86: 547-551.

Broadbent, P. & Baker, K. F. 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Australian Journal of Agricultural Research. 25: 121-137.

Burgess, M. H., Miller, P. R. & Jones, C. A. 2012. Pulse crops improve energy intensity and productivity of cereal production in Montana, USA. Journal of Sustainable Agriculture. 36: 699-718.

Chatterton, S., Bowness, R. & Harding, M. 2016. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Disease. 100: 1965-1978.

El-Mohamedy, R. S., Abdel-Kader, M., Abd-El-Kareem, F. & El-Mougy, N. S. 2013. Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens in vitro. Journal of Agricultural Technology. 9: 1507-1520.

Fox, J. & Weisberg, S. 2011. An R companion to applied regression. Thousand Oaks, CA: SAGE Publications.

Gangneux, C., Cannesan, M.-A., Bressan, M., Castel, L., Moussart, A., Vicré-Gibouin, M., Driouich, A., Trinsoutrot-Gattin, I. & Laval, K. 2014. A sensitive assay for rapid detection and quantification of *Aphanomyces euteiches* in soil. Phytopathology. 104: 1138-1147.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Molecular Plant Pathology. 8: 539-548.

Hamon, C., Coyne, C. J., Mcgee, R. J., Lesné, A., Esnault, R., Mangin, P., Hervé, M., Le Goff, I., Deniot, G. & Roux-Duparque, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biology. 13: 45.

Harrell Jr, F. & Dupont, C. 2019. Hmisc: Harrell Miscellaneous. R Package Version 4.2-0.

Hoitink, H., Madden, L. & Boehm, M. 1996. Relationships among organic matter decomposition level, microbial species diversity, and soilborne disease severity. Principles and Practice of Managing Soilborne Plant Pathogens. 4: 237-249.

Holub, E. & Grau, C. 1990. Specificity of resistance to *Aphanomyces euteiches* in seedling alfalfa. Plant Disease. 74: 164-168.

Jee, H.-J., Cho, W.-D. & Kim, C.-H. 2002. Effect of potassium phosphonate on the control of Phytophthora root rot of lettuce in hydroponics. The Plant Pathology Journal. 18: 142-146.

Karppinen, E. M., Payment, J., Chatterton, S., Bainard, J. D., Hubbard, M., Gan, Y. & Bainard, L. D. 2020. Distribution and abundance of *Aphanomyces euteiches* in agricultural soils: effect of land use type, soil properties, and crop management practices. Applied Soil Ecology. 150: 103470.

Li, Y. P., You, M. P. & Barbetti, M. J. 2014. Species of *Pythium* associated with seedling root and hypocotyl disease on common bean (*Phaseolus vulgaris*) in Western Australia. Plant Disease. 98: 1241-1247.

Long, J. A., Lawrence, R. L., Miller, P. R. & Marshall, L. A. 2014. Changes in field-level cropping sequences: Indicators of shifting agricultural practices. Agriculture, Ecosystems & Environment. 189: 11-20.

Ma, Y., Marzougui, A., Coyne, C. J., Sankaran, S., Main, D., Porter, L. D., Mugabe, D., Smitchger, J. A., Zhang, C. & Amin, M. 2020. Dissecting the genetic architecture of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. International Journal of Molecular Sciences. 21: 2129.

Malvick, D., Grau, C. & Percich, J. 1998. Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. Mycological Research. 102: 465-475.

Mazerolle, M. J. 2020. AICcmodavg: Model selection and multimodel inference based on AIC. R package version 2.3-1 ed.

Mcgee, R. J., Eigenbrode, S., Nelson, H. & Schillinger, W. 2017. Re-inventing Austrian winter pea towards developing food quality winter peas. Crops & Soils. 50: 4-46.

Mckinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Research. 26: 195-217.

Miller, P. R., Bekkerman, A., Jones, C. A., Burgess, M. H., Holmes, J. A. & Engel, R. E. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. Agronomy Journal. 107: 541-550.

Moussart, A., Wicker, E., Le Delliou, B., Abelard, J.-M., Esnault, R., Lemarchand, E., Rouault, F., Le Guennou, F., Pilet-Nayel, M.-L. & Baranger, A. 2009. Spatial distribution of *Aphanomyces euteiches* inoculum in a naturally infested pea field. European Journal of Plant Pathology. 123: 153-158.

Olsson, Å., Persson, L. & Olsson, S. 2011. Variations in soil characteristics affecting the occurrence of Aphanomyces root rot of sugar beet–risk evaluation and disease control. Soil Biology and Biochemistry. 43: 316-323.

Olsson, Å., Persson, L. & Olsson, S. 2019. Influence of soil characteristics on yield response to lime in sugar beet. Geoderma. 337: 1208-1217.

Persson, L., Larsson-Wikström, M. & Gerhardson, B. 1999. Assessment of soil suppressiveness to Aphanomyces root rot of pea. Plant Disease. 83: 1108-1112.

Pfender, W., Delwiche, P., Grau, C. & Hagedorn, D. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Disease. 68: 845-847.

Pfender, W. & Hagedorn, D. 1982. *Aphanomyces euteiches* f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology. 72: 306-310.

Pilet-Nayel, M., Muehlbauer, F., Mcgee, R., Kraft, J., Baranger, A. & Coyne, C. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology. 95: 1287-1293.

R Core Team, R. 2018. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bulletin. Virginia Agricultural Experiment Station. 151.

Sherwood, R. & Hagedorn, D. 1962. Studies on biology of *Aphanomyces euteiches*. Phytopathology. 52: 150-154.

Stone, A., Traina, S. & Hoitink, H. 2001. Particulate organic matter composition and Pythium damping-off of cucumber. Soil Science Society of America Journal. 65: 761-770.

Tofte, J., Smith, R. & Grau, C. 1992. Reaction of red clover to *Aphanomyces euteiches*. Plant Disease.

Tu, J. C. 1994. Effects of soil compaction, temperature, and moisture on the development of the Fusarium root rot complex of pea in southwestern Ontario. Phytoprotection. 75: 125-131.

Tweedy, B. G. 1981. Inorganic sulfur as a fungicide. Residue Reviews. 78: 43-68.

Vandemark, G., Kraft, J., Larsen, R., Gritsenko, M. & Boge, W. 2000. A PCR-based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. Phytopathology. 90: 1137-1144.

Vandemark, G. & Porter, L. 2010. First report of lentil root rot caused by *Aphanomyces euteiches* in Idaho. Plant Disease. 94: 480-480.

Williams, J. & Cooper, R. 2004. The oldest fungicide and newest phytoalexin–a reappraisal of the fungitoxicity of elemental sulphur. Plant Pathology. 53: 263-279.

Willsey, T., Patey, J., Vucurevich, C., Chatterton, S. & Carcamo, H. 2021. Evaluation of foliar and seed treatments for integrated management of root rot and pea leaf weevil in field pea and faba bean. Crop Protection. 143: 105538.

Wu, L., Chang, K.-F., Conner, R. L., Strelkov, S., Fredua-Agyeman, R., Hwang, S.-F. & Feindel, D. 2018. *Aphanomyces euteiches*: a threat to Canadian field pea production. Engineering. 4: 542-551.

Wu, L., Chang, K.-F., Hwang, S.-F., Conner, R., Fredua-Agyeman, R., Feindel, D. & Strelkov, S. E. 2019. Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. The Crop Journal. 7: 38-48.

Yokosawa, R., Kuninaga, S., Sakushima, A. & Sekizaki, H. 1995. Induction of oospore formation of *Aphanomyces euteiches* Drechsler by calcium ion. Japanese Journal of Phytopathology. 61: 434-438.

Zitnick-Anderson, K., Luis, E., Forster, S. & Pasche, J. S. 2020. Associations among the communities of soil-borne pathogens, soil edaphic properties and disease incidence in the field pea root rot complex. Plant and Soil. 457: 339-354.

Zitnick-Anderson, K. & Pasche, J. 2016. First report of Aphanomyces root rot caused by *Aphanomyces euteiches* on field pea in North Dakota. Plant Disease. 100: 522.

Zitnick-Anderson, K., Porter, L. D., Hanson, L. E. & Pasche, J. S. 2021b. Identification, laboratory, greenhouse, and field handling of *Aphanomyces euteiches* on pea (*Pisum sativum*). Plant Health Progress. PHP-01-21-0011-FI.

CHAPTER THREE

MANAGEMENT OF *APHANOMYCES EUTEICHES* WITH GREENHOUSE CROP ROTATION

Abstract

Aphanomyces root rot has been a rising concern in pulse growing areas of the Northern Great Plains since its first report in Idaho in 2010. Disease development is dependent on environmental factors favoring pathogen development, and the total number of years growing susceptible host crops is highly indicative of disease risk. This study investigated the impact of plant species in plant rotations relevant to the Northern Great Plains on soil inoculum of *A. euteiches* under greenhouse conditions. Infested soil was planted with treatment crops, harvested, and then dry pea was planted into the treatment soil to measure the impact of crop type on disease risk. Data was analyzed using ordinal regression. Dry pea and lentil increased root rot symptoms on the subsequent dry pea test crop compared to non-host plants ($P < 0.05$). Chickpea, a non-host pulse crop, reduced inoculum in a similar manner to cereal crops, flax, oilseeds, and brassicas. Growing cultivated oat and wild oat reduced root rot symptoms on dry pea. This research reiterates the importance of crop rotation in managing Aphanomyces root rot and provides insight into potential crop rotations for field applications.

Introduction

Montana is relatively new to pulse production compared to neighboring states and provinces. Acreage increased in the mid-1990s and were further increased after pulse crop commodity programs were included in the 2014 USDA Farm Bill. High market demand, a recognized fit in the cropping system, and increased domestic marketing have sustained and increased production. After several rotations of pulse crops in the same field, root rots are starting to become problematic in the Pacific Northwest and Northern Great Plains regions of the United States as well as the prairie provinces of Canada (Papavizas and Ayers 1974; Wu et al. 2018; Esmaeili Taheri et al. 2017). Key players in the root rot complex include *Aphanomyces euteiches*, *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani,* and nematodes from the genera *Pratylenchus*, *Ditylenchus*, *Paratylenchus*, and *Tylerchorhynchus* (Upadhaya et al. 2019; Schroeder et al. 2006; Hajihassani et al. 2016; Akhter et al. 2015; Smiley et al. 2013; Zitnick-Anderson et al. 2021a; Zitnick-Anderson et al. 2020). The pathogen complex varies over geographical area and time.

The highly damaging oomycete pathogen, *Aphanomyces euteiches*, has been a concern in pulse crops due to limited options and a thick-walled survival oospore that can live for approximately ten years without a host plant (Gaulin et al. 2007). In the absence of other effective management strategies, this requires lengthy rotations out of susceptible crops to avoid increasing inoculum. Research is underway to find solutions to protect dry pea and lentil roots, with a focus on seed treatments and resistant varieties. Under greenhouse conditions, Aphanomyces seedling blight was reduced with a variety of seed

treatment fungicides, however, yield increases are not consistent under field conditions (Wu et al. 2019; Papavizas and Lewis 1971; Gundersen et al. 2006). In Canadian field trials testing ethaboxam, dry pea roots were not protected from *A. euteiches* (Willsey et al. 2021). Biocontrol products incorporating *Trichoderma* fungal strains were tested on lentil genotypes, and also did not provide protection against *A. euteiches* (Prashar and Vandenberg 2017). Although warm, wet springs favor *A. euteiches* development, the pathogen can invade at any time during the growing season and seed treatments have limited residual activity after planting. Foliar fungicides do not transport to roots. Major QTLs have been found in dry pea and lentil varieties for Aphanomyces resistance in germplasm, providing hope for the future (Pilet-Nayel et al. 2005; McGee et al. 2012; Hamon et al. 2013; Malvick and Percich 1999; Ma et al. 2020; Desgroux et al. 2016).

There are over fifty known hosts documented for *A. euteiches* (Zitnick-Anderson et al. 2021b). In a French study, simulated greenhouse rotations of legumes for ten cycles revealed that susceptible legumes increased Aphanomyces root rot severity, while resistant or non-host legumes reduced root rot severity (Moussart et al. 2013). This study used French isolates of *A. euteiches*, and an assessment of popular rotational crops in Montana will offer more insight to potential pathogen fluctuations in response to grasses, oilseeds, and fiber crops grown in the greenhouse in addition to legumes. Selecting a non-host legume such as chickpea, or a resistant faba bean cultivar, may be appropriate in a North American crop rotation to combat Aphanomyces root rot. Susceptible field crops like dry pea, lentil, common bean, and alfalfa should be avoided. In Montana, cover crop blends often include Aphanomyces-susceptible legumes such as clovers or vetch for

nitrogen fixation benefits and could maintain or increase risk of disease for the dry pea or lentil crop. In addition, genetic variation of *A. euteiches* strains and races leads to variability in pathogenicity depending on region of origin (Malvick and Grau 2001; Malvick et al. 2009; Grünwald and Hoheisel 2006; Le May et al. 2018).

Non-host crops can reduce the potential inoculum of Aphanomyces in fields, however, the relative effect of each crop has not been quantified. For instance, an oat pre-crop helped to improve yield of dry pea when *A. euteiches* was widespread in a field (Allmaras et al. 2003). Greenhouse trials investigating green manures for Aphanomyces root rot reduction found that the green manure oat (cv. Troy) reduced inoculum compared to fallow (Williams-Woodward et al. 1997). Reports indicate that disease propagules (hyphae, oospores, and zoospores) germinate in response to oat, though it is not a host to the disease, so the pathogen does not survive (Chandler et al. 2004). It has also been reported that the oat compound avenacin destroys zoospores by lysing them (Deacon and Mitchell 1985). Multiple reports of the efficacy of oat for root rot reduction indicate its usefulness for disease suppression, and further investigation is required for use in Montana. Wild oat (*Avena fatua*) is a common weed species in Montana, and herbicide resistance is a known problem, leading to a high prevalence of this weed in Montana counties (Keith et al. 2015). It is not known, however, if wild oat also reduces Aphanomyces root rot in the same fashion as cultivated oat.

The aim of these experiments was to test the impact of crop species of interest in Montana agricultural production, and the weed wild oat, on disease severity of *A. euteiches.* It was hypothesized that host legumes will increase the soil inoculum of *A.*

euteiches, as measured by a greenhouse bioassay, while growing non-host plants will reduce soil inoculum.

Materials and Methods

Greenhouse trials were established to determine the impact of various plant species on the inoculum potential of *A. euteiches*. Trials were organized as a completely randomized design, with four inoculated and two un-inoculated pots per plant species, repeated once. An isolate of *A. euteiches* (AE1.1MT18) collected in Roosevelt County, Montana was selected for use in greenhouse trials, based on preliminary experiments indicating it was pathogenic on dry pea and lentil. This isolate was collected in 2018 from a field where dry pea crop failure had occurred due to severe root rot. Three trials classified as 'legumes', 'cereals', and 'other crops' were conducted with a total of twenty plant species tested (Table 3.01). In each experiment, dry pea was included as a positive control, and wheat was included as a non-host control.

Soil inoculum was started thirty days prior to experiments, using the homogenized oatmeal broth method designed for *A. cochlioides* in sugar beet (Schneider 1978; Willsey et al. 2018). After thirty days, the mixture of hyphal mats and oat water was blended and filtered through four layers of cheesecloth. Oospores were counted at 40x magnification using a hemocytometer with a 0.2mm cell depth (Malassez-Assistent, Germany) and the solution was diluted to desired spore concentration based on soil volume to be inoculated. Approximately 500 oospores / gram of soil was used in experiments.

Table 3.01. Plant species grown in greenhouse pots inoculated with *A. euteiches* for assessing their impact on soil inoculum

#	Common name	Scientific name	Cultivar	Trial[z]
1	Alfalfa	*Medicago sativa*	Ladak 65	Legumes
2	Chickpea	*Cicer arietinum*	Orion	Legumes
3	Faba bean	*Vicia faba*	MSU 14-24 SB	Legumes
4	Field spring dry pea	*Pisum sativum*	Majoret	All
5	Austrian winter pea	*Pisum sativum* var. arvense	Melrose	Legumes
6	Lentil	*Lens culinaris*	Avondale	Legumes
7	Vetch	*Lathyrus sativus*	AC Greenfix	Legumes
8	Wheat	*Tritcum aestivum*	Vida	All
9	Annual ryegrass	*Lolium multiflorum*	Gulf	Cereals
10	Barley	*Hordeum vulgare*	Hayes	Cereals
11	Corn	*Zea mays*	Painted hill	Cereals
12	Oat	*Avena sativa*	Monida	Cereals
13	Triticale	*X Triticosecale* Wittmack	AC Ultima	Cereals
14	Wild oat	*Avena fatua*	N/A	Cereals
15	Mustard	*Brassica carinata*	ACCA110	Other
16	Camelina	*Camelina sativa*	Suneson	Other
17	Chia	*Salvia hispanica*	JC19001	Other
18	Flax	*Linum usitatissimum*	Pembina	Other
19	Safflower	*Carthamus tinctorius*	MonDak	Other
20	Winter canola	*Brassica napus*	DKW-41-10	Other

[z]Three trials were conducted with 'legumes' indicating legume crops of interest for Montana production. The 'cereal' trial is primarily grass crops except for the weed, wild oat. Wild oat seeds were collected in Montana in 2017. The 'other' trial included oilseeds, brassicas, and fiber crops, that are not legumes or cereals.

A Kimwipe™ (Kimtech, Roswell, GA) was placed at the bottom of each five-inch pot. Control pots not requiring *A. euteiches* inoculum were filled with MSU mix (1:1:1 Canadian sphagnum peat moss, mineral soil mix, and Aquagro 2000G [Aquatrols, NJ]). The volume of MSU mix for inoculation treatment was placed in a utility tote (36.5 cm H x 52 cm W, x 78 cm D, Homz Durabilt, Chicago, IL) and the Aphanomyces inoculum was incorporated by thorough hand mixing. Treatment pots were filled with the

inoculated soil, and five seeds of each crop treatment (Table 3.01) were planted in four treatment pots and two control pots.

All pots were placed in 15 cm heavy duty plant saucers (Vigoro, Northbrook, IL) and placed 10 cm apart to avoid cross contamination between pots. Pots were watered daily or as necessary and harvested after 21 days. Greenhouse conditions were 22/18°C, and a 16/8 h day/night. All plants were harvested by emptying pots into a tray and washing plant roots for assessment using a root rot severity score described in chapter two (Figure 2.02). After harvest of the crop, soil was mixed and returned to its original pot using clean techniques to avoid contamination. Five *P. sativum* cv. Majoret seeds were planted to conduct a root rot bioassay. Dry pea plants were grown for 14 days and watered daily. At harvest, each plant was rated using a root rot score from 0-5, and scores were calculated for each species treatment based on the symptoms on the dry pea bait plants. Based on results from these trials, a subsequent trial was conducted focusing on the impact of oat (cv. Monida) on Aphanomyces root rot of both dry pea (cv. Majoret) and lentil (cv. Avondale) (Appendix Fig.A.01). Two treatments were tested with three plant cycles grown in greenhouse pots, dry pea-oat-dry pea, and lentil-oat-lentil. Each treatment had four inoculated replications, and two un-inoculated control pots.

Crop Cycling Trials

As an extension of this research, greenhouse trials were conducted following the methodology outlined above, with five cycles of plants grown in greenhouse pots instead of two with five separate ‘rotation’ treatments (Table 3.02), repeated once.

Table 3.02. Crop cycles selected for greenhouse trials

	Cycle				
Treatment	1	2	3	4	5
A	Wheat	Dry pea	Wheat	Wheat	Dry pea
B	Wheat	Lentil	Wheat	Wheat	Lentil
C	Dry pea	Wheat	Wheat	Chickpea	Dry pea
D	Dry pea	Wheat	Dry pea	Wheat	Dry pea
E	Dry pea	Oat	Dry pea	Oat	Dry pea

Statistical Analysis

Control pots without inoculum were removed from the dataset prior to analysis, as the role of these pots was to check for cross-contamination. Disease severity scores were analyzed using ANOVA with ordinal logistic regression in R version 4.1.2, using the 'MASS' package with the 'polr' function, with root rot disease severity score as the response variable and plant species as the independent variable (Venable and Ripley 2002; R Core Team 2018). Assumptions were checked with a nominal test from the 'ordinal' package, and tests for skewness and kurtosis with the 'psych' package (Revelle 2022; Christensen 2019). A post-hoc Dunn test was run using the 'FSA' R package to compare treatments for differences in root rot score, and mean comparison letters were generated for incorporation in figures (Ogle et al. 2021). Trial repetitions were not combined due to differences in treatments between trials. Quantitative measurements such as plant height and root length were analyzed using the linear model functions in the 'lme4' package in R (Bates et al. 2015), when additional variables were of interest (data not shown) (Bates et al. 2015).

Results

Legume Rotation Treatments

The dry pea, lentil, Austrian winter pea, alfalfa, and vetch treatments in the legume trial had an average disease severity score above three, while the wheat, faba bean, and chickpea treatments were below three (Fig. 3.01). In the repetition of this trial, the same trends were observed, although some treatments varied between repeats, for instance, the wheat score on dry pea bait plants was above two in the first trial, and below one in the second (Fig. 3.02).

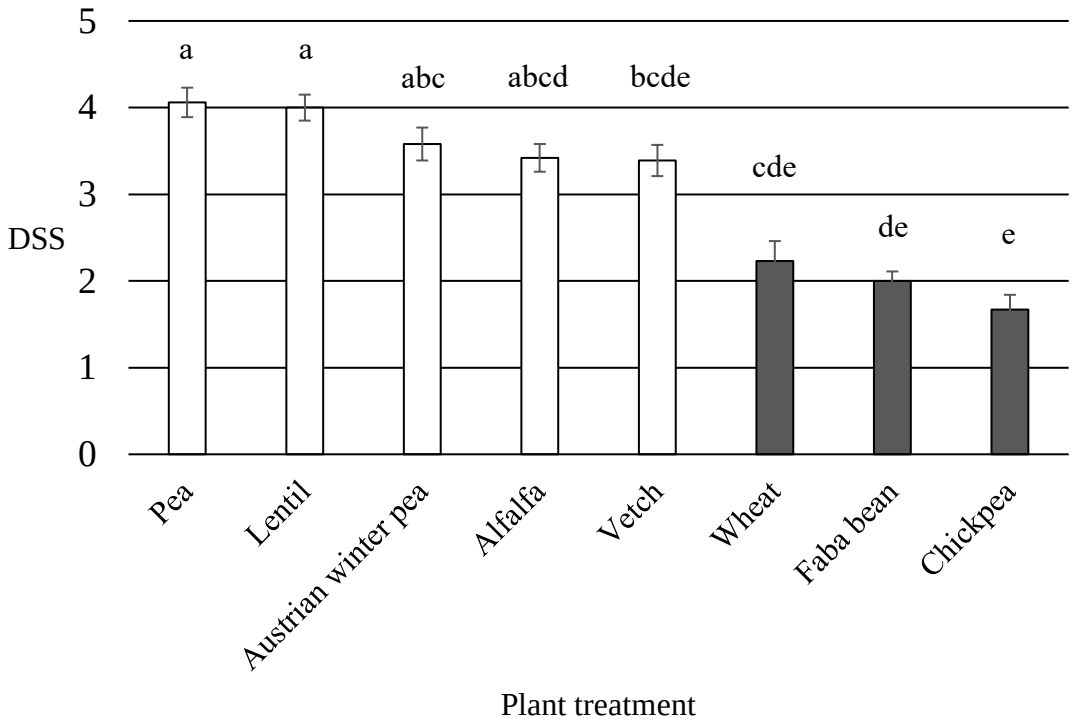


Fig. 3.01. Average disease severity score on dry pea bait plants in trial 1 after legume plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

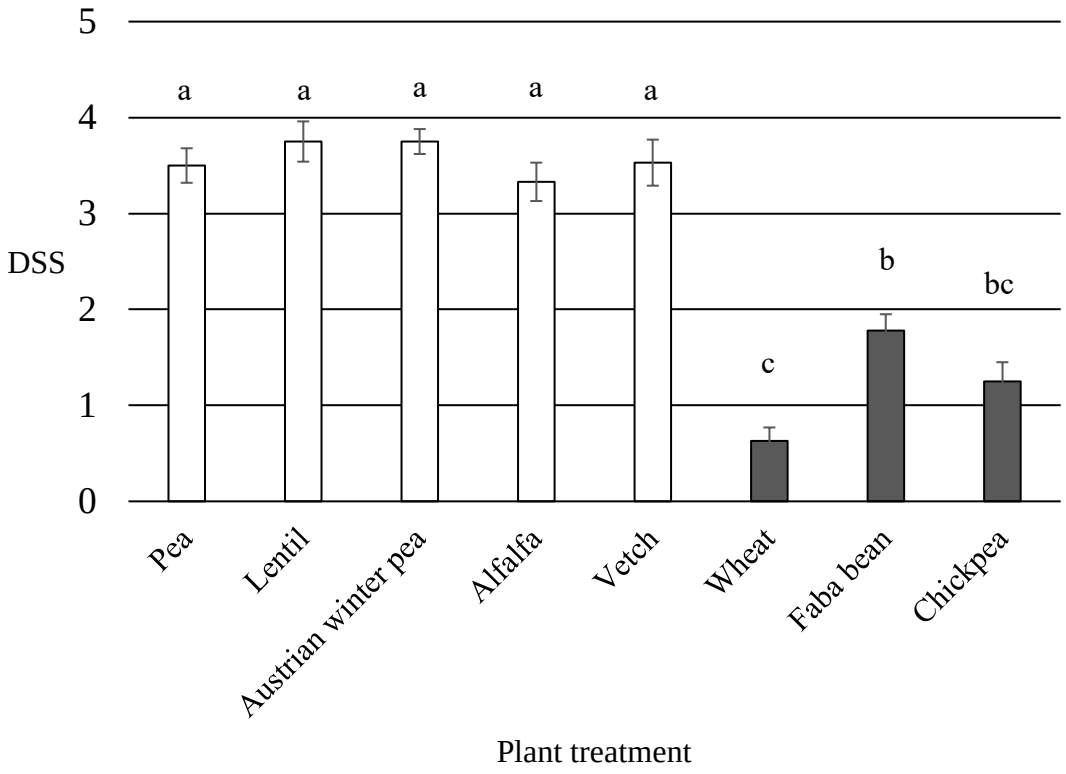


Fig. 3.02. Average disease severity score on dry pea bait plants in trial 2 after legume plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

Cereal Rotation Treatments

The dry pea test plants from the cereal trials showed a marked difference between the control dry pea treatment and grasses. The control dry pea treatment had moderate to severe root rot, while grassy treatment disease severity was between one and two (Fig. 3.03, 3.04). Wild oat and oat treatments differed between dry pea bait disease scores, although both had no to mild root rot symptoms. In trial 1, no root rot was detected on dry pea test plants in the oat treatment.

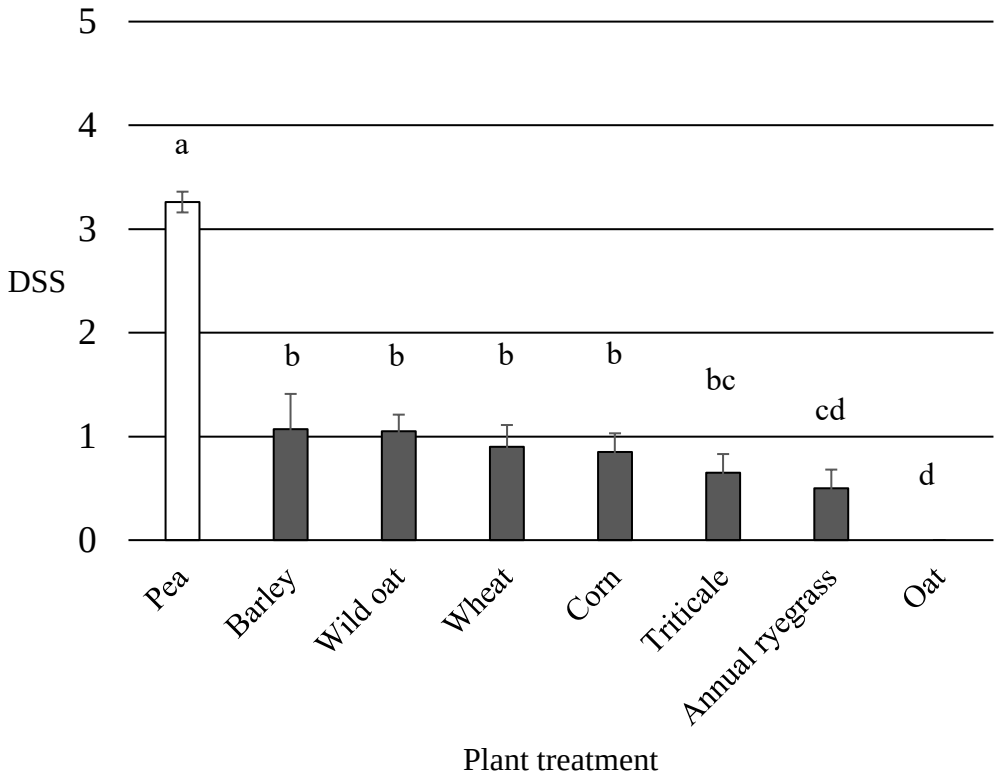


Fig. 3.03. Average disease severity score on dry pea bait plants in trial 1 after cereal plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

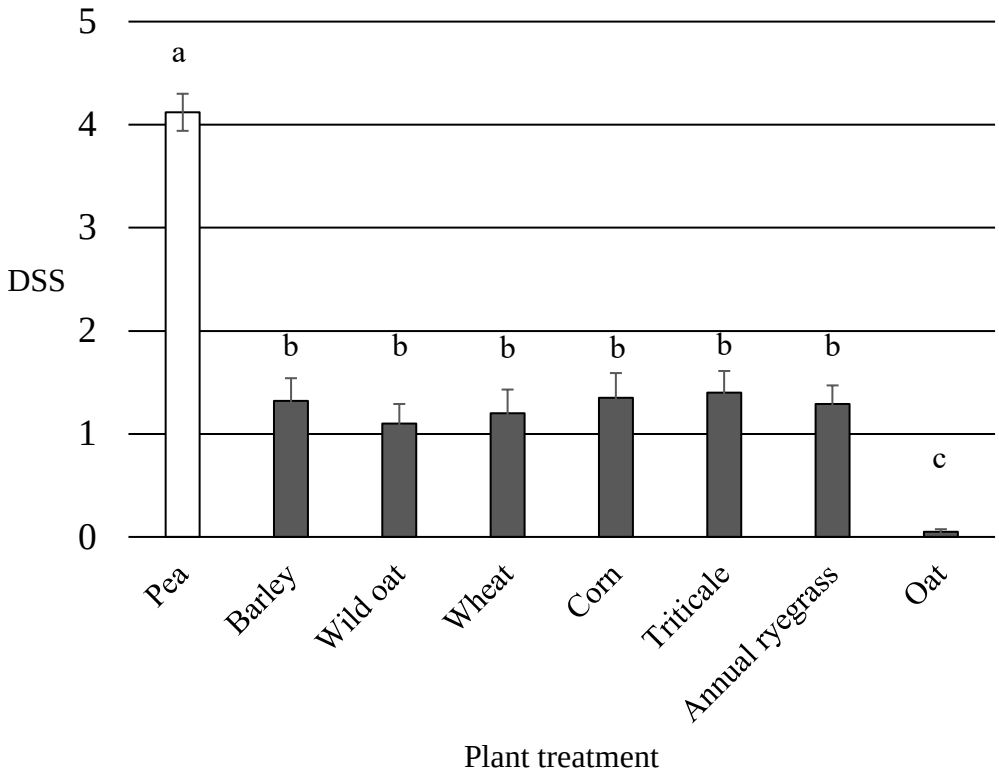


Fig. 3.04. Average disease severity score on dry pea bait plants in trial 2 after cereal plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

‘Other’ Rotation Treatments

The ‘other’ greenhouse experiment treatments had variable results between trial repeats. Root rot on dry pea bait plants was moderate to severe for the dry pea, flax, and chia treatments in trial 1 (Fig. 3.05). Canola, mustard, camelina, wheat, and safflower treatments had mild to moderate root rot on dry pea, on average below a three rating. In the second trial, root rot was reduced by treatment species (Fig. 3.06). The dry pea control treatment had severe root rot, while the other treatments had mild root rot

symptoms. This is quite apparent in the photo of plant roots from the dry pea-dry pea control treatment and the safflower-dry pea treatment (Fig. 3.07).

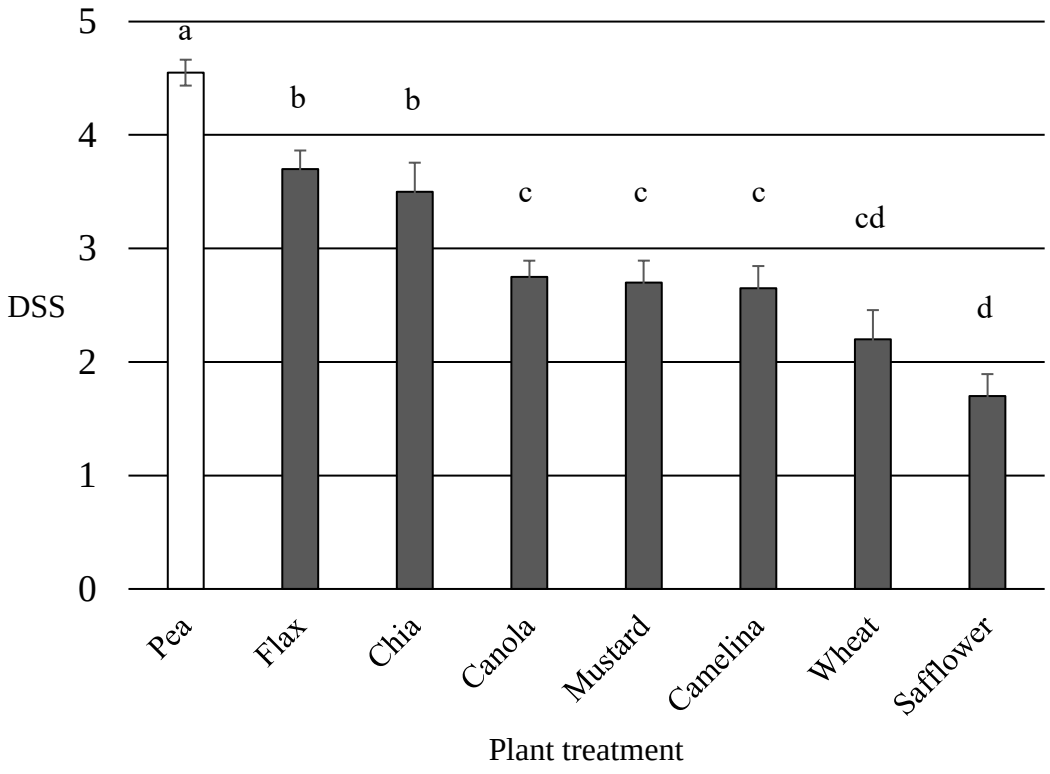


Figure 3.05. Average disease severity score on dry pea bait plants in trial 1 after oilseed, brassica, and fiber plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

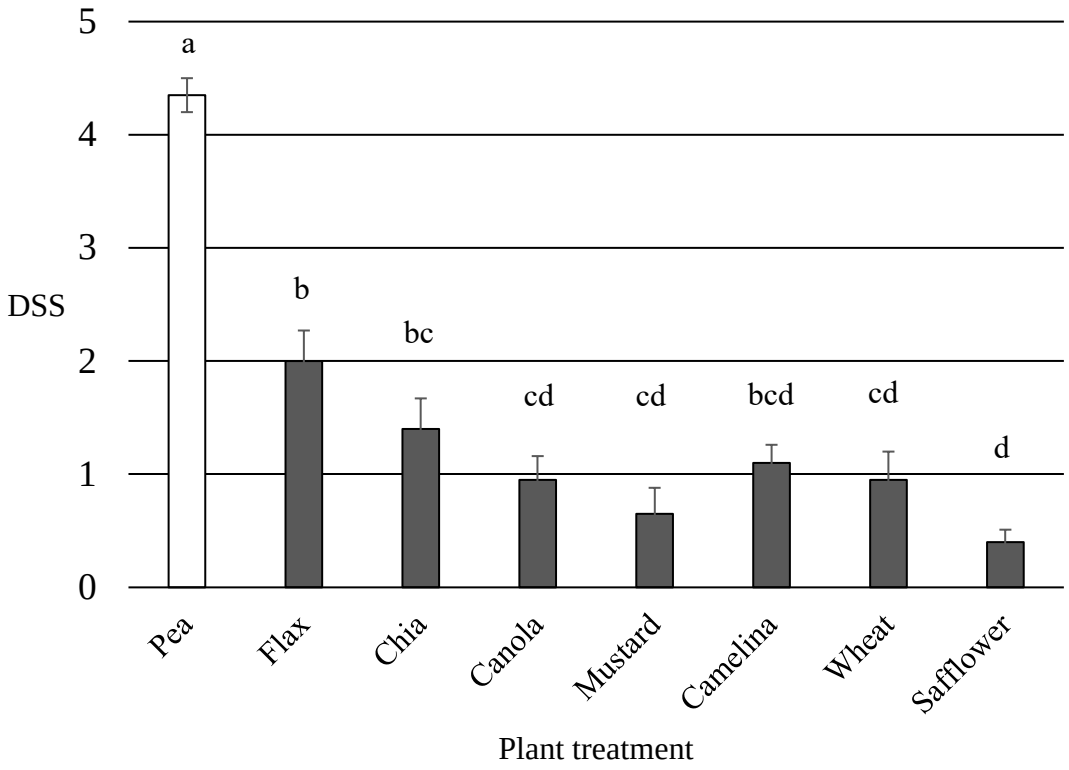


Figure 3.06. Average disease severity score on dry pea bait plants in trial 2 after oilseed, brassica, and fiber plant treatments in the greenhouse. Error bars are standard error of the mean. The white bar indicates *A. euteiches* host plants, dark gray bars indicate non-host species and resistant cultivars.

Figure 3.07. Photo showing the difference between severe Aphanomyces root rot in the control dry pea-dry pea treatment and milder root rot on dry pea in the safflower-dry pea treatment from trial 2 of the 'other' experiment. The first two plant reps in each photo beside yellow tags are un-inoculated controls, while the four replicates beside red tags are dry pea plants grown in inoculated pots, photo: C. Murphy

Crop Rotation Simulation Trial

In the first cycling trial, there was little evidence to suggest a difference between treatments in the final disease severity scores on dry pea and lentil at experiment termination (P = 0.05, Table 3.03). Fluctuations in root rot occurred between cycles, with mild to moderate symptoms for susceptible hosts in cycle two and three, versus the severe root rot symptoms on dry pea plants in treatments C, D, and E, at cycle one.

Table 3.03. Average disease severity score of Aphanomyces root rot on dry pea and lentil plants in trial 1 with different greenhouse cycles of crops of interest

Trt.	Cycle 1	DSS	Cycle 2	DSS	Cycle 3	DSS	Cycle 4	DSS	Cycle 5	DSS
A	Wheat	0	Dry pea	2.7	Wheat	0	Wheat	0	Dry pea	3.3
B	Wheat	0	Lentil	2.8	Wheat	0	Wheat	0	Lentil	3.8
C	Dry pea	4.7	Wheat	0	Wheat	0	Chickpea	0	Dry pea	3.5
D	Dry pea	4.7	Wheat	0	Dry pea	2.3	Wheat	0	Dry pea	3.7
E	Dry pea	4.7	Oat	0	Dry pea	2.4	Oat	0	Dry pea	3.3

In the second cycling trial, there was evidence that treatment C reduced root rot more effectively than treatments B, D, and E ($P = 0.03$) (Table 3.04). Overall root rot was lower in trial 2 compared to trial 1, with final root ratings all above three in the first trial, and below three in the second.

Table 3.04. Average disease severity score of Aphanomyces root rot on dry pea and lentil plants in trial 2 with different greenhouse cycles of crops of interest

Trt.	Cycle 1	DSS	Cycle 2	DSS	Cycle 3	DSS	Cycle 4	DSS	Cycle 5	DSS
A	Wheat	0	Dry pea	2.6	Wheat	0	Wheat	0	Dry pea	1.8
B	Wheat	0	Lentil	4.1	Wheat	0	Wheat	0	Lentil	2.2
C	Dry pea	3.2	Wheat	0	Wheat	0	Chickpea	0	Dry pea	1.4
D	Dry pea	3.4	Wheat	0	Dry pea	1.9	Wheat	0	Dry pea	2.4
E	Dry pea	4	Oat	0	Dry pea	1.9	Oat	0	Dry pea	2.2

Discussion

Aphanomyces root rot has caused major concerns in the Northern Great Plains and other geographic regions where dry pea, lentil, and other susceptible legumes are grown. The goal of this study was to determine if certain plant species lead to an increase, decrease, or static concentration of *A. euteiches* propagules in inoculated greenhouse soils. Growing susceptible legumes led to an increase in inoculum potential in greenhouse pots, as measured by a disease severity score on dry pea roots. All cereals tested significantly reduced root rot on dry pea bait plants compared to controls, with oat the most effective in one trial, with no evidence of root rot at all after oat. This corroborates the literature that oat is useful in managing *A. euteiches*, whether as a pre-crop or a green manure (Allmaras et al. 2003; Williams-Woodward et al. 1997). The appropriateness of cultivated oat in rotation for disease management in Montana needs to be weighed against the no tolerance policy of oat contamination in other grains for export. The weed species wild oat had a similar impact on root rot as the other grasses tested. Due to issues with herbicide resistance in Montana and other regions of the Northern Great Plains, the prevalence of wild oat may have an impact on root rot pathogen populations, although the issues with weed competition likely outweigh any disease suppression benefits for crops.

The other grasses reduced the *A. euteiches* inoculum in the greenhouse, however, it is not known if they also cause the disease propagules to germinate or lyse, as reported with oat and the compound avenacin (Chandler et al. 2004; Deacon and Mitchell 1985). Certain plant species do have chemistry known to help break pest cycles. For example,

Brassicaceae species, such as the tested mustard treatment (*Brassica carinata*), have sulfur-containing secondary metabolites, called glucosinolates. Sulfur is a useful fungicide for a number of plant diseases (Tweedy 1981). With the addition of water, glucosinolates can also release other compounds known to impact plant disease, in the process known as biofumigation (Matthiessen and Kirkegaard 2006). These compounds include nitriles, epithionitriles, and isothiocyanates (Papavizas and Lewis 1971; Fahey et al. 2001). Although there is evidence that the Brassicaceae do suppress *A. euteiches* infection on dry pea, it varies with the severity of the pathogen, the quality of the biomass, and the quantity of the biomass retained in the field (Hossain et al. 2012).

Other plant species can avoid infection with *A. euteiches* due to conditions not being favorable for pathogen development. Due to the colder temperatures that do not favor Aphanomyces development, Austrian winter pea is often able to avoid root disease (McGee et al. 2017). In the greenhouse, this avoidance was not seen as conditions were favorable for pathogen development, and the result was dry pea test plants with moderate to severe root rot symptoms after winter pea. Severity of Aphanomyces root rot symptoms on dry pea bait plants did not differ comparing winter pea and the other susceptible legume species tested.

In general, trends between trial repetitions in the greenhouse were similar, with reduced root rot severity on dry pea bait plants after non-hosts, however, differences were observed. In one trial with the oilseeds, mustards, and fiber crops, reduction in root rot was less than the other trial when non-host crops were grown. While an estimate of oospores per gram of soil was calculated, variance may be due to changing oospore

viability in inoculum mixes, levels of other propagules (hyphae) not being accounted for, or slight greenhouse light and temperature condition changes due to the season the trials were conducted in, as some trials were run in summer and others over winter. In the field, further variability will be present due to the *A. euteiches* isolates in the system and their responses to host plants. For example, *A. euteiches* cross-pathogenicity on alfalfa and dry pea vary depending on original host, with most isolates from dry pea causing disease on alfalfa, and only 20% of isolates from alfalfa causing disease on dry pea in one study (Holub and Grau 1990). Geographic origin can also impact the aggressiveness of *A. euteiches* isolates, although the most aggressive virulence type is also the most commonly found type in the major dry pea growing regions in Europe, North America, and New Zealand (Wicker et al. 2001).

Additional factors in field settings will be other fungi, oomycetes, and nematodes, some of which are secondary, opportunistic invaders increasing disease severity alongside *A. euteiches* (Peters and Grau 2002; Zitnick-Anderson et al. 2020). Environmental conditions and soil edaphic properties will also impact the disease cycle, such as moisture availability, soil texture, water-holding capacity, organic fractions, and micronutrients (Zitnick-Anderson et al. 2020; Allmaras et al. 2003; Bailey and Lazarovits 2003; Bødker et al. 1998; Esmaeili Taheri et al. 2017).

In the crop cycling trial, where five rounds of plants were grown in the same greenhouse pot, there was no difference between rotation treatment in the first trial repetition for final disease severity scores on dry pea and lentil. Disease severity was moderate (DSS 3-4) at the trial conclusion. In the second repetition of the trial, disease

severity at the commencement of the trial was less than the initial rep, and this was reflected in mild root rot symptoms at the trial conclusion. Treatment ‘C’ with a rotation of dry pea-wheat-wheat-chickpea-dry pea was lower compared to the other treatments in the second rep, however, all treatments had mild root rot on average at the end of the trial. There were issues with this experiment in practice, including the loss of soil in greenhouse pots, due to the removal of five root systems. In field settings, root residues also often harbor *A. euteiches* and other soilborne pathogens between seasons (Chan and Close 1987; Larsson 1994). For future experiments, returning roots to the pots after disease analysis, or taking only root subsamples would help rectify this issue.

Field trials with Aphanomyces root rot at the current time are attempted with excessive caution, often designating land for that purpose only, or conducting on-farm trials where the pathogen is already present. This is due to the long-lived nature of the oospore and difficulty managing it in soils after inoculation. This study supports the finding that incorporating crops that are not hosts to *A. euteiches* in crop rotations in Montana is important for reducing the soil inoculum of the pathogen.

References

Akhter, W., Bhuiyan, M. K. A., Sultana, F. & Hossain, M. M. 2015. Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani*) and improving yield in pea (*Pisum sativum* L.). Comptes Rendus Biologies. 338: 21-28.

Allmaras, R., Fritz, V., Pfleger, F. & Copeland, S. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil and Tillage Research. 70: 41-52.

Bailey, K. & Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil and Tillage Research. 72: 169-180.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software.

Bødker, L., Kjøller, R. & Rosendahl, S. 1998. Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza. 8: 169-174.

Chan, M. & Close, R. 1987. Aphanomyces root rot of peas 1. Evaluation of methods for assessing inoculum density of *Aphanomyces euteiches* in soil. New Zealand Journal of Agricultural Research. 30: 213-217.

Chandler, M. A., Fritz, V. A. & Allmaras, R. R. 2004. Response of *Aphanomyces euteiches* mycelia, zoospore, and oospore to oat extract. Canadian Journal of Plant Science. 84: 687-690.

Christensen, R. 2019. Ordinal - regression models for ordinal data. R Package Version 2019.12-10.

Deacon, J. & Mitchell, R. 1985. Toxicity of oat roots, oat root extracts, and saponins to zoospores of *Pythium* spp. and other fungi. Transactions of the British Mycological Society. 84: 479-487.

Desgroux, A., L'anthoëne, V., Roux-Duparque, M., Rivière, J.-P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P. & Piriou, C. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics. 17: 1-21.

Esmaeili Taheri, A., Chatterton, S., Foroud, N., Gossen, B. & Mclaren, D. 2017. Identification and community dynamics of fungi associated with root, crown, and foot rot of field pea in western Canada. European Journal of Plant Pathology. 147: 489-500.

Fahey, J. W., Zalcmann, A. T. & Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 56: 5-51.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Molecular Plant Pathology. 8: 539-548.

Grünwald, N. J. & Hoheisel, G.-A. 2006. Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete *Aphanomyces euteiches*. Phytopathology. 96: 1134-1141.

Gundersen, B., Inglis, D. A. & Porter, L. 2006. Evaluation of seed, in-furrow and foliar treatments for control of root rot on peas. Fungicide Nematic Tests. 61: V130.

Hajihassani, A., Tenuta, M. & Gulden, R. H. 2016. Host preference and seedborne transmission of *Ditylenchus weischeri* and *D. dipsaci* on select pulse and non-pulse crops grown in the Canadian Prairies. Plant Disease. 100: 1087-1092.

Hamon, C., Coyne, C. J., Mcgee, R. J., Lesné, A., Esnault, R., Mangin, P., Hervé, M., Le Goff, I., Deniot, G. & Roux-Duparque, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biology. 13: 45.

Holub, E. & Grau, C. 1990. Specificity of resistance to *Aphanomyces euteiches* in seedling alfalfa. Plant Disease. 74: 164-168.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. & Persson, P. 2012. *Aphanomyces* pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science. 62: 477-487.

Keith, B., Lehnhoff, E., Burns, E., Menalled, F. & Dyer, W. 2015. Characterisation of *Avena fatua* populations with resistance to multiple herbicides. Weed Research. 55: 621-630.

Larsson, M. 1994. Pathogenicity, morphology and isozyme variability among isolates of *Aphanomyces* spp. from weeds and various crop plants. Mycological Research. 98: 231-240.

Le May, C., Onfroy, C., Moussart, A., Andrivon, D., Baranger, A., Pilet-Nayel, M. & Vandemark, G. 2018. Genetic structure of *Aphanomyces euteiches* populations sampled from United States and France pea nurseries. European Journal of Plant Pathology. 150: 275-286.

Ma, Y., Marzougui, A., Coyne, C. J., Sankaran, S., Main, D., Porter, L. D., Mugabe, D., Smitchger, J. A., Zhang, C. & Amin, M. 2020. Dissecting the genetic architecture

of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. International Journal of Molecular Sciences. 21: 2129.

Malvick, D. & Grau, C. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Disease. 85: 740-744.

Malvick, D. & Percich, J. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Disease. 83: 51-54.

Malvick, D. K., Grünwald, N. J. & Dyer, A. T. 2009. Population structure, races, and host range of *Aphanomyces euteiches* from alfalfa production fields in the central USA. European Journal of Plant Pathology. 123: 171.

Matthiessen, J. N. & Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences. 25: 235-265.

Mcgee, R. J., Coyne, C. J., Pilet-Nayel, M.-L., Moussart, A., Tivoli, B., Baranger, A., Hamon, C., Vandemark, G. & Mcphee, K. 2012. Registration of pea germplasm lines partially resistant to *Aphanomyces* root rot for breeding fresh or freezer pea and dry pea types. Journal of Plant Registrations. 6: 203-207.

Mcgee, R. J., Eigenbrode, S., Nelson, H. & Schillinger, W. 2017. Re-inventing Austrian winter pea towards developing food quality winter peas. Crops & Soils. 50: 4-46.

Moussart, A., Even, M.-N., Lesne, A. & Tivoli, B. 2013. Successive legumes tested in a greenhouse crop rotation experiment modify the inoculum potential of soils naturally infested by *Aphanomyces euteiches*. Plant Pathology. 62: 545-551.

Ogle, D., Doll, J., Wheeler, P. & Dinno, A. 2021. FSA: Fisheries Stock Analysis. R package version 0.9.1.

Papavizas, G. & Lewis, J. 1971. Effect of amendments and fungicides on Aphanomyces root rot of peas. Phytopathology. 61: 215-220.

Papavizas, G. C. & Ayers, W. A. 1974. Aphanomyces species and their root diseases in pea and sugarbeet. United States Department of Agriculture, Economic Research Service.

Peters, R. & Grau, C. 2002. Inoculation with nonpathogenic *Fusarium solani* increases severity of pea root rot caused by *Aphanomyces euteiches*. Plant Disease. 86: 411-414.

Pilet-Nayel, M., Muehlbauer, F., Mcgee, R., Kraft, J., Baranger, A. & Coyne, C. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology. 95: 1287-1293.

Prashar, P. & Vandenberg, A. 2017. Genotype-specific responses to the effects of commercial *Trichoderma* formulations in lentil (*Lens culinaris* ssp. *culinaris*) in the presence and absence of the oomycete pathogen *Aphanomyces euteiches*. Biocontrol Science and Technology. 27: 1123-1144.

R Core Team, R. 2018. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Revelle, W. 2022. psych: Procedures for Personality and Psychological Research. 2.2.5 ed. Northwestern University, Evanston, Illinois, USA.

Schneider, C. 1978. Use of oospore inoculum of *Aphanomyces cochlioides* to initiate black root disease in sugar beet seedlings. Journal of the American Society of Sugar Beet Technologists. 20: 55-62.

Schroeder, K., Okubara, P., Tambong, J., Lévesque, C. & Paulitz, T. 2006. Identification and quantification of pathogenic *Pythium* spp. from soils in eastern Washington using real-time polymerase chain reaction. Phytopathology. 96: 637-647.

Smiley, R. W., Machado, S., Gourlie, J. A., Pritchett, L. C., Yan, G. & Jacobsen, E. E. 2013. Effects of crop rotations and tillage on *Pratylenchus* spp. in the semiarid Pacific Northwest United States. Plant Disease. 97: 537-546.

Tweedy, B. G. 1981. Inorganic sulfur as a fungicide. Residue Reviews. 78: 43-68.

Upadhaya, A., Yan, G., Pasche, J. & Kalil, A. 2019. Occurrence and distribution of vermiform plant-parasitic nematodes and the relationship with soil factors in field pea (*Pisum sativum*) in North Dakota, USA. Nematology. 21: 445-457.

Venable, W. N. & Ripley, B. D. 2002. Modern applied statistics with S. New York: Springer.

Wicker, E., Hullé, M. & Rouxel, F. 2001. Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. Plant Pathology. 50: 433-442.

Williams-Woodward, J. L., Pfleger, F., Fritz, V. A. & Allmaras, R. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Plant and Soil. 188: 43-48.

Willsey, T., Chatterton, S., Heynen, M. & Erickson, A. 2018. Detection of interactions between the pea root rot pathogens *Aphanomyces euteiches* and *Fusarium* spp. using a multiplex qPCR assay. Plant Pathology. 67: 1912-1923.

Willsey, T., Patey, J., Vucurevich, C., Chatterton, S. & Carcamo, H. 2021. Evaluation of foliar and seed treatments for integrated management of root rot and pea leaf weevil in field pea and faba bean. Crop Protection. 143: 105538.

Wu, L., Chang, K.-F., Conner, R. L., Strelkov, S., Fredua-Agyeman, R., Hwang, S.-F. & Feindel, D. 2018. *Aphanomyces euteiches*: a threat to Canadian field pea production. Engineering. 4: 542-551.

Wu, L., Chang, K.-F., Hwang, S.-F., Conner, R., Fredua-Agyeman, R., Feindel, D. & Strelkov, S. E. 2019. Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. The Crop Journal. 7: 38-48.

Zitnick-Anderson, K., Gargouri Jbir, T., Carlson, A., Postovit, S., Pasche, J. & Kalil, A. 2021a. *Fusarium* species associated with root rot of lentil (*Lens culinaris*) in North Dakota. Plant Health Progress.

Zitnick-Anderson, K., Luis, E., Forster, S. & Pasche, J. S. 2020. Associations among the communities of soil-borne pathogens, soil edaphic properties and disease incidence in the field pea root rot complex. Plant and Soil. 457: 339-354.

Zitnick-Anderson, K., Porter, L. D., Hanson, L. E. & Pasche, J. S. 2021b. Identification, laboratory, greenhouse, and field handling of *Aphanomyces euteiches* on pea (*Pisum sativum*). Plant Health Progress. PHP-01-21-0011-FI.

CHAPTER FOUR

EFFECT OF ACIDITY *IN VITRO* ON *APHANOMYCES EUTEICHES* ISOLATES

Abstract

Soil properties including structure, texture, and water holding capacity are known to greatly influence the likelihood of root disease outbreak in susceptible legumes caused by the damaging soilborne oomycete, *Aphanomyces euteiches.* Associations between Aphanomyces root rot and soil pH have been found in soil samples from dry pea and lentil fields, however relationships have varied based on geographic origin. The aim of this study was to determine if radial growth of *A. euteiches* on acidic plates was suppressed or enhanced compared to neutral plates for isolates originating from Montana and North Dakota. The study found differences between isolates, with some isolates suppressed in vitro with acidic conditions, and other isolates with more sustained growth at pH 4. This study indicates that the risk of Aphanomyces root rot on susceptible legumes under acidic conditions may vary with isolates present in the soil environment. Disease risk modelling will require further pH testing of *A. euteiches* isolates in greenhouse and field settings to explore this finding.

Introduction

Aphanomyces euteiches Drechs. is the causal agent of Aphanomyces root rot on the pulse crops dry pea, lentil, common bean, faba bean, and other susceptible legume species including alfalfa, vetch, clovers, and cowpea (Gaulin et al. 2007; Malvick et al. 1998; Pfender and Hagedorn 1982; Tofte et al. 1992; Wu et al. 2018; Vandemark and Porter 2010). *A. euteiches* is an oomycete soilborne pathogen, with its lifecycle occurring in the soil system, within plant roots, and in plant root debris. Due to such a lifecycle, soil conditions and chemical factors can greatly influence the ability of the pathogen to infect, reproduce, and spread. In the literature, several soil properties have been associated with Aphanomyces root rot presence and severity.

Soils with a high clay content and increased water holding capacity have been correlated with *A. euteiches* and other oomycete pathogens, such as *Pythium* spp. (Papavizas and Ayers 1974; Stone et al. 2001). Soil moisture impacts the secondary spread of oomycetes to neighboring plants in a field, as free water is necessary for their motile biflagellate zoospores to swim using chemotaxis (Scott 1961). In contrast to the short-lived zoospore, the oospore is the survival spore of *A. euteiches* and resilient giving changing soil conditions, including soil dryness, able to survive for a documented ten years in the absence of a host (Gaulin et al. 2007; Pfender and Hagedorn 1983; Sherwood and Hagedorn 1962). Therefore, soil moisture is necessary for secondary spread of the pathogen, but not necessary for pathogen survival.

In addition to soil moisture, total nitrogen and organic carbon were positively correlated with the abundance of *A. euteiches* in soil surveys of dry pea and lentil in

Saskatchewan (Karppinen et al. 2020). The type of nitrogen applied for fertilization may influence the incidence of root rot. For example, a positive correlation between ammonium application and *Fusarium oxysporum* colonization, and a negative correlation with soil nitrate application was documented in cucumber (Wang et al. 2019). Organic matter does not always positively correlate with disease and has been shown to enhance microbial diversity and competition (Raaijmakers and Mazzola 2016; Trivedi et al. 2015; Broadbent and Baker 1974). Calcium in soils has identified to be suppressive to *A. euteiches*, due to inhibition of zoospore production (Heyman et al. 2007). Surveys of dry pea fields in North Dakota revealed negative associations with *A. euteiches* and potassium soil levels (Zitnick-Anderson et al. 2020). Potassium salts are known to be useful for managing other root rot pathogens by inhibiting hyphal growth (El-Mohamedy et al. 2013; Jee et al. 2002).

The impact of pH on *Aphanomyces euteiches* disease progression is not well understood. From the North Dakota survey, pH and ferrous iron were positively associated with *A. euteiches* presence, although specific pH values of fields were not stated (Zitnick-Anderson et al. 2020). Soil pH was negatively correlated with abundance of *A. euteiches* found in roadsides in Saskatchewan, however a relationship was not seen in annually cropped fields between pH and pulse crops in the rotation (Karppinen et al. 2020).

Due to the intensifying issue of soil acidification in Montana and other areas of semiarid crop production in North America, it is of interest how *A. euteiches* responds to acidic soil conditions (Jones et al. 2019). Closely related oomycetes, such as *Pythium*

spp., had variable pathogen abundance at pH 7 to 8, dependent on which species was present in soybean fields (Rojas et al. 2017). *Aphanomyce cochlioides*, the causal agent of Aphanomyces root rot on sugar beet, was suppressed in alkaline soils, and overall health of sugar beet plants was improved at a pH over 6.5 (Bresnahan et al. 1998; Bresnahan et al. 2001; Olsson et al. 2011; Olsson et al. 2019). The same finding may be true for dry pea and lentil, which yield well if soil pH is not in the extremes (Rice et al. 2000; Mohebbi and Mahler 1989). For *A. euteiches* isolates from dry pea, isolates grew well at a pH of 6.5 on petri plates, however extremely acidic conditions were not imposed on isolates (Papavizas and Davey 1960). To investigate the growth response of *A. euteiches* isolates collected in Montana and North Dakota under in vitro acidic conditions, experiments were conducted in the lab.

Materials and Methods

To determine if *A. euteiches* isolates were suppressed by acidic conditions, petri plate experiments were conducted, comparing petri plates with a pH of 4 to a control neutral pH of 7. Trials used four *A. euteiches* isolates, with six petri plate replicates for each isolate and pH level, initially conducted with plates of water agar amended with granular tartaric acid (Fisher chemical, Houston, TX) and 1M sodium hydroxide (Fisher chemical, Houston, TX) adjusting the media to pH of 4 and 7 (data not shown). In subsequent trials, water agar was replaced with corn meal agar (CMA, PhytoTech Labs, Lenexa, KS) as most *A. euteiches* isolates did not grow on the water agar medium. Small hyphal squares (3mm x 3mm) from 5-day old *A. euteiches* cultures growing on CMA

plates were plated onto the experimental plates and stored in an incubator at 21°C with 24 h light. Radial growth measurements were taken daily, until plate fill (80mm), or experiment termination at 8 weeks when all hyphal growth had ceased. Three isolates collected from Montana dry pea fields (AE1.1MT17, AE1.1MT18, AE2.1MT19) and one isolate from a North Dakota dry pea field (AE36.1ND18) were used in trials. Trials were repeated once.

Statistical Analysis

Radial growth on petri plates was analyzed using the linear model function in the 'lme4' package with the 'lme' function in R version 4.1.2 for growth after 7 and 14 days, with pH and *A. euteiches* isolate as fixed effects and trial repetition as a random effect in the model (Bates et al. 2015; R Core Team 2018). A Tukey HSD test was conducted using the 'multcomp' package when evidence suggested a difference between isolates (Hothorn et al. 2008).

Results

For isolate AE1.1MT17, hyphal growth reached plate fill by day 12 on pH 7 plates, while in comparison hyphal growth was slower and suppressed on pH 4 plates, with *A. euteiches* hyphae ceasing to grow before plates were filled (Fig. 4.01). In contrast, the isolate AE1.1MT18 had faster hyphal growth on acidic plates, reaching 75% of plate fill after 21 days, while control plates stalled with approximately 25% plate fill (Fig. 4.02).

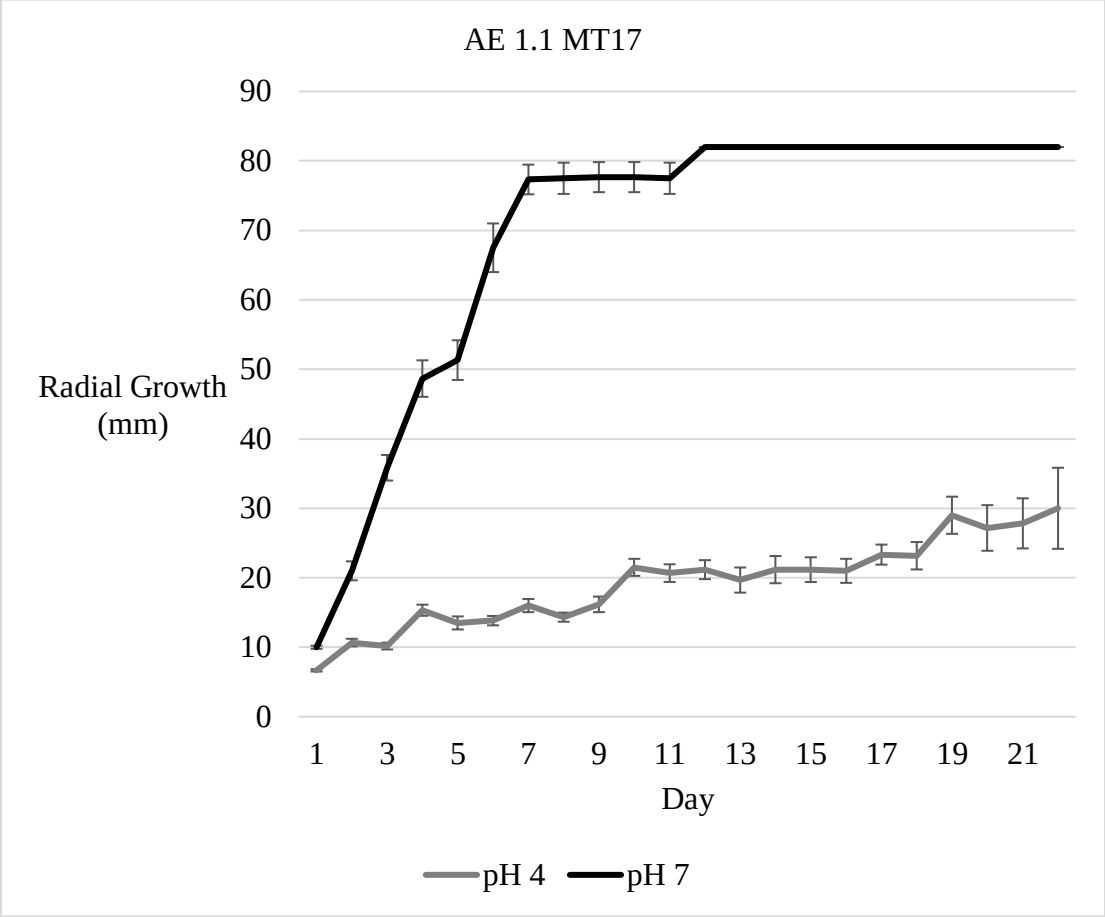


Figure 4.01 Average radial growth (mm) over time of isolate AE1.1MT17 on CMA plates adjusted to pH 4 and 7. Error bars represent standard error of the mean.

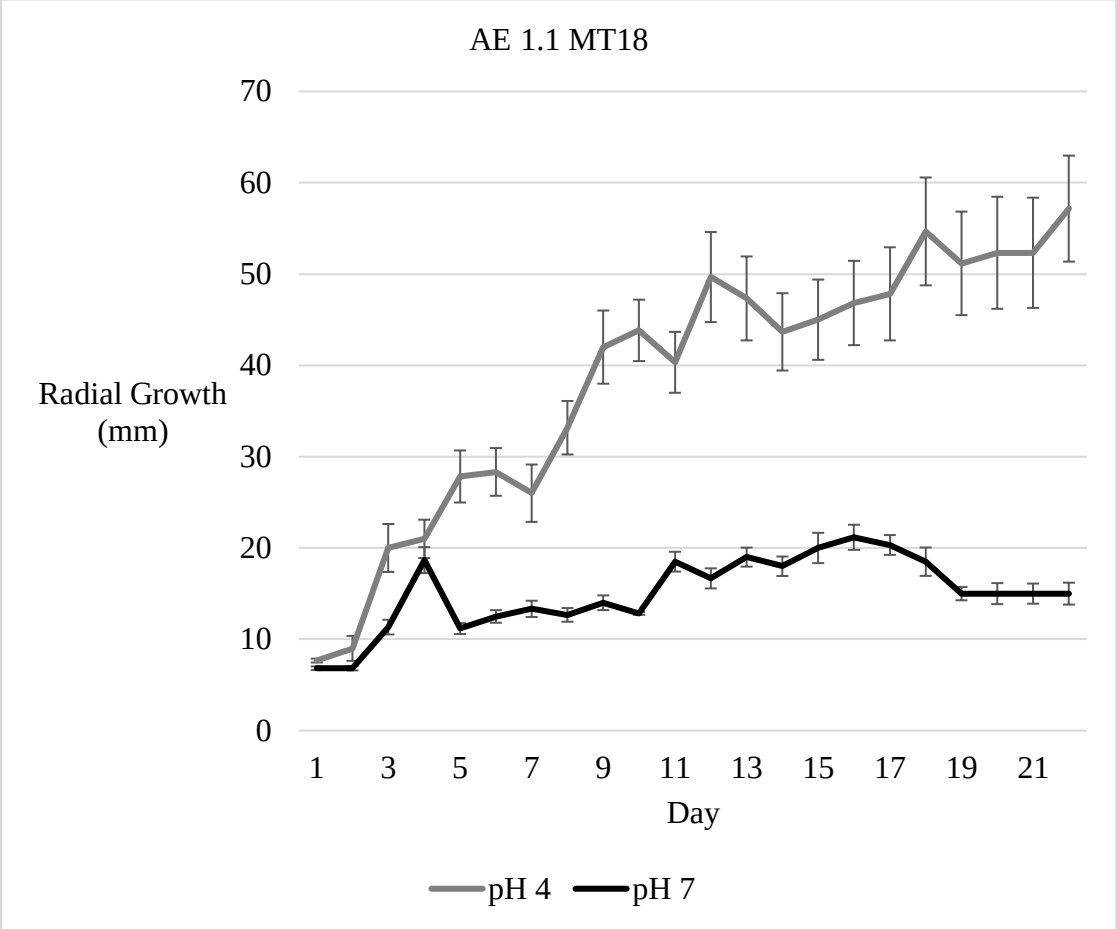


Figure 4.02 Average radial growth (mm) over time of isolate AE1.1MT18 on CMA plates adjusted to pH 4 and 7. Error bars represent standard error of the mean.

The isolate AE2.1MT19 grew faster on acidic plates compared to neutral plates, with plate fill not reached after 21 days for either treatment (Fig. 4.03). The *A. euteiches* isolate from North Dakota (AE36.1ND18) was suppressed by the acidic pH plates, with growth stalling at approximately 20% of the plate. In contrast, hyphal growth on neutral plates reached plate fill by day 17 (Fig. 4.04). There was strong evidence to suggest radial growth differences between isolates ($P < 0.001$) and pH level ($P < 0.001$). After 7 days of growth, isolates AE1.1MT18 and AE2.1MT19 were not statistically different, and

isolates AE1.1MT17 and AE36.1ND18 did not differ. The same results were seen when radial growth on day 14 was analyzed.

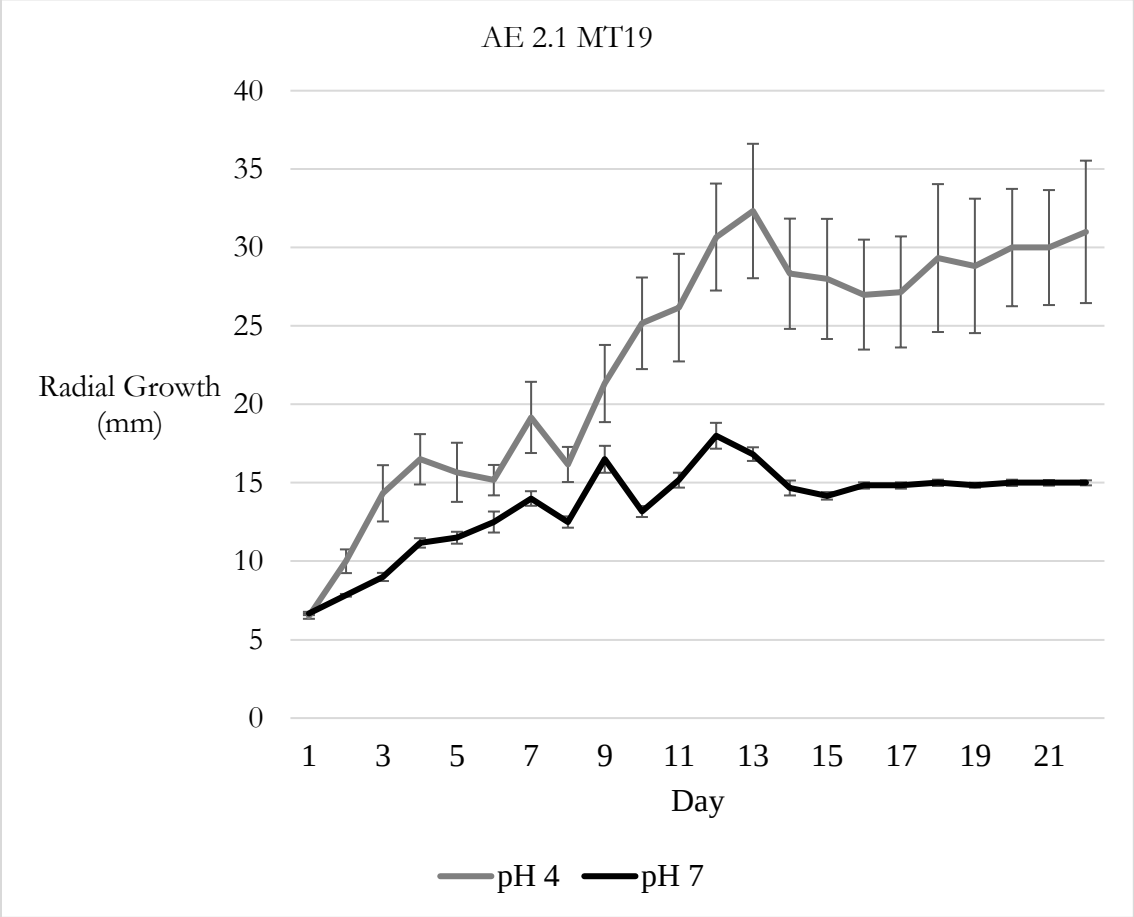


Figure 4.03 Average radial growth (mm) over time of isolate AE2.1MT19 on CMA plates adjusted to pH 4 and 7. Error bars represent standard error of the mean.

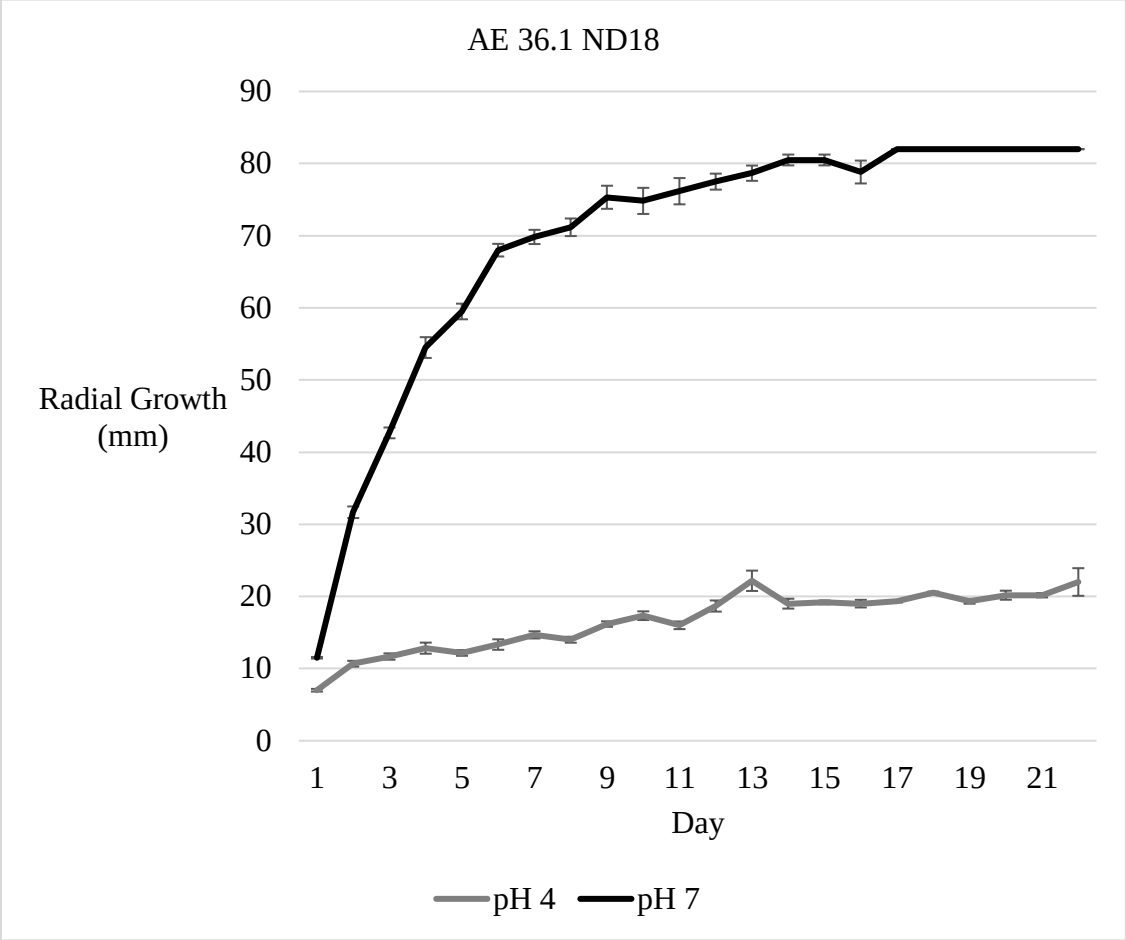


Figure 4.04 Average radial growth (mm) over time of isolate AE36.1ND18 on CMA plates adjusted to pH 4 and 7. Error bars represent standard error of the mean.

Discussion

The implementation of no-till farming practices in the Northern Great Plains has helped to increase stubble matter in fields, protect soils from erosion, promote biodiversity, and conserve soil nutrients and moisture (Mijangos et al. 2006; Mitchell et al. 2017; Peterson et al. 1996; House and Parmelee 1985). Although there are numerous benefits to reduced tillage, it has also been linked to decreasing soil pH compared to when conventional tillage is employed (Zhao et al. 2022). In Montana, reduced tillage practices and other issues such as the overuse of nitrogen fertilizer and low rainfall accelerating the process have led to increasing issues with soil acidification (Jones et al. 2019). Acidification is of concern for dry pea producers, as dry pea plants perform the best with neutral to marginally alkaline soils (Rice et al. 2000). The growth and production of lentil is also constrained by low pH conditions (Tang and Thomson 1996; Mahler and McDole 1987). Root rot issues are also increasing in the state on pulse crops, and the impact of soil acidification on soilborne disease populations is not always clear. The soil environment plays a significant role in *A. euteiches* development, reproduction, and spread, as its lifecycle occurs primarily in the rhizosphere (Allmaras et al. 2003; Heyman et al. 2007; Karppinen et al. 2020; Persson et al. 1999).

In this study, it was found that some isolates of *A. euteiches* grew faster on amended acidic petri plates, compared to the neutral pH control plates. For other isolates, growth was suppressed by the acidic plate conditions. Although growth on petri plates does not consider the soil and environmental factors influencing the disease cycle of *A.*

euteiches under field conditions, this data indicates that further research may reveal that different isolates respond differently to soil pH in the rhizosphere.

Research conducted in fresh pea fields in Sweden revealed that the disease severity of *A. euteiches* was reduced in alkaline soils compared to slightly acidic soils (Persson et al. 1999). The same has been seen with studies on the root rot pathogen of sugar beet, *Aphanomyces cochlioides*. Aphanomyces root rot of sugar beet was suppressed with increasing pH, and liming soils helped to increase yield when the pathogen was present (Olsson et al. 2011; Olsson et al. 2019).

Large scale surveys of field soil where pulses have been grown have been undertaken in different geographic regions. These surveys have revealed variable responses in *A. euteiches* to soil pH, and this could be due to the isolates or pathotypes of the pathogen present in the region. In one study of 142 annually cropped fields in Saskatchewan, there was no correlation between soil pH on Aphanomyces root rot. However, *A. euteiches* abundance was negatively correlated with soil pH when it was identified in roadside soils (Karppinen et al. 2020). In a survey of 60 dry pea fields in North Dakota, there was a positive relationship between soil pH and *A. euteiches* presence (Zitnick-Anderson et al. 2020). When large scale surveys are conducted of soilborne pathogens in pulse fields, dividing soils into pH categories for analysis may be necessary to reveal nuanced relationships with pathogen abundance and soil pH.

In conclusion, the effect of low soil pH on *A. euteiches* may be difficult to ascertain as isolates may respond differently to acidic soil conditions. Future research would be beneficial to determine if the results seen here are replicated *in vivo* and under

field conditions. Isolate and pathotype variability may explain why conflicting reports of the relationship of soil pH and *A. euteiches* abundance occur in the literature. Soil pH may prove to be a useful indicator of soil suppressiveness to Aphanomyces root rot, and disease prediction modelling may need to consider the isolates and pathotypes present in the geographic area when considering risk of root rot disease.

References

Allmaras, R., Fritz, V., Pfleger, F. & Copeland, S. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil and Tillage Research. 70: 41-52.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software.

Bresnahan, G., Dexter, A. & Koskinen, W. 1998. The effect of soil pH on sugarbeet yield and herbicide degradation. Sugarbeet Research and Extension Reports. 29: 82-88.

Bresnahan, G. A., Dexter, A. G., Windels, C. E., Brantner, J. R. & Luecke, J. L. 2001. Influence of soil pH on *Aphanomyces cochlioides* in sugarbeet. Sugarbeet Research and Extension Reports. 32: 264-268.

Broadbent, P. & Baker, K. F. 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Australian Journal of Agricultural Research. 25: 121-137.

El-Mohamedy, R. S., Abdel-Kader, M., Abd-El-Kareem, F. & El-Mougy, N. S. 2013. Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens in vitro. Journal of Agricultural Technology. 9: 1507-1520.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Molecular Plant Pathology. 8: 539-548.

Heyman, F., Lindahl, B., Persson, L., Wikström, M. & Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea. Soil Biology and Biochemistry. 39: 2222-2229.

Hothorn, T., Bretz, F. & Westfall, P. 2008. Simultaneous inference in general parametric models. Biometrical Journal.

House, G. J. & Parmelee, R. W. 1985. Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. Soil and Tillage Research. 5: 351-360.

Jee, H.-J., Cho, W.-D. & Kim, C.-H. 2002. Effect of potassium phosphonate on the control of Phytophthora root rot of lettuce in hydroponics. The Plant Pathology Journal. 18: 142-146.

Jones, C., Engel, R. & Olson-Rutz, K. 2019. Soil acidification in the semiarid regions of North America's Great Plains. Crops & Soils. 52: 28-56.

Karppinen, E. M., Payment, J., Chatterton, S., Bainard, J. D., Hubbard, M., Gan, Y. & Bainard, L. D. 2020. Distribution and abundance of *Aphanomyces euteiches* in agricultural soils: effect of land use type, soil properties, and crop management practices. Applied Soil Ecology. 150: 103470.

Mahler, R. & Mcdole, R. 1987. Effect of soil pH on crop yield in northern Idaho. Agronomy Journal. 79: 751-755.

Malvick, D., Grau, C. & Percich, J. 1998. Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. Mycological Research. 102: 465-475.

Mijangos, I., Pérez, R., Albizu, I. & Garbisu, C. 2006. Effects of fertilization and tillage on soil biological parameters. Enzyme and Microbial Technology. 40: 100-106.

Mitchell, J. P., Shrestha, A., Mathesius, K., Scow, K. M., Southard, R. J., Haney, R. L., Schmidt, R., Munk, D. S. & Horwath, W. R. 2017. Cover cropping and no-tillage improve soil health in an arid irrigated cropping system in California's San Joaquin Valley, USA. Soil and Tillage Research. 165: 325-335.

Mohebbi, S. & Mahler, R. 1989. The effect of soil pH on wheat and lentils grown on an agriculturally acidified northern Idaho soil under greenhouse conditions. Communications in Soil Science and Plant Analysis. 20: 359-381.

Olsson, Å., Persson, L. & Olsson, S. 2011. Variations in soil characteristics affecting the occurrence of Aphanomyces root rot of sugar beet–risk evaluation and disease control. Soil Biology and Biochemistry. 43: 316-323.

Olsson, Å., Persson, L. & Olsson, S. 2019. Influence of soil characteristics on yield response to lime in sugar beet. Geoderma. 337: 1208-1217.

Papavizas, G. & Davey, C. 1960. Some factors affecting growth of *Aphanomyces euteiches* in synthetic media. American Journal of Botany. 47: 758-765.

Papavizas, G. C. & Ayers, W. A. 1974. Aphanomyces species and their root diseases in pea and sugarbeet. United States Department of Agriculture, Economic Research Service.

Persson, L., Larsson-Wikström, M. & Gerhardson, B. 1999. Assessment of soil suppressiveness to Aphanomyces root rot of pea. Plant Disease. 83: 1108-1112.

Peterson, G. A., Schlegel, A. J., Tanaka, D. L. & Jones, O. R. 1996. Precipitation use efficiency as affected by cropping and tillage systems. Journal of Production Agriculture. 9: 180-186.

Pfender, W. & Hagedorn, D. 1982. *Aphanomyces euteiches* f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology. 72: 306-310.

Pfender, W. & Hagedorn, D. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology. 73: 1109-1113.

R Core Team, R. 2018. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Raaijmakers, J. M. & Mazzola, M. 2016. Soil immune responses. Science. 352: 1392-1393.

Rice, W., Clayton, G., Olsen, P. & Lupwayi, N. 2000. Rhizobial inoculant formulations and soil pH influence field pea nodulation and nitrogen fixation. Canadian Journal of Soil Science. 80: 395-400.

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., Esker, P. D., Giesler, L. J., Jardine, D. J. & Malvick, D. K. 2017. Oomycete species associated with soybean seedlings in North America—Part II: Diversity and ecology in relation to environmental and edaphic factors. Phytopathology. 107: 293-304.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bulletin. Virginia Agricultural Experiment Station. 151.

Sherwood, R. & Hagedorn, D. 1962. Studies on biology of *Aphanomyces euteiches*. Phytopathology. 52: 150-154.

Stone, A., Traina, S. & Hoitink, H. 2001. Particulate organic matter composition and Pythium damping-off of cucumber. Soil Science Society of America Journal. 65: 761-770.

Tang, C. & Thomson, B. 1996. Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legume species. Plant and Soil. 186: 321-330.

Tofte, J., Smith, R. & Grau, C. 1992. Reaction of red clover to *Aphanomyces euteiches*. Plant Disease.

Trivedi, P., Rochester, I. J., Trivedi, C., Van Nostrand, J. D., Zhou, J., Karunaratne, S., Anderson, I. C. & Singh, B. K. 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. Soil Biology and Biochemistry. 91: 169-181.

Vandemark, G. & Porter, L. 2010. First report of lentil root rot caused by *Aphanomyces euteiches* in Idaho. Plant Disease. 94: 480-480.

Wang, M., Gu, Z., Wang, R., Guo, J., Ling, N., Firbank, L. G. & Guo, S. 2019. Plant primary metabolism regulated by nitrogen contributes to plant–pathogen interactions. Plant and Cell Physiology. 60: 329-342.

Wu, L., Chang, K.-F., Conner, R. L., Strelkov, S., Fredua-Agyeman, R., Hwang, S.-F. & Feindel, D. 2018. *Aphanomyces euteiches*: a threat to Canadian field pea production. Engineering. 4: 542-551.

Zhao, X., He, C., Liu, W. S., Liu, W. X., Liu, Q. Y., Bai, W., Li, L. J., Lal, R. & Zhang, H. L. 2022. Responses of soil pH to no-till and the factors affecting it: A global meta-analysis. Global Change Biology. 28: 154-166.

Zitnick-Anderson, K., Luis, E., Forster, S. & Pasche, J. S. 2020. Associations among the communities of soil-borne pathogens, soil edaphic properties and disease incidence in the field pea root rot complex. Plant and Soil. 457: 339-354.

CHAPTER FIVE

SUMMARY OF APHANOMYCES RESEARCH AND FUTURE WORK

The production of dry pea and lentil has been constrained by a root rot complex, consisting of multiple pathogens. The complex includes *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani* Kühn, nematodes, and *Aphanomyces euteiches* Drechs. This dissertation presented research on the soilborne oomycete pathogen, *A. euteiches*, in Montana. Prior to this research, knowledge of *A. euteiches* in the state was very limited. It was initially detected in 2016 in two dry pea fields when a survey of dry pea diseases was conducted. Due to the importance of this pathogen in other dry pea growing regions, it was suspected that Aphanomyces root rot would become problematic in Montana as acres of pulse crops increased. The popularity of dry pea and lentil was increasing as producers moved away from traditional cereal-fallow rotations, creating conducive crop rotations for *A. euteiches* disease propagules to proliferate.

Due to collaborations with the Schutter Diagnostic Lab (SDL) at Montana State University, root samples of dry pea and lentil, as well as soil samples, were provided to the author to conduct initial soil bioassays and molecular checks for *A. euteiches*. A soil bioassay was designed for pathogen detection, testing a variety of procedures to find the quantity of soil needed, seed treatments to increases specificity of the test to *A. euteiches*, and determining sterile techniques to avoid contamination between samples. It became apparent from bioassay testing of SDL samples that Aphanomyces root rot was

widespread in the state, and its presence had likely been attributed to other issues prior to molecular testing, due to the pathogen being difficult to isolate and easily overlooked.

The pathogen was detected in five Montana counties, in over twenty fields. Due to this discovery, twelve fields were selected for further investigation of the horizontal distribution of the pathogen and soil properties that may contribute to disease, with permission from the producers and landowners. In many fields, it was found that the horizontal distribution of Aphanomyces root rot was highly sporadic. Some fields had only one quadrat out of thirty-five quadrats showing significant root rot. In contrast, two fields had very consistent root rot pressure in all or almost all quadrats, with the producer of one field experiencing complete crop loss of dry pea, and the other reporting significant yield loss. The chance of detecting *A. euteiches* in pulse fields will therefore be enhanced by multiple sampling sites in a field.

Correlations were found between Aphanomyces root rot with soil pH, organic matter, potassium, and sulfur; although, correlations were not seen in all fields tested. Future field tests should check for soil moisture saturation at the time of sampling, and take measures of soil bulk density, as these factors contribute to the lifecycle of *A. euteiches*. Using in vitro tests with acidic versus neutral pH plates, it was found that isolates of *A. euteiches* responded differently to acidic plate conditions, with some isolates impeded by low pH, and others thriving.

A future direction of this work would be correlating disease severity scores in greenhouse soil bioassays with the relative quantification of pathogen DNA from soil samples, to determine if real-time quantitative PCR (qPCR) can be used instead of soil

bioassays for faster detection of *A. euteiches*. Although qPCR testing using the ITS region can detect the pathogen in roots and soil, relative DNA is difficult to link to disease severity scores disease. This is because ITS regions vary in number in cells of *A. euteiches*, and relative DNA does not consistently correlate with number of disease propagules. Soil conditions can also impact the ability of qPCR to detect the pathogen in soil. This work may therefore require designing new primers that do not use the ITS region or conducting both molecular tests and soil bioassays for disease risk prediction.

Future research beneficial to Montana pulse producers would be designing and confirming soil bioassays and molecular tests for other pathogens in the pulse root rot complex, including *Fusarium* spp., *Pythium* spp., and any new or emerging pathogens. *Phytophthora* spp. on chickpea was recently discovered causing root rot in Montana fields and further work is required to determine how virulent this genus is on pulse crops in the state. A beneficial outcome would be the establishment of a soil testing service for multiple pulse root rot pathogens at the Regional Pulse Crop Diagnostic Laboratory, Bozeman Montana, where growers could submit samples for root rot assessment prior to planting pulses.

Other findings from this dissertation indicate that host legumes in greenhouse soils proliferate Aphanomyces root rot, while non-host plants typically reduce disease severity on subsequent dry pea test plants. This research could be expanded to include more than one isolate of *A. euteiches*. In addition, future research into crop rotations under field conditions would be useful to check these findings with variable environmental conditions and with other root rot pathogens present. Of particular interest

is how Aphanomyces root rot responds after an oat crop is grown, using Montana isolates. Field trials can be difficult to conduct, as inoculating *A. euteiches* in research plots may mean the land is contaminated with the pathogen for many years to come, due to a lack of available management methods.

In conclusion, maintaining the sustainability of pulse crop production in Montana requires knowledge of the pathogens threatening production, the environmental conditions conducive to the pathogen, and the management strategies available to combat yield loss. The research presented here confirms that the soilborne oomycete *A. euteiches* is present in many fields with a history of dry pea and lentil in Montana, and crop rotation is an important tool for its management. It also confirms that environmental factors in the soil system play a role in the disease lifecycle, and future disease risk prediction models needs to consider soil edaphic properties and isolate preferences as factors influencing disease.

CHAPTER SIX

IMPACT OF CROPPING SYSTEM AND COVER CROP TERMINATION METHOD ON DISEASE OF LENTIL AND WHEAT FIELD PLOTS

Abstract

Cover crops are a useful alternative to fallowing the land in the Northern Great Plains, to protect soils from erosion and improve soil structure and health. Cover crop management requires timely termination to mitigate water losses. This trial assessed the impact of termination method of a cover crop on foliar, crown, and root diseases of wheat and lentil grown in a five-year rotation (safflower under-sown with sweet clover, a sweet clover cover crop, winter wheat, lentil, and winter wheat). There were three cover crop termination methods tested: sheep grazing in an organic treatment with minimal tillage, tandem-disk tillage in an organic treatment, and herbicide burn-down in a conventionally managed treatment. Plant diseases of lentil did not differ between the management treatments, although several root rot pathogens were isolated including *Rhizoctonia solani*, *Fusarium* spp., and *Pythium* spp. Root rot pathogens in wheat plots also did not differ between treatments. The severity of stripe rust on wheat was higher in tilled organic plots than conventional plots, without the application of foliar fungicide in the conventional treatment. Leaf spots, primarily identified as tan spot of wheat, were highest in conventional plots compared to the two organic treatments. Foliar fungicides were not sprayed to target leaf spot pathogens. Management style in crop production has implications for plant diseases and the effect of environment needs to be considered.

Introduction

In the semiarid Northern Great Plains region of the United States, sustainable crop production is hindered by diminishing soil fertility and water constraints (Nielsen et al. 2011). The region is impacted by periodic drought conditions, high temperature fluctuations between seasons with cold and dry winters, and areas impacted by extreme wind conditions that exacerbate soil erosion (Hansen et al. 2012; Liebig et al. 2004). Zero-till crop production with fallow has been popular in Montana and the Northern Great Plains to increase the retention of annual precipitation by maintaining crop residues and soil structure (Nielsen and Vigil 2010). In recent years, cover cropping has been proposed instead of periods of fallow to add soil cover to fields. This helps protect soils from erosion during periods when the land would otherwise be bare. Cover cropping can also improve soil quality, soil fertility, and soil structure (Mitchell et al. 2017; Ghimire et al. 2018). It allows nutrient cycling, particularly if a legume is used in the mixture. However, cover crops can become a water sink, using precious soil water stores that would otherwise be available for the next crop. For example, winter wheat yield was reduced by 10% following cover crops compared to fallow in the Great Plains, worsened by dry conditions (Nielsen et al. 2016). In dryland agriculture, cover crops need to be managed in a way that diminishes water loss.

Since cover crops are not grown for grain production, the timing of termination is typically conducted when plants are in the vegetative stage. Alternative methods have been proposed for cover crop termination, such as terminating the crop by animal grazing. In a recent study in the Great Plains, this was tested with sheep (*Ovis aries*)

grazing. Using sheep improved nutrient cycling compared to harvesting with equipment (Carr et al. 2021). Cattle (*Bos taurus*) have been shown to successfully terminate cover crops; however, can increase soil compaction and reduce water infiltration, which can cause challenges for the root health and yield of future crops (Franzluebbers and Stuedemann 2008). While a lofty ideal, adding animals to a system can help provide system self-sufficiency, through animal manure inputs, recycling nutrients, soil conservation benefits, and the revenue from animal production in addition to crops (Franzluebbers and Stuedemann 2014; Wilkins 2008). It is important to note there are increased costs of an integrated system, with increased labor and equipment needs compared to systems with cropping alone (Poffenbarger et al. 2017).

Animal grazing was proposed for cover crop termination as an alternative to tillage, particularly for use in organic systems where chemical burndown of cover crops is not possible. There are known problems with tilling the soil, such as decreasing organic matter, reduced soil moisture, potentially decreased soil microorganism diversity, and reductions in beneficial arthropod density (Liebig et al. 2004; House and Parmelee 1985; Peterson et al. 1998). Tilling, however, can be useful to break plant disease cycles, particularly for residue-borne and soil-borne pathogens that are difficult to manage in organic systems that rely heavily on cultural management of disease (Bockus and Shroyer 1998).

Plant disease management strategies are contingent on the cropping system adopted. For example, organic systems do not have synthetic fungicides at their disposal when disease outbreaks occur and must rely primarily on cultural practices or organic

products, if available. According to the latest survey available, Montana has over 200 certified organic agriculture farms with 350,000 acres of organic crops (NASS 2019a). Organic agriculture is an important contributor to the state economy. The focus of this manuscript was to monitor for differences in foliar, crown, and root diseases of wheat and lentil under three system treatments in a field trial: chemically managed with no tillage, organically managed with tillage, and organically managed with sheep grazing for cover crop termination.

Materials and Methods

Field Site

In 2017 the final year of a five-year research trial was conducted at the Fort Ellis agricultural research station in Bozeman, Montana. Methodology followed that of the first four years of the trial (Ranabhat 2017). The trial ran from 2013-2017 and investigated cropping system and cover crop termination methods on plant diseases of wheat and lentil. The field site was previously used for animal grazing prior to 2004, and a wheat-fallow trial was conducted in the years 2004 to 2008 (Lenssen et al. 2013). Soil at the location is a blackmore silt loam with an approximate pH of 6.7 in the top 15 cm of the soil profile (Barsotti et al. 2013).

Experimental Design and Sampling

The experiment was designed as a split plot, with main plots having an area of 0.55 ha. Cropping system was randomly assigned to main plots: chemically managed with no tillage, organically managed with tillage, and organically managed with sheep grazing cover crop termination. Five split plots of different crops (13 x 90 m) were randomly assigned within main plots. All split plot treatments were present in each year (2013-2017) and spit-plot treatments were safflower under-sown with sweet clover, a sweet clover cover crop, winter wheat, lentil, and winter wheat. The sweet clover cover crop was terminated at the late bud stage, a few days prior to first bloom. Termination of sweet clover was conducted with glyphosate in the chemically managed plots, using a tandem-disk in tilled plots, and via sheep grazing in grazed plots. Buffer zones between main plots were ten meters, and between split-plots were one meter. Organic plot seeding density was doubled compared to other plots.

Wheat and lentil whole plant samples were collected from plots in a ‘W’ sampling pattern from the north third of the plots in a designated destructive sampling area to avoid tampering with yield results. Five plants were sampled per point, for a total of 25 plants sampled per plot. Wheat plots were sampled at tiller, flag, and maturity. Lentil plots were sampled when the sixth multifoliate leaf had unfolded at the sixth node, and at full bloom. Samples were refrigerated in paper bags (AJM MI, USA) and processed within 7 days of collection. Foliar diseases were scored as the percentage of leaf covered by disease. Foliar disease severity for plants was calculated from disease severity of all leaves, and averages calculated from severity of all plants in each plot. Stems were also

checked for evidence of disease, although none was found. Root disease severity was scored as the percentage of root lesion covering the root system (Fernandez and Jefferson 2004). Disease incidence for whole plants for each pathogen was designated with a '1' if disease was present, and a '0' if absent, for statistical analysis. Diseased samples were placed into a humid chamber. Hyphal growth was transferred to potato dextrose agar, and to selective media if required, for further identification of pathogens present.

Statistical Analysis

Sampling times and plant species were analyzed separately, due to different plant diseases being present. The one exception to this was leaf spots in wheat, as leaf spot severity did not differ between tiller and flag samplings. The 'lme4' package in R version 4.1.2 was used for generalized linear mixed-effects modeling of disease incidence and severity (Bates et al. 2015; R Core Team 2018). For wheat plots, binary disease incidence data for the 25 plants was analyzed as the response variable with fixed effects (treatment and split-plot ID) and random effects (block and plot), using the 'glmer' function with a binomial distribution. 'Split-plot ID' was necessary as there were two wheat split-plot treatments. When there was no difference in disease incidence or severity between the two wheat split plot treatments, 'split-plot ID' was included in the model as a random effect instead of a fixed effect.

Disease incidence in lentil was analyzed in the same way, however no 'split-plot ID' fixed effect was included as only one year of lentil was present in the crop rotation. Evidence for a difference between main plot treatments was tested using the 'Anova' function (Fox and Weisberg 2011). The 'r.squaredGLMM' function was used to compute

R-squared values. When differences occurred between main plots (α=0.05) a Tukey's HSD test was run using the 'multcomp' package (Hothorn et al. 2008).

Disease severity was also analyzed as a response variable for modeling pathogens of wheat and lentil. The 'lmer' function was used and wheat plots were modelled with fixed effects treatment and split-plot ID, (with split-plot included as a random effect instead if no difference was found between the wheat split-plots) and random effects block and plot. Disease severity of lentil was modelled in the same fashion, without the 'split-plot ID' fixed effect. Analysis of variance and post-hoc testing was completed as described for disease incidence. To test for homogeneity of variance and normality, residual and Q-Q plots were viewed.

Results

Foliar diseases detected in wheat plots included stripe rust (*Puccinia striiformis*), powdery mildew (*Blumeria graminis*), and leaf spots, primarily identified as tan spot (*Pyrenophora tritici-repentis*). Root and crown rot pathogens identified in wheat included *Rhizoctonia solani*, *Fusarium* spp., *Cochliobolus sativus,* and *Pythium* spp. Disease severity results more accurately reflected disease pressure in the field plots and is reported here instead of disease incidence. Wheat samples collected at flag differed in stripe rust severity for main plots (P = 0.01), although did not differ between the two split-plot wheat treatments (P = 0.26). Tilled organic plots (4.6% ± 1.2) had higher stripe rust severity at flag than chemical plots (1.4% ± 0.8), while stripe rust severity in grazed organic plots did not differ from the other two main plot treatments (3.6% ± 0.8). Foliar

diseases were not detected at the third sampling, at wheat maturity, due to plant senescence impacting foliar disease visibility. Wheat leaf spots sampled at tiller and flag differed between main plot treatments ($P < 0.001$). There was little evidence of a difference between split-plot wheat rotation ($P = 0.06$), or between tiller and flag sampling times on wheat leaf spot severity ($P = 0.97$). Severity of wheat leaf spots was higher in chemical plots (6.5% ± 1.3) compared to tilled organic (2.7% ± 1.1) and grazed organic plots (2.2% ± 0.6). There were no differences detected between wheat main plots for root and crown rot pathogens at any sampling time.

Lentil plots overall had minimal plant disease incidence and severity. However, several root rot pathogens were isolated from a small number of root samples. These were *Rhizoctonia solani*, *Fusarium* spp., and *Pythium* spp. There was no difference in incidence or severity of main plot treatments for lentil plant pathogens.

Discussion

The aim of this study was to determine if there were notable differences in disease pressure in wheat and lentil plots with different management techniques: tilled organic, chemical no-till, and organic plots with sheep grazing for cover crop termination. Stripe rust severity of wheat was higher in tilled organic plots than chemically managed plots, however this cannot be attributed to a chemical treatment as foliar fungicides were not sprayed in chemical plots during the growing season for management of rust. In previous years of this trial, stripe rust incidence was higher in chemical plots than organic plots (Ranabhat 2017). The rust pathogen is widely dispersed, primarily by wind transported urediniospores, with over summering occurring on wheat and alternate hosts in surrounding areas (Jin et al. 2010; Shaner and Powelson 1973). Therefore, it is likely that stripe rust severity was impacted most by plot location in the field and alternate host management (such as herbicide application in chemical plots for weeds). If foliar fungicides are utilized in chemical plots, stripe rust severity in chemical plots would likely be reduced even more in comparison to organic plots (Carmona et al. 2020; Chen 2014).

Leaf spots in wheat were higher in chemical plots without tillage compared to organic plots with tillage or grazing. This is likely due in part because tan spot is a residue-borne pathogen, and tillage helps to disturb and bury the residue in a system (Bockus 1992). In years where tan spot severity is high, applying a foliar fungicide in fields using chemical reduced tillage or no tillage systems can help to mitigate losses to the pathogen (Jørgensen and Olsen 2007). Tillage practices, while useful in pathogen

management, have negative side effects on the rhizosphere soil structure, water holding capacity, organic matter, and soil biodiversity (Liebig et al. 2004; Peterson et al. 1998; Peterson et al. 1996; Mijangos et al. 2006). In the Northern Great Plains, many growers have shifted to minimal tillage practices due to these concerns.

In contrast to foliar diseases, crown and root rot diseases did not differ between management systems for wheat or lentil plots in the crop rotation. In a similar study conducted in Saskatchewan, with a crop rotation including wheat, lentil, and dry pea, root rot severity was reduced with the use of tillage (Bailey et al. 2000). It is possible that the time frame of this trial was not sufficient for differences to be detected between the management styles for crown and root rot pathogen severity. It can take a long time to see the beneficial effect of organic amendments, crop residue strategies, and disease suppressive soils on pathogen populations (Bailey and Lazarovits 2003).

Managing diseases in any system can have its downsides, whether it be cultural control techniques such as tillage with side-effects to soil health, the added input cost to apply fungicide treatments, the unintended side effects of chemical treatments to beneficial organisms or natural predators, and more. In this field trial, there was evidence to suggest that chemical plots had reduced diversity and species richness of beneficial insects (ground beetles (Carabidae)) compared to organically managed plots (Adhikari and Menalled 2020). In similar studies, recorded beneficial microbial activity was increased under organic systems compared to conventional production with chemical treatments, which could have consequences for competition with pathogen populations (Van Bruggen 1995).

For future trials, inoculating with plant diseases of interest may be necessary to elucidate a pronounced response to plant pathogens within a crop management system, as natural disease presence and severity was low overall. The impact of the environment for each pathogen of interest also needs to be considered, as pathogens will also vary in a system based on temperature, moisture conditions, and soil factors (Bailey et al. 2000; Bailey et al. 2001; Duveiller et al. 2007). The use of foliar fungicides for targeting tan spot and stripe rust would also lead to differences between chemically managed plots and organic plots, however this may not necessarily equate to a yield benefit for the crops. Integrated management approaches for plant pathogens in each system therefore need to be assessed on a case-by-case basis.

References

Adhikari, S. & Menalled, F. D. 2020. Supporting beneficial insects for agricultural sustainability: the role of livestock-integrated organic and cover cropping to enhance ground beetle (Carabidae) communities. Agronomy. 10: 1210.

Bailey, K., Gossen, B., Derksen, D. & Watson, P. 2000. Impact of agronomic practices and environment on diseases of wheat and lentil in southeastern Saskatchewan. Canadian Journal of Plant Science. 80: 917-927.

Bailey, K., Gossen, B., Lafond, G., Watson, P. & Derksen, D. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. Canadian Journal of Plant Science. 81: 789-803.

Bailey, K. & Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil and Tillage Research. 72: 169-180.

Barsotti, J. L., Sainju, U. M., Lenssen, A. W., Montagne, C. & Hatfield, P. G. 2013. Crop yields and soil organic matter responses to sheep grazing in US northern great plains. Soil and Tillage Research. 134: 133-141.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software.

Bockus, W. & Shroyer, J. 1998. The impact of reduced tillage on soilborne plant pathogens. Annual Review of Phytopathology. 36: 485-500.

Bockus, W. W. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Disease. 633.

Carmona, M., Sautua, F., Pérez-Hérnandez, O. & Reis, E. M. 2020. Role of fungicide applications on the integrated management of wheat stripe rust. Frontiers in Plant Science. 11: 733.

Carr, P. M., Bell, J. M., Boss, D. L., Delaune, P., Eberly, J. O., Edwards, L., Fryer, H., Graham, C., Holman, J. & Islam, M. A. 2021. Annual forage impacts on dryland wheat farming in the Great Plains. Agronomy Journal. 113: 1-25.

Chen, X. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. Canadian Journal of Plant Pathology. 36: 311-326.

Duveiller, E., Singh, R. P. & Nicol, J. M. 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica. 157: 417-430.

Fernandez, M. & Jefferson, P. 2004. Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. Canadian Journal of Plant Pathology. 26: 325-334.

Fox, J. & Weisberg, S. 2011. An R companion to applied regression. Thousand Oaks, CA: SAGE Publications.

Franzluebbers, A. J. & Stuedemann, J. A. 2008. Soil physical responses to cattle grazing cover crops under conventional and no tillage in the Southern Piedmont USA. Soil and Tillage Research. 100: 141-153.

Franzluebbers, A. J. & Stuedemann, J. A. 2014. Crop and cattle production responses to tillage and cover crop management in an integrated crop–livestock system in the southeastern USA. European Journal of Agronomy. 57: 62-70.

Ghimire, R., Ghimire, B., Mesbah, A. O., Idowu, O. J., O'neill, M. K., Angadi, S. V. & Shukla, M. K. 2018. Current status, opportunities, and challenges of cover cropping for sustainable dryland farming in the Southern Great Plains. Journal of Crop Improvement. 32: 579-598.

Hansen, N. C., Allen, B. L., Baumhardt, R. L. & Lyon, D. J. 2012. Research achievements and adoption of no-till, dryland cropping in the semi-arid US Great Plains. Field Crops Research. 132: 196-203.

Hothorn, T., Bretz, F. & Westfall, P. 2008. Simultaneous inference in general parametric models. Biometrical Journal.

House, G. J. & Parmelee, R. W. 1985. Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. Soil and Tillage Research. 5: 351-360.

Jin, Y., Szabo, L. J. & Carson, M. 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. Phytopathology. 100: 432-435.

Jørgensen, L. & Olsen, L. 2007. Control of tan spot (*Drechslera tritici-repentis*) using cultivar resistance, tillage methods and fungicides. Crop Protection. 26: 1606-1616.

Lenssen, A. W., Sainju, U. M. & Hatfield, P. G. 2013. Integrating sheep grazing into wheat–fallow systems: crop yield and soil properties. Field Crops Research. 146: 75-85.

Liebig, M. A., Tanaka, D. & Wienhold, B. J. 2004. Tillage and cropping effects on soil quality indicators in the northern Great Plains. Soil and Tillage Research. 78: 131-141.

Mijangos, I., Pérez, R., Albizu, I. & Garbisu, C. 2006. Effects of fertilization and tillage on soil biological parameters. Enzyme and Microbial Technology. 40: 100-106.

Mitchell, J. P., Shrestha, A., Mathesius, K., Scow, K. M., Southard, R. J., Haney, R. L., Schmidt, R., Munk, D. S. & Horwath, W. R. 2017. Cover cropping and no-tillage improve soil health in an arid irrigated cropping system in California's San Joaquin Valley, USA. Soil and Tillage Research. 165: 325-335.

Nass 2019a. 2019 Organic Survey. In: AGRICULTURE, U. S. D. (ed.). National Agricultural Statistics Service.

Nielsen, D. C., Lyon, D. J., Higgins, R. K., Hergert, G. W., Holman, J. D. & Vigil, M. F. 2016. Cover crop effect on subsequent wheat yield in the central Great Plains. Agronomy Journal. 108: 243-256.

Nielsen, D. C. & Vigil, M. F. 2010. Precipitation storage efficiency during fallow in wheat-fallow systems. Agronomy Journal. 102: 537-543.

Nielsen, D. C., Vigil, M. F. & Benjamin, J. G. 2011. Evaluating decision rules for dryland rotation crop selection. Field Crops Research. 120: 254-261.

Peterson, G., Halvorson, A., Havlin, J., Jones, O., Lyon, D. & Tanaka, D. 1998. Reduced tillage and increasing cropping intensity in the Great Plains conserves soil C. Soil and Tillage Research. 47: 207-218.

Peterson, G. A., Schlegel, A. J., Tanaka, D. L. & Jones, O. R. 1996. Precipitation use efficiency as affected by cropping and tillage systems. Journal of Production Agriculture. 9: 180-186.

Poffenbarger, H., Artz, G., Dahlke, G., Edwards, W., Hanna, M., Russell, J., Sellers, H. & Liebman, M. 2017. An economic analysis of integrated crop-livestock systems in Iowa, USA. Agricultural Systems. 157: 51-69.

R Core Team, R. 2018. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ranabhat, N. 2017. Effect of agronomic practices on disease incidence, severity, and impacts in Montana cropping systems. Master of Science, Montana State University.

Shaner, G. & Powelson, R. L. 1973. The oversummering and dispersal of inoculum of *Puccinia striiformis* in Oregon. Phytopathology. 63: 13-17.

Van Bruggen, A. H. 1995. Plant disease severity in high-input compared to reduced-input and organic farming systems. Plant Disease. 79: 976-984.

Wilkins, R. J. 2008. Eco-efficient approaches to land management: a case for increased integration of crop and animal production systems. Philosophical Transactions of the Royal Society B: Biological Sciences. 363: 517-525.

REFERENCES CITED

REFERENCES CITED

Adhikari, S. & Menalled, F. D. 2020. Supporting beneficial insects for agricultural sustainability: the role of livestock-integrated organic and cover cropping to enhance ground beetle (Carabidae) communities. Agronomy. 10: 1210.

Akhter, W., Bhuiyan, M. K. A., Sultana, F. & Hossain, M. M. 2015. Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani*) and improving yield in pea (*Pisum sativum* L.). Comptes Rendus Biologies. 338: 21-28.

Allmaras, R., Fritz, V., Pfleger, F. & Copeland, S. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil and Tillage Research. 70: 41-52.

Allmaras, R., Kraft, J. & Miller, D. 1988. Effects of soil compaction and incorporated crop residue on root health. Annual Review of Phytopathology. 26: 219-243.

Almquist, C., Persson, L., Olsson, Å., Sundström, J. & Jonsson, A. 2016. Disease risk assessment of sugar beet root rot using quantitative real-time PCR analysis of *Aphanomyces cochlioides*. European Journal of Plant Pathology. 145: 731-742.

Appiah, A. A., Van West, P., Osborne, M. C. & Gow, N. A. 2005. Potassium homeostasis influences the locomotion and encystment of zoospores of plant pathogenic oomycetes. Fungal Genetics and Biology. 42: 213-223.

Bailey, K., Gossen, B., Derksen, D. & Watson, P. 2000. Impact of agronomic practices and environment on diseases of wheat and lentil in southeastern Saskatchewan. Canadian Journal of Plant Science. 80: 917-927.

Bailey, K., Gossen, B., Lafond, G., Watson, P. & Derksen, D. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. Canadian Journal of Plant Science. 81: 789-803.

Bailey, K. & Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil and Tillage Research. 72: 169-180.

Banniza, S., Bhadauria, V., Peluola, C., Armstrong-Cho, C. & Morrall, R. 2013. First report of *Aphanomyces euteiches* in Saskatchewan. Canadian Plant Disease Survey. 93: 163-164.

Barsotti, J. L., Sainju, U. M., Lenssen, A. W., Montagne, C. & Hatfield, P. G. 2013. Crop yields and soil organic matter responses to sheep grazing in US northern great plains. Soil and Tillage Research. 134: 133-141.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software.

Beale, J. W., Windels, C. E. & Kinkel, L. L. 2002. Spatial distribution of *Aphanomyces cochlioides* and root rot in sugar beet fields. Plant Disease. 86: 547-551.

Bockus, W. & Shroyer, J. 1998. The impact of reduced tillage on soilborne plant pathogens. Annual Review of Phytopathology. 36: 485-500.

Bockus, W. W. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Disease. 633.

Bødker, L., Kjøller, R. & Rosendahl, S. 1998. Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza. 8: 169-174.

Boosalis, M. & Scharen, A. L. 1959. Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. Phytopathology. 49: 192-198.

Bowers, J. & Parke, J. 1993. Epidemiology of Pythium damping-off and Aphanomyces root rot of peas after seed treatment with bacterial agents for biological control. Phytopathology. 83: 1466-1473.

Bresnahan, G., Dexter, A. & Koskinen, W. 1998. The effect of soil pH on sugarbeet yield and herbicide degradation. Sugarbeet Research and Extension Reports. 29: 82-88.

Bresnahan, G. A., Dexter, A. G., Windels, C. E., Brantner, J. R. & Luecke, J. L. 2001. Influence of soil pH on *Aphanomyces cochlioides* in sugarbeet. Sugarbeet Research and Extension Reports. 32: 264-268.

Broadbent, P. & Baker, K. F. 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Australian Journal of Agricultural Research. 25: 121-137.

Burgess, M. H., Miller, P. R. & Jones, C. A. 2012. Pulse crops improve energy intensity and productivity of cereal production in Montana, USA. Journal of Sustainable Agriculture. 36: 699-718.

Cannesan, M. A., Gangneux, C., Lanoue, A., Giron, D., Laval, K., Hawes, M., Driouich, A. & Vicré-Gibouin, M. 2011. Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. Annals of Botany. 108: 459-469.

Carmona, M., Sautua, F., Pérez-Hérnandez, O. & Reis, E. M. 2020. Role of fungicide applications on the integrated management of wheat stripe rust. Frontiers in Plant Science. 11: 733.

Carr, P. M., Bell, J. M., Boss, D. L., Delaune, P., Eberly, J. O., Edwards, L., Fryer, H., Graham, C., Holman, J. & Islam, M. A. 2021. Annual forage impacts on dryland wheat farming in the Great Plains. Agronomy Journal. 113: 1-25.

Chan, M. & Close, R. 1987. Aphanomyces root rot of peas 1. Evaluation of methods for assessing inoculum density of *Aphanomyces euteiches* in soil. New Zealand Journal of Agricultural Research. 30: 213-217.

Chandler, M. A., Fritz, V. A. & Allmaras, R. R. 2004. Response of *Aphanomyces euteiches* mycelia, zoospore, and oospore to oat extract. Canadian Journal of Plant Science. 84: 687-690.

Chatterton, S., Bowness, R. & Harding, M. 2016. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Disease. 100: 1965-1978.

Chatterton, S., Harding, M. W., Bowness, R., Mclaren, D. L., Banniza, S. & Gossen, B. D. 2019. Importance and causal agents of root rot on field pea and lentil on the Canadian prairies, 2014–2017. Canadian Journal of Plant Pathology. 41: 98-114.

Chen, X. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. Canadian Journal of Plant Pathology. 36: 311-326.

Christensen, R. 2019. Ordinal - regression models for ordinal data. R Package Version 2019.12-10.

Deacon, J. & Mitchell, R. 1985. Toxicity of oat roots, oat root extracts, and saponins to zoospores of *Pythium* spp. and other fungi. Transactions of the British Mycological Society. 84: 479-487.

Deacon, J. & Saxena, G. 1998. Germination triggers of zoospore cysts of *Aphanomyces euteiches* and *Phytophthora parasitica*. Mycological Research. 102: 33-41.

Desgroux, A., L'anthoëne, V., Roux-Duparque, M., Rivière, J.-P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P. & Piriou, C. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics. 17: 1-21.

Djébali, N., Jauneau, A., Ameline-Torregrosa, C., Chardon, F., Jaulneau, V., Mathé, C., Bottin, A., Cazaux, M., Pilet-Nayel, M.-L. & Baranger, A. 2009. Partial resistance of *Medicago truncatula* to *Aphanomyces euteiches* is associated with protection of

the root stele and is controlled by a major QTL rich in proteasome-related genes. Molecular Plant-Microbe Interactions. 22: 1043-1055.

Duveiller, E., Singh, R. P. & Nicol, J. M. 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica. 157: 417-430.

El-Mohamedy, R. S., Abdel-Kader, M., Abd-El-Kareem, F. & El-Mougy, N. S. 2013. Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens in vitro. Journal of Agricultural Technology. 9: 1507-1520.

Esmaeili Taheri, A., Chatterton, S., Foroud, N., Gossen, B. & Mclaren, D. 2017. Identification and community dynamics of fungi associated with root, crown, and foot rot of field pea in western Canada. European Journal of Plant Pathology. 147: 489-500.

Fahey, J. W., Zalcmann, A. T. & Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 56: 5-51.

Fernandez, M. & Jefferson, P. 2004. Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. Canadian Journal of Plant Pathology. 26: 325-334.

Fox, J. & Weisberg, S. 2011. An R companion to applied regression. Thousand Oaks, CA: SAGE Publications.

Franzluebbers, A. J. & Stuedemann, J. A. 2008. Soil physical responses to cattle grazing cover crops under conventional and no tillage in the Southern Piedmont USA. Soil and Tillage Research. 100: 141-153.

Franzluebbers, A. J. & Stuedemann, J. A. 2014. Crop and cattle production responses to tillage and cover crop management in an integrated crop–livestock system in the southeastern USA. European Journal of Agronomy. 57: 62-70.

Gangneux, C., Cannesan, M.-A., Bressan, M., Castel, L., Moussart, A., Vicré-Gibouin, M., Driouich, A., Trinsoutrot-Gattin, I. & Laval, K. 2014. A sensitive assay for rapid detection and quantification of *Aphanomyces euteiches* in soil. Phytopathology. 104: 1138-1147.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Molecular Plant Pathology. 8: 539-548.

Ghimire, R., Ghimire, B., Mesbah, A. O., Idowu, O. J., O'neill, M. K., Angadi, S. V. & Shukla, M. K. 2018. Current status, opportunities, and challenges of cover

cropping for sustainable dryland farming in the Southern Great Plains. Journal of Crop Improvement. 32: 579-598.

Gossen, B. D., Conner, R. L., Chang, K.-F., Pasche, J. S., Mclaren, D. L., Henriquez, M. A., Chatterton, S. & Hwang, S.-F. 2016. Identifying and managing root rot of pulses on the Northern Great Plains. Plant Disease. 100: 1965-1978.

Grünwald, N. J. & Hoheisel, G.-A. 2006. Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete *Aphanomyces euteiches*. Phytopathology. 96: 1134-1141.

Gundersen, B., Inglis, D. A. & Porter, L. 2006. Evaluation of seed, in-furrow and foliar treatments for control of root rot on peas. Fungicide Nematic Tests. 61: V130.

Hajihassani, A., Tenuta, M. & Gulden, R. H. 2016. Host preference and seedborne transmission of *Ditylenchus weischeri* and *D. dipsaci* on select pulse and non-pulse crops grown in the Canadian Prairies. Plant Disease. 100: 1087-1092.

Hamon, C., Coyne, C. J., Mcgee, R. J., Lesné, A., Esnault, R., Mangin, P., Hervé, M., Le Goff, I., Deniot, G. & Roux-Duparque, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biology. 13: 45.

Hansen, N. C., Allen, B. L., Baumhardt, R. L. & Lyon, D. J. 2012. Research achievements and adoption of no-till, dryland cropping in the semi-arid US Great Plains. Field Crops Research. 132: 196-203.

Harrell Jr, F. & Dupont, C. 2019. Hmisc: Harrell Miscellaneous. R Package Version 4.2-0.

Heyman, F., Lindahl, B., Persson, L., Wikström, M. & Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea. Soil Biology and Biochemistry. 39: 2222-2229.

Hoitink, H., Madden, L. & Boehm, M. 1996. Relationships among organic matter decomposition level, microbial species diversity, and soilborne disease severity. Principles and Practice of Managing Soilborne Plant Pathogens. 4: 237-249.

Holub, E. & Grau, C. 1990. Specificity of resistance to *Aphanomyces euteiches* in seedling alfalfa. Plant Disease. 74: 164-168.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. & Persson, P. 2012. *Aphanomyces* pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science. 62: 477-487.

Hothorn, T., Bretz, F. & Westfall, P. 2008. Simultaneous inference in general parametric models. Biometrical Journal.

House, G. J. & Parmelee, R. W. 1985. Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. Soil and Tillage Research. 5: 351-360.

Hwang, S., Lopetinskyz, K. & Evans, L. 1991. Effects of seed infection by *Ascochyta* spp., fungicide seed treatment, and cultivar on yield parameters of field pea under field conditions. Canadian Plant Disease Survey. 71: 169-172.

Jee, H.-J., Cho, W.-D. & Kim, C.-H. 2002. Effect of potassium phosphonate on the control of Phytophthora root rot of lettuce in hydroponics. The Plant Pathology Journal. 18: 142-146.

Jin, Y., Szabo, L. J. & Carson, M. 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. Phytopathology. 100: 432-435.

Jones, C., Engel, R. & Olson-Rutz, K. 2019. Soil acidification in the semiarid regions of North America's Great Plains. Crops & Soils. 52: 28-56.

Jørgensen, L. & Olsen, L. 2007. Control of tan spot (*Drechslera tritici-repentis*) using cultivar resistance, tillage methods and fungicides. Crop Protection. 26: 1606-1616.

Joshi, M., Timilsena, Y. & Adhikari, B. 2017. Global production, processing and utilization of lentil: a review. Journal of Integrative Agriculture. 16: 2898-2913.

Karppinen, E. M., Payment, J., Chatterton, S., Bainard, J. D., Hubbard, M., Gan, Y. & Bainard, L. D. 2020. Distribution and abundance of *Aphanomyces euteiches* in agricultural soils: effect of land use type, soil properties, and crop management practices. Applied Soil Ecology. 150: 103470.

Keith, B., Lehnhoff, E., Burns, E., Menalled, F. & Dyer, W. 2015. Characterisation of *Avena fatua* populations with resistance to multiple herbicides. Weed Research. 55: 621-630.

Kjøller, R. & Rosendahl, S. 1998. Enzymatic activity of the mycelium compared with oospore development during infection of pea roots by *Aphanomyces euteiches*. Phytopathology. 88: 992-996.

Kraft, J., Marcinkowska, J. & Muehlbauer, F. 1990. Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet-sieving/baiting technique. Plant Disease. 74: 716-718.

Kraft, J. M. & Pfleger, F. L. 2001. Compendium of pea diseases and pests, American Phytopathological Society (APS Press).

Kumar, S., Barpete, S., Kumar, J., Gupta, P. & Sarker, A. 2013. Global lentil production: constraints and strategies. SATSA Mukhapatra-Annual Technical. 17: 1-13.

Larsson, M. 1994. Pathogenicity, morphology and isozyme variability among isolates of *Aphanomyces* spp. from weeds and various crop plants. Mycological Research. 98: 231-240.

Le May, C., Onfroy, C., Moussart, A., Andrivon, D., Baranger, A., Pilet-Nayel, M. & Vandemark, G. 2018. Genetic structure of *Aphanomyces euteiches* populations sampled from United States and France pea nurseries. European Journal of Plant Pathology. 150: 275-286.

Lenssen, A. W., Sainju, U. M. & Hatfield, P. G. 2013. Integrating sheep grazing into wheat–fallow systems: crop yield and soil properties. Field Crops Research. 146: 75-85.

Levenfors, J. P., Wikström, M., Persson, L. & Gerhardson, B. 2003. Pathogenicity of *Aphanomyces* spp. from different leguminous crops in Sweden. European Journal of Plant Pathology. 109: 535-543.

Lewis, J. & Papavizas, G. 1971. Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. Phytopathology. 61: 208-214.

Li, Y. P., You, M. P. & Barbetti, M. J. 2014. Species of *Pythium* associated with seedling root and hypocotyl disease on common bean (*Phaseolus vulgaris*) in Western Australia. Plant Disease. 98: 1241-1247.

Liebig, M. A., Tanaka, D. & Wienhold, B. J. 2004. Tillage and cropping effects on soil quality indicators in the northern Great Plains. Soil and Tillage Research. 78: 131-141.

Lockwood, J. L. 1960. Pea introductions with partial resistance to Aphanomyces root rot. Phytopathology. 50: 621-4.

Long, J. A., Lawrence, R. L., Miller, P. R. & Marshall, L. A. 2014. Changes in field-level cropping sequences: Indicators of shifting agricultural practices. Agriculture, Ecosystems & Environment. 189: 11-20.

Ma, Y., Marzougui, A., Coyne, C. J., Sankaran, S., Main, D., Porter, L. D., Mugabe, D., Smitchger, J. A., Zhang, C. & Amin, M. 2020. Dissecting the genetic architecture of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. International Journal of Molecular Sciences. 21: 2129.

Mahler, R. & Mcdole, R. 1987. Effect of soil pH on crop yield in northern Idaho. Agronomy Journal. 79: 751-755.

Malvick, D. & Grau, C. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Disease. 85: 740-744.

Malvick, D., Grau, C. & Percich, J. 1998. Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. Mycological Research. 102: 465-475.

Malvick, D. & Percich, J. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Disease. 83: 51-54.

Malvick, D., Percich, J., Pfleger, F., Givens, J. & Williams, J. 1994. Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Disease. 78: 361-365.

Malvick, D. K., Grünwald, N. J. & Dyer, A. T. 2009. Population structure, races, and host range of *Aphanomyces euteiches* from alfalfa production fields in the central USA. European Journal of Plant Pathology. 123: 171.

Matthiessen, J. N. & Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences. 25: 235-265.

Mazerolle, M. J. 2020. AICcmodavg: Model selection and multimodel inference based on AIC. R package version 2.3-1 ed.

Mcgee, R. J., Coyne, C. J., Pilet-Nayel, M.-L., Moussart, A., Tivoli, B., Baranger, A., Hamon, C., Vandemark, G. & Mcphee, K. 2012. Registration of pea germplasm lines partially resistant to *Aphanomyces* root rot for breeding fresh or freezer pea and dry pea types. Journal of Plant Registrations. 6: 203-207.

Mcgee, R. J., Eigenbrode, S., Nelson, H. & Schillinger, W. 2017. Re-inventing Austrian winter pea towards developing food quality winter peas. Crops & Soils. 50: 4-46.

Mckinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Research. 26: 195-217.

Mijangos, I., Pérez, R., Albizu, I. & Garbisu, C. 2006. Effects of fertilization and tillage on soil biological parameters. Enzyme and Microbial Technology. 40: 100-106.

Miller, P. R., Bekkerman, A., Jones, C. A., Burgess, M. H., Holmes, J. A. & Engel, R. E. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. Agronomy Journal. 107: 541-550.

Mitchell, J. E., Balla, H. S. & Yang, G. 1969. An approach to the study of the population dynamics of *Aphanomyces euteiches* in soil. Phytopathology. 59: 206-212.

Mitchell, J. P., Shrestha, A., Mathesius, K., Scow, K. M., Southard, R. J., Haney, R. L., Schmidt, R., Munk, D. S. & Horwath, W. R. 2017. Cover cropping and no-tillage improve soil health in an arid irrigated cropping system in California's San Joaquin Valley, USA. Soil and Tillage Research. 165: 325-335.

Mohebbi, S. & Mahler, R. 1989. The effect of soil pH on wheat and lentils grown on an agriculturally acidified northern Idaho soil under greenhouse conditions. Communications in Soil Science and Plant Analysis. 20: 359-381.

Moussart, A., Even, M.-N., Lesne, A. & Tivoli, B. 2013. Successive legumes tested in a greenhouse crop rotation experiment modify the inoculum potential of soils naturally infested by *Aphanomyces euteiches*. Plant Pathology. 62: 545-551.

Moussart, A., Even, M.-N. & Tivoli, B. 2008. Reaction of genotypes from several species of grain and forage legumes to infection with a French pea isolate of the oomycete *Aphanomyces euteiches*. European Journal of Plant Pathology. 122: 321-333.

Moussart, A., Wicker, E., Le Delliou, B., Abelard, J.-M., Esnault, R., Lemarchand, E., Rouault, F., Le Guennou, F., Pilet-Nayel, M.-L. & Baranger, A. 2009. Spatial distribution of *Aphanomyces euteiches* inoculum in a naturally infested pea field. European Journal of Plant Pathology. 123: 153-158.

Nass 2019a. 2019 Organic Survey. In: AGRICULTURE, U. S. D. (ed.). National Agricultural Statistics Service.

Nass 2019b. National Agricultural Statistics Service, Commodity Statistics. In: AGRICULTURE, U. S. D. O. (ed.).

Navarro, F., Sass, M. E. & Nienhuis, J. 2008. Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Science. 48: 962-972.

Nielsen, D. C., Lyon, D. J., Higgins, R. K., Hergert, G. W., Holman, J. D. & Vigil, M. F. 2016. Cover crop effect on subsequent wheat yield in the central Great Plains. Agronomy Journal. 108: 243-256.

Nielsen, D. C. & Vigil, M. F. 2010. Precipitation storage efficiency during fallow in wheat-fallow systems. Agronomy Journal. 102: 537-543.

Nielsen, D. C., Vigil, M. F. & Benjamin, J. G. 2011. Evaluating decision rules for dryland rotation crop selection. Field Crops Research. 120: 254-261.

Ogle, D., Doll, J., Wheeler, P. & Dinno, A. 2021. FSA: Fisheries Stock Analysis. R package version 0.9.1.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annual Review of Phytopathology. 25: 125-143.

Olsson, Å., Persson, L. & Olsson, S. 2011. Variations in soil characteristics affecting the occurrence of Aphanomyces root rot of sugar beet–risk evaluation and disease control. Soil Biology and Biochemistry. 43: 316-323.

Olsson, Å., Persson, L. & Olsson, S. 2019. Influence of soil characteristics on yield response to lime in sugar beet. Geoderma. 337: 1208-1217.

Papavizas, G. & Davey, C. 1960. Some factors affecting growth of *Aphanomyces euteiches* in synthetic media. American Journal of Botany. 47: 758-765.

Papavizas, G. & Lewis, J. 1971. Effect of amendments and fungicides on Aphanomyces root rot of peas. Phytopathology. 61: 215-220.

Papavizas, G. C. & Ayers, W. A. 1974. Aphanomyces species and their root diseases in pea and sugarbeet. United States Department of Agriculture, Economic Research Service.

Persson, L., Larsson-Wikström, M. & Gerhardson, B. 1999. Assessment of soil suppressiveness to Aphanomyces root rot of pea. Plant Disease. 83: 1108-1112.

Peters, R. & Grau, C. 2002. Inoculation with nonpathogenic *Fusarium solani* increases severity of pea root rot caused by *Aphanomyces euteiches*. Plant Disease. 86: 411-414.

Peterson, G., Halvorson, A., Havlin, J., Jones, O., Lyon, D. & Tanaka, D. 1998. Reduced tillage and increasing cropping intensity in the Great Plains conserves soil C. Soil and Tillage Research. 47: 207-218.

Peterson, G. A., Schlegel, A. J., Tanaka, D. L. & Jones, O. R. 1996. Precipitation use efficiency as affected by cropping and tillage systems. Journal of Production Agriculture. 9: 180-186.

Pfender, W., Delwiche, P., Grau, C. & Hagedorn, D. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Disease. 68: 845-847.

Pfender, W. & Hagedorn, D. 1982. *Aphanomyces euteiches* f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology. 72: 306-310.

Pfender, W. & Hagedorn, D. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology. 73: 1109-1113.

Pfender, W., Rouse, D. & Hagedorn, D. 1981. A "most probable number" method for estimating inoculum density of *Aphanomyces euteiches* in naturally infested soil. Phytopathology. 71: 1169-1172.

Pilet-Nayel, M., Muehlbauer, F., Mcgee, R., Kraft, J., Baranger, A. & Coyne, C. 2002. Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theoretical and Applied Genetics. 106: 28-39.

Pilet-Nayel, M., Muehlbauer, F., Mcgee, R., Kraft, J., Baranger, A. & Coyne, C. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology. 95: 1287-1293.

Poffenbarger, H., Artz, G., Dahlke, G., Edwards, W., Hanna, M., Russell, J., Sellers, H. & Liebman, M. 2017. An economic analysis of integrated crop-livestock systems in Iowa, USA. Agricultural Systems. 157: 51-69.

Prashar, P. & Vandenberg, A. 2017. Genotype-specific responses to the effects of commercial *Trichoderma* formulations in lentil (*Lens culinaris* ssp. *culinaris*) in the presence and absence of the oomycete pathogen *Aphanomyces euteiches*. Biocontrol Science and Technology. 27: 1123-1144.

Quillévéré-Hamard, A., Le Roy, G., Lesné, A., Le May, C. & Pilet-Nayel, M.-L. 2021. Aggressiveness of diverse French *Aphanomyces euteiches* isolates on pea near isogenic lines differing in resistance quantitative trait loci. Phytopathology. 111: 695-702.

R Core Team, R. 2018. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Raaijmakers, J. M. & Mazzola, M. 2016. Soil immune responses. Science. 352: 1392-1393.

Ranabhat, N. 2017. Effect of agronomic practices on disease incidence, severity, and impacts in Montana cropping systems. Master of Science, Montana State University.

Revelle, W. 2022. psych: Procedures for Personality and Psychological Research. 2.2.5 ed. Northwestern University, Evanston, Illinois, USA.

Rice, W., Clayton, G., Olsen, P. & Lupwayi, N. 2000. Rhizobial inoculant formulations and soil pH influence field pea nodulation and nitrogen fixation. Canadian Journal of Soil Science. 80: 395-400.

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., Esker, P. D., Giesler, L. J., Jardine, D. J. & Malvick, D. K. 2017. Oomycete species associated with soybean seedlings in North America—Part II: Diversity and ecology in relation to environmental and edaphic factors. Phytopathology. 107: 293-304.

Saharan, K. & Khetarpaul, N. 1994. Protein quality traits of vegetable and field peas: varietal differences. Plant Foods for Human Nutrition. 45: 11-22.

Schneider, C. 1978. Use of oospore inoculum of *Aphanomyces cochlioides* to initiate black root disease in sugar beet seedlings. Journal of the American Society of Sugar Beet Technologists. 20: 55-62.

Schroeder, K., Okubara, P., Tambong, J., Lévesque, C. & Paulitz, T. 2006. Identification and quantification of pathogenic *Pythium* spp. from soils in eastern Washington using real-time polymerase chain reaction. Phytopathology. 96: 637-647.

Scott, W. W. 1961. A monograph of the genus *Aphanomyces*. Technical Bulletin. Virginia Agricultural Experiment Station. 151.

Sekizaki, H. & Yokosawa, R. 1988. Studies on zoospore-attracting activity. I. Synthesis of isoflavones and their attracting activity to *Aphanomyces euteiches* zoospore. Chemical and Pharmaceutical Bulletin. 36: 4876-4880.

Shaner, G. & Powelson, R. L. 1973. The oversummering and dispersal of inoculum of *Puccinia striiformis* in Oregon. Phytopathology. 63: 13-17.

Shang, H., Grau, C. & Peters, R. 2000. Oospore germination of *Aphanomyces euteiches* in root exudates and on the rhizoplanes of crop plants. Plant Disease. 84: 994-998.

Shekib, L. A., Zoueil, M., Youssef, M. & Mohamed, M. S. 1986. Amino acid composition and *in vitro* digestibility of lentil and rice proteins and their mixture (*Koshary*). Food Chemistry. 20: 61-67.

Sherwood, R. & Hagedorn, D. 1962. Studies on biology of *Aphanomyces euteiches*. Phytopathology. 52: 150-154.

Sivachandra Kumar, N. T., Caudillo-Ruiz, K. B., Chatterton, S. & Banniza, S. 2021. Characterization of *Aphanomyces euteiches* pathotypes infecting peas in Western Canada. Plant Disease. PDIS-04-21-0874-RE.

Smiley, R. W., Machado, S., Gourlie, J. A., Pritchett, L. C., Yan, G. & Jacobsen, E. E. 2013. Effects of crop rotations and tillage on *Pratylenchus* spp. in the semiarid Pacific Northwest United States. Plant Disease. 97: 537-546.

Smýkal, P., Aubert, G., Burstin, J., Coyne, C. J., Ellis, N. T., Flavell, A. J., Ford, R., Hýbl, M., Macas, J. & Neumann, P. 2012. Pea (*Pisum sativum* L.) in the genomic era. Agronomy. 2: 74-115.

Stone, A., Traina, S. & Hoitink, H. 2001. Particulate organic matter composition and Pythium damping-off of cucumber. Soil Science Society of America Journal. 65: 761-770.

Tang, C. & Thomson, B. 1996. Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legume species. Plant and Soil. 186: 321-330.

Thygesen, K., Larsen, J. & Bødker, L. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. European Journal of Plant Pathology. 110: 411-419.

Tofte, J., Smith, R. & Grau, C. 1992. Reaction of red clover to *Aphanomyces euteiches*. Plant Disease.

Trivedi, P., Rochester, I. J., Trivedi, C., Van Nostrand, J. D., Zhou, J., Karunaratne, S., Anderson, I. C. & Singh, B. K. 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. Soil Biology and Biochemistry. 91: 169-181.

Tu, J. 1987. Integrated control of the pea root rot disease complex in Ontario. Plant Disease (USA). 71: 9-13.

Tu, J. C. 1994. Effects of soil compaction, temperature, and moisture on the development of the Fusarium root rot complex of pea in southwestern Ontario. Phytoprotection. 75: 125-131.

Tweedy, B. G. 1981. Inorganic sulfur as a fungicide. Residue Reviews. 78: 43-68.

Upadhaya, A., Yan, G., Pasche, J. & Kalil, A. 2019. Occurrence and distribution of vermiform plant-parasitic nematodes and the relationship with soil factors in field pea (*Pisum sativum*) in North Dakota, USA. Nematology. 21: 445-457.

Van Bruggen, A. H. 1995. Plant disease severity in high-input compared to reduced-input and organic farming systems. Plant Disease. 79: 976-984.

Vandemark, G. & Ariss, J. 2007. Examining interactions between legumes and *Aphanomyces euteiches* with real-time PCR. Australasian Plant Pathology. 36: 102-108.

Vandemark, G., Kraft, J., Larsen, R., Gritsenko, M. & Boge, W. 2000. A PCR-based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. Phytopathology. 90: 1137-1144.

Vandemark, G. & Porter, L. 2010. First report of lentil root rot caused by *Aphanomyces euteiches* in Idaho. Plant Disease. 94: 480-480.

Venable, W. N. & Ripley, B. D. 2002. Modern applied statistics with S. New York: Springer.

Wakelin, S. A., Walter, M., Jaspers, M. & Stewart, A. 2002. Biological control of *Aphanomyces euteiches* root-rot of pea with spore-forming bacteria. Australasian Plant Pathology. 31: 401-407.

Walker, C. A. & Van West, P. 2007. Zoospore development in the oomycetes. Fungal Biology Reviews. 21: 10-18.

Wang, M., Gu, Z., Wang, R., Guo, J., Ling, N., Firbank, L. G. & Guo, S. 2019. Plant primary metabolism regulated by nitrogen contributes to plant–pathogen interactions. Plant and Cell Physiology. 60: 329-342.

Wicker, E., Hullé, M. & Rouxel, F. 2001. Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. Plant Pathology. 50: 433-442.

Wicker, E., Moussart, A., Duparque, M. & Rouxel, F. 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. European Journal of Plant Pathology. 109: 47-60.

Wilkins, R. J. 2008. Eco-efficient approaches to land management: a case for increased integration of crop and animal production systems. Philosophical Transactions of the Royal Society B: Biological Sciences. 363: 517-525.

Williams-Woodward, J. L., Pfleger, F., Fritz, V. A. & Allmaras, R. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Plant and Soil. 188: 43-48.

Williams, J. & Cooper, R. 2004. The oldest fungicide and newest phytoalexin–a reappraisal of the fungitoxicity of elemental sulphur. Plant Pathology. 53: 263-279.

Willsey, T., Chatterton, S., Heynen, M. & Erickson, A. 2018. Detection of interactions between the pea root rot pathogens *Aphanomyces euteiches* and *Fusarium* spp. using a multiplex qPCR assay. Plant Pathology. 67: 1912-1923.

Willsey, T., Patey, J., Vucurevich, C., Chatterton, S. & Carcamo, H. 2021. Evaluation of foliar and seed treatments for integrated management of root rot and pea leaf weevil in field pea and faba bean. Crop Protection. 143: 105538.

Windels, C. 1996. Limitations of a greenhouse assay for determining potential of Aphanomyces root rot in sugarbeet fields. Journal of Sugar Beet Research. 33: 1-13.

Wu, L., Chang, K.-F., Conner, R. L., Strelkov, S., Fredua-Agyeman, R., Hwang, S.-F. & Feindel, D. 2018. *Aphanomyces euteiches*: a threat to Canadian field pea production. Engineering. 4: 542-551.

Wu, L., Chang, K.-F., Hwang, S.-F., Conner, R., Fredua-Agyeman, R., Feindel, D. & Strelkov, S. E. 2019. Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. The Crop Journal. 7: 38-48.

Xue, A. 2003a. Efficacy of *Clonostachys rosea* strain ACM941 and fungicide seed treatments for controlling the root rot complex of field pea. Canadian Journal of Plant Science. 83: 519-524.

Xue, A. G. 2003b. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. Phytopathology. 93: 329-335.

Yokosawa, R., Kuninaga, S., Sakushima, A. & Sekizaki, H. 1995. Induction of oospore formation of *Aphanomyces euteiches* Drechsler by calcium ion. Japanese Journal of Phytopathology. 61: 434-438.

Zhao, X., He, C., Liu, W. S., Liu, W. X., Liu, Q. Y., Bai, W., Li, L. J., Lal, R. & Zhang, H. L. 2022. Responses of soil pH to no-till and the factors affecting it: A global meta-analysis. Global Change Biology. 28: 154-166.

Zhou, Y., Zhu, H., Fu, S. & Yao, Q. 2017. Variation in soil microbial community structure associated with different legume species is greater than that associated with different grass species. Frontiers in Microbiology. 8: 1007.

Zitnick-Anderson, K., Gargouri Jbir, T., Carlson, A., Postovit, S., Pasche, J. & Kalil, A. 2021a. *Fusarium* species associated with root rot of lentil (*Lens culinaris*) in North Dakota. Plant Health Progress.

Zitnick-Anderson, K., Luis, E., Forster, S. & Pasche, J. S. 2020. Associations among the communities of soil-borne pathogens, soil edaphic properties and disease incidence in the field pea root rot complex. Plant and Soil. 457: 339-354.

Zitnick-Anderson, K. & Pasche, J. 2016. First report of Aphanomyces root rot caused by *Aphanomyces euteiches* on field pea in North Dakota. Plant Disease. 100: 522.

Zitnick-Anderson, K., Porter, L. D., Hanson, L. E. & Pasche, J. S. 2021b. Identification, laboratory, greenhouse, and field handling of *Aphanomyces euteiches* on pea (*Pisum sativum*). Plant Health Progress. PHP-01-21-0011-FI.

APPENDICES

APPENDIX A

ADDITIONAL FIELDS SAMPLED

Field 5, located in Daniels County, had notable waterlogging and compaction issues at the field entryway. The producer had reported root rot issues on dry pea in years prior to the year of sampling. Field transects displayed mild root rot, with some quadrats free of disease based on soil bioassays and PCR testing. Two quadrats in entrance line one had moderate root rot on dry pea (Table A.01). The low spot quadrats had very mild root rot (Table A.02).

Table A.01. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 5 sampled in 2018 in Daniels County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	2	0.7	1	0.1	0	0.1
20	1	0.0	1	0.1	1	0.1
30	2	0.1	1	0.1	1	0.1
40	2	0.0	1	0.1	1	0.1
50	1	0.8	1	0.1	0	0.1
60	2	0.3	1	0.1	1	0.1
70	3	0.4	1	0.2	1	0.1
80	1	0.3	0	0.1	1	0.1
90	1	0.0	0	0.1	1	0.1
100	3	0.0	0	0.1	0	0.1

Table A.02. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 5 sampled in 2018 in Daniels County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	1	0.2
2	10	1	0.1
3	0	2	0.1
4	10	1	0.1
5	20	1	0.1

Fields 6 and 7 were neighboring fields sampled in Valley County, with different crop rotation management. Field 6 was sown to dry pea more frequently than Field 7, and the latter had a creek waterway with runoff where the low spot transect was sampled from. Although the producer had witnessed root rot on dry pea and lentil in previous years, and the presence of *A. euteiches* was confirmed with PCR, disease severity was low, with only two quadrats in the entryway of Field 6 showing moderate disease severity in the greenhouse bioassay (Table A.03). The low spot in Field 6 was dry at the time of sampling, and root rot severity was minimal (Table A.04).

Table A.03. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 6 sampled in 2018 in Valley County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	1	0.1	1	0.4	0	0.0
20	1	0.1	0	0.2	1	0.0
30	1	0.0	1	0.2	0	0.0
40	1	0.2	1	0.2	2	0.2
50	1	0.0	1	0.1	1	0.6
60	2	0.4	0	0.1	3	0.3
70	2	0.3	3	0.6	1	0.4
80	0	0.2	1	0.1	1	0.2
90	0	0.5	1	0.1	2	0.6
100	1	0.3	1	0.1	0	0.2

Table A.04. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 6 sampled in 2018 in Valley County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	1	0.2
2	10	2	0.3
3	0	1	0.2
4	10	2	0.3
5	20	0	0.1

Field 7 had little to no root rot disease in the entrance lines (Table A.05), however there were two quadrat hotspots with moderate root rot severity on the edge of the low spot transect (Table A.06).

Table A.05. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 7 in 2018 in Valley County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	0	0.2	0	0.2	1	0.1
20	1	0.1	1	0.1	0	0.1
30	1	0.2	0	0.1	1	0.1
40	0	0.2	1	0.1	1	0.1
50	0	0.1	1	0.1	1	0.1
60	1	0.1	0	0.2	1	0.1
70	1	0.2	1	0.1	1	0.1
80	1	0.1	1	0.1	0	0.1
90	1	0.2	0	0.1	1	0.2
100	0	0.1	1	0.1	1	0.2

Table A.06. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 7 sampled in 2018 in Valley County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	3	0.3
2	10	1	0.2
3	0	1	0.2
4	10	2	0.3
5	20	3	0.3

Field 8, located in Sheridan County, had only one entrance line quadrat, and one low spot quadrat with moderate Aphanomyces root rot. Sixty percent of transects had no detectable root rot, confirmed with PCR (Table A.07, A.08).

Table A.07. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 8 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	0	0.1	1	0.2	1	0.2
20	0	0.1	0	0.1	1	0.1
30	0	0.1	1	0.2	1	0.2
40	0	0.1	0	0.1	1	0.1
50	0	0.1	0	0.1	0	0.1
60	2	0.2	0	0.1	0	0.2
70	1	0.2	0	0.1	0	0.1
80	0	0.1	3	0.2	0	0.2
90	0	0.1	1	0.2	0	0.1
100	0	0.2	0	0.1	0	0.2

Table A.08. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 8 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	0	0.1
2	10	2	0.3
3	0	2	0.2
4	10	3	0.2
5	20	0	0.1

Field 9 sampled in Sheridan County had minimal root rot, with the only quadrat with a moderate DSS of 3 in the low spot transect line (Table A.09, A.10).

Table A.09. *Aphanomyces euteiches* median disease severity scores of entrance line transects of Field 9 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Distance from entrance (m)	DSS Median Line 1	DSS SE	DSS Median Line 2	DSS SE	DSS Median Line 3	DSS SE
10	0	0.2	1	0.2	1	0.1
20	2	0.3	1	0.2	0	0.2
30	0	0.3	1	0.2	1	0.2
40	1	0.2	1	0.2	1	0.2
50	2	0.2	1	0.2	1	0.2
60	0	0.1	1	0.1	1	0.2
70	1	0.3	1	0.1	1	0.3
80	1	0.2	0	0.1	0	0.2
90	0	0.2	1	0.1	1	0.2
100	1	0.2	2	0.3	1	0.1

Table A.10. *Aphanomyces euteiches* median disease severity scores of low spot transects of Field 9 sampled in 2019 in Sheridan County, Montana, as measured by a greenhouse bioassay

Low spot quadrat #	Distance from low spot center (m)	DSS Median	DSS SE
1	20	1	0.3
2	10	1	0.2
3	0	3	0.2
4	10	2	0.3
5	20	1	0.2

Three fields were sampled in 2020, with reduced sampling due to extremely dry soil conditions and restrictions on travel. Moderate to severe root rot was found in the entrance samplings of the three fields sampled in 2020, except for one quadrat in Field 10, with only slight root discoloration in the soil bioassay (Table A.11). Low spot transects of the three fields sampled were highly variable, Field 11 soil samples exhibited slight to moderate root rot symptoms on dry pea, and the other two fields had barely detectable root rot (Table A.12).

Table A.11. *Aphanomyces euteiches* median disease severity scores as measured by a greenhouse bioassay of entrance line transects of Field 10 in Daniels County, Field 11 in Sheridan County, and Field 12 in Valley County, sampled in 2020.

Distance from entrance (m)	DSS Median Field 10	DSS SE	DSS Median Field 11	DSS SE	DSS Median Field 12	DSS SE
10	3	0.2	4	0.5	3	0.9
20	4	0.3	3	0.5	4	0.3
30	4	0.3	3	0.6	4	0.0
40	4	0.5	3	0.3	4	0.3
50	1	0.2	3	0.3	4	0.3

Table A.12. *Aphanomyces euteiches* median disease severity scores as measured by a greenhouse bioassay of low spot transects of Field 10 in Daniels County, Field 11 in Sheridan County, and Field 12 in Valley County sampled in 2020.

Low spot quadrat #	Distance from low spot center (m)	DSS Median Field 10	DSS Median Field 11	DSS Median Field 12
1	20	1	3	0
2	10	0	1	1
3	0	0	3	0
4	10	0	3	0
5	20	0	2	0

A greenhouse trial was conducted, where the impact of growing oat between rounds of dry pea (dry pea-oat-dry pea) and rounds of lentil (dry pea-lentil-dry pea) was analyzed. Oat reduced disease severity on both dry pea and lentil from severe root rot to mild or moderate root rot ($P = 0.03$, Fig. A.01).

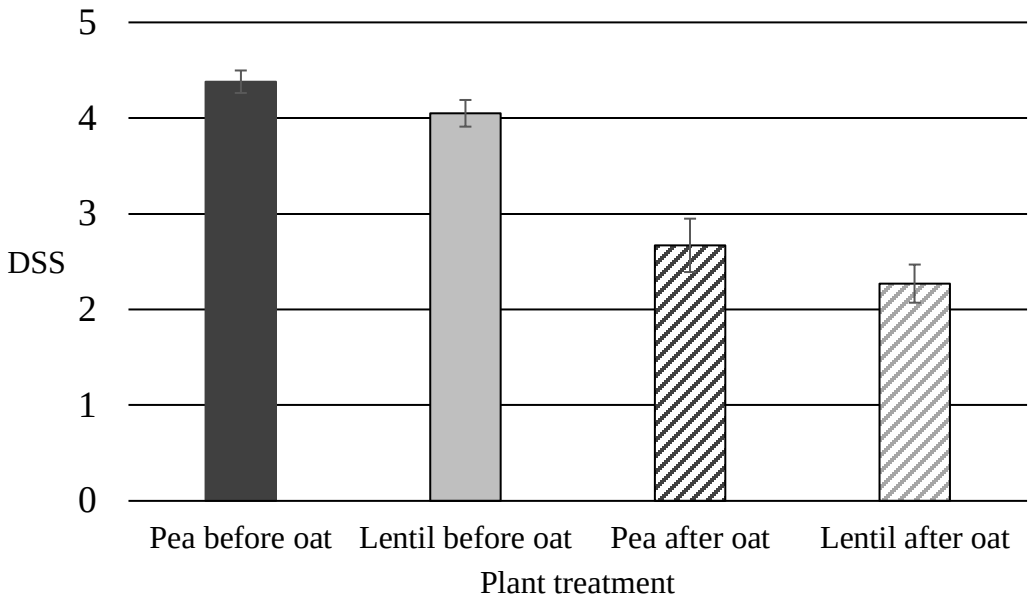


Figure A.01. Average disease severity score of *A. euteiches* on dry pea and lentil bait plants before and after growing oats in the greenhouse. Error bars are standard error of the mean. The filled dark and light gray bars are disease scores on dry pea and lentil plants before oats were grown, respectively. The hatched dark and light gray bars are disease scores on dry pea and lentil bait plants after oats were grown, respectively.