ROLE OF CROP FERTILITY AND SEED TREATMENTS IN MANAGING FUSARIUM ROOT ROT OF LENTIL (*LENS CULINARIS* MEDIKUS) IN THE NORTHERN GREAT PLAINS

by

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DEDICATION

I would like to dedicate this thesis my parents, who had always fostered me to grow and challenge myself. The support of my friends and family gave me the confidence to go to graduate school. I would also like to dedicate this to all the pulse growers out there. I hope this research provides some insight, and key management tools that could be used to improve lentil crop management.

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ABSTRACT

Lentil is a relatively new but economically important crop for the state of Montana, along with surrounding states in the northern Great Plains. Comparatively little is known about the basic fertility of lentil, and importance of inoculant type on lentil. Additionally, the rise of pulse crop acres in the northern Great Plains, has given rise to root rot pathogens, such as Fusarium root rot. Fusarium root rot of pulses, has a wide host range, limiting the efficacy of rotation in its management. This research is comprised of two main studies. Field trials occurred at sites in Bozeman, Havre, Moccasin, and Sidney in 2019 and 2020.

The objective of chapter two was to evaluate the effect of rhizobial inoculant formulations (granular vs. seed-coat/peat-powder) and nutrient additions (potassium, sulfur, and a micronutrient fertilizer), on lentil establishment, growth, seed protein, and yield. For chapter two, in six of eight site-years there was no yield difference between inoculant types. Applications of sulfur (S) fertilizer increased yield at three of eight site-years by an average of 303 kg ha^{-1} (17%) compared to treatments without S. Results from this study further suggest the importance of S fertilization for lentil. The objective for chapter three was to evaluate seed treatments' ability to control Fusarium root rot on lentil establishment, growth, disease severity and yield. In three of eight site-years, the inoculated control had a relatively high disease severity compared to other seed treatments. In general, treatment responses varied across site-year due to low disease pressure. Additionally, *F. graminearum* and *F. oxysporum* were isolated at a high frequency from control plots at sites in 2019. Data from 2020 is pending.

CHAPTER ONE

LITERATURE REVIEW

Adaptation, Economic and Ecological Importance of Pulse Crops in the NGP

A legume refers to any plant from the Fabaceae family (Chan, 2021). Pulses are a subgroup of legumes that are harvested for dry edible seed. Pulses include crops such as chickpea, dry bean, dry pea, faba bean and lentil (Hall et al., 2017). Pulses improve global food security challenges by providing a shelf table, sustainable source of protein for developing countries (Xipsiti et al., 2017). The majority of pulse crops harvested have been exported to developing countries as a staple protein source for human consumption (Considine et al., 2017; Joshi & Rao, 2017). The nutritional value of pulses has led to an increase in production of pulses intended for domestic consumption including hummus, pet food, alternative meat products, and protein supplementation of processed snack foods.

Pulses are well adapted to arid and semi-arid regions, as they originated in the Middle East (Ladizinsky, 1987). Now pulses are commercially produced in other countries such as Argentina, Australia, Brazil, Canada, China, France, Russia, and the USA. A critical challenge for dryland farming systems in the semi-arid northern Great Plains (NGP) is the variability of spring precipitation and snow melt (Whitlock et al., 2017). Summer fallow has traditionally been practiced in this region to preserve moisture in the soil profile (Tanaka et al., 2010). Summer fallow area in the NGP peaked in 1971 (Tanaka et al., 2010); however, due to several agronomic and economic factors, the area in fallow has since steadily decreased (Carlyle, 1997). Studies with pulses in the cropping rotation in the NGP reported important increases in overall profit compared with fallow-wheat systems (Zentner et al., 2001; Burgess et al., 2012; Miller et al., 2015). The integration of pulse crops into a crop rotation by farmers in the NGP can protect wheat yield and protein levels under variable N management and reduce exposure to economic variability (Miller et al., 2015). Various research efforts with pulses allowed farmers to adopt a different cropping system in Montana replacing fallow with pulse crops (Long et al., 2014).

While many grain producers in the NGP have included pulse crops in their cropping systems due to increased farm-scale economic security, the use of pulse crop species in dryland farming systems has positively benefited field-scale environmental parameters such as soil fertility (Grant et al., 2002). Other environmental benefits of pulses include decreasing greenhouse gas emissions as a result of a decrease in fertilizer use associated with pulse crops being used in the rotation (Lemke et al., 2007; MacWilliam et al., 2014). In addition to their grain value, and contributing to soil nitrogen, pulse crops increase cropping system water use efficiency, and yield of subsequent crops due to both nitrogen and non-nitrogen benefits (e.g. disease breaks, less deep water use) (Miller et al., 2002; Miller et al., 2003; Lupwayi & Kennedy, 2007; Kirkegaard et al., 2008)

Lentil

Domestication, Uses, and Economic Importance of Lentil

Lentil (*Lens culinaris* Medikus) is one of the oldest cultivated pulse crop species (Sandhu & Singh, 2007). Lentil is produced in over 50 countries, with the largest 2018 production (Mg ha^{-1}) in Canada (1,897,954), India (1,471,281), USA (345,982), Australia (325,965), and Turkey (320,236) (FAOSTAT, 2019). India accounts for a quarter of the lentil production worldwide, but usually must import lentils to meet domestic demand. Canada is the largest global lentil exporter in the world with the majority of the production occurring in Saskatchewan (Singh & Singh, 2014). Due to increased global consumption of lentil, total planted area across the globe has steadily increased (Erskine et al., 2011; Lal, 2017).

Lentil is a vital source of protein (Erskine et al., 2011) and contains additional health properties to improve food nutritional quality (Warne et al., 2019). There have been various studies throughout the world that have shown that lentil contain anticarcinogenic properties (Ragg et al., 2006), and have the potential to lower blood pressure, reduce risk of coronary heart disease, type 2 diabetes, and obesity (Faris et al., 2013; Lakkakula et al., 2017). Consumer preference is also shifting towards plant-based protein sources like lentil in the USA (Lakkakula et al., 2017).

In North America, lentil is a commercially important crop in Montana, Idaho, North Dakota, and Washington (USDA-NASS, 2020b), along with the prairie region in Canada including southern Saskatchewan, and southern Alberta and Manitoba in Canada (Agriculture and Agri-Food Canada, 2020). In Montana, lentil production increased at an average annualized rate of 20% from 1998 to 2012. In 2012, Montana was the largest lentil-producing state based on area planted and total grain harvested, accounting for 38% of U.S. lentil grain production (Janzen et al., 2014). Since then, the area planted to lentil in Montana peaked in 2017, with the majority of lentil production in northeastern Montana (Figure 1-1) (USDA-NASS, 2019). After 2017, U.S. lentil production decreased due to a prolonged softening of international export markets related to trade policies in India (Branson, 2017).

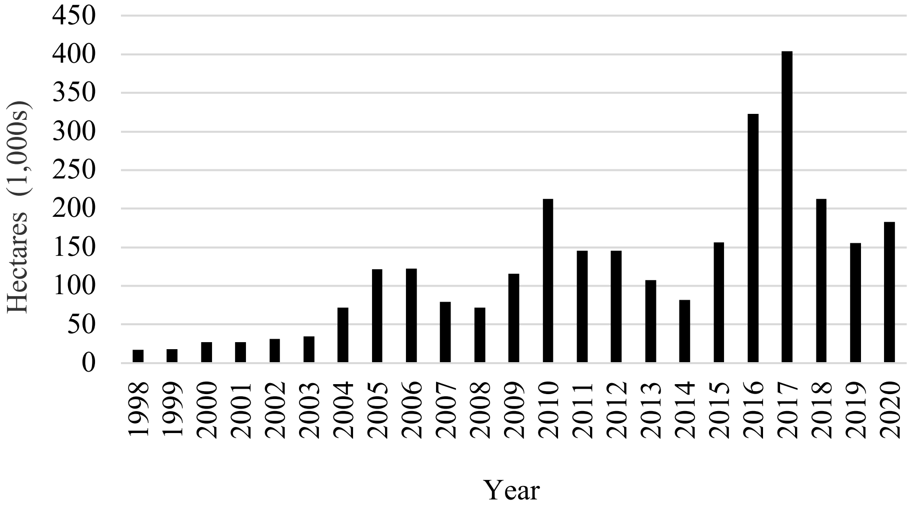

Figure 1-1. Lentil hectares planted in the northern Great Plains from 1998 to 2020 – Montana and North Dakota (USDA-NASS, 2019)

Lentil Crop Characteristics, and Management

Lentil is an annual cool season legume with moderate resistance to drought and high temperature stress (Sita et al., 2017). Drought (water deficit) and heat (high air temperature) stresses are major abiotic factors that influence the productivity of lentil throughout the world (Johansen et al., 1994; Sehgal et al., 2018). Miller et al. (2002) compared lentil yields from locations across the NGP in relation to total rainfall during the growing season and average daily maximum temperature. Lentil yield had no relationships with either rainfall or average daily maximum temperature, suggesting lentil are well adapted to the dryland conditions in this region. Lentil, like most legumes, can have a poor tolerance for high temperatures during flowering and early pod set (Erskine et al., 1993). Heat stress, when linked to low moisture during the flowering and pod fill stage, can drastically reduce seed yield due to injury of the reproductive organs and accelerated development/shortened reproductive period throughout seed maturation (Siddique et al., 2003).

The rooting depth of lentil was observed to be less than 0.9 m in a multi-year study at Bozeman, MT, in deep silt loam soil (Miller & Holmes, 2012), agreeing with earlier research. At Swift Current, SK, on silty clay loam soils, lentil had a relatively high-water use efficiency with ability to utilize soil water in the upper 0.9 m (Angadi et al., 2008). This relatively shallow effective rooting depth can conserve soil water at depth for a subsequent crop, and plant decomposition can increase soil available nitrogen (Miller et al., 2003). Lentil can add 10-15 kg ha^{-1} of nitrogen mineralized over the course of the next growing season (i.e. ‘nitrogen credit’) (Miller et al., 2015). In the NGP, lentil is sown in April to early May. Early seeding will increase the height and size of the plant at first bloom, while planting after April can lower grain quality (e.g. seed size) and seed yield (Miller et al., 2006).

Lentil is suitable for planting in no-till systems in the NGP. Cutforth et al. (2002) found that lentil yields increased as wheat stubble height increased in southern Saskatchewan. Lentil is short (25-50 cm); therefore land rolling after planting is a common practice to push rocks down and improve harvestability (Miller et al., 2002). The optimal timing for lentil rolling has never been reported in no-till systems, but current research underway in MT indicates lentil yield is insensitive to roll timing until the 10-lf stage or later (pers. comm., P. Miller).

Lentil Nutritional Content

With respect to lentil grain, nutritional quality and consumption is important (Warne et al., 2019). Lentil is a great source of protein, fiber, carbohydrates, fats, and essential minerals (Meiners et al., 1976; Urbano et al., 2007). Grusak (2009) reviewed 17 publications regarding lentil seed protein content and found that lentil grain averaged 28.3% protein and ranged from 15.9 to 31.3%. Several studies have been conducted to better understand factors that influence lentil crude protein concentration. Huang et al. (2016) evaluated the response of lentil to commercial peat-powder rhizobia inoculant and starter N fertilizers (urea vs. environmentally safe nitrogen ESN) in field conditions in central Montana. Compared to the non-inoculated control, the application of rhizobia inoculant plus urea and ESN enhanced lentil protein content by 34%. However, the effect of different rhizobial inoculant types on lentil protein content has not been investigated. Wang and Daun (2006) collected lentil samples from the Canadian Grain Commission survey of commercially grown lentil, and found that variation in crude protein content was due to a combination of genetic (e.g. variety) and environmental factors. Environmental factors such as drought (Tao et al., 2017; Bestwick et al., 2018b), and agronomic factors such as crop fertility drive differences in pea protein (McLean et al., 1974). Bestwick et al. (2018b) found that pea protein content in the NGP was increased by granular rhizobia inoculant formulations, compared with seed coated inoculants, and by sulfur (S) in dry years, yet work on lentil is more limited.

Pulse Crop Nutrient Management

Nitrogen Fixation and Rhizobia Inoculants

The symbiotic relationship between *Rhizobium* bacteria and legumes help the plant meet 80 – 90% of N requirements by fixing atmospheric dinitrogen (N_2) gas through biological N fixation (BNF) into a plant-usable form, ammonia (NH_3) (Bohlool et al., 1992). Factors that limit rhizobial survival and activity include low pH soil, nutrient availability, issues with inoculation source, or an increase in the concentration of soil nitrate due to excessive tillage, extended fallow, and synthetic nitrogen fertilizer (Peoples et al., 2009). Understanding the conditions that benefit the *Rhizobium* bacteria will directly benefit BNF and plant growth (Graham & Vance, 2003).

The increase in pulse acreage in the NGP has led to reassessments of inoculant formulations and methods of delivery for nitrogen fixing bacteria (Lupwayi & Kennedy, 2007). Inoculation is the process of introducing a rhizobial bacteria strain appropriate for the specific crop to infect the legume roots to create nodules that facilitate nitrogen fixation. The benefit of promoting BNF in grain and forage legumes is the enhancement of yield potential without the use of nitrogen fertilizers (Stephens & Rask, 2000). Differences in crop genotype, rhizobial strain, inoculant formulation, inoculation rate, and growing season conditions of the crop affect nodulation and N-fixation (Lupwayi et al., 2006; Abi-Ghanem et al., 2011). Abi-Ghanem et al. (2011) tested nine strains of *Rhizobium leguminosarum* bv. *viciae* with five different lentil varieties in the greenhouse and found that total BNF was influenced by both variety and rhizobial strain. Rhizobial inoculants have the potential to increase nodule number, N accumulation in biomass, and N-fixation of dry pea (Clayton et al., 2004). However, yield response to inoculation is not consistent. In a review of field studies in the Canadian provinces, Vessey (2004) reported that lentil had a positive yield response to rhizobia inoculant in 16 of 34 site-years. In fields that have grown the same legume, populations of rhizobia in the soil (e.g. native and naturalized strains) rhizobia can survive in the soil for several years after harvest of a legume crop. Kucey and Hynes (1989) showed bean and pea rhizobia can survive in the soil at least five years after planting. However, rhizobia concentrations in the soil generally decline over time by two orders of magnitude, due to the introduced rhizobia strains not being competitive as other native bacteria in the soil (Kucey & Hynes, 1989). Chemining'wa and Vessey (2006) conducted a multiple site study in the eastern Canadian prairie. Study sites had either grown lentil or pea in the past 10 years or had not been cultivated for 25-50 years. Uninoculated pea without fertilizer nitrogen were used to assess the presence of infective native strains of *Rhizobium leguminosarum* bv. *viciae* (Rlv). Chemining'wa and Vessey (2006) found pea rhizobia in fields that had not grown inoculated pea crops for up to 25 years. McKenzie et al. (2001c) assessed four pea cultivars, rhizobia inoculation, and starter fertilizer N across 58 field trials in Alberta from 1995 to 1998 and discovered that inoculation slightly increased yield by an average of 14% in 40% of field trials. In addition to this finding, the frequency of inoculation benefit was slightly higher (48% vs. 38%) on land with no history of legumes than on land that had grown a legume, but the average inoculation benefit was greater (19% vs. 5%) on land with no history of legumes. Furthermore, McKenzie et al. (2001c) found that when spring soil nitrate nitrogen (NO_3-N) at a soil depth of 30 cm was less than 20 kg N ha^{-1}, the application of N fertilizer (20, 40, or 60 kg ha^{-1}) increased pea yield in 33% of the trials, by an average of 11%. Modest benefits of rhizobial inoculation were attributed to adequate nodulation of pea by indigenous rhizobia populations in the soil.

Inoculant Types

Inoculants are commercially available in the three forms; granular, powder, and liquid (Stephens & Rask, 2000). These inoculants can either be applied directly to the seed or applied in the seed furrow. Each inoculant type has advantages and disadvantages (Table 1-1) (Stephens & Rask, 2000; Bashan et al., 2014). The carrier type is referring to the abiotic substrate (solid, liquid, or gel) in the form of peat, clay, or water solution. Some seed-applied fungicides or insecticides can be toxic to rhizobia in slurries inoculated on the seed, whereas granules are unlikely to be affected by chemicals applied to the seed-coat (Denton et al., 2009). The physical separation of the seed and in-furrow granular inoculants means that the inoculant can be supplied with minimal concern for seed-applied chemicals (Stephens & Rask, 2000). Seed coat inoculants are applied to the seed prior to sowing and either require a nontoxic sticking agent or a static charge to ensure adhesion to the seed surface. Conversely, soil-applied inoculants are applied in the seed furrow as granules during seeding.

Table 1-1. Rhizobium inoculant types and characteristics

Formula type	Granular	Powder	Liquid
Application:	Soil	Seed	Seed
Carrier type:	Peat or clay	Peat or clay	Water solution
Survivability in adverse conditions:	Best	Good	Poor
Frequency used:	Least used	Most used	Most used
Cost:	Most expensive	Inexpensive	Inexpensive

Note. Table made from literary work of (Deaker et al., 2004; Jones & Olson-Rutz, 2018).

Several studies have been conducted to assess the influence of different rhizobia inoculant formulations on dry pea. In Alberta, Clayton et al. (2004) assessed inoculant types (e.g. peat-based granules and seed-coatings and liquid inoculants). Seed yield, seed protein, nodule number, and N-fixation was greater in the order: granular > peat power > liquid = uninoculated control. Clayton et al. (2004) also compared different starter N fertilizer rates and found that fertilizer N application rates 20 and 40 kg N ha^{-1} had no effect on biomass N, indicating starter N was not necessary, whereas application rates greater than 40 kg ha^{-1} reduced nodulation. In Montana and North Dakota, granular inoculants also produced higher pea seed protein content compared to seed applied peat-powder under moderate drought (Bestwick et al., 2018b). Rice et al. (2000) compared the ability of liquid inoculant applied to the seed, powder inoculant applied to the seed, and granular in a band to establish nodules at soil pH levels of 4.4, 5.4, and 6.6. Granular inoculant was successful at establishing nodules at soil pH levels of 4.4 and had greater N-fixation and biomass compared to other formations at soil pH of 5.4. In Montana, soil acidification is becoming a constraint to field crop production in Montana, caused by high application of urea nitrogen fertilizers (Jones, 2019). In Montana, in 2020, 24 of 56 counties have been identified with soil pH below 5.5, with some fields as low as 3.8 (Jones, unpub). A decrease in soil pH increases hydrogen ion (H^+) concentrations in the soil and increases the solubility of toxic metal ions such as Al^{3+}, and Mn^{2+} leading to inhibited nodule growth (Ferguson et al., 2013). Granular soil inoculant can promote rhizobia populations throughout the root, whereas seed-coat inoculants tend to form nodules in clusters near the cotyledon (Rice et al., 2000). Spring soil nitrate levels and cropping history are important factors to consider to achieve the benefits of inoculation. McKenzie et al. (2001c) assessed 22 fields in Alberta and found that inoculation was more likely to boost protein of pea on fields with no pulse crop history than those with crop history and when spring soil nitrate-N levels are less than 20 kg N ha^{-1}.

Nodulation Failure

Nodulation failure can occur from environmental factors. For example, the success of inoculation can depend on the ratio of the inoculant cells to indigenous rhizobia (Vlassak et al., 1997), which may also be competitive to inoculated rhizobia populations (Chemining'wa & Vessey, 2006). Along with indigenous rhizobia population, the BNF relationship and survival of the rhizobia on the seed may decrease due to environmental conditions. The main environmental factors that will affect rhizobia survival in the soil include high air temperatures (tropical regions), low temperature (temperate regions), soil acidity and low soil moisture (Lupwayi et al., 2006). Tillage operations and inconsistent spring moisture can cause warm and dry soils leading to rhizobia desiccation, decreasing the survival before the chance of infection (Torabian et al., 2019). Dry seedbeds are a common occurrence in the NGP but their effect on nodulation has not been assessed here. Water is required to export N products from the nodules to the rest of the plant; when nodule water supply decreases, N products build up in the nodule and inhibit further N-fixation (Serraj et al., 1999; Kasper et al., 2019).

Nitrogen Management

Lentil require optimal amounts of nutrients to obtain high grain yield and protein content. Even though legumes can fix their own nitrogen, soil nitrate is required to promote sufficient seedling growth for the symbiosis to initiate. Several studies focused on starter nitrogen rates and inoculant types to better understand effects on seed protein content and yield. In general, starter N does not consistently increase protein content of pea and the use of granular inoculants can provide adequate nitrogen supply to meet protein and yield potential under normal soil conditions (McKenzie et al., 2001c; Clayton et al., 2004). However, if nodulation fails, applying sufficient N fertilizer within six weeks after seeding may secure pulse crop yield (McConnell et al., 2002).

Phosphorus, Potassium, Sulfur and Micronutrient Deficiencies and Management

Plants involved in BNF are particularly sensitive to phosphorus (P), potassium (K), and sulfur (S) deficiencies. Maximum nitrogen uptake occurs during the branching and early bud formation stage of lentil, therefore early growing season availability of P and K are critical for incorporation into plant biomass and to ensure early N-fixation (Malhi et al., 2007; Jones et al., 2011).These nutrients affect nitrogen fixation by modulating growth of rhizobia, nodule formation and functions, or indirectly by affecting the growth of the host plant; however, several processes and mechanisms still remain uncertain (Divito & Sadras, 2014).

Phosphorus. Phosphorus is used in molecular and biochemical plant processes, particularly in the energy transformation in nodules (Mitran et al., 2018). Nodules themselves are strong sinks for phosphorus. Thus, nodulation and N-fixation are strongly influenced by P availability (Hart, 1989; Weisany et al., 2013). Plants that are deficient in P produce less green leaf area and biomass due to impaired shoot metabolism (Jakobsen, 1985). Phosphorus is a mobile nutrient in the plant affecting older/lower leaves. McKenzie et al. (2001b) found triple superphosphate (TSP) in pea increased yield by 52% in Alberta when soil test P levels were less than 30 kg P ha^{-1}. A starter application of triple superphosphate at the rate of 13.1 kg P ha^{-1} was sufficient to achieve maximum yield and protein content. A study in Alberta had an increase in dry pea yield in 50% of field trials with five different application rates of TSP ranging from 1 to 26 kg P ha^{-1} (Karamanos et al., 2003). Notably, the application of 19.5 kg P ha^{-1} for all soil types increased maximum average yield by 438 kg ha^{-1} in soils with $\leq$ 10 mg of Modified Kelowna (MK) extractable P kg^{-1} (Karamanos et al., 2003). A yield response with 19.5 kg P ha^{-1}

was higher on loam (535 kg ha^{-1}) than clay loam soils (285 kg ha^{-1}) (Karamanos et al., 2003). In low Olsen P soils ($\leq$ 12 ppm) of central MT, 33.6 kg P_2O_5 ha^{-1} improved grain yield of dry pea, lentil, and chickpea (Chen et al., 2006).

<u>Potassium</u>. There are few reports that detail lentil response to K fertilizer on lentil. Potassium plays a vital role in water uptake by plant roots, photosynthesis, assimilation transport and enzyme activation (Pettigrew, 2008). Potassium is a mobile nutrient in the plant with deficiency symptoms of chlorosis that are more localized with symptoms of burning on the leaf margin. Pulse crops remove more than twice the amount of K concentration per unit of grain from the soil than wheat (12 g kg^{-1} and 3.7 g kg^{-1}), respectively (Jacobsen et al., 2003). Montana soils generally have high K, often exceeding 250 ppm, therefore the need for K applications may be minimal. Pea yield or protein limitation by K deficiency has not been documented in Montana (Jones & Olson-Rutz, 2018).

<u>Sulfur</u>. Sulfur is important for nitrogen uptake and protein production. If the plant cannot form protein due to low S, nodules will not be stimulated to fix nitrogen (Scherer, 2008). Sulfur is an immobile nutrient in the plant often affecting newer or younger leaves with symptoms sometimes localized. Deficiency symptoms include upper leaves turning light to yellow green with small and thin stems. In Montana, pulse crops remove almost twice the amount of S concentration per unit of harvested grain (2.49 g kg^{-1}) than wheat (1.35 g kg^{-1}) (Jacobsen et al., 2003). Sulfur deficiencies are common in soils with low organic matter that will not release significant amounts of S over the growing season through mineralization (Harward & Reisenauer, 1966; Rehm & Caldwell, 1968). Deficiencies are also common in coarse soils where S has leached out from the rooting zone over time, and is more common in coarse textured soils (Sawyer & Barker, 2002; Mascagni Jr et al., 2008).

Responses to S fertilization on pulses in the northern Great Plains has been limited. However, Bestwick et al. (2018a) found fertilizer S increased pea protein content by 0.8% units in samples from Montana and North Dakota under moderate drought conditions. In a northern Idaho fertilizer guide for lentil, Mahler (2005) recommends soils testing less than 4 ppm S should receive 16.8 kg S ha^{-1} but the research basis for this was not presented. Responses to S is inconsistent due to highly variable concentrations of gypsum (calcium sulfate) below typical soil sampling depths (Jones & Dinkins, 2019). Sulfur is released from soil organic matter or gypsum (calcium sulfate). When soil SO_4-S is tested in a lab, gypsum dissolves in saturated solution but not necessarily in dry soils in the field. In addition, since SOM provides a major source of S through mineralization especially in higher SOM soils, standard sulfate-S tests that generally do not involve an incubation can underestimate true growing season availability. This makes standard soil tests generally an unreliable predictor of S needs in the state of Montana. Other studies have found inconsistencies with soil sulfate testing and grain yield. In Iowa, corn showed no response to S fertilizer despite having low soil extractable sulfate-S levels (Sawyer & Barker, 2002). Therefore, crop S removal rates, and tissue concentrations can be used in combination to evaluate the need for S fertilization (Jones & Olson-Rutz, 2018).

Micronutrients. Micronutrients are necessary for plant growth and N-fixation. An adequate supply of micronutrients is especially important in low fertile soils. A study by Islam et al. (2018) tested the effects of B, Mo, and Zn on the growth and yield of lentil in Bangladesh. Results showed that the application of micronutrients, singly or in combinations, had significant effects on all yield components with maximum seed production in plots with Zn, B, and Mo combined. In Montana, Mohammed and Chen (2018) assessed micronutrient fertilization of B, Cu, Fe, Mn, Mo and Zn on pea yield through foliar applications. All micronutrient application increased pea yield than the control in two of four site-years. For example, the application of 0.6-1.2 kg Cu ha^{-1} had a higher yield than the control by an average of 941 kg ha^{-1} (Mohammed & Chen, 2018). Micronutrient applications with soil test results above critical values, still resulted in an increase in yield from Fe and Mn applications, further suggesting that critical levels should not be a sole factor for micronutrient fertilization (Mohammed & Chen, 2018).

Soil pH on Nodulation and N-Fixation

Lentil grows well on slightly acidic to moderately alkaline soils (5.7–7.2 pH) (Mahler et al., 1988). Acidic soils (pH < 5) can hamper performance of lentil by causing deficiencies of some nutrients and toxic levels for others (Yadav et al., 2009). Acidic soils can also lead to inhibited nodule growth/development, resulting in reduced N benefit to the following crop and possible loss of lentil yield potential (Ferguson et al., 2013). Mohebbi and Mahler (1989) assessed various soil pH levels from soils in northern Idaho on lentil. In greenhouse studies, the lowest acceptable pH value for lentil was 4.5 which was indicated by stunted growth and necrotic leaf tissue. Nodulation of lentil roots by *Rhizobium leguminosarum* was also affected. Nodules were inhibited when pH < 5.5, to zero nodulation below pH 4. In field trials in northern Idaho, a reduction in plant biomass was observed blow pH 5.6 (Mohebbi & Mahler, 1989).

Nodulation is more sensitive to acidic soil conditions than other aspects of legume plant growth (Evans et al., 1990). Low pH conditions have also been shown to affect rhizobia attachment to root hairs and colonization leading to reduce nodule formation (Caetano-Anollés et al., 1989; Taylor et al., 1991; Miransari et al., 2006). To alleviate the unfavorable effects of acidic soils, agriculture lime can increase soil pH, reduce extractable aluminum concentrations below the phytotoxic levels and increase plant nodulation (Unkovich et al., 1996). Studies show that only one application of lime can have a long term carry over effect of more than 20 years (Lukin & Epplin, 2003). Other than liming, applications of micronutrients that are limited in acid soils such as zinc, boron, and molybdenum can improve maximum nutrient uptake, final yield, and nodulation of lentil (Hossain et al., 2020).

Disease Constraints to Lentil Production

Root Rot Complex

Lentil (*lens culinaris)* is susceptible to production constraints from biotic and abiotic stresses. Abiotic factors include low and high temperatures at seeding and flowering growth stages, intermittent drought throughout the crop season, cold and dry or wet soils at planting, terminal drought, and heat. Major biotic factors causing yield losses in the NGP and globally include Fusarium wilt, root rot, Ascochyta blight, rusts, nematodes, aphids and bruchids (Chen et al., 2011; Kumar et al., 2013). Spring-planted lentil in high elevation regions frequently experience terminal drought and heat stress with a high incidence of root disease (Erskine et al., 2011). The root rot disease complex in pulse crops in the NGP consist of *Rhizoctonia solani, Pythium* spp., *Aphanomyces euteiches,* and *Fusarium* spp. (Gossen et al., 2016). Initial symptoms of each root rot pathogen are distinct, but usually do not occur in isolation. Therefore, accurately identifying the causal agents based on visual symptoms is difficult. General below ground symptoms include reduced root system and root discoloration varying from golden brown to black. Above ground symptoms include yellowing of leaves, wilting, stunting and reduced yield (Kalil et al., 2020). Other symptoms caused by the disease complex include damping off, seedling blight, root rot, and a reduction in plant stand, and a decrease of seedling vigor leading to delayed emergence (Hwang et al., 2001; Gossen et al., 2016). Uneven plant stand is also observed from poor germination and seedling blight (Hwang et al., 2007). Symptoms are first visible on the roots which are often overlooked, but progress over time and are more noticeable in favorable disease years (Gossen et al., 2016). Often growers notice declining yields or foliar symptoms but attribute it to other causes such as fertility.

Among the four root rot pathogens that comprise the root rot complex, *Fusarium* spp. is the most frequently isolated in pea and lentil fields (Chatterton et al., 2015a; Chatterton et al., 2019). Fusarium root rot of peas and lentil is caused by multiple species of *Fusarium* including *F. avenaceum, F. solani, F. oxysporum, F. acuminatum, F. redolens, F. sporotrichiodes, F. equiseti, F. culmorum,* and *F. graminearum* (Kalil et al., 2020). *Rhizoctonia* is a genus of ascomycetous fungi, and *R. solani* can cause seedling blight and root rot of lentil (Chang et al., 2008). *R. solani* can be divided into 14 anastomosis groups (AG) that are semi specific to crops (Anderson, 1982; Kalil et al., 2020). Chickpeas, dry beans, soybeans, lentil and wheat are susceptible to AG-4 and AG-8 (Mathew et al., 2012; Sharma-Poudyal et al., 2015; Kalil et al., 2020). *Pythium* spp. and *A. eutiches* are in the genus of oomycete fungi that are fungal-like water molds. Oomycetes produce resting structures called oospores that germinate in the presence of a susceptible host or under wet soil conditions. Germinating oospores can directly infect plants roots and zoospores are produced that can swim in waterlogged conditions towards other host roots (Kalil et al., 2020). Pythium root rot of lentil is caused by the species *P. ultimum* (Ingram & Cook, 1990). This species also has a wide host range. Unlike *Fusarium* spp., *Rhizoctonia solani*, and *A. euteiches*, the symptoms of Pythium infection include seed rot and damping off which result in a reduced stand and bare patches (Kalil et al., 2020). *Aphanomyces euteiches* was first reported on dry peas in Canada (2013), North Dakota (2014) and in Idaho (2008) (Chatterton et al., 2015a; Zitnick-Anderson & Pasche, 2016; Zitnick-Anderson et al., 2020b) and was identified in north east Montana in 2017 (Murphy, unpub).The effects of the disease caused by *A. euteiches* often weakens plants allowing opportunistic pathogens such as *Fusarium* spp. to also invade the roots (Willsey et al., 2018). *A euteiches* oospores can persist in the soil for many years without host crops, and at high levels can remain harmful to susceptible species for up to ten years (Pfender & Hagedorn, 1983; Willsey et al., 2018; Karppinen et al., 2020).

Fusarium Root Rot in Pulse Crops

Root rots are a major concern in pulse production in the NGP, and several surveys in the past few years show that *Fusarium* spp. are often the most frequently isolated root rot pathogen (Chatterton et al., 2019; Zitnick-Anderson et al., 2020a). Commercial pea and lentil fields were surveyed between 2014 to 2017 across the Canadian prairies of Alberta, Saskatchewan and Manitoba. Root rot was present in every pea and lentil field surveyed in the three provinces. *F. avenaceum* was the most common pathogen identified by culturing and PCR analyses (Chatterton et al., 2019). Similar results were found in dry pea surveys in North Dakota during 2004, 2005, 2008, and 2009. The most frequently isolated fungal species from infected pea roots were *F. oxysporum* and *F. avenaceum* (Chittem et al., 2015). In Montana several *Fusarium* spp. cause root rot on both pulse and cereal crops (Moparthi et al., 2020). A survey of *Fusarium* spp. species associated with root rot in lentil was conducted in Montana, North Dakota, and

Washington in 2019 and 2020 (Fonseka & Bugingo, unpub). From field surveys in Montana, *F. oxysporum* was the predominant Fusarium. spp. found in lentil fields along with *F. graminearum*, and *F. redolens* (Bugingo, unpub).

Fusarium Root Rot Taxonomy, Symptoms, and Lifecycle

Fusarium is a large genus of ascomycetous fungi that includes many plant pathogens. Morphological features of *Fusarium* spp. include septate hyphae, presence/absence of spores called macroconidia and microconidia, presence/absence of chlamydospores, fungal morphology and presence and shape of spore phylliades (Leslie & Summerell, 2008). Differences in the shape of the macroconidia are central to the identification of *Fusarium* species, although other characteristics are also used, and in some cases are critical to distinguish similar species. Cultures of *Fusarium* spp. differ on their growth rate and vary in color from whitish to yellow, pink, red or purple shades (Leslie & Summerell, 2008; Ellis, 2020). One of the most critical ways to identify species is by molecular methods, since there can be variability between isolates within a species (shape and size of conidia, color, absence of macroconidia), this can result to misidentification (Leslie & Summerell, 2008; Ellis, 2020). For sequencing the translation elongation factor 1 alpha (EF-1α), RPB1 and/or RPB2 with at least two independent loci is used for accurate identification of *Fusarium* spp. Internet validated databases such as Fusarium MLST (Pennsylvania State University) or FUSARIUM-ID (CBS-KNAW Fungal Biodiversity Centre) are sequence databases for Fusarium identification and are more reliable compared to Genbank. (O'Donnell et al., 2015; Ellis, 2020).

The common intraspecific designation currently used with Fusarium is form species, or *formae speciales (f. sp.).* Within *Fusarium*, *formae speciales* is applied to *F. oxysporum* (Leslie & Summerell, 2008). Certain *Fusarium* spp. are host specific (*F. oxysporum)* and infect a certain crop type hence the pathogen has *formae specialis,* while others are generalists and have a wide host range. In general, *Fusarium* spp. are opportunistic pathogens on pulse crops and require some type of plant stress to cause infection (Bogdan, 2019). The primary inoculum source for infection are chlamydospores, which are asexual survival spores. Mycelium also infect seedlings.

Symptoms of *Fusarium* spp. root rot include reddish brown to blackish brown lesions on the roots (Gossen et al., 2016). If the disease has progressed further in the plant root system a reddish color can be found in the root of the vascular system. The taproot may be discolored and intact, but the fine roots can be destroyed along with a decrease in root nodulation (Hwang et al., 1994). Some of the above ground symptoms include stunting, yellowing of leaf tissue, decay and poor emergence creating bare patches in the field. In general, *Fusarium* spp. are opportunistic pathogens on pulse crops and require some type of plant stress to cause infection. The environmental conditions that increase plant stress and chance for infection include poor or inadequate soil drainage, low organic matter, low soil fertility, high soil compaction, high soil temperatures, flooding, and drought (Harveson et al., 2005; Larkin, 2015; Gossen et al., 2016; Chatterton et al., 2020). These abiotic soil characteristics can delay germination and ultimately impede seedling growth and favor Fusarium root rot development.

The lifecycle of *Fusarium* spp. is similar among species, but some may differ by absence of chlamydospores (e.g. *Fusarium avenaceum)*. The cycle starts with thick -walled spores called chlamydospores, that overwinter in infested soil and crop residue are the primary source of root rot disease inoculum (Figure 1- adopted from Gossen et al., (2016)). Exudates from germinating host plant seeds signal these resting spores to germinate and produce hyphae. The hyphae then infect the developing hypocotyl (below the cotyledon) and epicotyl (above the cotyledon), at the point of seed attachment causing secondary infection (Bogdan, 2019). Hyphae can also produce macroconidia (asexual spores) which can directly infect developing roots. At the end of the growing season, overwintering chlamydospores are produced in infected plant tissue and the spores can persist in the soil for several years (Nash et al., 1961; Leslie & Summerell, 2008).

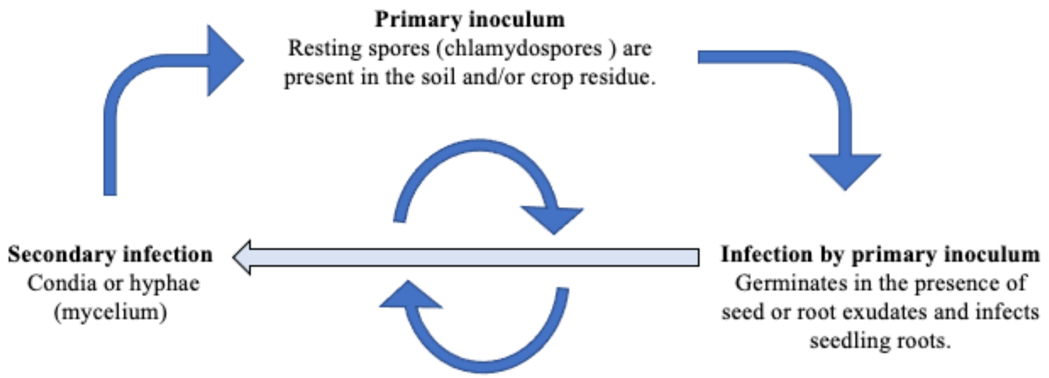

Figure 1-2. Fusarium lifecycle adopted from Gossen et al., (2016).

Species of Fusarium Affecting Lentil in the NGP

Fusarium avenaceum

F. avenaceum (teleomorph *Gibberella avenacea*) is a soil borne saprophyte and is a pathogen to legumes, carnations, and other perennial plants (monocots and dicots) (Leslie & Summerell, 2008). *F. avenaceum* may also be seed borne for some legumes and can cause Fusarium head blight of wheat (Kellock et al., 1978; Kang et al., 2005). The morphological characteristics on PDA vary, producing mycelium from white to light yellow, pink, and grayish rose in color (Leslie & Summerell, 2008).

Two types of conidia are formed by *F. avenaceum,* macroconidia and microconidia. The macroconidia are found on sporodochia and are pale orange in color with three to five septa and are long and slender, tapering to a point. The microconidia can be formed on monophialides or polyphialides in aerial mycelia (Leslie & Summerell, 2008). However, the microconidia typically have one to two septa. Macroconidia are formed more often than microconidia, but conditions have to be favorable for conidia to form. Compared to other *Fusarium* spp., *F. avenaceum* does not produce chlamydospores. *F. avenaceum* also lacks a perfect sexual state (Leslie & Summerell, 2008). *F. avenaceum* can only live as a saprophyte, and if factors favor the pathogen, then *Fusarium* will continue to infect living and dead host plants.

F. avenaceum is the most common species, and the ability to cause infection in wide host range of cereals, canola and pulse crops has allowed the pathogen to maintain and increase its prevalence in the NGP (Fernandez et al., 2007; Zhou et al., 2014; Chatterton et al., 2019). In Montana, cross pathogenicity has been observed in trials where *F. avenaceum* was highly aggressive on pulses and cereals. This indicates that rotations between these crops can still lead to an increase in inoculum of *F. avenaceum,* making crop rotation limited in efficacy as a disease management strategy (Moparthi et al., 2020).

Fusarium oxysporum

The causal organism of Fusarium wilt of lentil is *Fusarium oxysporum f.* sp. *lentis*. This is an important disease which has been reported on every continent where lentil production occurs (Belabid et al., 2010). *F. oxysporum* is just one of two of the nine species descriptions of Snyder and Hansen that remains in general use (Leslie & Summerell, 2008). Disease symptoms include stunting, wilting, marked reduction of root system, and internal vascular discoloration of the lower stem (Li et al., 2015).

In general, cultures of *F. oxysporum* grown on potato dextrose agar (PDA) vary in color from pale to dark violet and dark magenta, but some isolates produce no pigment at all. Some *F. oxysporum* isolates mutate readily to the pionnotal form or to a flat “wet” mycelial colony with a yellow to orange appearance when cultured on PDA (Leslie & Summerell, 2008). On PDA *F. oxysporum f.* sp. *lentis* (Fol) cultures vary in color from yellowish brown, dark brown, creamy white, to dark tan (Saxena et al., 2019). Fol produces three kinds of spores called microconidia, macroconidia and chlamydospores. The microconidia have no septae and are oval or kidney shaped. Macroconidia have three septae, a distinct foot cell and pointed apical cell (Khare, 1980). The sexual stage for this species has not been found. Fol is a soilborne pathogen, although seed infestation and infection are common. The mycelium or chlamydospores can survive in the soil up to six years either in dormant form or saprophytically without a suitable host (Lodhi et al., 2006; Taylor et al., 2007).

Specific races of Fusarium wilt caused by Fol have been reported in Italy, Syria, Algeria, India, Iran, and Australia but have not been characterized yet in North America (Belabid & Fortas, 2002; Belabid et al., 2004; Datta et al., 2009; Lindbeck, 2009; Husien Al-Husien et al., 2017; Nourollahi & Madahjalali, 2017). Fusarium wilt can cause complete crop failure, especially if environmental conditions consist of a warm spring and hot dry summer. Environmental factors that induce maximum disease severity caused by Fusarium wilt include soil temperatures ranging from 24 – 27 º C, air temperature of 17 – 18 º C, 60 - 78% relative humidity and a soil pH ranging from 5.2 - 5.8 (Kaushal & Sharma, 1998; Fatima et al., 2015).

Fusarium redolens

Fusarium redolens is reported to be a root and crown pathogen on a wide range of plants (Esmaeili Taheri et al., 2011). Characteristics of *F. redolens* on PDA include colonies with flat aerial mycelium that are white to pink with some brown pigmentation in the agar (Leslie & Summerell, 2008). In Europe and the United States, *F. redolens* has been associated with vascular wilt of lentil, asparagus, and soybeans (Baayen et al., 2000; Riccioni et al., 2008; Bienapfl et al., 2010). In all parts of the world, *F. redolens* causes crown rot of wheat (Moya-Elizondo et al., 2011; Gebremariam et al., 2018). At first, Fol was reported as the cause of Fusarium wilt of lentil. In Italy, *F. redolens* was reported as an important causal organism for Fusarium wilt (Riccioni et al., 2008). Safarieskandari et al. (2020) found that *F. redolens* was a relatively weaker root rot pathogen compared to other *Fusarium* spp. causing mild root rot symptoms in pulses. Symptoms on field-grown pulses include brown to black discoloration and/or lesions on the crown, main and lateral roots. In contrast, cereal’s adventitious roots were the main target of *F. redolens* where it caused light brown discolorations and lesions (Esmaeili Taheri et al., 2011).

Fusarium acuminatum

Fusarium acuminatum can be found in temperate regions as a saprophyte, as is associated with root and crown diseases of a variety of hosts, with pulses being especially susceptible (Leslie & Summerell, 2008). Cultures on PDA vary, slower growing cultures are white mycelium with a rose to burgundy pigmentation that can be grayish-rose; sporodochia form in the center of the colony in a small central spore mass and are pale orange to dark brown (Leslie & Summerell, 2008). Red pigments to brown can sometimes form in agar (Logrieco et al., 1992).

F. acuminatum produces all three spore types (microconidia, macroconidia and chlamydospores). The microcondia is not a reliable taxonomic indicator for this species since it usually has 1 - septate, but occasionally 0 – septate (Leslie & Summerell, 2008). The macroconidia is most distinct compared to other taxonomic features and has more dorsiventral curvature and has thick walls and a distinct foot-shape to the base of the basal cell (Leslie & Summerell, 2008).

In North Dakota, Chittem et al. (2015) found that *F. acuminatum* was the third most prevalent species isolated in 57% of fields planted in dry pea surveyed in 2008 and 2009. Isolates were then tested on their pathogenicity to dry pea and were found to be less virulent. In Montana, Moparthi et al. (2020) tested Fusarium species associated with root rot of pulses and their cross pathogenicity to cereal crops. Overall, *F. acuminatum* was the second most abundant species isolated from roots and seeds of lentil, chickpeas, and dry peas, although it was not found to be highly aggressive on pulses or cereal crops.

Fusarium culmorum

Fusarium culmorum is found in temperature regions and is often associated with cereal foot rots and head blights (Scherm et al., 2013). The pathogen has also been associated with seeds and/or roots of pulses (Moparthi et al., 2020). *F. culmorum* grows rapidly producing abundant sporodochia in a large central spore mass (1 to 2 cm diameter) that is initially pale orange, but masses may be formed by some isolates (Leslie & Summerell, 2008). Rings of spore masses may be formed by some isolates under alternating conditions of light and dark (Leslie & Summerell, 2008). Most strains form a red pigment in the agar, but some may be olive brown mycelium and olive brown pigment in the agar (Leslie & Summerell, 2008). Isolates of *F. culmorum* can be highly aggressive in lentil, barley, and wheat. Isolates of *F. culmorum* from chickpea and lentil can also be cross pathogenic with barley and wheat, suggesting multiple *Fusarium* spp. from seeds and roots can cause root rot on both pulses and cereals (Moparthi et al., 2020).

Management of Fusarium Root Rot

Cultural Practices

Some cultural management practices can be implemented to prevent disease incidence from Fusarium root rot. Cultural practices such as time of seeding, crop rotations, and tillage are important factors to consider, as well as cropping history. Regarding plant resistance, only lentil have been screened for vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis* (Kamboj et al., 1990; Eujayl et al., 1998; Mohammadi et al., 2012). However, little research has been conducted on sources of root rot resistance for lentil.

The timing of lentil seeding is very important to ensure full growth to crop maturation in the northern Great Plains region. Planting earlier in the spring in colder soils can slow germination and predispose seedlings to infection caused by the soil-borne pathogens. Planting mid-May improved seedling establishment compared to early or late May in trials inoculated with *F. avenaceum*. In greenhouse studies, the most severe root rot infection occurred in lentil at soil temperatures 20-27.5 º C (Hwang et al., 2000).

Extended crop rotations and minimal soil tillage have been known to improve overall soil health and structure (Larkin, 2015). Minimal tillage causes an accumulation of crop residues in which the pathogen survives on the soil surface. An increase in yield and reduction in root rot severity can be achieved by deep tillage compared to no-till to breakdown crop residues (De Jensen et al., 2004; Váňová et al., 2011). In dry pea, extended rotation intervals of four to five years still experienced root rot injury from *F. solani* f. sp. *pisi* and *F. avenaceum* (Chatterton et al., 2015b). Symptoms of Fusarium root rot injury in fields without a history of pea or lentil in the rotation can also be found and supports the hypothesis that rotation alone will not be an effective tool for managing Fusarium root rot (Gossen et al., 2016).

Seed Treatments

In addition to the cultural management techniques previously described, root rot is commonly managed through chemical control with seed treatment fungicides. The use of fungicide seed treatments is a common practice worldwide. Fungicide seed treatments have often been used to control (1) fungal pathogens located on the seed surface, such as cover smuts of cereals, (2) internal seedborne pathogens such as loose smut of cereals and (3) soil-borne pathogens attacking germinating seeds/seedlings (Khanzada et al., 2002; Bradley, 2008; Arshdeep et al., 2014). The wide host ranges of *Fusarium* spp. limit the effectiveness of crop rotations. Although not always consistently, fungicides seed treatments have shown to provide protection against stand losses in pulses for 2-3 weeks after planting (Chang et al., 2013; Chang et al., 2014). Seed treatments fungicides differ in their mobility, mode of action, and their activity on different pathogens in the root rot complex. The use of biocontrol seed treatments has also shown to have some activity against pathogens in the Fusarium genus (Xue, 2003).

Fungicide modes of action fall into three groups according to their mobility. The first is contact fungicides. These are surface protectants that target seed surface-borne and soilborne pathogens. Because they remain on the surface, protectants lose activity after being washed off and active ingredients have broken down within a few weeks of planting (Damicone, 2014). The second group is locally systemic, which target both the seed surface-borne and internally seedborne pathogens. The third group is fully systemic, these are xylem mobile and systemically translocated throughout the plant. Locally and fully systemic fungicides are penetrants and are translocated within plant tissues after the initiation of plant growth or application to growing plant tissues (Damicone, 2014). There are fungicides that may have more than one type of mobility (Lamichhane et al., 2020). Under field conditions, all of these fungicide groups when used as a seed treatment target pathogens that attack germinating seeds or emerging seedlings for up to 2-3 weeks from sowing and 3-4 weeks of protection with some newer chemistries (Kazda et al., 2005; Bogdan, 2019).

Among the mobility types, fungicides are grouped by chemical composition (modes of action) by the Fungicide Resistance Action Committee (FRAC) (Damicone, 2014; Lamichhane et al., 2020). Common seed treatments for lentil include chemicals in combination or alone of fludioxonil, mefenoxam, and metalaxyl (McMullen & Lamey, 2000). A list of other mode of actions of fungicide seed treatments used for lentil that are routinely used are listed in (The Northcentral IMP Center - Pulse Crop Working Group, 2020). Fludioxonil provides a broad-spectrum protection against Rhizoctonia and Fusarium seedling blight. Mefenoxam and metalaxyl provide protection against *Pythium* spp. (Hwang et al., 2001). Metalaxyl and mefanoxam belong to the phenylamides (PA) group. This group of fungicides are xylem-mobile and systemically move from roots to shoots but typically have a residual affect 2-3 weeks after planting (Damicone, 2014). PA fungicides inhibit RNA biosynthesis and have a high risk of resistance development and control oomycete fungi. Fludioxonil belong to the phenylpyrroles (PP) group and interferes with the transportation of amino acids and sugars in the fungal membrane. There is a low to moderate risk of resistance development (Damicone, 2014). A list of FRAC groups, names and common active ingredients is also listened in (Burrows et al., 2017; FRAC - Fungicide Resistance Action Committee, 2021). Most seed treatments are effective against seed decay and damping off (Gossen et al., 2016). Fungicide seed treatments have shown to be effective at managing Fusarium seedling blight, decay and damping off of pulse crops, but have shown to have little effect on root rot (Hwang et al., 2000; Chang et al., 2013). Studies have shown that the efficacy of seed treatment fungicides on root rot management varies due timing of disease onset (Hwang et al., 2000).

Hwang et al. (2000) assessed different seed treatments on lentil in Alberta, CA in *F. avenaceum*-inoculated field trials. Hwang et al. (2000) found that seed treatments with Crown® (thiabendazole and carbathiin) on lentil consistently improved seedling survival, reduced root rot severity and increased seed yield relative to the inoculated control. Additionally, the two seed treatments performed similarly to the non-inoculated controls across varieties CDC milestone, CDC Redwing and Laird and three different planting dates. Whereas treatments with Vitaflo® (thiram and carbathiin) and Raxil® (tebuconazole) also improved lentil establishment and yield but less consistently. In another study, mefenoxam and fludioxonil (Apron Maxx®) seed treatments improved seedling emergence, and yield for faba bean compared to a non-treated inoculated control (Chang et al., 2014). Similar results were found in field plots with Apron Maxx® + Rhizobium (inoculant) planted in dry pea inoculated with *F. avenaceum* and *R. solani*. The addition of the rhizobium increased seedling survival shoot dry weight, nodulation and reduced root rot severity relative to treatments without inoculant for both Apron Maxx® and non-treated seeds (Chang et al., 2013). A study assessing management strategies for rhizoctonia seed blight of dry pea found that seed treatments fungicides with Vitaflo® + Apron® (carbathiin + thiram, metalaxyl) improved plant establishment and reduced *R.solani* injury under field conditions in Alberta, Canada (Hwang et al., 2007). Godebo et al. (2020) assessed soil bacteria as biocontrol agents of Aphanomyces root rot of dry pea. They found that the addition of *Rhizobium* significantly suppressed Aphanomyces root rot dry pea grown in the greenhouse. Overall, fungicide seed treatments in combination with rhizobia inoculation is beneficial for management of root rot and enhancing overall root nodulation of pulses (Muthomi et al., 2007).

Seed treatment alternatives to fungicides include biological control agents or biofungicides that are either bacterial or fungal (Burrows et al., 2017; Ons et al., 2020). An antagonist biological control for root rot of dry pea is *Clonostachys rosea,* strain (ACM941). *Clonostachys rosaea* as a seed treatment managed pathogens associated with the pea root rot complex and decreased root rot severity by 76% (Xue, 2003). Wang et al. (2003) assessed bacterial antagonists against Aschochyta blight, seedling blight and root rot of peas caused by (*Pythium ultimum, Rhizoctonia solani, Fusarium avenaceum,* and *Ascochyta pisi).* Out of 301 strains of *Pseudomonas fluorescens*, *Serratia* spp., and *Bacillus* spp., 30 strains were found to inhibit *P. ultimum, F. avenaceum, R. solani,* and *A. pisi* according to results from in vitro plate bioassays. In greenhouse evaluations for Fusarium root rot, only *Bacillus* spp. reduced disease severity. This study showed that some bacteria isolates obtained from pea fields in Alberta display antifungal properties.

Summary

The production of lentil is important to Montana and other growing regions in the NGP. Studies have been conducted to test rhizobial inoculant formulations, and the nutrient provisions of nitrogen and phosphorus on other pulse crops such as dry pea (McKenzie et al., 2001b; Karamanos et al., 2003; Clayton et al., 2004). However, there has been little research effort on advantages of potassium (K) and sulfur (S) on lentil yield and growth. Research has also been conducted assessing agronomic factors that influence or increase pea protein formation (McLean et al., 1974; Bestwick et al., 2018a); however, there is little to no research on starter fertilizer and inoculant type that specifically maximize lentil protein.

The rise of pulse crop production has led to an increasing incidence of root rot. There have been studies testing efficacy of fungicide seed treatments in dry pea and faba bean, but very little research has been conducted in lentil. Root rots are becoming a constraint to lentil production in the Northern Great Plains, and preliminary reports in Montana suggest that *F. oxysporum* was the predominant pathogens associated with root rot in lentil (Bugingo, unpub). *Fusarium* root rot is made up of several different species, many of which have a wide host range, and is difficult to manage in susceptible crops. Since the system is very complex, developing resistant varieties to the entire species complex will be very difficult. Multiple methods of disease management are needed including extended crop rotations, timely planting, increased tillage, and seed treatment fungicides, which do not provide satisfactory management under high disease pressure from *Fusarium* spp. since seed treatments typically last 2-3 weeks after planting.

The efficacy of the active ingredients in seed treatments fungicides vary according to the species of soilborne plant pathogen targeted. Therefore, it is important to discern the treatments that specifically target and decrease symptoms of *Fusarium* root rot. There may be differences in efficacy of a single product against multiple species within the genus Fusarium, as well, although that question is beyond the scope of this project. The best method for disease management of root rot in lentil includes some cultural preventative measures. These could include (1) extended cropping rotations, which helps decrease the inoculum in soil by planting non-host crops; (2) optimal seeding date, which provides seedlings the optimal temperature for growth and decreases seedling stress; (3) development of resistant varieties (4) increase in tillage operations to bury crop residue; (5) improving crop fertility (e.g. rhizobia inoculants and nutrient provisions) and (6) fungicide seed treatments.

Objectives

The studies presented in this thesis focus on agronomic practices for crop fertility and Fusarium root rot management of lentil. In addition to the resulting impact on plant health, seed yield, and seed nutritional quality (protein). Since plant health in lentil is expected to interact with root rots, it was important to study the influence of nutrient provision in the presence and absence of disease. Chapter one evaluates rhizobial inoculants in the form of granular and seed-coat (peat-powder) and potassium, sulfur and a micronutrient foliar application. Chapter two assesses the efficacy of seed treatment fungicides. Understanding the aspects of lentil fertility through rhizobia inoculant formulations and nutrient provisions (with potassium, sulfur, and micronutrients), and chemical control through fungicidal seed treatments is important to develop an integrated management approach for Fusarium root rot.

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CHAPTER TWO

RHIZOBIAL INOCULANT FORMULATIONS AND NUTRIENT PROVISIONS FOR LENTIL (*LENS CULINARIS* MEDIKUS)

Abstract. Relatively little is known about inoculant and fertility response in lentil (*Lens culinaris* Medikus). Lentil is an important crop in the northern Great Plains, grown on 183,400 ha^{-1} in 2020 (USDA-NASS, 2020a). The objective of this experiment was to evaluate the effect of peat rhizobial inoculant formulations (granular vs. seed-coat) and nutrient additions (potassium, sulfur, and micronutrients), on lentil establishment, growth, seed protein, and yield. This study was conducted at four Montana locations in 2019 and 2020: Bozeman, Havre, Moccasin and Sidney. In six of eight site-years, there was no yield difference between inoculant types, with opposing results in two site-years. Application of S fertilizer increased seed protein in one site-year by 0.7 %-units and seed yield in three of eight site-years, by an average of 303 kg ha^{-1} (16.8%) in those site-years. Sites with low and high sulfate-S had a positive yield response from S fertilizer, revealing the unreliability of standard sulfate-S testing. Application of K fertilizer had no effect on seed yield. However, in one-site-year, the combination of K + S fertilizer increased seed yield by 260 kg ha^{-1} (10% of average yield for that site) over unfertilized treatments, when K or S alone did not. Results from this study further suggest the potential importance of S fertilization for lentil.

Introduction

Climatic conditions in the NGP include long winters, short, warm summers, large diurnal ranges in temperature, frequent strong winds, and variable spring and summer precipitation (Padbury et al., 2002). These climatic conditions increase the risk for agricultural production. Lentil is most often grown in place of fallow in dryland wheat rotations in Montana due to increased profit. In addition, the shallow root depth for lentil increase precipitation use efficiency when grown in crop rotation with wheat (Long et al., 2014). Regions in western Canada have found similar economic and environmental benefits with dry pea and lentil in oilseed-cereal rotations (MacWilliam et al., 2014). Lentil serves to diversify dryland cropping systems, mitigating environmental and economic risk (Zentner et al., 2001; Miller et al., 2002). Demand for lentil is increasing due to consumer preference shifting towards plant-based protein sources (Lakkakula et al., 2017; Warne et al., 2019). This has led to an increase in production area of lentil across the U.S. NGP at approximately 183,400 ha^{-1} in 2020, with the majority of acres planted in Montana at 150,000 ha^{-1} (USDA-NASS, 2020c) (Figure 2-1).

Achieving optimal yield of lentil begins with proper crop fertility management practices. Lentil is capable of biological N fixation (BNF) through a symbiotic relationship with rhizobia bacteria on the plants' roots. The formation of root nodules provides a low oxygen environment, necessary for the enzymatic conversion of atmospheric N_2 to plant-available NH_3. Farmers introduce commercially available rhizobia inoculants to the soil with lentil seed (Denton et al., 2009; Peoples et al., 2009). The benefit of promoting the BNF process in lentil is that it supports plant growth, yield, and the production of high protein seed without the use of nitrogen fertilizers (Stephens & Rask, 2000; Peoples et al., 2009). Factors that determine the amount of N_2 fixed include cultural practices that limit the presence of rhizobia (e.g. problems with inoculation source, excessive tillage, extended fallow periods and fertilizer nitrogen) (Peoples et al., 2009). Plants that are involved in BNF are also particularly sensitive to phosphorus (P), potassium (K), and sulfur (S) deficiencies by modulating growth of rhizobia, nodule formation/functions, or indirectly affecting the growth of the plant (Divito & Sadras, 2014). Among the three nutrients listed, phosphorus is already known to promote seedling health to pulses in the region (McKenzie et al., 2001b; Karamanos et al., 2003). However, research of K, S, and micronutrient requirements for grain legumes has been limited (Mahler et al., 1988). In regard to micronutrients, zinc, boron, and molybdenum are the most frequently cited to limit plant growth in lentil (McKenzie et al., 2007).

Inoculants come in three forms: granular, powder and liquid. Each form has advantages and disadvantages that are important to consider. Granular soil inoculant can promote rhizobia populations throughout the upper root zone, whereas seed-coat inoculants tend to form nodules in clusters near the cotyledon. Cropping history is important to consider, and efficacy of inoculants was generally greater in Alberta, Canada farm fields with no pulse history (McKenzie et al., 2001c). In Montana and North Dakota, Bestwick et al. (2018b) found that granular rhizobia inoculants provided higher pea seed protein content than seed-coat peat-powder in drier than normal years. Other studies in Alberta assessed the influence of different rhizobia inoculant formulations on pea (Rice et al., 2000; McKenzie et al., 2001c; Clayton et al., 2004), but there is very little inoculant research data on lentil.

Similarly, research on lentil response to potassium, sulfur, and micronutrients is scant. In Montana, soils generally have average K levels above 250 ppm, therefore the need for potassium applications may be minimal (Jones & Olson-Rutz, 2018). In Montana, 19 kg K ha^{-1} is suggested for lentil if K levels in the top 15 cm are < 250 mg kg^{-1} (Jacobsen et al., 2003). Sulfur deficiencies are common on Montana soil with low, or no, gypsum (calcium sulfate) levels (Jacobsen et al., 2003). Even worse, sulfate-S often does not accurately reflect S availability (Franzen, 2018), and there are no S fertilizer guidelines for lentil in Montana. Sulfate-S can be in calcium sulfate forms that dissolve during lab testing under saturated, well mixed conditions, but dissolve slower and result in relatively low sulfate availability when soils are at or below field moisture capacity. Thus, sulfate-S availability may be overestimated by lab tests. Conversely, soil organic matter mineralization can release substantial quantities of sulfate during the growing season (McGill & Cole, 1981), preventing a S response even when sulfate-S is low (Sawyer & Barker, 2002). In northern Idaho, S applications of 16.8 kg per ha^{-1} are recommended for lentil if soil test less levels in the 0-61 cm depth are <4 mg kg^{-1} (i.e. < 32 lb/ac) for sulfate-S (Mahler, 2005). Lentil response to micronutrients has not been well studied, but reports from Bangladesh and Montana, showed that applications of foliar micronutrients with Zn, B, and Mo increased lentil (Islam et al., 2018) and dry pea yield (Mohammed & Chen, 2018).

To fill a research void on lentil fertility and inoculants, in the northern Great Plains, we initiated a study at four sites in The objectives of this study were to: (1) compare granular vs seed-coat inoculant formulation for efficacy, and (2) determine if potassium, sulfur, or micronutrients enhance lentil growth, seed protein and yield.

Materials and Methods

Experimental Design

The experiments occurred at sites in southwestern (Bozeman), central (Moccasin), northern (Havre), and eastern (Sidney) Montana in 2019 and 2020. Specific field locations, precipitation, growing season temperature, plot sizes, and soil series are listed in Table 2-1. All sites were managed under no-till systems and were planted with *Avondale*, a medium sized green lentil cultivar (PVP # 201400092) (USDA PLC, 2014). The experimental design was a randomized complete block with eight treatments and five blocks.

Treatments consisted of contrasting peat-based inoculant formulations (granular and seed-coat) with combinations of potassium, sulfur, and a micronutrient fertilizer application (Table 2-2). Treatments in 2019 and 2020 were the same except for the micronutrient treatment, which was applied well above label rates in 2019, resulting in plant injury, and therefore discarded from all 2019 analyses. Granular rhizobial inoculant (*Rhizobium leguminosarum biovar viceae* 1×10^8 CFU g^{-1}) was applied at 6.7 kg ha^{-1} in the seed furrow (Verdesian Life Sciences, Cary, NC). Seed-coat inoculant (*Rhizobium leguminosarum biovar viceae* 2×10^8 CFU/g^{-1}) was applied at 0.31 kg per 100 kg of seed. *Micro 1000* contains a combination of seven micronutrients essential for plant growth plus cobalt, which is necessary for BNF (Table 2-3). Although the *Micro 1000* contained three macronutrients (S, Ca, Mg), the rates of these at 2.3 L ha^{-1} was about 0.03 kg ha^{-1}, a negligible fraction of lentil needs, and hence we call this a micronutrient solution hereafter.

Field Operations

Field sites were soil sampled in the spring prior to planting to measure baseline levels of pH, salts, OM, Olsen-P, K, Zn, sulfate-S, and NO_3-N. Brennan et al. (2001) found lentil and wheat require high amounts of Zn. Therefore, soil Zn levels were added as a soil test parameter. The seeding rate at all sites targeted 120 plants m^{-2}, resulting in seeding rates of 62 and 75 kg ha^{-1}, in 2019 and 2020, respectively. Mono-ammonium phosphate (MAP; 11-52-0) was applied at a rate of 50.4 kg ha^{-1} at planting. In 2020 at Moccasin, no MAP fertilizer was applied to the fertility trial. At planting, control plots without inoculant were seeded first, then all treatments containing granular inoculant, and then lastly treatments containing seed-coat inoculant to minimize contamination from the peat powder associated with the seed-coat inoculant. Treatments with the micronutrient foliar application were applied at late bud or early flowering stage with a backpack sprayer. Herbicide management varied by site (Table 2-4). Important field dates such as planting, emergence, stand count, harvest and data collection dates were noted (Table 2-5). Relevant cropping history was recorded from each site to understand the inoculant effect of introduced rhizobia (Table 2-6). Five site-years had a history of pulses, in the previous six years, while three did not.

Soil Data

Soil samples were analyzed by Agvise Laboratories© (0.25 M KCl method) except Moccasin 2020 samples were sent to Ward Laboratories, Inc.© (Table 2-7). In general, all sites had soil pH levels ≥ 6.5 and were within the optimal range for lentil growth and nutrient availability (Mahler et al., 1988). Soil potassium levels ranged from 214 to 414 ppm and were sufficient at most sites, except three of eight site-years had soil K levels < 250 ppm (Jacobsen et al., 2003) for lentil soil K. Sulfate-S levels in the 0–15 cm depth ranged from 2.1 to 16 mg kg^{-1}. Sites at Moccasin in 2019 and at Bozeman in 2020 exhibited low sulfate-S (< 4 ppm in 0-15 cm soil depth) (Mahler, 2005). In 2019, the Sidney site was not tested for sulfate-S. Sulfate-S levels from the 15-61 cm depth ranged from 2 to 17.4 mg kg^{-1}. Low sulfate-S levels

(0-4 ppm) in the 15-61 cm depth were observed in six of eight sites. Soil samples from Moccasin and Havre in 2020 were relatively high for sulfate-S. Sulfate-S also varied substantially between depths at Sidney in 2020. Moccasin soil samples for sulfate-S were assessed by the Mehlich-3 method for extractable S used by Ward Laboratories, Inc.© which could overestimate sulfate-S availability because the extractant is acidic (pH ~2.3), dissolving carbonates that contain sulfate making it available for measurement (Cihacek et al., 2015). Soils at all other sites were extracted with 0.25 M KCl for sulfate-S method. Soil nitrate-N levels from 0 to 15 cm depth ranged from 2.1 to 5.2 mg kg^{-1}. Havre in 2019 had high nitrate-N levels from the 15 to 61 cm depth of 27.2 mg kg^{-1}. Other site-years had lower levels < 8 mg kg^{-1}.

Crop Data Collection

Data collection included plant stand density, plot vigor, percent canopy cover, final yield, and seed protein. Plant stand density was measured 21-28 days after seeding by counting all plants in 1 m of two different rows, approximately 2.5 m from the front and rear of the plot (4 m-row total). Stand counts were then converted to plant population in plants m^{-2}.

Plant growth was assessed two ways; visual assessment of ‘plot vigor’ and image-based canopy cover. Plot vigor was recorded as a percent and was determined by assigning 100% to the most vigorous appearing plot in each replicate, then assigning rates within the replicate compared to the highest rated plot. Fractional green canopy cover percent (FGCC) was assessed using the Canopeo App © (Oklahoma State University). The Canopeo algorithm uses color values in red-green-blue spectrum to determine fractional green canopy cover (Patrignani & Ochsner, 2015). Three pictures were taken perpendicular to the plot (front, middle, and back each about 2.5 m apart) and the values recorded and averaged. When taking pictures, the arm of the assessor was equally extended level with the shoulder and the picture was taken to achieve a uniform distance to the canopy greater than 0.6 m, as recommended. Plots were also hand-weeded before data collection for consistent canopy cover results.

All plots were harvested with a combine and lentil seeds were then cleaned and weighed. Seed moisture content of each plot was determined using a seed moisture analyzer. Grain yield in kg ha $^{-1}$ was determined on a dry matter basis accounting for the seed moisture content of each plot. The N concentrations of all 2019 grain samples was measured by a Kjeltec 2003 Analyzer Unit (Foss Analytical, Hilleroed, Denmark). At Bozeman in 2020, lentil grain samples were measured by automated dry combustion analysis (LECO Corp., St. Joseph, MI). A conversion factor of N × 6.25 was used to report 'protein'(Coyne et al., 2005).

At Bozeman, in 2020, NDVI maps were made on 9 July (Barnes and Powell, unpub), for a separate study assessing fertility treatments on N-fixation (Baber, unpub). The map showed Normalized Difference Vegetation Index (NDVI) percentage (Gandhi et al., 2015). The NDVI is a spectral index used to assess plant vigor, greenness, and percent canopy cover (*personal communication*, S. Powell). Estimates from NDVI were compared with the Canopeo application at Bozeman 2020 for estimations of FGCC. Fractional canopy cover data was collected on the same day the NDVI imagery was taken. The NDVI estimates were calculated from 0.66 m^{-2} of each plot from pre-flagged locations used for corresponding biomass sampling (Baber, unpub data).

Statistical Analyses

All parameters were analyzed with R 4.0.3 (R Core Team, 2021). Treatment responses varied among site-years; therefore, the data were analyzed individually by location. A linear model was fit using the lme in the *nlme* package for each response variable of interest within each location and year (Pinheiro et al., 2021). Block was considered as a random effect while treatment was considered a fixed effect. Plots using intplot function were made to assess Treatment * Block interactions (Greenwood, 2019). The analysis of variance (type III ANOVA) test was performed to examine differences in treatment means. Differences were considered significant at $P<0.1$.

Diagnostic plots were to assess normality of fixed and random effects, along with Cook's Distance plots for possible outliers using the *predictmeans* package (Luo et al., 2020). Cook's Distance plots indicated influential points and observations greater than 0.5 were cross-referenced with field notes for possible explanations such as large rodent holes or errors at planting. No data transformations were needed. All diagnostic plots showed constant variance and the residuals did not deviate far from normality. Estimated treatments means for each model were obtained from the *lsmeans* package (Length, 2016). In 2020 at Bozeman, a correlation analysis was performed using the *ggpubr* package with fraction green canopy cover (FGCC) percentage of each plot and NDVI percentage of each plot, to measure collinearity between these different plant growth assessment methods (Kassambara, 2020).

Pre-planned contrast comparisons were conducted to parse out specific treatment effects using the same *lsmeans* package. Linear contrasts were used to answer these six research questions, (1) If inoculant (Control vs. Granular, Seed-coat) have an effect when other fertilizer treatments are not present; (2) If inoculant types (Granular vs. Seed-coat) have an effect, regardless of other fertilizer treatments; (3) Compare the mean fertilizer effect of treatments with or without the addition of potassium fertilizer, regardless of rhizobial inoculant; (4) Compare the mean fertilizer effect of treatments with or without sulfur fertilizer, regardless of rhizobial inoculant or potassium fertilizer; (5) Compare the mean fertilizer effects of treatments with or without potassium and sulfur fertilizer, regardless of rhizobial inoculant, (6) If micronutrients (Granular+K+S vs. Granular+K+S+Micronutrient) have an effect when inoculant and fertilizer treatments are not present.

Results and Discussion

Site Characterization

Locations in this experiment covered a range of growing season conditions across the state of Montana. Total precipitation during the growing season (May-July) varied from 102 to 366 mm among locations and years (Table 2-1). According to National Drought Mitigation Center (NDMC) et al. (2020), in early July 2019, all sites in this study were not experiencing drought, a rarity in Montana (Figure 2-2). A more seasonal view of the weather departure from normal May-July precipitation generally agreed with the snapshot drought assessment of early July. In 2019, all sites had near normal May-July precipitation (Figure 2-3). In 2020, three of four sites were classified as abnormally dry, with Bozeman even drier under moderate drought (Figure 2-4). All sites in 2020 had below normal precipitation with Sidney tending even drier than the other sites (Figure 2-5).

Plant Stand

Mean plant density ranged from -47 to +14 plants m^{-2} of the target density of 120 plants m^{-2} among sites. Plant stand density exceeded the target density in only two of eight site-years, but densities were sufficient to avoid important yield loss at all other site-years (Table 2-8 and 2-9). For example, nearby seeding rate studies at Bozeman showed no yield loss in 2019 until densities dropped to 54 plants m^{-2}, and in 2020, no yield loss was observed despite the lowest seeding rate having a density of only 43 plants m^{-2} (Miller, unpub). In 2020 at Havre, lentil yield at 66 plants m^{-2} yielded 91% of a density of 109 plants m^{-2} (Miller, unpub). In 2019 at Bozeman, inoculated treatments had a higher plant stand by 18 plants m^{-2} over the uninoculated control. In 2019 at Sidney, seed-coat inoculant treatments had 15 plants m^{-2} more than granular inoculant treatments. However, there were no consistent effects of treatments on plant stand and plant stand differences were not sufficiently large to be biologically important.

Plant Growth

Inoculant. Pea, lentil and faba bean require the same rhizobium species, *Rhizobium leguminosarum* for effective nodulation. Rhizobia inoculant treatments had a higher stand vigor rating at the R1 (i.e. early bloom) growth stages in two of eight site-years (Tables 2-10 and 2-11). In 2019 at Bozeman and 2020 at Sidney, treatments with inoculant treatments increased stand vigor ratings by 8.6% and 4.5%, respectively. Both sites had a history of pulses. Interestingly, assessment of plant growth by fractional green canopy cover (FGCC) also indicated a positive effect of inoculated treatments in two of eight site-years, of similar magnitude, but they were not the same site-years as for stand vigor at R1 growth stages (Tables 2-12 and 2-13). Inoculated treatments were 10.9% greater for FGCC than the uninoculated control at Havre in 2019 and 4.2% greater at Moccasin in 2020. Both sites had recent pulse crop history. In contrast, the three site-years without recent pulse crop history showed no response to inoculant. Thus, recent cropping history of pulses appeared to have had little to no effect on inoculant performance through plant growth assessments in this two-year study.

Inoculant formulation had little effect on stand vigor rating. Conversely, assessment by FGCC revealed clear differences between granular and seed coat formulations at two site-years, but in opposing directions. In 2019, at Havre, FGCC was lower by 6.9% with granular inoculant, while at Sidney, FGCC was 7.5% higher with granular inoculant. In 2020 at Bozeman correlation between FGCC and NDVI estimates showed that the Canopeo application agreed well with the NDVI imagery ($P<0.001$) (Figure 2-6). Other studies have found that NDVI estimates of wheat and different forage legumes on the plot level were linearly correlated with FGCC (Goodwin et al., 2018; Jáuregui et al., 2019).

Fertilizer. Potassium fertilizer showed no effect on FGCC, unsurprising given soil K levels in soil were generally above Montana's critical level. Conversely, treatments with S fertilizer increased FGCC in three of eight site-years, in 2019 at Sidney by 2.7%, and in 2020 by 7.0% at Bozeman and 6.8% at Moccasin (Table 2-13). There was no effect of the micronutrient application on FGCC at any site in 2020. Low soil sulfate-S was measured at Bozeman in both years of this study, but only in 2020 was there an observed S response. The observed difference in response may be due to the moderate drought conditions that occurred in 2020 at Bozeman, making sulfate-S less available. Protein responses to S in moderate drought conditions have been documented in Montana in pea (Bestwick et al., 2018b), and yield responses to S observed in winter wheat (Jackson & Engel, 2006) were attributed to lower S mineralization in dry soils. Moccasin has very shallow soils with coarse fragments 20 to 80 cm below the soil surface, increasing the likelihood of sulfate leaching and increasing the likelihood of observing an S response. Soils at Sidney were medium-textured loams, that coupled with greater than normal precipitation in 2019, could have leached soil sulfate below the rooting zone, as noted by (Franzen & Grant, 2008), possibly explaining the small S response with FGCC observed at Sidney.

In greenhouse studies, Zhao et al. (1999) found that + S treatments increased chlorophyll content in pea. In 2020 at Bozeman, plots with S fertilizer were notably greener than -S treatments (Figure 2-7). Combined with our observations in lentil at three of eight site-years with high FGCC from S fertilization, this suggests lentil may have a high demand for S.

Yield

Inoculant. Inoculant increased lentil yield by an average of 510 kg ha^{-1} (43% of site means) at two of eight site-years and trended similarly (*P*=0.12-0.14) for two additional site years, with an average yield increase of 205 kg ha^{-1} (7.8% of site means) (Tables 2-14 and 2-15). These inoculant responses appeared unrelated to recent pulse history.

Inoculant formulation varied in yield response, with granular inoculant increasing yield at two of eight site-years and decreasing yield at one site-year. The response at Sidney in 2019 was very large; granular inoculant increased yield by 1110 kg ha^{-1} (84% of site mean) compared to seedcoat, which was not different from the uninoculated control. The other two responses, positive and negative, were small. In summary, application of inoculant increased lentil yield in four of eight site-years, with little regard to recent pulse history.

As noted by Vessey (2004), yield response to inoculation is not consistent, even in one small part of the NGP – the eastern Canadian prairie. In our study, six of eight site-years did not have a yield response to inoculant versus the uninoculated control, including two of three site-years without recent pulse crop history. Studies have been conducted for pea to assess whether rhizobial inoculation will make the crop self-sufficient for N, or if N supplementation is needed to maximize yield and protein (McKenzie et al., 2001c; Clayton et al., 2004). For pea, granular inoculant provided adequate N supply to meet protein and yield potential, and the application of starter N had inconsistent impact. Inoculant-induced BNF generally supplied enough N to meet yield potential at these sites. Some other reasons for lack of response to inoculation may include high soil mineral N, drought stress, other nutrient limitations, or native and naturalized populations of rhizobia strains in the soil (Kucey & Hynes, 1989; McKenzie et al., 2001c; Vessey, 2004). In 2020, Bozeman and Sidney experienced moderate drought coupled with possible S nutrient limitations, perhaps causing little response to inoculant. In 2019 at Sidney, the seed-coat inoculant performed similarly to the uninoculated control, signifying inoculant failure. Seed-coat inoculants are more challenging to care for before and during the application process in the field. Other explanations for limited inoculant response include high nitrate-N in spring soil sampling. In 2020 at Havre, nitrate-N levels from 15 to 61 cm depth were high at 27.2 mg kg^{-1}, which would indicate > 160 kg NO_3-N ha^{-1}, in that soil depth alone, in addition to NO_3-N in other depths. High nitrate levels can inhibit nodulation and N-fixation (Voisin et al., 2003; Lupwayi et al., 2006).

Fertilizer. Potassium fertilizer elicited a small yield response (84 kg ha^{-1}) only at one site-year, in 2019 at Sidney, which was an unusually responsive site overall. That site had the lowest soil K level (214 mg kg^{-1}) among eight site-years, somewhat below Montana's critical level of 250 mg kg^{-1} (Jacobsen et al, 2003), so it was not surprising that there was a K response there. Conversely, S fertilizer increased yield at three of eight site-years by an average of 304 kg ha^{-1} (17% of site means). Fertilizer S increased yield in 2019 at Sidney by 370 kg ha^{-1}, and in 2020 at Bozeman by 278 kg ha^{-1}, and Moccasin 265 kg ha^{-1} (Table 2-15). In one case, Bozeman 2019, the combination of K + S increased yield by 153 kg ha^{-1} (5.8%) that was not apparent with K or S alone. The micronutrient application had no effect on yield at any site in 2020 (Table 2-15).

Soils were generally satisfactory in potassium; however, sulfate-S levels before planting did not accurately predict S response observed in the field. For example, in 2020 at Moccasin, sulfate-S levels were 7.9 mg S kg^{-1} and treatments with S increased yield by 265 kg ha^{-1} over treatments without S. Conversely, in 2019 at Bozeman and Moccasin, sulfate-S levels were low, <4.4 mg kg^{-1} in the 0-15 cm soil depth, and no S response was observed.

There have been very few studies assessing the advantages of potassium and sulfur in pulses. McKenzie et al. (2001c) found no increase in yield or protein of dry pea among starter fertilizer K rates up to 50 kg ha^{-1}, and S rates ranging up to 20 kg ha^{-1}. However, soil test levels were not reported in that study. McKenzie et al. (2001a) also found little to no response from K fertilization to dry bean in southern Alberta, with soil K levels ranging from 447 to 893 kg K ha^{-1}. The S responses observed at sites in Montana identifies a need to find improvements to the current soil or tissue testing procedural methods. The research sites that had a S response on yield were the same sites that exhibited a strong S response in FGCC. The explanation for yield responses to S were likely due to environmental factors such as moderate drought (2020 in Bozeman), shallow and somewhat rocky soils causing sulfate leaching (2020 in Moccasin), as well as higher than average rainfall with medium-textured soils (2019 in Sidney). In addition to the S response observed in 2020 at Bozeman, treatments with S fertilizer increased plant biomass, and BNF of lentil by 38 kg N ha^{-1} (Baber unpub data). The addition of K and S to the seed-coat treatment helped increase yield in 2019 at Sidney, whereas seed-coat alone performed similarly to the uninoculated control.

Seed Protein

Seed protein data was collected for all sites in 2019 and additional data from Bozeman and Sidney in 2020 was also analyzed on a priority basis. Protein data will be reported for all 2020 sites but was not available at the time of this thesis submission. Strong contrasting visual differences between + and – S treatments in 2020 at Bozeman raised curiosity about the potential for a large difference in seed protein. In this study, the site with the greatest mean protein concentration was observed in 2019 at Bozeman (254 g kg^{-1}), whereas the lowest protein concentration occurred in Sidney in 2019 (207 g kg^{-1}).

Inoculant. Inoculant increased seed protein concentration in only one of six site-years, in 2019 at Havre, by 20 g kg^{-1} compared to the uninoculated control (Table 2-16). This site had a recent field history of pulse crops in 2016. However, two other site-years without a recent history of pulses, were not affected by inoculant. Also in 2019 at Havre, seed-coat inoculant had a higher protein concentration by 10 g kg^{-1} over granular, whereas in 2019 at Sidney, the granular was 17 g kg^{-1} higher than seed-coat.

Study sites in Alberta had greater average protein in granular treatments than peat powder, 194 vs. 176 g kg^{-1}, respectively (Clayton et al., 2004). Bestwick et al. (2018b) found that inoculant type (peat granular vs. peat-powder) was a key management factor for yellow pea protein concentration in Montana and North Dakota, where granular inoculant produced a higher average protein (245 g kg^{-1}) than peat powder seed-coat (229 g kg^{-1}).

Fertilizer. Treatments with S fertilizer increased seed protein in two of six site-years; in 2019 at Bozeman by 7 g kg^{-1} and at Moccasin by 4 g kg^{-1} (Table 2-16). The site in 2019 at Moccasin was generally unresponsive in all other parameters such as plant stand, growth by vigor rating and FGCC, and yield. Even though Bozeman in 2020 exhibited a striking visual response to S application (Figure 2-7), it did not manifest in increased protein (Table 2-17). Thus, S fertility was more important for lentil seed yield than protein concentration in this visually responsive site-year. There was no effect of the either site in 2020.

It is not surprising that protein concentration was not affected from K fertilization, since sites were generally above Montana's critical levels. Bestwick et al. (2018b) also found that K fertilizer did not correlate with yellow pea protein concentration across farm fields in Montana and North Dakota. Additionally, the report from Bestwick et al. (2018b) found that S fertilizer management was a key management factor for yellow pea protein concentration in Montana. Sulfur increased protein content by 8 g kg^{-1} in moderately drought stressed environments. Of the 149 surveys reported in the study, only 51

(34%) reported farm fields reported having used S fertilizer, with the highest proportion of applied S at rates ranging from 3.4 to 9.0 kg ha^{-1} (Bestwick et al., 2018b). This suggests that more research is urgently needed to understand the importance of S fertility, and the likely extent of economic response in the NGP. In this study, S fertilizer treatment cost approximately \$10 ha^{-1} but in 37% of the site=years generated an average yield increase that would be worth more than \$100 ha^{-1} in many recent market years for lentil.

Conclusion

Overall, the effect of inoculant versus the untreated control was inconsistent across response variables at all sites, but when differences occurred, they favored inoculation. In this two-year study, sites with a recent history of pulses did not predict well lentil response to rhizobial inoculant compared to the untreated control. Response to granular vs seed-coat inoculant was very large (84% of overall average site yield) in only one site-year, Sidney 2019. Potassium (K) fertilizer had little to no important effects on lentil in this study where soil K test levels at all sites exceeded 214 ppm. Conversely, S fertilizer caused many positive effects on lentil in this study, increasing seed yield by an average of 300 kg ha^{-1} in three of eight site-years. If this percentage response can be extrapolated to all lentil fields in Montana, the profitability of lentil production could be increased, importantly addressing this one aspect of soil fertility. This study also revealed the unreliability of standard soil test procedures for S. These results raise an important S fertility issue for pulse crops in Montana that merits further attention.

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Tables

Table 2-1. Site characteristics at study sites in 2019 and 2020.

Site	Bozeman		Havre		Moccasin		Sidney	
Year	2019	2020	2019	2020	2019	2020	2019	2020
Decimal Degrees ºN	45.67		48.50		47.07		47.78	
Decimal Degrees ºW	-111.15		-109.80		-109.95		-104.24	
Ave. temperature ºC[a]	14.4	15.0	15.6	16.7	13.3	14.4	17.2	18.9
Total precip. (mm)[a]	366	127	102	102	178	203	203	127
Plot length m	7.6	7.6	6.7	6.7	4.6	4.6	6.1	6.1
Width m	2.4	2.4	1.5	1.5	1.5	1.5	1.5	1.5
Rows	8	8	5	5	5	7	6	6
Row width cm	22.0	22.0	30.5	30.5	30.5	22.0	23.0	23.0
Soil series[b]	Amsterdam		Telstad		Danvers		Williams	
Soil subgroup[b]	Typic Haplustolls		Aridic Argiustolls		Typic Argiborolls		Typic Argiustolls	
Texture[b]	Silt loam		Clay loam		Silty clay loam		Loam	

[a] Ave. Temperature and total precipitation (May-July) from High Plains Regional Climate Center CLIMOD (2020).

[b] Soil profile information from the University of California Davis - California Soil Resource Lab et al. (2019).

Table 2-2. Lentil fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2019 - 2020.

Treatment	Fertilizer Source	Fertilizer Rate	K	S
		------------- kg ha^{-1}	-------------	
1. Control	-	-		
2. Granular inoculant	-	-		
3. Seed-coat inoculant	-	-		
4. Granular with K	KCl	28.0	14	
5. Seed-coat with K	KCl	28.0	14	
6. Granular with K+S	Potassium sulfate	33.6	14	5.6
7. Seed-coat with K+S	Potassium sulfate	33.6	14	5.6
8.Granular+K+S+Micro1000[a]	Potassium sulfate + Micro1000	33.6	14	5.6

Note. Mono-ammonium phosphate (11-52-0) was used at planting at a rate of 50.4 kg ha^{-1} to supply 25.8 kg P_2O_5 ha^{-1}.

[a] Micro1000 was applied at first flower at 2.3 L ha^{-1}.

Table 2-3. Nutrient concentrations in *Micro 1000* foliar product AgroLiquid, St. Johns, MI).

Micronutrient	Micronutrient %	kg ha^{-1}
Sulfur[a]	-[b]	-
Boron	0.02	0.001
Copper	0.25	0.007
Iron	0.37	0.010
Manganese[a]	1	0.028
Zinc	1	0.028
Calcium[a]	1	0.028
Magnesium	0.5	0.014
Cobalt	0.1	0.003
Molybdenum	0.1	0.003
Nickel	0.001	< 0.001[c]

Note. Micro1000 was applied at first flower at rate of 2.3 L ha^{-1}.

[a] Macronutrients in *Micro 1000*

[b] There is also sulfur in this product, although it is not listed on the label.

[c] Nickel = 0.00003 kg ha^{-1}

Table 2-4. Pre and in-season pesticide applications at Bozeman, Havre, Moccasin, and Sidney in 2019 and 2020.

Site-Year	Application	Use	Date	CO	Brand	Active Ingredient	Product rate mL ha^{-1}	ai rate mL ha^{-1}
Bozeman 2019	Pre-plant	Herbicide	23 Apr	Loveland	Makaze	Glyphosate	2339	959
	Pre-plant	Herbicide	3 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	Herbicide	3 Apr	BASF	Sharpen	Saflufenacil	73	22
	In-crop	Insecticide	31 May	Syngenta	Warrior II	Lambda-cyhalothrin	143	33
Bozeman 2020	Pre-plant	Herbicide	20 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	Herbicide	20 Apr	BASF	Sharpen	Saflufenacil	73	22
Havre 2019	None	-	-	-	-	-	-	-
Havre 2020	Preplant	Herbicide	20 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	Herbicide	20 Apr	BASF	Sharpen	Saflufenacil	73	22
	In-crop	Herbicide	4Jun	AgraCity	Quiz	Quizalofop p-ethyl	731	75
Moccasin 2019	Pre-plant	Herbicide	30 Apr	Bayer	RT3	Glyphosate	2339	1141
Moccasin 2020	Pre-plant	Herbicide	20 Apr	Bayer	RT3	Glyphosate[a]	2339	1141
Sidney 2019	Pre-plant	Herbicide	25 Apr	Corteva	Durango	Glyphosate	1754	881
	Pre-plant	Herbicide	22 Apr	BASF	Outlook	Dimethenamid-P	877	560
Sidney 2020	Pre-emerg.	Herbicide	22 Apr	Bayer	PowerMax	Glyphosate	1754	854
	Pre-emerg.	Herbicide	22 Apr	BASF	Outlook	Dimethenamid-P	877	560

[a] Moccasin spot sprayed for creeping thistle (*Cirsium arvense* L.) with glyphosate 6 June 2020

Table 2-5. Field operation and data collection dates at Bozeman, Havre, Moccasin, and Sidney 2019 and 2020.

Site	Bozeman		Havre		Moccasin		Sidney	
Year	2019	2020	2019	2020	2019	2020	2019	2020
Planting	3 May	21 Apr	22 Apr	25 Apr	30 Apr	29 Apr	19 Apr	21 Apr
Date of emergence	1 June	18 May	10 May	10 May	20 May	25 May	6 May	2 May
Stand count	5 June	26 May	10 May	26 May	23 May	1 June	20 May	15 May
Vigor rating	1 July	9 July	25 June	23 June	11 June	15 July	17 June	28 June
Percent canopy cover	1 July	9 July	25 June	23 June	11 June	15 July	17 June	28 June
Micro application	NA[b]	2 July	NA	19 June	NA	1 July	NA	17 June
Flower date	2 July	2 July	20 June	19 June	12-14 July	28 June	24 June	16 June
Days to flower[a]	61	75	60	56	76	61	67	57
Harvest	26 Aug	24 Aug	1 Aug	2 Aug	28 Aug	6 Aug	16 Aug	6 Aug

[a] Days to flower calculated from planting date to start of flowering (50% of plants with open flowers) in calendar days.

[b] NA – Micro application in 2019 was taken out of dataset due to application rates above the label rate causing plant injury.

Table 2-6. Site cropping history in Bozeman, Havre, Moccasin, and Sidney in 2019 and 2020.

Site	Bozeman		Havre		Moccasin		Sidney	
Year	2019	2020[a]	2019	2020	2019[a]	2020	2019[a]	2020
2019	-	Barley	-	Spring barley	-	Spring wheat	-	Spring wheat
2018	Chem fallow	Spring wheat	Spring wheat	Chem fallow	Spring wheat	Spring wheat	Spring wheat	Spring wheat, barley and durum
2017	Faba bean (partial)	Chem Fallow	Chem fallow	Winter wheat	Barley	Spring wheat	Tilled fallow	Spring wheat
2016	Spring Wheat	Winter wheat	Spring pea	Chickpea and lentil	Barley	Winter wheat	Spring wheat	Spring pea
2015	Chem Fallow	Chem Fallow	Chem fallow	Spring barley	Fallow	Barley	Safflower	Tilled fallow
2014	Winter wheat	Oat	Spring wheat	Chem fallow	Spring wheat	Spring pea	Spring wheat, barley and durum	Spring wheat

[a] Research sites without a history of pulses.

Table 2-7. Soil pre-plant nutrient levels in Bozeman, Havre, Moccasin, and Sidney in 2019 and 2020.

Site	Bozeman		Havre		Moccasin		Sidney	
Year	2019	2020	2019	2020	2019	2020[d]	2019	2020
Date of sampling	25 Apr	10 Apr	15-Feb	20 Apr	14 Feb	20 Apr	16 Apr	16 Apr
pH	7.7	7.9	8.1	6.5	8.0	7.3	7.3	7.3
OM %	2.8	2.5	2.1	1.5	3.3	3.5	-	2.5
Salts dS m^{-1}	0.3	0.3	0.3	0.6	-	0.2	-	-
Olsen-P mg kg^{-1}[a]	9.3	12.3	13.5	30.5	16.0	30.0	23.3	22.5
Potassium mg kg^{-1}[a]	414	334	366	308	274	243	214	242
Zinc mg kg^{-1}[a]	0.4	-	0.4	-	-	0.9	-	0.4
Sulfate-S mg kg^{-1} 0-15[a]	4.4	3.3	9.3	16.0	2.1	7.9	-	9.0
Sulfate-S mg kg^{-1} 15-61[b]	2.0	2.3	3.9	17.4	2.2	11.9	-	2.5
Nitrate-N mg kg^{-1} 0-15[a]	2.2	5.2	3.1	3.5	2.1	2.9	2.8	4.6
Nitrate-N mg kg^{-1} 15-61[b]	1.8	1.8	3.1	27.2	1.4	2.0	7.2	2.4
Nitrate-N mg kg^{-1} 61-91[c]	-	0.7	-	8.4	-	-	-	-

[a] Soil depth analyses of 0-15 cm for (pH, OM, Salt, P, K, Zn, S, N)

[b] Soil depth analyses of 15-61 cm for (S and Nitrate-N)

[c] Soil depth analyses of 61-91 cm for (Nitrate-N)

[d] Soils were analyzed through Agvise Laboratories© except Moccasin 2020 was sent to Ward Laboratories, Inc.©.

Table 2-8. Plant stand density of lentil pre-planned contrasts among fertility treatments at Bozeman, Moccasin, and Sidney, MT, 2019.

	Site	Bozeman	Moccasin	Sidney
Number	Treatment	------- Plants m^{-2} -------		
1	Control	70	76	87
2	Granular	86	78	78
3	Seed-coat	88	70	80
4	Granular+K	85	66	69
5	Seed-coat+K	84	75	84
6	Granular+K+S	82	71	63
7	Seed-coat+K+S	74	75	90
Mean		**81**	**73**	**79**
p-value		0.051	0.537	0.058
Linear Contrasts (P - Values)				
1 vs. 2, 3	Control vs. Inoculant	0.004	0.991	0.736
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.961	1.000	0.018
2, 3 vs. 4, 5	W/out K vs. w/ K	0.961	0.923	0.995
4, 5 vs. 6,7	W/out S vs. w/ S	0.445	0.998	1.000
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.146	0.791	0.997
Linear Contrast Estimated Differences[a]				
1 vs. 2, 3	Control vs. Inoculant	-17.6	2.3	8.5
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	2.1	0.7	-15
2, 3 vs. 4, 5	W/out K vs. w/ K	2.6	3.4	2.2
4, 5 vs. 6,7	W/out S vs. w/ S	6.5	1.3	-0.2
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	9.1	4.7	2.0

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-9. Plant stand density of lentil pre-planned contrasts among fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2020.

	Site	Bozeman	Havre	Moccasin	Sidney
Number	Treatment	-------- Plants m^{-2} --------			
1	Control	78	77	138	121
2	Granular	76	83	137	126
3	Seed-coat	74	74	136	121
4	Granular+K	80	88	128	113
5	Seed-coat+K	73	83	123	124
6	Granular+K+S	78	77	131	125
7	Seed-coat+K+S	82	83	151	109
8	Gran+K+S+Micro1000	67	88	131	119
Mean		**76**	**82**	**134**	**120**
p-value		0.659	0.011	0.191	0.717
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.985	0.974	1.000	0.998
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.992	0.773	0.868	0.958
2, 3 vs. 4, 5	W/out K vs. w/ K	0.999	0.060	0.357	0.942
4, 5 vs. 6,7	W/out S vs. w/ S	0.935	0.223	0.094	0.999
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.833	0.967	0.949	0.844
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	3.0	-1.8	1.6	-2.6
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	-1.7	2.4	-4.7	3.7
2, 3 vs. 4, 5	W/out K vs. w/ K	-1.3	-6.9	11.3	4.9
4, 5 vs. 6,7	W/out S vs. w/ S	-3.7	5.4	-15.6	1.8
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-5.0	-1.6	-4.4	6.7

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-10. Plant growth assessed by stand vigor ratings of lentil with pre-planned contrasts among fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2019.

		Bozeman	Havre	Moccasin	Sidney
Number	Treatment	-------- Vigor (0-100%) --------			
1	Control	78.4	92.0	75.6	93.4
2	Granular	87.8	93.0	69.4	94.4
3	Seed-coat	86.2	92.3	69.4	93.2
4	Granular+K	85.4	95.5	74.0	94.4
5	Seed-coat+K	83.4	93.8	67.8	93.6
6	Granular+K+S	86.4	91.8	70.0	96.0
7	Seed-coat+K+S	82.4	94.3	75.8	93.2
Mean		**84.3**	**93.2**	**71.7**	**94.0**
p-value		0.001	0.192	0.741	0.288
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	<0.001	0.982	0.685	0.993
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.093	1.000	1.000	0.111
2, 3 vs. 4, 5	W/out K vs. w/ K	0.215	0.237	0.995	0.999
4, 5 vs. 6,7	W/out S vs. w/ S	1.000	0.437	0.985	0.937
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.215	0.994	0.891	0.841
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	-8.6	-0.6	6.2	-0.4
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	2.5	0.0	0.1	1.6
2, 3 vs. 4, 5	W/out K vs. w/ K	2.6	-2.0	-1.5	-0.2
4, 5 vs. 6,7	W/out S vs. w/ S	0.0	1.6	-2.0	-0.6
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	2.6	-0.4	-3.5	-0.8

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-11. Plant growth assessed by stand vigor ratings of lentil with pre-planned contrasts among fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2020.

		Bozeman	Havre	Moccasin	Sidney
Number	Treatment	-------- Vigor (0-100%) --------			
1	Control	93.2	83.2	95.4	89.0
2	Granular	94.8	82.2	96.4	93.8
3	Seed-coat	92.4	83.6	97.2	93.8
4	Granular+K	94.0	87.6	96.8	93.2
5	Seed-coat+K	93.0	81.2	96.4	93.2
6	Granular+K+S	95.2	84.6	97.8	95.1
7	Seed-coat+K+S	94.2	86.6	98.5	95.4
8	Gran+K+S+Micro1000	94.6	82.6	97.6	95.4
Mean		**93.9**	**84.0**	**97.0**	**93.6**
p-value		0.225	0.904	0.251	0.107
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.991	1.000	0.497	0.040
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.113	0.995	0.970	1.000
2, 3 vs. 4, 5	W/out K vs. w/ K	1.000	0.989	0.998	0.991
4, 5 vs. 6,7	W/out S vs. w/ S	0.464	0.995	0.233	0.565
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.549	0.909	0.362	0.819
6 vs. 8	W/ out Micro vs. w/ Micro	0.989	0.997	1.000	1.000
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	-0.4	0.3	-1.4	-4.8
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	1.5	1.0	-0.4	-0.1
2, 3 vs. 4, 5	W/out K vs. w/ K	0.1	-1.5	0.2	0.6
4, 5 vs. 6,7	W/out S vs. w/ S	-1.2	-1.2	-1.6	-2.1
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-1.1	-2.7	-1.4	-1.5
6 vs. 8	W/ out Micro vs. w/ Micro	0.6	2.0	0.2	-0.3

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-12. Plant growth assessed by percent canopy cover of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2019.

		Bozeman	Havre	Moccasin[b]	Sidney
Number	Treatment	--------- Canopy cover (0-100%) ---------			
1	Control	63.3	18.3	3.6	23.3
2	Granular	62.7	25.4	3.4	26.7
3	Seed-coat	58.6	33.1	3.9	22.1
4	Granular+K	59.6	27.5	3.6	28.1
5	Seed-coat+K	58.2	31.1	2.8	21.7
6	Granular+K+S	60.9	22.9	2.9	33.3
7	Seed-coat+K+S	61.2	32.1	2.9	21.9
Mean		**60.6**	**27.2**	**3.3**	**25.3**
p-value		0.636	<0.001	0.525	<0.001
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.844	<0.001	1.000	0.898
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.814	<0.001	0.995	<0.001
2, 3 vs. 4, 5	W/out K vs. w/ K	0.904	1.000	0.843	0.989
4, 5 vs. 6,7	W/out S vs. w/ S	0.811	0.688	0.938	0.092
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	1.000	0.704	0.400	0.030
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	2.7	-10.9	-0.1	-1.1
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	1.7	-6.9	0.1	7.5
2, 3 vs. 4, 5	W/out K vs. w/ K	1.7	0.0	0.4	-0.5
4, 5 vs. 6,7	W/out S vs. w/ S	-2.1	1.8	0.3	-2.7
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-0.4	1.8	0.7	-3.2

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

[b] Values are low at this site due to date of data collection. Lentil was at V4 (4th node stage). At other sites data collection occurred during early bloom.

Table 2-13. Plant growth assessed by percent canopy cover of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2020.

		Bozeman	Havre	Moccasin	Sidney
Number	Treatment	-------- Canopy cover (0-100%) --------			
1	Control	69.2	44.4	79.6	74.1
2	Granular	72.0	46.9	84.7	80.5
3	Seed-coat	69.3	44.4	82.8	77.5
4	Granular+K	71.4	44.1	83.1	78.4
5	Seed-coat+K	70.9	43.9	82.3	78.2
6	Granular+K+S	79.1	44.6	89.4	79.2
7	Seed-coat+K+S	77.2	42.9	89.6	79.4
8	Gran+K+S+Micro1000	80.1	47.7	91.4	79.5
Mean		**73.6**	**44.9**	**85.4**	**78.4**
p-value		<0.001	0.565	<0.001	0.344
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.838	0.971	0.015	0.104
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.383	0.787	0.864	0.942
2, 3 vs. 4, 5	W/out K vs. w/ K	0.993	0.825	0.835	0.990
4, 5 vs. 6,7	W/out S vs. w/ S	<0.001	1.000	<0.001	0.973
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	<0.001	0.735	<0.001	1.000
6 vs. 8	W/ out Micro vs. w/ Micro	0.990	0.659	0.698	1.000
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	-1.5	-1.3	-4.2	-5.0
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	1.7	1.4	0.8	1.0
2, 3 vs. 4, 5	W/out K vs. w/ K	-0.5	1.7	1.1	0.7
4, 5 vs. 6,7	W/out S vs. w/ S	-7.0	0.3	-6.8	-1.0
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-7.5	1.9	-5.8	-0.3
6 vs. 8	W/ out Micro vs. w/ Micro	-0.9	-3.2	-2.0	-0.3

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-14. Seed yield of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2019.

		Bozeman	Havre	Moccasin	Sidney
TrtNumber	Trt	--------- kg ha^{-1} ---------			
1	Control	2549	721	1179	625
2	Granular	2803	1094	1246	1831
3	Seed-coat	2692	1154	1164	673
4	Granular+K	2677	1097	1178	1827
5	Seed-coat+K	2604	1124	1177	846
6	Granular+K+S	2571	1003	1322	2300
7	Seed-coat+K+S	2404	1153	1207	1111
Mean		**2614**	**1049**	**1210**	**1316**
p-value		0.027	<0.001	0.54	<0.001
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.121	<0.001	0.995	<0.001
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.209	0.017	0.574	<0.001
2, 3 vs. 4, 5	W/out K vs. w/ K	0.486	0.990	0.986	0.077
4, 5 vs. 6,7	W/out S vs. w/ S	0.160	0.801	0.502	<0.001
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.002	0.528	0.802	<0.001
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	-198.3	-403.4	-25.9	-626.5
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	117.1	-78.9	65.7	1108.8
2, 3 vs. 4, 5	W/out K vs. w/ K	107.3	13.6	27.3	-84.2
4, 5 vs. 6,7	W/out S vs. w/ S	152.7	32.6	-86.9	-369.3
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	260.0	46.3	-59.5	-453.5

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-15. Seed yield of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2020.

	Site	Bozeman	Havre	Moccasin	Sidney
Number	Treatment	--------- kg ha^{-1} ---------			
1	Control	1975	2295	2387	2160
2	Granular	2016	2321	2676	2353
3	Seed-coat	1923	2242	2523	2213
4	Granular+K	1926	2253	2596	2221
5	Seed-coat+K	1898	2252	2492	2333
6	Granular+K+S	2251	2230	2798	2388
7	Seed-coat+K+S	2129	2258	2819	2287
8	Gran+K+S+Micro1000	2239	2190	2877	2378
Mean		**2045**	**2255**	**2646**	**2292**
P - value		<0.001	0.482	0.002	0.225
CV %		0.03	3.32	6.13	5.65
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	1.000	0.715	0.140	0.487
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.013	0.987	0.690	0.917
2, 3 vs. 4, 5	W/out K vs. w/ K	0.307	0.959	0.940	1.000
4, 5 vs. 6,7	W/out S vs. w/ S	<0.001	1.000	0.010	0.871
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	<0.001	0.901	0.060	0.908
6 vs. 8	W/ out Micro vs. w/ Micro	0.999	0.982	0.963	1.000
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	5.4	-66.7	-212.3	-123.0
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	80.9	17.3	78.4	42.8
2, 3 vs. 4, 5	W/out K vs. w/ K	57.6	29.3	55.5	6.1
4, 5 vs. 6,7	W/out S vs. w/ S	-278.2	8.6	-264.7	-60.8
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-220.6	38.0	-209.2	-54.7
6 vs. 8	W/ out Micro vs. w/ Micro	12.2	40.0	-79.1	10.3

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-16. Seed protein concentration of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, Havre, Moccasin, and Sidney, MT, 2019.

		Bozeman	Havre	Moccasin	Sidney
Number	Treatment	---------- g kg^{-1} ----------			
1	Control	250	196	236	203
2	Granular	252	211	239	219
3	Seed-coat	252	222	240	197
4	Granular+K	252	219	238	212
5	Seed-coat+K	252	225	235	199
6	Granular+K+S	258	217	240	217
7	Seed-coat+K+S	260	230	242	202
Mean		**254**	**217**	**238**	**207**
p-value		<0.001	<0.001	0.083	<0.001
Linear Contrasts (P - Values)					
1 vs. 2, 3	Control vs. Inoculant	0.788	<0.001	0.438	0.231
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	1.000	<0.001	1.000	<0.001
2, 3 vs. 4, 5	W/out K vs. w/ K	0.998	0.242	0.214	0.825
4, 5 vs. 6,7	W/out S vs. w/ S	<0.001	0.994	0.029	0.310
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	<0.001	0.114	0.889	0.895
Linear Contrast Estimated Differences[a]					
1 vs. 2, 3	Control vs. Inoculant	-2	-20	-3	-5
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0	-10	0	17
2, 3 vs. 4, 5	W/out K vs. w/ K	0	-6	3	2
4, 5 vs. 6,7	W/out S vs. w/ S	-7	-1	-4	-3
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-7	-7	-1	-2

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Table 2-17. Seed protein concentration of lentil with pre-planned contrasts amongst fertility treatments at Bozeman, and Sidney, MT, 2020.

		Bozeman	Sidney
Number	Treatment	--- g kg^{-1} ---	
1	Control	254	233
2	Granular	258	234
3	Seed-coat	258	232
4	Granular+K	259	235
5	Seed-coat+K	264	234
6	Granular+K+S	260	235
7	Seed-coat+K+S	266	234
8	Gran+K+S+Micro1000	264	234
Mean		**260**	**234**
p-value		0.42	0.929
Linear Contrasts (P - Values)			
1 vs. 2, 3	Control vs. Inoculant	0.899	1.000
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	0.639	0.845
2, 3 vs. 4, 5	W/out K vs. w/ K	0.864	0.869
4, 5 vs. 6,7	W/out S vs. w/ S	0.988	0.999
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	0.593	0.726
6 vs. 8	W/ out Micro vs. w/ Micro	0.952	0.961
Linear Contrast Estimated Differences[a]			
1 vs. 2, 3	Control vs. Inoculant	-4	0
2, 4, 6 vs. 3, 5, 7	Granular vs. Seed-coat	-4	1
2, 3 vs. 4, 5	W/out K vs. w/ K	-3	-1
4, 5 vs. 6,7	W/out S vs. w/ S	-2	0
2, 3 vs. 6, 7	W/out K+S vs. w/ K+S	-5	-2
6 vs. 8	W/ out Micro vs. w/ Micro	-4	-2

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$

[a] Estimates with a positive number indicate that treatments to left (or w/out a fertilizer) had higher estimated means than treatments to the right. A negative number indicates that treatments to the right (or w/ a fertilizer) had higher estimated means.

Figures

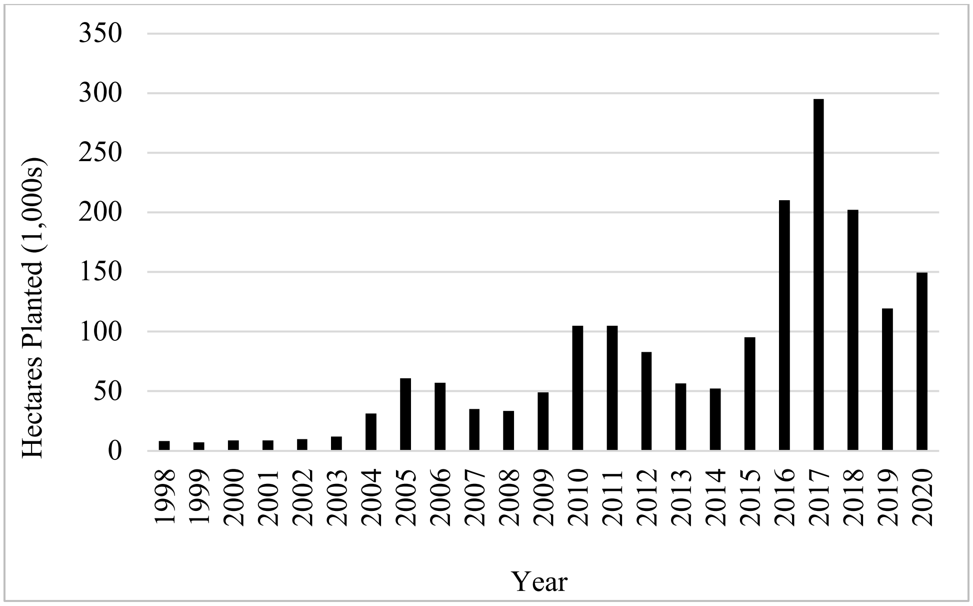

Figure 2-1. Total hectares planted of lentil in Montana from 1998 to 2020 (NASS - Quick Stats, 2020).

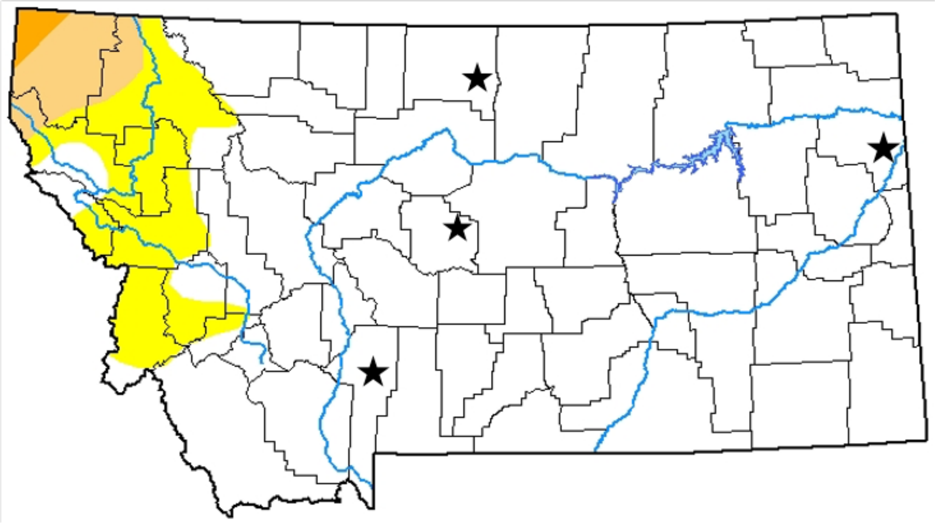

Figure 2-2. Drought monitor map of Montana on 2, July 2019 (National Drought Mitigation Center (NDMC) et al., 2019). Yellow is classified as D0 - abnormally dry. Light orange is classified as D1 – moderate drought, and dark orange classified as D2 – severe drought. Research sites are denoted with a star.

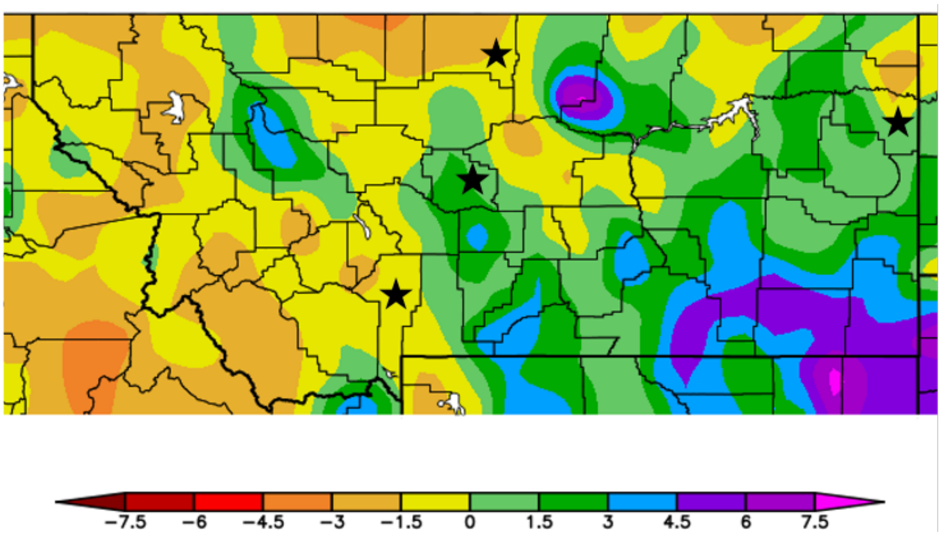

Figure 2-3. Departure from normal precipitation (in) of Montana from 1 May 2019 to 31 July 2019. Departure from normal was determined from 30 years of data from the High Plains Regional Climate Center (HPRCC) (2019). Research sites are denoted with a star.

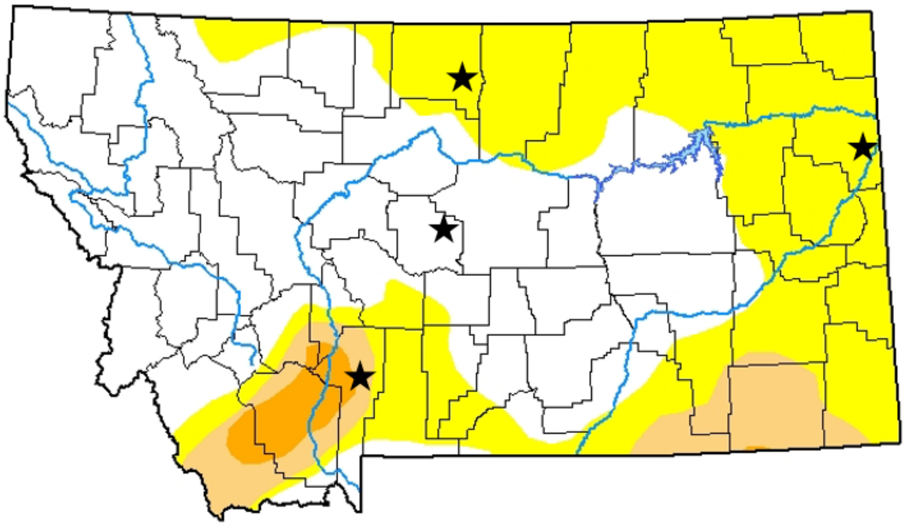

Figure 2-4. Drought monitor map of Montana on 7, July 2020 (National Drought Mitigation Center (NDMC) et al., 2020). Yellow is classified as D0 - abnormally dry. Light orange is classified as D1 – moderate drought, and dark orange classified as D2 – severe drought. Research sites are denoted with a star.

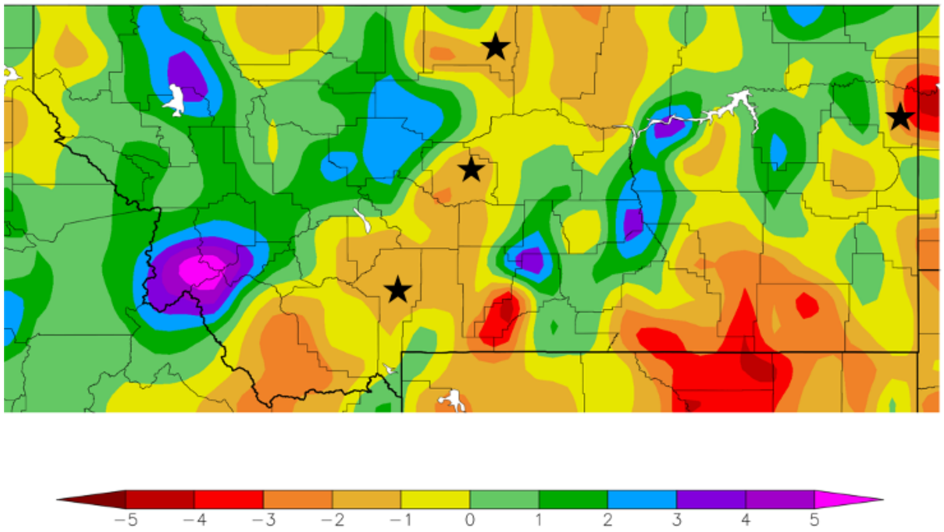

Figure 2-5. Departure from normal precipitation (in) of Montana from 1 May 2020 to 31 July 2020. Departure from normal was determined from 30 years of data from the High Plains Regional Climate Center (HPRCC) (2020). Departure from normal was determined from 30 years of data from the . Research sites are denoted with a star.

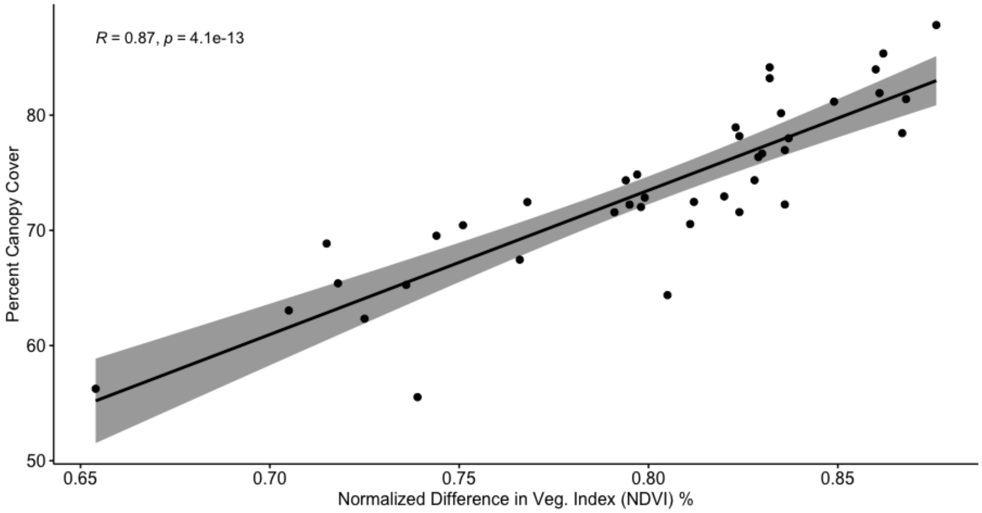

Figure 2-6. Linear correlation relationship of percent canopy cover and NDVI % in 2020 at Bozeman.

Figure 2-7. Visual response of S application on lentil in 2020 at Bozeman. Plot on the left received inoculant plus potassium fertilizer, plot on the right received the same inoculant and potassium rates with additional 5.6 kg S ha $^{-1}$ fertilizer (photo by Kaleb Baber).

CHAPTER THREE

EFFICACY OF SEED-TREATMENTS FOR MANAGEMENT OF LENTIL ROOT ROT CAUSED BY *FUSARIUM* SPP.

Abstract. There is little known about seed treatment efficacy to control Fusarium root rot of lentil (*Lens culinaris* Medikus). The objective of this experiment was to evaluate the efficacy of seed treatments for management of root rot caused by *Fusarium* spp. on lentil establishment, growth, disease severity and yield. This study included four Montana locations in 2019 and 2020: Bozeman (Post Farm), Moccasin (CARC), Havre (NARC), and Sidney (EARC). All trials were inoculated with Fusarium except for Havre. Overall disease severity was relatively low at all sites ranging from 5-29%. There were differences in plant stand among seed treatment in two of eight site-years. Assessments of lentil growth by stand vigor and percent green canopy cover differed among treatment in one of eight site-years, but at different sites, in 2019 at Havre (*P*=0.018) and in 2020 at Bozeman (*P*=0.017). In Havre treatments with Rizolex (Tolclofos-methyl) decreased vigor and plant stand in 2019 and 2020, indicating some phytotoxic effects on lentil. Disease severity differed among seed treatments in three of eight site years. The inoculated control had a high DSI (9%) compared to CruiserMaxx Vibrance Pulse (3%) and Obvius (2%) in 2020 at Havre. Even greater differences were observed in Bozeman in 2019 and 2020; the control had a DSI of (20%) in 2019 compared to CruiserMaxx Vibrance Pulse (11%) and Obvius (13%). There were differences in yield among seed treatments in two of eight site-years, however, the effect of treatment on yield was inconsistent. There were no consistent effects of seed treatments in this two-year research trial. Disease pressure was inconsistent and relatively low. However, the seed treatments CruiserMaxx Vibrance Pulse and Obvius improved canopy cover and lowered disease severity at sites with low disease pressure from Fusarium. Treatments with Rizolex + Apron XL decreased lentil yield and plant vigor at a site that was not inoculated with Fusarium.

Introduction

Montana accounts for 60-70% of the total United States lentil production in hectares planted from 2019- 2020. Other major lentil production states in the U.S. include North Dakota, Washington and Idaho (USDA-NASS, 2020c). The northeast region of the state produces most of the lentils in Montana, with Sheridan county leading production. As of 2020, a total of 360,000 acres of lentil were harvested with a value of production of $90.6 million (USDA-NASS, 2020a). Although lentil production is increasing in the northern Great Plains (NGP), the root rot disease complex is a growing concern on lentil due to its high prevalence in surveys along the NGP region in the United States and pulse growing regions in Canada.

The root rot disease complex includes *Fusarium* spp., *Pythium* spp., *Aphanomyces euteiches*, and *Rhizoctonia solani*. In 2019 and 2020 field surveys were conducted from lentil plants from commercial grower fields in the states of Montana, North Dakota, and Washington. *Fusarium oxysporum* was the predominant *Fusarium* spp. isolated across all three states in 2019 (Bugingo & Fonseka, unpub). In Montana, *F. graminearum* and *F. redolens* were also isolated from diseased lentil roots collected from the survey (Bugingo & Fonseka, unpub). Among the four genera that make up the root rot complex, *Fusarium* spp. is the most frequently isolated in pea and lentil fields from field surveys in the Canadian prairies (Chatterton et al., 2015b; Chatterton et al., 2019). *Fusarium* spp. root rot is also a major concern to dry pea production in North Dakota. The *Fusarium* spp. most frequently isolated from pea roots in 2008 and 2009 field surveys include *F. oxysporum* (67-95% of fields) and *F. avenaceum* (72-90% of fields) (Chittem et al., 2015). Among the frequency of Fusarium isolated from pea and lentil fields in North Dakota, *Aphanomyces euteiches* has also been reported (Zitnick-Anderson & Pasche, 2016; Zitnick-Anderson et al., 2020b). The effects of *A. euteiches* weakens plant roots, allowing for opportunistic pathogens such as Fusarium to co-invade plant roots (Willsey et al., 2018).

The initial symptoms of disease caused by each genus is distinct, however they are challenging to visually distinguish in the field and co-infections are common. Below ground symptoms include a reduced root system and root discoloration varying from golden brown to black. Above ground foliar symptoms include yellowing of leaves, wilting, stunting and reduced yield (Gossen et al., 2016; Kalil et al., 2020). Symptoms specific to *Fusarium* spp. root rot include reddish brown to blackish brown lesions on the roots (Gossen et al., 2016). The taproot may also be discolored and intact, but the fine lateral roots can be destroyed along with a decrease in root nodulation (Hwang et al., 1994). *Fusarium* spp. infect the developing hypocotyl and epicotyl at the point of seed attachment (Bogdan, 2019). Symptoms are visible on the roots, but require uprooting of plants, and therefore may be overlooked in the field (Gossen et al., 2016). *Fusarium* spp. are opportunistic pathogens on pulse crops and require some type of plant stress to cause infection (Bogdan, 2019). The environmental conditions that increase the chance of infection include poor and inadequate soil drainage, low organic matter, low soil fertility, high soil compaction, high soil temperatures, flooding, and drought (Harveson et al., 2005; Larkin, 2015; Gossen et al., 2016; Chatterton et al., 2020).

Due to the complexity of the disease, host, and environmental influences on the development of root rot, it can be difficult to manage. For management of Fusarium root rot in lentil, there is no complete plant resistance. Root rot management recommendations include extended crop rotations (4-5 years out of pulses), timely planting, increased tillage, and fungicide seed treatments (Hwang et al., 2000; Váňová et al., 2011; Chatterton et al., 2015b). Growing evidence suggests that the species causing root rot have wide host ranges including cereals, which may limit the utility of crop rotation as a management tool (Moparthi et al., 2020). Seed treatment fungicides have been effective in faba bean and lentil to assist with crop establishment under disease pressure (Hwang et al., 2000; Hwang et al., 2007; Chang et al., 2013; Chang et al., 2014). Seed treatment fungicides reduced disease severity and, increased final yield in faba bean and lentil by managing the pathogens *F. avenaceum* and *R. solani*. Biological control agents such as *Clonostachys rosea* have also been effective in decreasing root rot severity and improving plant stand in dry peas (Xue, 2003). Fungicide efficacy varies among the different root rot pathogens and can change rapidly if a pathogen becomes resistant to a fungicide (Kalil et al., 2020).

The objective of this research was to further elucidate the efficacy of seed treatment fungicides for management of lentil root rot caused by Fusarium. Trials were conducted to 1) determine the most pathogenic isolate of Fusarium to use in field studies and 2) determine the most effective seed treatment for Fusarium in a four location, two-year uniform study.

Materials and Methods

Isolate Source and Screening for Pathogenicity

In 2019 and 2020 *Fusarium* spp. isolates were obtained from lentil from prior field surveys in Montana and North Dakota (Bugingo, Fonseka, unpub). In 2019, 33 Fusarium isolates from were tested for pathogenicity on lentil from disease surveys in 2016; five isolates that were tested to be the most aggressive to lentil were used for inoculum preparation (Fonseka, unpub). In 2020 isolates from Montana and North Dakota screened for pathogenicity and virulence of lentil the variety, Avondale. North Dakota screened 72 isolates from 2019 North Dakota disease surveys (*personal communication*, D. Fonseka). In Montana 24 isolate were screened for pathogenicity of lentil. The top three most virulent isolates from each state in Montana and North Dakota were used for field inoculum production in 2020 at Montana, using six isolates (Table 3-1).

Isolate Screening for Pathogenicity of Lentil in 2020 at Montana. Isolates used for pathogenicity screening in 2020 at Montana were obtained from field surveys from lentil roots or seeds from field surveys in 2019 at Montana (Bugingo, unpub) and pea roots (Moparthi et al., 2020). Two isolates used in 2019 field trials from North Dakota were screened again for pathogenicity. For culturing, ½ strength potato dextrose agar (Difco, Spark, MD amended with streptomycin and neomycin at a concentration of 50 mg L^{-1} (Zitnick-Anderson et al., 2018) was used. Isolates were hyphal tipped three times to ensure a pure culture. Fusarium isolates were grown in alternate light and dark for a 12h-photoperiod at room temperature to promote spore production. Cultures that grew the most quickly (one week), were used in the first pathogenicity trial. Cultures that needed more time to grow were used in a second trial of the study. Each trial was performed in a randomized complete block design (RCBD) with five replicates in the first trial and six replicates in the second trial. In the first trial 13 isolates were tested. In the second trial 12 isolates were used. Both studies had two shared treatments, a control (½ strength PDA) and *F. oxysporum* (DIV-L9) (Table 3-2). Isolates were identified using the internal transcriber spacer (ITS) region and the translation elongation factor primers (EF-1α) in *Fusarium*-ID database. The ITS and (EF-1α) were amplified with the primers sets ITS4/ITS5 and EF-1/EF-2, respectively (White et al., 1990; O'Donnell et al., 1998).

For the pathogenicity study in the greenhouse, methods for hyphal inoculation of cultures were adopted from Parikh et al. (2018). Mycelium plugs on the agar were cut in a sterile flow hood from the outer most edge of the culture using a 0.5 cm brass cork borer. Four-inch pots were prepared and filled ¾ full of media Sunshine Mix (Sun Gro Horticulture Distribution Inc., Agawam, MA). Five seeds were planted at an even distance apart in a hexagon pattern. Two mycelium plugs were placed next to each lentil seed at planting. The final 1/4th of the pot was lightly filled with soil and watered. After 14 days, plants were removed, and roots were washed and assessed. Root rot severity was measured using a linear scale of 0 to 5 adopted from Ondrej et al. (2008) (Figure 3-1). A score of 0 was a healthy root with no visible symptoms, whereas a score of 5 had a tap root that was completely rotted. (Figure 3-1).

Inoculum Preparation

Isolates were obtained from Montana and North Dakota fields. Pathogenicity testing in the greenhouse indicated virulence on lentil. The most virulent *Fusarium* spp. isolates were obtained from lentil and pea in North Dakota and Montana (Table 3-2). Some of these isolates in 2019 and 2020 were representative isolates from field surveys. All isolates were from roots except one isolate, *F. avenaceum*

(GH-36SL16-1735-38A), was from lentil seed. All isolates used in field trials were from lentil, except two isolates used in 2020 were from pea. Isolates from pea were determined to be cross pathogenic on lentil from a pathogenicity trial in 2020 (Moparthi et al., 2020).

All isolates were replated three times, hyphal tipped to ensure a pure culture on petri plates on ½ strength potato dextrose agar (Difco, Spark, MD amended with streptomycin and neomycin at a concentration of 50 mg L^{-1} (Zitnick-Anderson et al., 2018). In 2019 *Fusarium* spp. isolates were grown at 20 ºC with a 12-hour photoperiod in an incubator. In 2020 Fusarium isolates were grown in alternate light and dark for 12-photoperiod at room temperature to promote spore production. After 14 days of growth, a plate uniformly covered in hyphae was used to inoculate a 2 L Erlenmeyer flask containing 1 L of carboxylmethylcellulose (CMC) broth amended with amended streptomycin and neomycin at a concentration of 50 mg L^{-1} (Zitnick-Anderson et al., 2018) and left on a shaker table at 150 rpm for seven to nine days. The Sigma-Alrich formulation of CMC (C-4888; St. Louis, MO) was prepared following manufacturer instructions (Tuite, 1969).

Pearl white, proso millet (Montana Milling, Inc. Great Falls, MT) was prepared in metal trays (32.4 × 26.4 × 5.5 cm), each containing 3 kg of millet grain. Distilled water was added to trays covered with two layers of tinfoil and left overnight to soak. Water from each tray was drained using a strainer and covered with two layers of tinfoil. Trays were autoclaved for two hours at 121 ºC. The trays were left to cool overnight and then autoclaved for an additional two hours the following day. In a laminar flow hood, 250 mL of inoculum prepared as described above was poured on millet seed and stirred to ensure uniform colonization. Inoculum was allowed to colonize the millet for 10-14 days, and every three days trays were opened and stirred under a laminar flow hood. Once the pathogen fully colonized the grain with mycelia, each tray was dumped onto pans with butcher paper and left to dry for seven to ten days in an empty greenhouse. Clumps of millet were broken up by steel mesh sieves small enough for pearl white millet seed, and Fusarium inoculum was stirred every other day to dry the inoculum. Once fully dry, the inoculum was weighed and bagged per plot with an equal proportion of each isolate to achieve 1 g of inoculum per 0.3 linear row meter (Table 3-3). In 2020, *F. avenaceum* isolate WIL-19-21 R1S1 was slower growing, therefore higher proportions of North Dakota isolates of *F. culmorum* and *F. oxysporum* were added to field inoculum in 2020 (Table 3-4). Montana isolates were already packaged in equal proportion prior to adding isolates from North Dakota, thus proportions of North Dakota isolates were adjusted.

Virulence Trials of Field Inoculum in 2019 and 2020

The virulence and aggressiveness of isolates used in 2019 and 2020 in field inoculum production were tested in the greenhouse to determine which isolate grown on the millet inoculum successfully caused the highest disease symptoms exhibited on lentil roots. To determine which isolate was the most virulent for lentil, greenhouse trials were conducted in 2019 and 2020 with a medium green lentil cultivar, Avondale. Each trial was performed in a randomized complete block design (RCBD) with six replicates. In 2019 five isolates were tested individually and in 2020, six isolates were individually tested as well as the combination of all isolates to mimic field conditions.

Four-inch square pots were prepared and filled ¾ full with the media Sunshine Mix (Sun Gro Horticulture Distribution Inc., Agawam, MA). In 2019 six lentil seeds and in 2020, five seeds were planted an even distance apart in a hexagon shape. A random number generator was used for treatment randomization and 0.5g of millet inoculum was added evenly to the pots. The final quarter of the pot was filled with media and lightly watered. Pots were sufficiently watered every other day. After 14 days, plants were removed, and roots were washed and assessed. Root severity was measured using a linear scale of 0 to 5 (Figure 3-1). A score of 0 was a healthy root with no visible symptoms, whereas a score of 5 had a tap root that was completely rotted.

Colony Forming Units on Millet

A serial dilution was used as a quantitative estimate of the concentration of *Fusarium* spp. on the grain. In 2019 dilutions from 10^1 up to 10^7 were prepared. In 2020 only dilutions 10^3 to 10^5 were prepared. The process started with pipetting 1 mL from the stock solution, to the series of dilutions and vortexed for 5 seconds immediately after to homogenize the dilution mixture. Final dilutions in 2019 included four replicates, in 2020 six replicates. Final dilutions were plated on ½ strength potato dextrose agar (Difco, Spark, MD), amended with streptomycin and neomycin at a concentration of 50 mg L^{-1} (Zitnick-Anderson et al., 2018).

In 2019 and 2020, the optimal dilution for quantification of isolates grown on millet grain was 10^4, between 30-300 CFU. In 2019, *F. avenaceum* (WIL-L15) had the highest colony forming units of (5.6×10^7 CFU g^{-1}), whereas *F. oxysporum* (MOU-19-1*)* had the lowest colony forming units of (1.4×10^7 CFU g^{-1}) (Table 3-5). In 2020, *F. avenaceum* (GH-37PUL-17-034) and *F. oxysporum* (MOU-19-1) colonized millet grain the most compared to other isolates at (5.3×10^7 CFU g^{-1} and 3.9×10^7 CFU g^{-1}), respectively (Table 3-5).

Field Trials

Experimental Design. Field trials occurred at sites in Post Farm (Bozeman), Center Agricultural Research Center (Moccasin), Northern Agricultural Research Center (Havre), and Eastern Agricultural Research Center (Sidney) in Montana in 2019 and 2020. Specific field locations, precipitation, growing season temperature, plot sizes, and soil series are listed in (Table 3-6). Average growing season temperature and total precipitation was retrieved from (High Plains Regional Climate Center CLIMOD, 2020). Soil series information of sites was obtained from (University of California Davis - California Soil Resource Lab et al., 2019). Relevant cropping history of the past five years was recorded from each site (Table 3-7). All sites were under no-till management and were planted with Avondale, a medium green lentil cultivar (PVP # 201400092) (USDA PLC, 2014). A total of ten seed treatments were evaluated for seed treatment efficacy against lentil root rot over two growing seasons (Table 3-8). In 2020, six of the same seed treatments used in 2019 were used again with three new seed treatments. Each trial was conducted in a RCBD design with four replicates. All seed treatments were applied by MSU plant pathology and cropping systems lab except for treatments from Valent, which was applied by the manufacturer (Valent, U.S.A., LLC).

An inoculated control was used with mefenoxam (Apron XL, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.13 mL kg^{-1} of seed to control *Pythium* spp. An additional treatment of thiamethoxam (Cruiser5FS, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.83 mL kg^{-1} of seed was included as an insecticide. All seed treatments included mefenoxam or metalaxyl to control *Pythium* spp. as well as thiamethoxam or imidacloprid as an insecticide. The study in 2020 assessed two essential oil treatments (Oregano) with mefenoxam and without mefenoxam. The essential oil treatment has controlled *Pythium* spp. in lab testing (Parikh, unpub). Fungicide seed treatments without mefenoxam or metalaxyl as an active ingredient were treated with mefenoxam (Apron XL at 0.13 kg ha^{-1} of seed). Except one essential oils treatment did not include Apron XL as an organic option. A baseline insecticide treatment of thiamethoxam (Cruiser5FS at 0.83 kg ha^{-1} of seed) was also added to treatments. Imidacloprid was added as an insecticide to EverGol Energy, due to registrant specifications for product compatibility. Imidacloprid (Gaucho, Bayer CropScience, Campbellville, ON) was applied with EverGol Energy at 1.56 kg ha^{-1} seed. The treatment, Valent 1 (V-10465), was a proprietary product, courtesy of Valent in 2019 (Valent, U.S.A. LLC), that was also treated with thiamethoxam at 0.83 kg ha^{-1} seed (Cruiser5FS, Syngenta Crop Protection). The Arysta Biological treatment used in 2019 was a new product being tested, designated with an arbitrary number (GDC S3202AA) (*personal communication*, Sambeek) (Arysta LifeSciences, Cary, NC). At planting, all sites except for Havre, MT were supplemented with *Fusarium* spp. infested millet inoculum. In 2020 Moccasin received a new seeder before planting, with the capacity to plant seven rows instead of five thus the inoculum rate was lower at that site compared to other sites in 2020. Trials were planted in the same footprint as 2019 with new randomization of treatments.

The seeding rate at all sites were targeted at 120 plants m^{-2}. In 2019, target plant density was not reached, therefore planting weights were adjusted accordingly. Seed weights in 2019 and 2020 were 62 and 75 kg ha^{-1}, respectively. In 2019 and 2020 mono-ammonium phosphate (MAP; 11-52-0) was applied at a rate of 50.4 kg ha^{-1} at planting at all sites except for Moccasin in 2020, which received a starter application of 20-30-20-10 at a rate of 56 kg ha^{-1}. Granular inoculant (*Rhizobium leguminosarum biovar viceae* 1×10^8 CFU g^{-1}, PRIMO GX2), was applied at a rate of 6 - 6.7 kg ha^{-1} in the seed furrow at planting (Verdesian Life Sciences, Cary, NC).

Data Collection. In 2019 plots were seeded on 04 May (Bozeman), 25 Apr. (Moccasin), 03 May (Havre) and 26 Apr. (Sidney). In 2020 plots were seeded on 27 Apr. (Bozeman), 28 Apr. (Moccasin), 24 Apr. (Havre) and 22 Apr. (Sidney). Plant stand density was measured 21-28 days after seeding by counting all plants in 1 m of two different rows, approximately 2.5 m from the front and rear of plot (4 m-row total). Stand counts were then converted to plant population in plants m^{-2}.

In season field assessments for plant growth and root evaluations occurred just before or during first flowering stage. Except in 2019 at Moccasin, data collection was earlier due to oncoming rain. Plot vigor was recorded as a percent and was determined by assigning 100% to the most vigorous plot in each replicate, then assigning rates within the replicate compared to the highest rated plot. Fraction green canopy cover (FGCC) was assessed using the Canopeo App © (Oklahoma State University) which quantifies percent canopy cover and greenness. Three pictures were taken perpendicular to the plot (front, middle, and back, each about 2.5 m apart) and the values were recorded and averaged. When taking pictures, the arm of the assessor was equally extended level with the shoulder and the picture was taken to achieve a uniform distance to the canopy greater than 0.6 m, as recommended. Plots were also hand-weeded before data collection for increased accuracy of the crop canopy cover measurements.

Root evaluations were assessed in 2019 on 1 July (Bozeman), 13 June (Moccasin), 25 June (Havre) and 18 June (Sidney). In 2020 root rot evaluations were on 7 July (Bozeman), 14 July

(Moccasin), 22 June (Havre) and the 28 June (Sidney). For disease assessments, five plants in a cluster were sampled at five points in a "W" shaped pattern for each plot sample totaling 25 plants/plot. Outside rows were not sampled. Plant samples were collected in plastic bags and stored in coolers in a cold storage room until washed. Plants were rated for disease severity on a scale of 0-5. Lentil roots in the control samples were preserved on ice and sent directly to North Dakota State University after rating for *Fusarium* spp. characterization.

For final yield, all plots were harvested with a plot combine, cleaned via sieving and blowers, and weighed. Final dry matter yield in kg ha^{-1} was determined by accounting for seed moisture percentage at harvest from each plot. In 2019 plots were harvested on 26 Aug. (Bozeman), 28 Aug. (Moccasin), 20 Aug. (Havre) and the 16 Aug. (Sidney). In 2020 harvest dates were 14 Aug. (Bozeman), 6 Aug. (Moccasin), 31 July (Havre) and the 8 Aug. (Sidney).

Statistical Analyses

All parameters were analyzed with R 4.0.3 (R Core Team, 2021). Treatment responses varied by location and year; therefore, data were not combined. A linear mixed effects model was fit for each response variable of interest within each location and year using *lme* in the *nlme* package (Pinheiro et al., 2021). Block was considered as a random effect while treatment was considered a fixed effect. Interaction diagnostic plots were made using *intplot* were made to assess treatment * block interactions (Greenwood, 2019). Analysis of variance (type III ANOVA) tests were performed to examined differences in treatment means. Differences were considered significant at $P<0.1$. If a difference among treatments was detected, a Tukey's HSD test was performed at $P<0.1$ using the *multcomp* package (Hothorn et al., 2008). Diagnostic plots were used to assess normality of fixed and random effects, along with Cook's distance plots for possible outliers using the *predictmeans* package (Luo et al., 2020). Cook's distance plots were made to indicate influential points and observations greater than 0.5, then cross-referenced with field notes for possible explanations such as large rodent holes or errors at planting. No transformations were needed for the response variables of interest. Diagnostic plots showed constant variance and residuals that did not deviate far from normality. Estimated treatments means for each model were obtained from the *lsmeans* package (Length, 2016).

Categorical root rot severity data from greenhouse and field studies was converted to a disease severity index % (DSI%) using the formula adopted from Li et al. (2014).

$$DSI\ \% = \left[\frac{(a\ \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5)}{(a + b + c + d + e + f) \times\ g}\right] \times\ 100$$

where a, b, c, d, e, and f represent the number of plants with disease severity ratings of 0, 1, 2, 3, 4 and 5, respectively, with g the highest root rot rating of 5 always in the equation.

Results and Discussion

Greenhouse Trials in 2019 and 2020

Isolate Screening for Pathogenicity of Lentil in 2020 at Montana. The data from trial 1 and trial 2 were combined since they had two shared treatments, the control, and *F oxysporum* (DIV-L9), which performed similarly in each trial. There variance between trials was (σ^2 =0), therefore the data was combined. It is important to note that trial 1 consisted of six blocks, possibly leading to more power to detect differences of treatments whereas trial 2 consisted of five blocks (Goulet & Cousineau, 2019). Of the 24 Fusarium isolates screened for pathogenicity, three isolates were the most pathogenic to lentil with a high DSI compared to other isolates ($F_{24,114} = 6.88$, $P<0.001$, Table 3-9, Figure 3-2). *F. culmorum* (BUR-19-13-R2S1) had the highest DSI of (47% ± 4). Isolates *F. avenaceum* (GH-37PUL-17-034) and *F. avenaceum* (GH-36SL16-1735-38A) also had a high DSI of (40% ± 4) and (35% ± 4) respectively. The two isolates with the highest DSI, *F. culmorum* (BUR-19-13-R2S1) and *F. avenaceum* (GH-37PUL-17-034) were obtained from pea roots and showed cross pathogenicity to lentil, whereas *F. avenaceum* (GH-

36SL16-1735-38A) was obtain from lentil seeds. Moparthi et al. (2020) originally found that isolates of *F. culmorum* and *F. avenaceum* can be high aggressive in lentil, as well as cross pathogenic to cereal crops. These three isolates were used for field inoculum production along with three other Fusarium isolates from North Dakota in 2020.

Virulence Trials of Field Inoculum in 2019 and 2020. Fusarium isolates used in field inoculum production were tested for virulence on lentil variety Avondale. The composition of Fusarium isolates used varied from 2019 to 2020 that were representative of isolates found in field surveys in Montana and North Dakota. All *Fusarium* spp. isolates were pathogenic on lentil (Table 3-10). In 2019 and 2020, disease severity differed among Fusarium species on field inoculum ($F_{5,25} = 70.9$, $P<0.001$) and ($F_{7,35} = 70.9$, $P<0.001$). In 2019, *F. oxysporum* 2 (DIV-L9) had the highest disease severity compared to all other isolates with DSI of (54 % ± 2). All other isolates performed similarly except for the uninoculated control. In 2020 six treatments had a similar DSI. All Fusarium isolates in 2020 had a higher average DSI by 28% than the uninoculated control. *F. oxysporum* (MOU-19-1) had the highest DSI of (32% ± 4), although it was not statistically different from six other isolates with high disease severity percentages. The combination of all isolates used in the same proportions as field trials in 2020 had the lowest DSI% of inoculated treatments with a disease severity of (16% ± 4).

In 2019 Fusarium isolates were packaged in equal proportion. In 2020, the proportions changed due to the isolates' ability to colonize on millet seed. Higher proportions of *F. culmorum* (BUR-19-13-R2S1) and *F. oxysporum* (MOU-19-1) were used to make up for the slow growth of *F. avenaceum* (WIL-19-21-R1S1). Montana isolates were already cultured and packed at a rate divided equally among six isolates before incorporating the three North Dakota isolates. So higher proportions of two North Dakota isolates had to be packaged to achieve our target rate of 1 gram per linear 0.3 row m. In 2020, the combination of all isolates was tested in the same proportion as field trials. The combination of all isolates had a smaller DSI by 12% than Fusarium isolates inoculated individually. The total inoculum density for all treatments was the same, at 0.5 grams per pot. The combination of all Fusarium isolates grown on the millet could have had a smaller proportion of each isolate represented by less inoculum with the same inoculum density of 0.5 grams per pot compared to other treatments. Several pathogens together may also cause an antagonist interaction for pathogens competing for the same resources (Jesus Junior et al., 2014). In greenhouse experiments, competition was observed between some *Fusarium* spp. colonizing pea roots. Zitnick-Anderson et al. (2018) found that *F. graminearum* colonized more on dry pea root tissue compared when co-inoculated with multiple species. More research is needed on multiple versus single species inoculation methods and their interactive effects on lentil root rot.

Field Trial Results in 2019 and 2020

Site Characterization. Locations in this experiment covered a range of growing conditions across the state of Montana. Total precipitation during the growing season (May-July) varied from 102-336 mm among sites in 2019 and 102-203 mm among sites in 2020 (Table 3-6). Total growing season precipitation in Bozeman in 2019 was 366 mm while in 2020 it was just 127 mm. According to National Drought Mitigation Center (NDMC) et al. (2019), in early July of 2019, all sites in this study were not experiencing drought. However, in early July of 2020, all four sites were under typical drought conditions. Research sites in Moccasin, Havre and Sidney were classified as D0 by low soil moisture, poor dryland crop germination, hard soils and agriculture ponds and creeks beginning to dry out. In the Gallatin Valley of Bozeman, MT, drought conditions were much greater in the D1 drought classification range. At this level, field crops are damaged, streams and ponds begin to dry up, and fire potential increases (National Drought Mitigation Center (NDMC) et al., 2020)

Plant Stand. Plant density ranged from -45 plants m^{-2} to + 24 plants m^{-2} of our target density of 120 plants m^{-2}. Stand density exceeded the 120 m^{-2} threshold in only one of eight site-years. Plant density was sufficient to yield adequately at all other site-years (Table 3-11). A study in 2019 at Bozeman assessing seed rate of lentil showed no yield loss until plant stand density dropped below 54 m^{-2} (Miller, unpub). Lentil yield at a stand density of 66 m^{-2} yielded 91% of a stand density of 109 m^{-2} (Miller, unpub). Differences in plant stand among seed treatments were observed in two of seven site-years, in 2020 at Havre ($F_{9,27} = 3.38$, P=0.007) and Moccasin ($F_{9,27} = 0.063$, P=0.063) (Table 3-11). In 2020 at Havre, six seed treatments: Apron Maxx, CruiserMaxx Vibrance Pulse, EverGol Energy, Rancona Summit, Trilex 200 and essential oils, had a higher average plant stand density than Rizolex + Apron XL by 13 plants m^{-2} in a field with low disease pressure. In 2020 at Moccasin, Obvius had a higher plant stand density than essential oils by 33 plants m^{-2}. Other sites showed no differences in plant stand density among seed treatments. Plant stand was not recorded in 2019 at Havre due shortness of staff.

Havre is the only site that did not received Fusarium inoculum at planting. However, lentil roots still exhibited symptoms of Fusarium root rot. Rizolex + Apron XL had the lowest plant stand of 68 plants m^{-2} compared to other treatments, indicating possible phytotoxicity under very low disease pressure. The observation was not likely to be caused by Apron XL since other treatments such as essential oils also had Apron XL. Although more testing in the greenhouse or under low disease pressure would need to be conducted to further understand potential phytotoxicity on lentil.

Plant growth. Assessment of lentil growth by stand vigor ratings did not differ among seed treatments at all sites, except in 2019 at Havre ($F_{9,27} = 2.83$, P = 0.018) (Table 3-12). Four treatments, the control, Apron Maxx, Valent 1, and Arysta Biological had higher plot vigor compared to Rizolex + Valent 1 by an average of 5% in 2019 at Havre (Table 3-12). Lentil growth as measured by fraction green canopy cover (FRCC) did not differ among seed treatments at all sites except in 2019 at Bozeman ($F_{9,26} = 2.88$, P = 0.017) (Table 3-13). In 2019 at Bozeman, seed treatments CruiserMaxx Vibrance Pulse, Obvius and Valent 1 had a higher FGCC by 8.5% than EverGol Energy. Higher overall mean FGCC may be due to later date of data collection.

All data was collected during the R1 – early bloom stage. However, in 2019 at Moccasin, data was collected 17 days before early bloom during the V4 (4th node stage) due to anticipated weather events. Therefore, canopy cover values and vigor ratings were lower at that site year. In 2019 at Havre, the treatment Rizolex + Apron XL had a lower plant stand density. A similar decreasing trend was observed with vigor ratings in 2020 at Havre, where Rizolex + Valent 1 had lower vigor compared to the untreated control as well as the treatment with just Valent 1, further suggesting a possible phytotoxic effect of the chemistry tolclofos-methyl to lentil. Greenhouse studies with tomatoes grown in the greenhouse had vegetive growth that was negatively affected by tolclofos-methyl with phytotoxic effects on tomato height and leaf area at a rate of 4 g m^{-2} of soil (Selim & Selim, 2019). Since the treatment Rizolex + Valent 1 had lower vigor compared to Valent 1, the chemistry in Rizolex would need to be assessed further under low disease pressure. It is important to note that this observation occurred at a site with little to no disease pressure.

Disease Severity. Overall, all eight site-years had relatively low disease with a mean DSI ranging from 5-29% (Figure 3-3, Table 3-14). DSI above 60% is considered high, 40-60% is considered medium and anything less than 40% is considered low/insignificant disease pressure (*personal communications*, Fonseka and Pasche). Sites with the highest mean DSI in this two-year study were at Sidney in 2019 and 2020, with a DSI of 29 and 28%, respectively. Moccasin had a low DSI in 2019 at 27%, and in 2020 at 12%. Havre had the lowest disease pressure from not receiving Fusarium inoculum at planting, however lentil roots still exuded symptoms of natural field inoculum. Moccasin was inconsistent compared to other sites.

Disease severity differed among seed treatments in three of eight site-years (Table 3-14). In 2019 at Bozeman the control had a higher DSI than treatments with CruiserMaxx Vibrance Pulse, Obvius and

Rizolex+Valent 1 by an average of 7% ($F_{9,27} = 2.44$, $P = 0.035$) (Figure 3-4). In 2020 at Bozeman the control had a higher DSI than treatments CruiserMaxx Vibrance Pulse, Trilex 2000, Essential oils + Apron XL, Rizolex + Apron XL and Rancona Summit by an average of 16% ($F_{9,27} = 3.45$, $P = 0.006$) (Figure 3-5). In 2020 at Havre, there was also a difference in disease severity among seed treatments at a site with no Fusarium inoculum at planting ($F_{9,27} = 2.83$, $P = 0.018$). The control had the higher DSI than Obvius by 7%. The control also had a higher disease severity by 6% than CruiserMaxx Vibrance Pulse and EverGol Energy (Figure 3-6).

All sites had a lower DSI in 2020 compared to 2019, except for Bozeman, despite sites being planted in the same footprint to increase disease potential. Even though 2019 was not an ideal disease year for Fusarium root rot, the higher rainfall and lower temperatures may have favored symptom development compared to the drought conditions that occurred in 2020. Perhaps other field inoculation methods and rates should be explored to further assess seed treatment efficacy. The inoculation method of culturing Fusarium isolates on whole millet grain is not the only field inoculation method for culturing root rot pathogens. Chang et al. (2014) used powdered inoculum of *F. avenaceum* and *R. solani* at planting with lentil at different inoculum density rates at 15, 30 and 45 mL row $^{-1}$. Chang et al. (2014) found that seedling emergence and yield decreased with increasing rates inoculum density of *F. avenaceum*, and each fungicide improve seedling emergence compared with the non-treated inoculated control. However, none of the seed treatments reduce root rot severity at varying rates in inoculum density. Applications of Apron Maxx, Trilex EverGol reduced root rot severity compared to the non-treated control in fields inoculated with *R. solani* (Chang et al., 2014).

Sites in Bozeman and Sidney had a history of chickpeas in the rotation in the past five cropping seasons as of 2019, however Moccasin also had a higher DSI at a site without a history of pulses (Table 3-8). In three of eight site-years, the inoculated control, Cruiser + Apron XL, had a higher DSI compared to some seed treatments possibly indicating infection from the field inoculum at planting compounded with natural native Fusarium species and possibly. All seed treatments had a control for *Pythium* spp except for one essential oil treatment.

Final Yield. Average yield of lentil variety Avondale varied by site. The average yield across sites in 2020 were 16% higher compared to the average yield of sites in 2019. At Bozeman and Havre, the average yield decreased by 14% and 48% from 2019 to 2020, respectively. Yield increased at Moccasin by 51%, and two-fold at Sidney from 2019 to 2020. In relation to average yield performance of Avondale across the state of Montana, Bozeman, and Moccasin were the only sites with a similar yield performance compared to the variety evaluated at the same research locations (Franck et al., 2019; Franck et al., 2020) (Figure 3-3). However, it is important to note that the yield performance of Avondale at Sidney was on irritated lentil not dryland. Lower average yield at Havre and Sidney could be due to weed pressure at sites in 2019. In 2020 sites were managed more rigorously to suppress weeds. In 2020 at Havre, yields were greatly impacted due to damage from deer.

Final yield differed among seed treatments in two of eight site-years, both in 2019 and 2020 at the Moccasin site ($F_{9,27} = 1.96$, $P = 0.018$) and ($F_{9,27} = 3.24$, $P = 0.086$), respectively (Table3-15). In 2019 the control (Cruiser + Apron) had a higher yield when compared to Obvius by an average of 255 kg ha^{-1}. In 2020, the seed treatments Obvius had higher yield when compared to essential oils by 367 kg ha^{-1}. Other seed treatments such as Apron Maxx and EverGol Energy also had a higher yield compared to essential oils by an average of 341 kg ha^{-1}. Although it is important to note that the essential oils treatment was included as an organic seed treatment option. The yield response from the seed treatment Obvius differed by site-year at Moccasin. As previously stated, the Obvius treatment had the lowest yield in 2019, and was the highest yielding treatment in 2020 at Moccasin.

Fusarium spp. Characterization in Control Plots at MT Sites in 2019. *Fusarium* spp. characterization was conducted by sampling lentil roots in control plots in 2019 and 2020. Results from 2019 will be presented since *Fusarium* spp. isolated from plants in 2020 field trials are still being characterized. It is important to note that the isolates recovered from control plots in Havre consisted of natural inoculum since it was not infested with Fusarium. A total of 35 isolates were recovered from control plots at Montana sites (Table 3-16). The number of isolates recovered by site slightly differed. In Havre, a site without Fusarium inoculum at planting, the highest number of isolates were recovered (n=13). Sites in Bozeman and Sidney each had (n=10) isolates recovered from lentil roots. The number of each species recovered from lentil roots were *F. graminearum* (n=20), *F. oxysporum* (n=12), *and F. redolens* (n=3). In greenhouse pathogenicity trials, *F. oxysporum* and *F. graminearum* isolates had the highest average DSI of 54% and 53%, respectively. Whereas *F. redolens* isolates had an average DSI of 38% (Fonseka, unpub) (Table 3-16).

In 2019, two *F. oxysporum* isolates were used for field inoculum. One in particular had a DSI of 54% and was virulent on lentil in the greenhouse. However, it cannot be concluded that virulent *F. oxysporum* isolates from the field inoculum were recovered from plants in control plots. *F. oxysporum* was the predominant species isolated from diseased lentil roots across Montana, North Dakota, and Washington in 2019 (Fonseka & Bugingo, unpub) and may have originated from natural inoculum. *F. graminearum* was recovered at a high frequency in field surveys in 2019 in lentil fields in Montana (Bugingo & Fonseka, unpub), and from control plots in 2019. Chongo et al. (2001) found that *F. graminearum* can infect roots of pulses and the crown of wheat crops indicating a broad host range for this species. *F. graminearum* was not artificially inoculated, therefore isolations from control plots are likely were likely from existing populations of the pathogen

Conclusion

This study sought to determine the efficacy of seed treatments for the management of Fusarium root rot of lentil. This included a two-year study in four locations in Montana, with three sites inoculated by Fusarium species isolated from field surveys in Montana and North Dakota. In greenhouse studies, *Fusarium* spp. inoculated individually had a greater DSI compared to a combination of species (Table 3-11). The combination of Fusarium species grown on millet grain likely had a small portion of each isolate, thus were represented by less inoculum density colonized on the grain planted at rate of 1 gram per 0.3 linear row meter in field trials. However multiple samples from the field inoculum would need to be assessed in greenhouse trials with the multiple and single species inoculation to make a more definitive conclusion. In 2019, *F. oxysporum* isolate DIV-L9 had the greatest DSI. *F. oxysporum* was also the species that was most frequently isolated from control plots. *F. graminearum* was also frequently recovered from plants in control plots. *F. graminearum* was not used in the inoculum, indicating these isolates were from natural inoculum. Further research may need to be conducted on the yield loss and disease severity caused by multiple *Fusarium* spp. compared to single species inoculation, in order to clarify the effectiveness of inoculated field trials (Zitnick-Anderson et al., 2018).

There were no consistent effects of seed treatments in this two-year research trial. Results varied by site-year and disease pressure was low. Overall, all eight site-years had relatively low disease with a mean DSI ranging from 5-28%. Sites with a history of pulses in the rotation such as Bozeman and Sidney were generally high in DSI. However, Moccasin, still had a relatively high DSI without a history of pulse crops. In Havre, natural Fusarium inoculum led to Fusarium infection. In three of eight-site years, DSI was greater than 20% in 2019 and 2020 at Sidney and in 2020 at Moccasin. In 2019 and 2020 at Sidney there were no observed differences in seed treatments in all plant response parameters even though DSI was 29% in 2019 and 28% in 2020.

In 2019 at Moccasin the seed treatment Obvius had a higher plant stand with a mean DSI of 27%. Five of eight site-years DSI was less than 20%. In Bozeman 2019 and 2020, several seed treatments had a lower DSI than the inoculated control (Cruiser + Apron). In 2020 at Moccasin, the seed treatment Obvius had a higher plant stand and final yield compared to other seed treatments. In 2019 and 2020 at Havre, mean DSI was low, 8% and 5%, respectively, since the sites were not inoculated with *Fusarium* infested millet. In 2019 at Havre small differences in plant stand were observed in seed treatments, in 2020 the seed treatment Obvius had a lower DSI compared to the inoculated control (Cruiser + Apron) in 2020. This research indicates that seed treatment fungicides may be a viable option for management of root rot caused by *Fusarium* spp. even under low disease pressure. However more site-years of research would need to be conducted under higher disease pressure to further discern seed treatment effects on plant stand density, growth, disease severity and yield of lentil.

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Tables

Table 3-1. Isolates used for field and virulence greenhouse trials in 2019 and 2020.

Year	ID[b]	Species	County, State	Crop	Isolation source	EF1-a [a]
2019	BUR-L13	*F. oxysporum*	Burke, ND	Lentil	Roots	
2019	DIV-L9	*F. oxysporum*	Divide, ND	Lentil	Roots	
2019	WIL-L15	*F. avenaceum*	Williams, ND	Lentil	Roots	
2019	DIV-L13	*F. acuminatum*	Divide, ND	Lentil	Roots	
2019	DIV-L-16	*F. redolens*	Divide, ND	Lentil	Roots	
2020	WIL-19-21 R1S1	*F. avenaceum*	Williams, ND	Lentil	Roots	KU981027
2020	BUR-19-13-R2S1	*F. culmorum*	Burke, ND	Lentil	Roots	KU885985
2020	MOU-19-1	*F. oxysporum*	Mountrails, ND	Lentil	Roots	KU361471
2020	18SMB3-8F7/35b	*F. culmorum*	Valley, MT	Pea	Roots	MK816990
2020	GH-36SL16-1735-38A	*F. avenaceum*	Valley, MT	Lentil	Seed	MK817018
2020	GH-37PUL-17-034	*F. avenaceum*	Valley, MT	Pea	Roots	MK817004

Note. Isolates listed were used for Fusarium inoculum production for field trials in Bozeman, Moccasin and Sidney, MT in 2019 and 2020.

[a] GenBank Accession #: (Moparthi et al., 2020; and Fonseka, unpub)

[b] ID, is the designated isolate identification – source.

Table 3-2. Fusarium isolates screened for pathogenicity on lentil var. Avondale in the greenhouse, 2020.

Trial #	Trt Num[a]	ID[d]	*Fusarium* spp.[b]
1	1	19042 F4 IN 1.1	*F. redolens*
2	2	19042 F4 IN 1.2	*F. accuminatum*
1	3	19042 F4 IN 1.4	*F. oxysporum*
1	4	19042 F4 IN 1.5	*F. oxysporum*
1	5	19042 F4 IN 1.6	*F. oxysporum*
1	6	19042 F4 IN 2.1	*F. redolens*
2	7	19042 F4 IN 2.4	*F. lunatum*
2	8	19042 F4 IN 2.6	*F. acuminatum*
2	9	19042 F4 IN 3.1	*F. cortaderiae*
1	10	19042 F4 IN 3.5	*F. redolens*
1	11	19042 F4 IN 4.5	*F. Hostae, SC*
1	12	19042 F4 IN 4.6	*F. redolens*
2	13	19042 F5 IN 1.2	*F. cortaderiae*
2	14	19042 F5 IN 2.1	*F. cortaderiae*
1	15	19042 F5 IN 4.1	*F. Hostae, SC*
1	16	19042 F5 IN 4.2	*F. cortaderiae*
2	17	19042 F5 IN 4.3	*F. cortaderiae*
2	18	19042 F5 IN 4.5	*F. cortaderiae*
2	19	GH-36SL16-1735-38A	*F. avenaceum*
2	20	GH-37PUL-17-034	*F. avenaceum*
1	21	18SMB3-8F7/35b	*F. culmorum*
1	22	BUR-L13	*F. oxysporum*
1 & 2	23	DIV-L9	*F. oxysporum*
2	24	F. sol GH	*F. solani*

[a] Trt Num, Treatment number.

[b] Fusarium isolates species identification using elongation factor in *Fusarium*-ID database.

[d] ID, is the designated isolate identification – source.

Table 3-3. Field inoculum calculations for Bozeman, Moccasin, and Sidney, MT, 2019 and 2020.

Year	Calculations	Bozeman	Moccasin	Sidney
	Plots	40	40	40
	Rows	8	5	6
	Plot length (m)	7.6	4.6	6.1
2019[a]	grams/isolate/plot	40	15	24
2020[b]	grams/isolate/plot	33.3	12.5	20

Note. Isolates were packaged at a rate of 1 gram of inoculum per 0.3 linear row meter.

[a] Field inoculum rate applied equally among five isolates

[b] Field inoculum rate of six isolates packaged in different proportions due to slow growth of some Fusarium isolates.

Table 3-4. Grams of each isolate packaged per plot for field trials in 2019 and 2020.

	Isolate ID		Weight in grams		
Year	ID	Species	Bozeman	Moccasin	Sidney
2019	BUR-L13	*F. oxysporum*	40	15	24
	DIV-L9	*F. oxysporum*	40	15	24
	WIL-L15	*F. avenaceum*	40	15	24
	DIV-L13	*F. acuminatum*	40	15	24
	DIV-L-16	*F. redolens*	40	15	24
	Total grams/plot		200	75	120
2020	WIL-19-21 R1S1	*F. avenaceum*[a]	25	9.4	15
	BUR-19-13-R2S1	*F. culmorum*	37.5	14.1	22.5
	MOU-19-1	*F. oxysporum*	37.5	14.1	22.5
	18SMB3-8F7/35b	*F. culmorum*	33.3	12.5	20
	GH-36SL16-1735-38A	*F. avenaceum*	33.3	12.5	20
	GH-37PUL-17-034	*F. avenaceum*	33.3	12.5	20
	Total grams/plot		200	75	120

[a] Isolate was slower to colonize on millet, therefore higher proportions where added from North Dakota isolates.

Table 3-5. Colony forming units of Fusarium millet inoculum used in field trials in 2019 and 2020.

Year	ID	Species	10^4 Ave. CFU	SE	CFU g^{-1}
2019	BUR-L13	*F. oxysporum*	70	7	1.4E+07
	DIV-L9	*F. oxysporum*	115	4	2.3E+07
	WIL-L15	*F. avenaceum*	281	9	5.6E+07
	DIV-L13	*F. acuminatum*	121	6	2.4E+07
	DIV-L-16	*F. redolens*	39	1	7.8E+06
2020	WIL-19-21 R1S1	*F. avenaceum*	47	3	9.4E+06
	BUR-19-13-R2S1	*F. culmorum*	70	3	1.4E+07
	MOU-19-1	*F. oxysporum*	194	10	3.9E+07
	18SMB3-8F7/35b	*F. culmorum*	123	3	2.5E+07
	GH-36SL16-1735-38A	*F. avenaceum*	166	3	3.3E+07
	GH-37PUL-17-034	*F. avenaceum*	266	4	5.3E+07

Table 3-6. Site characteristic at study sites in 2019 and 2020.

Site	Bozeman	Havre	Havre	Sidney
	2019 Site Characterization			
Decimal degrees (DD)ºN	45.67	48.51	47.06	47.78
Decimal degrees (DD)ºW	-111.15	-109.77	-109.96	-104.24
Soil series [b]	Amsterdam	Telstad	Danvers	Williams
Soil subgroup [b]	Typic Haplustolls	Aridic Argiustolls	Borolls	Typic Argiustolls
Texture [b]	Fine silty loam	Fine loam	Silty clay loam	Fine loam
Plot length (m)	7.6	6.7	4.6	6.1
Width (m)	2.4	1.5	1.5	1.5
Rows (no.)	8	5	5, 7[c]	6
Row width (cm)	22	30.5	30.5	23
Ave. temperature ºC[a]	14.4	15.6	13.3	17.2
Total precip. (mm)[a]	366	102	178	203
	2020 Site Characterization			
Ave. temperature ºC[a]	15.0	16.7	14.4	18.9
Total precip. (mm)[a]	127	102	203	127

[a] Ave. Temperature and total precipitation during the lentil growing season (May-July) from the High Plains Regional Climate Center CLIMOD (2020).

[b] Soil profile information from the University of California Davis - California Soil Resource Lab et al. (2019).

[c] Five rows in 2019, seven in 2020 due to change in available equipment.

Table 3-7. Site cropping history at Bozeman, Havre, Moccasin, and Sidney in 2019 and 2020

Site	Bozeman[a]	Havre	Moccasin	Sidney[a]
2018	Chem fallow	Spring barley	Winter wheat	Spring wheat
2017	Chickpea/flax	Chem fallow	Barley	Chickpeas
2016	Chem fallow	Spring barley	Barley	Spring wheat
2015	Barley	Chem fallow	Chem fallow	Chickpeas
2014	Canola	Spring barley	Spring wheat	Safflower

Note. Sites in 2020 were planted on the same footprint as 2019 with new randomization of treatments.

[a] Sites with a history of pulses.

Table 3-8. Seed treatments used on lentil variety Avondale in 2019 and 2020 field trials at Bozeman, Moccasin, Havre, and Sidney.

Year	Treatment	Company	ai	FRAC	ai rate mL kg^{-1} seed	Product rate mL kg^{-1} seed
2019, 2020	Control – Apron XL[ac]	Syngenta	Mefenoxam	4	0.04	0.13
2019, 2020	Apron Maxx[a]	Syngenta	Mefenoxam Fludioxonil	4 12	0.11 0.8	3.26
2019, 2020	CruiserMaxx Vibrance Pulse	Syngenta	Thiamethoxam Thiabendazole Sedaxane Mefenoxam Fludioxonil	1 - 7 4 12	0.28 0.14 0.05 0.03 0.02	3.26
2019, 2020	EverGol Energy[b]	Bayer	Prothioconazole Penflufen Metalaxyl	3 7 4	0.05 0.02 0.04	0.65
2019, 2020	Obvius[a]	BASF	Fluxapyroxad Pyraclostrobin Metalaxyl	7 11 4	0.05 0.05 0.17	3
2019, 2020	Trilex 2000[b]	Bayer	Trifloxystrobin	11	0.14	0.65
2019	Valent 1[a]	Valent	V-1046[c] S-2399	NA[d]	NA[d]	3
2019	Arysta Biological[c]	Arysta LifeSciences	GDC S3202AA	-	-	1.3
2019	Rancona Summit + Arysta Biological[a]	Arysta LifeSciences	Ipconazole Metalaxyl GDC S3202AA	3 4 -	0.02 0.04	2.61 1.3

Note. Table 3-8 Continued to next page.

Table 3-8. Seed treatments used on lentil variety Avondale in 2019 and 2020 field trials at Bozeman, Moccasin, Havre, and Sidney, continued.

2019	Rizolex + Valent 1[a]	Valent	Tolclofos-methyl V-10465[c] S - 2399	14 NA[d]	0.08 NA[d]	0.2 3
2020	Essential Oils + Apron XL[a]	100PureEss. Syngenta	Oregano[d] Mefenoxam	- 4	0.003 0.04	1.54 0.13
2020	Essential Oils[a]	100PureEss.	Oregano[d]	-	0.003	1.54
2020	Rizolex + Apron XL[a]	Valent Syngenta	Tolclofos-methyl Mefenoxam	14 4	0.08 0.04	0.18 0.13
2020	Rancona Summit [a]	Arysta LifeSciences	Ipconazole Metalaxyl	3 4	0.02 0.04	2.61

[a] Treatments with baseline insecticide Cruiser5FS – thiamethoxam, ai conc. rate of 0.4 mL kg seed, product rate at 0.83 mL kg $^{-1}$ seed (Syngenta Crop Protection, inc., Greensboro, NC).

[b] Treatments with Gaucho – imidacloprid, ai conc. rate of 0.76 mL kg^{-1} seed, product rate at 1.56 mL kg $^{-1}$ seed (Bayer Crop Science, Campbellville, ON).

[c] Treatments with baseline treatment Apron XL – mefenoxam, ai conc. rate of 0.43 mL kg^{-1} seed, product rate at 1.3 mL kg $^{-1}$ seed. (Syngenta Crop Protection, inc., Greensboro, NC).

[d] Oregano oil dilution concentration of 1mL/500mL, 0.2% (100PureEssential oils, Harrisburg, PA).

[e] V-1046 contain ai of Ethaboxam (FRAC 22), Mandestrobin (FRAC 11), and Metalaxyl (FRAC 4) applied courtesy of Valent (Valent, U.S.A. LLC).

[d] NA, is unknown active ingredient % concentration and rate.

Table 3-9. Greenhouse pathogenicity screening of Fusarium isolates from 2019 field surveys in Montana.

Trial #	I.D. #	ID[b]	*Fusarium* spp.	DSI	SE
1	1	19042 F4 IN 1.1	*F. redolens*	9 b[a]	4
2	2	19042 F4 IN 1.2	*F. accuminatum*	11 b	4
1	3	19042 F4 IN 1.4	*F. oxysporum*	11 b	4
1	4	19042 F4 IN 1.5	*F. oxysporum*	13 b	4
1	5	19042 F4 IN 1.6	*F. oxysporum*	7 b	4
1	6	19042 F4 IN 2.1	*F. redolens*	7 b	4
2	7	19042 F4 IN 2.4	*F. lunatum*	5 b	4
2	8	19042 F4 IN 2.6	*F. acuminatum*	9 b	4
2	9	19042 F4 IN 3.1	*F. cortaderiae*	9 b	4
1	10	19042 F4 IN 3.5	*F. redolens*	7 b	4
1	11	19042 F4 IN 4.5	*F. Hostae, SC*	8 b	4
1	12	19042 F4 IN 4.6	*F. redolens*	8 b	4
2	13	19042 F5 IN 1.2	*F. cortaderiae*	10 b	4
2	14	19042 F5 IN 2.1	*F. cortaderiae*	11 b	4
1	15	19042 F5 IN 4.1	*F. Hostae, SC*	11 b	4
1	16	19042 F5 IN 4.2	*F. cortaderiae*	5 b	4
2	17	19042 F5 IN 4.3	*F. cortaderiae*	5 b	4
2	18	19042 F5 IN 4.5	*F. cortaderiae*	8 b	4
2	19	GH-36SL16-1735-38A	*F. avenaceum*	35 a	4
2	20	GH-37PUL-17-034	*F. avenaceum*	40 a	4
1	21	BUR-19-13-R2S1	*F. culmorum*	47 a	4
1	22	BUR-L13	*F. oxysporum*	11 b	4
1 & 2	23	DIV-L9	*F. oxysporum*	11 b	3
2	24	F. sol GH	*F. solani*	5 b	4
1 &2	25	Control (0.5 PDA)	-	0 b	3
p-value				<0.001	

Note. Significance of treatment effects was evaluated at a = 0.1.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] ID, is the designated isolate identification – source.

Table 3-10. Pathogenicity of *Fusarium* spp. from field inoculum on lentil var. Avondale in the greenhouse, 2019 and 2020.

Year	ID	Treatments	DSI	SE
2019	Uninoculated	Control	0 c	2
	BUR-L13	*F. oxysporum 1*	24 b[a]	2
	DIV-L9	*F. oxysporum 2*	54 a	2
	WIL-L15	*F. avenaceum*	23 b	2
	DIV-L13	*F. acuminatum*	24 b	2
	DIV-L-16	*F. redolens*	21 b	2
p-value			<0.001	
2020	Uninoculated	Control	0 b	4
	WIL-19-21 R1S1	*F. avenaceum 1*	25 a[a]	4
	BUR-19-13-R2S1	*F. culmorum 1*	31 a	4
	MOU-19-1	*F. oxysporum*	32 a	4
	18SMB3-8F7/35b	*F. culmorum 2*	25 a	4
	GH-36SL16-1735-38A	*F. avenaceum 2*	30 a	4
	GH-37PUL-17-034	*F. avenaceum 3*	25 a	4
	Combination	All (field inoculum)	16 ab	4
p-value			<0.001	

Note. Significance of treatment effects was evaluated at a=0.1.

[a] Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

Table 3-11. Plant stand density of lentil seed treatment trials in Bozeman, Moccasin, and Sidney in 2019 and Bozeman, Havre, Moccasin, and Sidney in 2020.

Year	2019			2020			
Site	Bozeman	Moccasin	Sidney	Bozeman	Havre	Moccasin	Sidney
				Plants m^{-2}			
Control	98	69	84	145	78 ab	104 ab[a]	102
Apron Maxx	84	64	58	146	84 a	95 ab	103
CruiserMaxx Vibrance Pulse	98	76	68	151	83 a	90 ab	110
EverGol Energy	88	77	70	148	81 a	104 ab	103
Obvius	91	83	79	149	73 ab	113 a	99
Trilex 2000	94	78	61	139	81 a	104 ab	98
Valent 1	98	75	58	-	-	-	-
Arsyta Biological	90	75	73	-	-	-	-
Rancona Summit + Arysta Bio.[b]	101	74	77	-	-	-	-
Rizolex + Valent1	102	77	63	-	-	-	-
Essential Oils + Apron XL	-	-	-	138	75 ab	103 ab	97
Essential Oils	-	-	-	131	84 a	80 b	97
Rizolex + Apron XL	-	-	-	140	68 b	84 ab	101
Rancona Summit	-	-	-	149	84 a	100 ab	112
Mean	94	75	69	144	79	98	102
p-value	0.301	0.792	0.217	0.318	0.007	0.063	0.704
CV%	9.5	14.7	19.1	6.6	6.3	12.4	10.3

Note. Significance of treatment effects were evaluated at a = 0.1. Columns with a dash - were treatments not used in that site-year. Insecticide treatments not included for clarity. Plant stand density was not collected at Havre in 2019.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] Arysta Bio., Arysta Biological (Arysta LifeScience, Cary, NC)

Table 3-12. Percent vigor of lentil seed treatment trials in 2019 and 2020 at Bozeman, Havre, Moccasin, and Sidney.

Year	2019				2020			
Site	Bozeman	Havre	Moccasin	Sidney	Bozeman	Havre	Moccasin	Sidney
				Vigor (0-100%)				
Control	94.3	96.0 a[a]	81.4	81.3	96.5	89.0	97.5	90.0
Apron Maxx	92.2	96.3 a	77.3	83.0	96.3	83.0	96.0	91.3
CruiserMaxx Vibrance Pulse	92.0	94.0 ab	83.0	85.0	99.3	81.0	96.3	91.3
EverGol Energy	93.5	93 ab	82.0	81.0	94.3	76.5	97.3	92.0
Obvius	94.8	95.5 ab	83.3	79.7	97.5	84.0	97.8	91.0
Trilex 2000	91.8	93.8 ab	73.0	84.0	96.0	74.0	95.3	89.0
Valent 1	90.8	97.0 a	74.3	82.3	-	-	-	-
Arsyta Biological	91.0	96.5 a	70	82.0	-	-	-	-
Rancona Summit + Arysta Bio.[b]	92.3	92.5 ab	83.3	81.3	-	-	-	-
Rizolex + Valent1	91.0	91.0 b	82.3	82.0	-	-	-	-
Essential Oils + Apron XL	-[a]	-	-	-	96.0	85.8	95.0	87.0
Essential Oils	-	-	-	-	95.0	77.3	96.5	87.0
Rizolex + Apron XL	-	-	-	-	94.0	83.3	96.8	93.0
Rancona Summit	-	-	-	-	95.7	75.5	96.0	91.7
Mean	92.3	94.5	79.0	82.1	96.0	80.9	96.4	90.3
p-value	0.183	0.018	0.079	0.784	0.285	0.787	0.646	0.218
CV%	2.2	2.1	7.3	3.1	2.3	13.4	1.8	2.2

Note. Significance of treatment effects were evaluated at a = 0.1. Columns with a dash - were treatments not used in that site-year. Insecticide treatments not included for clarity.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] Arysta Bio., Arysta Biological (Arysta LifeScience, Cary, NC)

Table 3-13. Percent canopy cover of lentil seed treatment trials in 2019 and 2020 at Bozeman, Havre, Moccasin, and Sidney.

Year	2019				2020			
Site	Bozeman	Havre	Moccasin[d]	Sidney	Bozeman	Havre	Moccasin	Sidney
				Canopy cover (0-100%)				
Control	75.4 ab[a]	50.6	1.9	19.5	94.3	57.7	90.0	66.6
Apron Maxx	76.5 ab	50.2	1.3	18.6	94.7	52.6	93.0	68.6
CruiserMaxx Vibrance Pulse	80.1 a	54.1	2.0	16.0	95.0	50.8	89.1	68.5
EverGol Energy	71.5 b	51.2	1.6	17.2	95.0	48.8	90.2	72.1
Obvius	80.8 a	51.8	1.8	19.5	95.1	58.1	91.7	69.0
Trilex 2000	75.1 ab	48.8	1.6	17.6	93.4	50.4	89.9	68.7
Valent 1	79.0 a	53.8	1.8	19.1	-	-	-	-
Arsyta Biological	77.6 ab	56.1	1.4	17.0	-	-	-	-
Rancona Summit + Arysta Bio.[b]	75.3 ab	55.3	2.0	17.6	-	-	-	-
Rizolex + Valent1	73.9 ab	52.5	1.5	16.5	-	-	-	-
Essential Oils + Apron XL	-[a]	-	-	-	94.6	58.9	88.7	65.0
Essential Oils	-	-	-	-	94.9	49.3	90.7	67.5
Rizolex + Apron XL	-	-	-	-	94.5	58.0	91.4	71.7
Rancona Summit	-	-	-	-	95.7	48.7	91.4	69.6
Mean	76.5	52.5	1.7	17.8	94.7	53.3	90.6	68.7
p-value	0.017	0.465	0.093	0.933	0.952	0.432	0.478	0.130
CV%	3.6	7.4	16	15.8	1.8	13.1	2.5	3.1

Note. Significance of treatment effects were evaluated at a = 0.1. Columns with a dash - were treatments not used in that site-year. Insecticide treatments not included for clarity.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] Arysta Bio., Arysta Biological (Arysta LifeScience, Cary, NC)

[d] Lower canopy cover due to early data collection at V4 growth stage.

Table 3-14. Disease severity index (%) of lentil seed treatment trials in 2019 and 2020 at Bozeman, Havre, Moccasin, and Sidney.

Year	2019				2020			
Site	Bozeman	Havre	Moccasin	Sidney	Bozeman	Havre	Moccasin	Sidney
					Disease severity index (%)			
Control	20 a	7	26	27	31 a	9 a	11	36
Apron Maxx	15 ab	5	23	31	21 ab	5 ac	16	26
CruiserMaxx Vibrance Pulse	11 b	11	28	24	16 b	3 bc	13	24
EverGol Energy	16 ab	10	23	30	22 ab	3 bc	14	25
Obvius	13 b	4	23	25	22 ab	2 c	13	31
Trilex 2000	16 ab	11	27	27	16 b	6 ac	11	28
Valent 1	14 ab	7	31	33				
Arsyta Biological	17 ab	8	28	24				
Rancona Summit + Arysta Bio.[b]	15 ab	8	29	32				
Rizolex + Valent1	14 b	7	28	32				
Essential Oils + Apron XL	-	-	-	-	14 b	7 ab	10	26
Essential Oils	-	-	-	-	26 ab	7 ab	12	28
Rizolex + Apron XL	-	-	-	-	14 b	5 ac	11	25
Rancona Summit	-	-	-	-	15 b	4 bc	13	27
Mean	15	8	27	29	20	5	12	28
p-value	0.035	0.742	0.719	0.438	0.006	0.003	0.692	0.256

Note. Significance of treatment effects were evaluated at a = 0.1. Columns with a dash - were treatments not used in that site-year. Insecticide treatments not included for clarity.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] Arysta Bio., Arysta Biological (Arysta LifeScience, Cary, NC)

Table 3-15. Seed yield of lentil seed treatment trials in 2019 and 2020 at Bozeman, Havre, Moccasin, and Sidney.

Year	2019				2020			
Site	Bozeman	Havre	Moccasin	Sidney	Bozeman	Havre	Moccasin	Sidney
					kg ha $^{-1}$			
Control	2696	637	1499 a	711	2449	347	2185 ac	1708
Apron Maxx	2678	647	1336 ab[a]	544	2361	342	2220 ab	1558
CruiserMaxx Vibrance Pulse	2902	762	1428 ab	835	2374	360	1978 ac	1708
EverGol Energy	2802	593	1468 ab	674	2371	361	2238 ab	1837
Obvius	2771	690	1244 b	990	2439	354	2255 a	1702
Trilex 2000	2710	591	1358 ab	673	2272	317	1941 bc	1792
Valent 1	2874	691	1297 ab	744	-	-	-	-
Arsyta Biological	2805	663	1386 ab	938	-	-	-	-
Rancona Summit + Arysta Bio.[b]	2912	725	1480 ab	811	-	-	-	-
Rizolex + Valent1	2732	661	1413 ab	740	-	-	-	-
Essential Oils + Apron XL	-	-	-	-	2398	377	2046 ac	1652
Essential Oils	-	-	-	-	2534	320	1888 c	1573
Rizolex + Apron XL	-	-	-	-	2377	386	2131 ac	1583
Rancona Summit	-	-	-	-	2326	325	2100 ac	1754
Mean	2788	666	1391	766	2390	349	2098	1687
p-value	0.291	0.525	0.086	0.424	0.455	0.52	0.009	0.589
CV%	4.4	14.1	7.4	28.7	5.1	11.8	5.8	10.2

Note. Significance of treatment effects were evaluated at a = 0.1. Columns with a dash - were treatments not used in that site-year. Insecticide treatments not included for clarity.

[a] Tukey's HSD: Means followed by the same letter in a column by site had treatments that are not statistically different from each other (p = 0.1)

[b] Arysta Bio., Arysta Biological (Arysta LifeScience, Cary, NC)

Table 3-16. Count and disease severity of Fusarium isolates recovered from control plots in 2019 at Montana sites.

Site	Bozeman	Havre	Moccasin	Sidney	Species data
		Count of species			Total count by species
F. graminearum	5	6	-	9	20
F. oxysporum	5	5	1	1	12
F. redolens	-	2	1	-	3
Total count from each site	10	13	2	10	**35**
		Ave. DSI by site			Ave. DSI by species
F. graminearum	46	48	-	60	53
F. oxysporum	50	55	56	61	54
F. redolens	-	58	0	-	38
Ave. DSI by site	48	52	28	61	**52**

Note. Fusarium isolates recovered from control plots consisted of two studies in four locations. Pathogenicity trials in 2019 was then conducted from isolates recovered in the greenhouse on lentil (Fonseka, unpub). Some samples were misplaced from Bozeman and Moccasin.

Figures

Figure 3-1. Root rot severity scale used for greenhouse and field trials adopted from Ondrej et al. (2008). Root rot scale with 0= healthy root with no visible symptoms; 1= hypocotyl lesions start appearing; 2 = lesions are starting to appear in both hypocotyl and epicotyl; 3 = lesions spreading out in hypocotyl and epicotyl from the point of seed attachment; 4 = epicotyl, hypocotyl, and root system almost completely infected and only a little amount of healthy tissue remaining; 5 = tap root reduced.

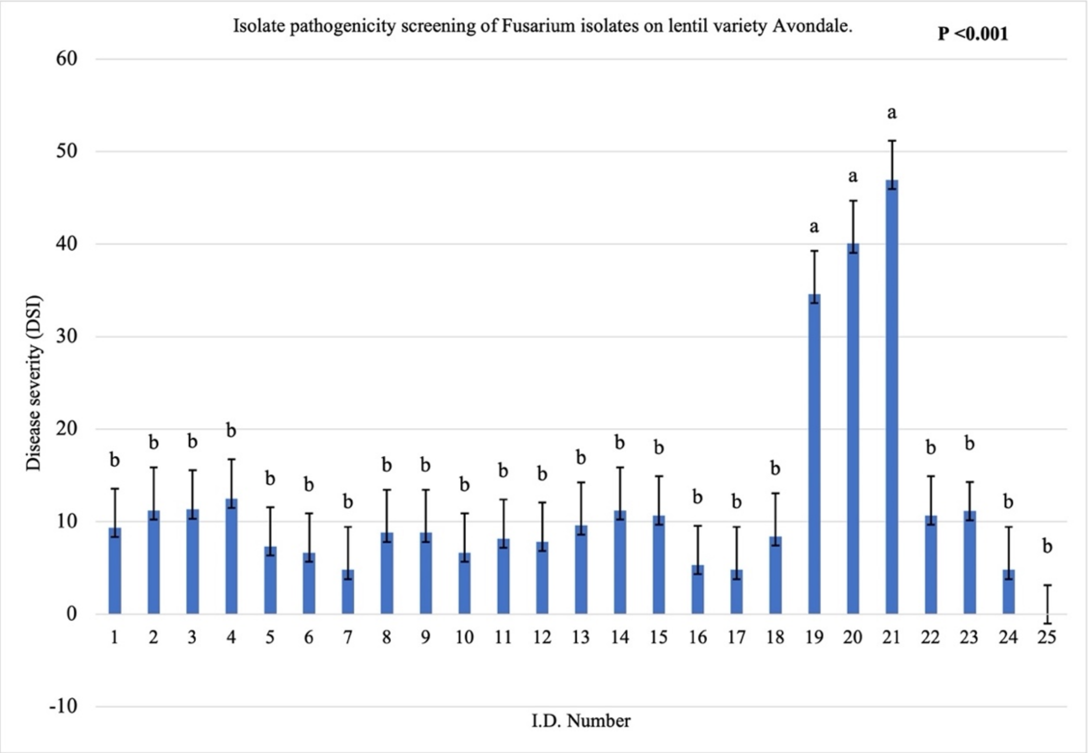

Figure 3-2. Greenhouse pathogenicity screening of Fusarium isolates from 2019 field surveys in Montana. Inoculation of pots was conducted through hyphal plugs. ID number 25 was the uninoculated control (0.5 strength PDA).

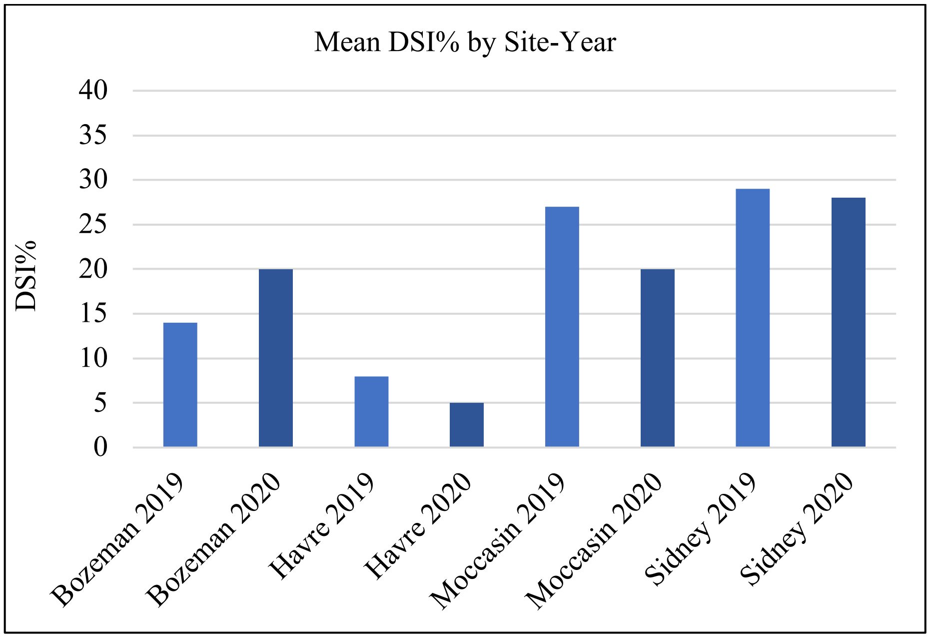

Figure 3-3. Mean disease severity by site-year in Montana.

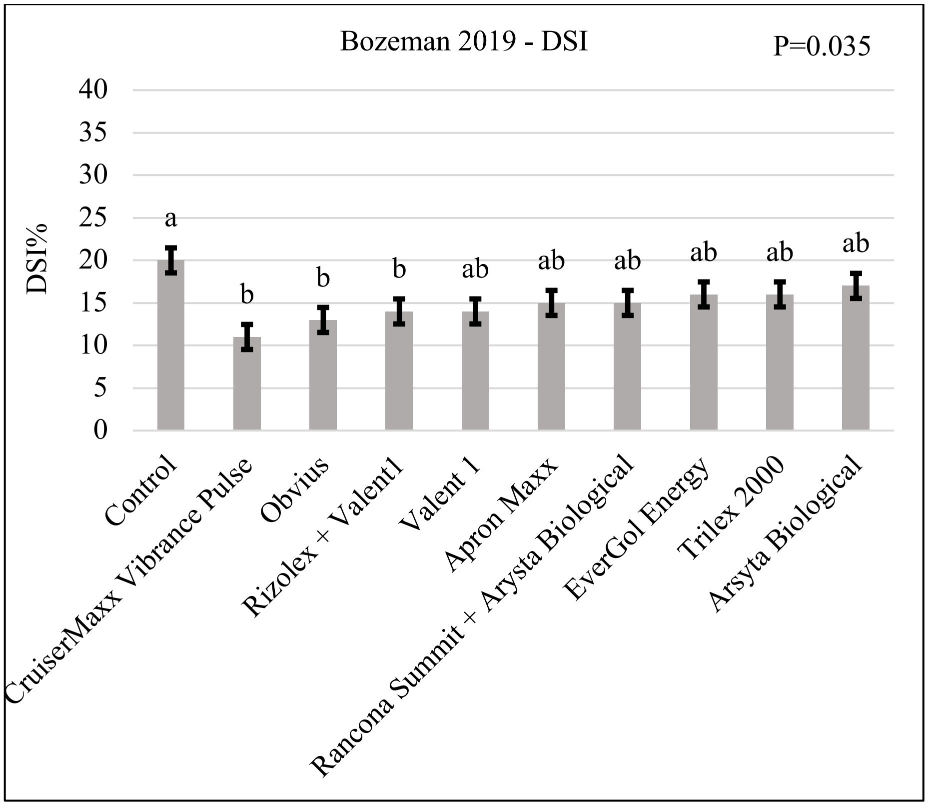

Figure 3-4. Disease severity among seed treatments in 2019 at Bozeman, MT.

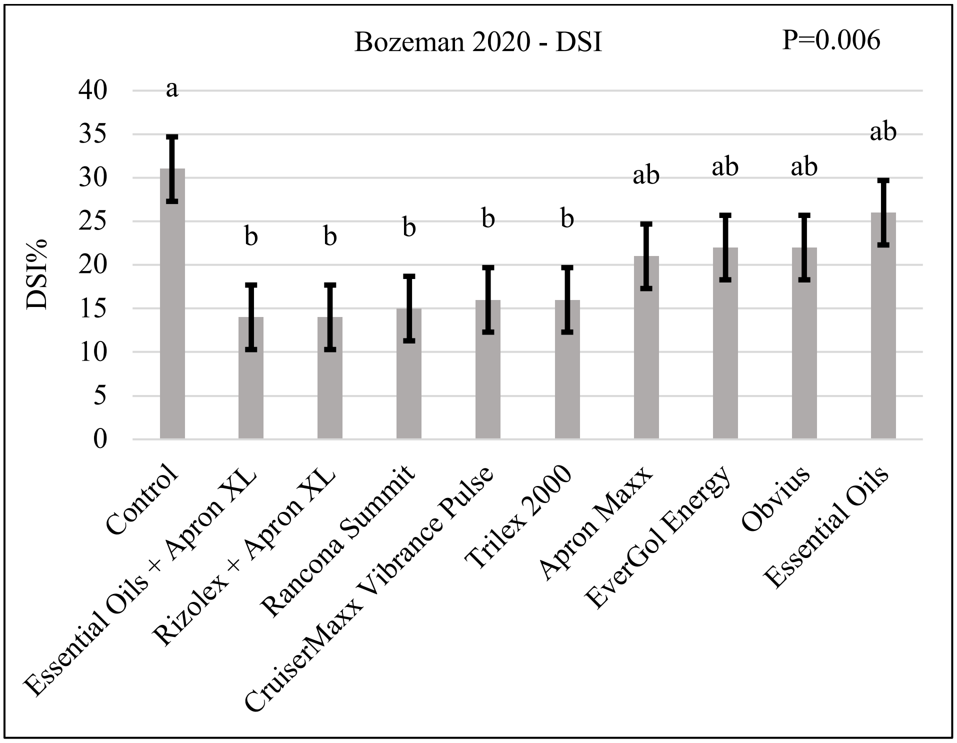

Figure 3-5. Disease severity among seed treatments in 2020 at Bozeman, MT.

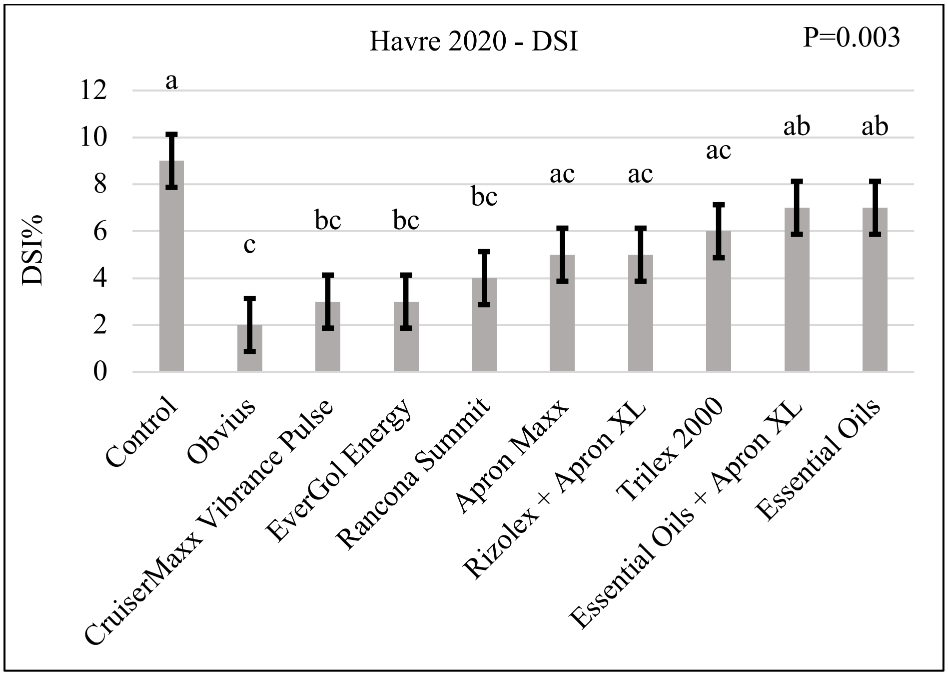

Figure 3-6. Disease severity among seed treatments in 2020 at Havre, MT.

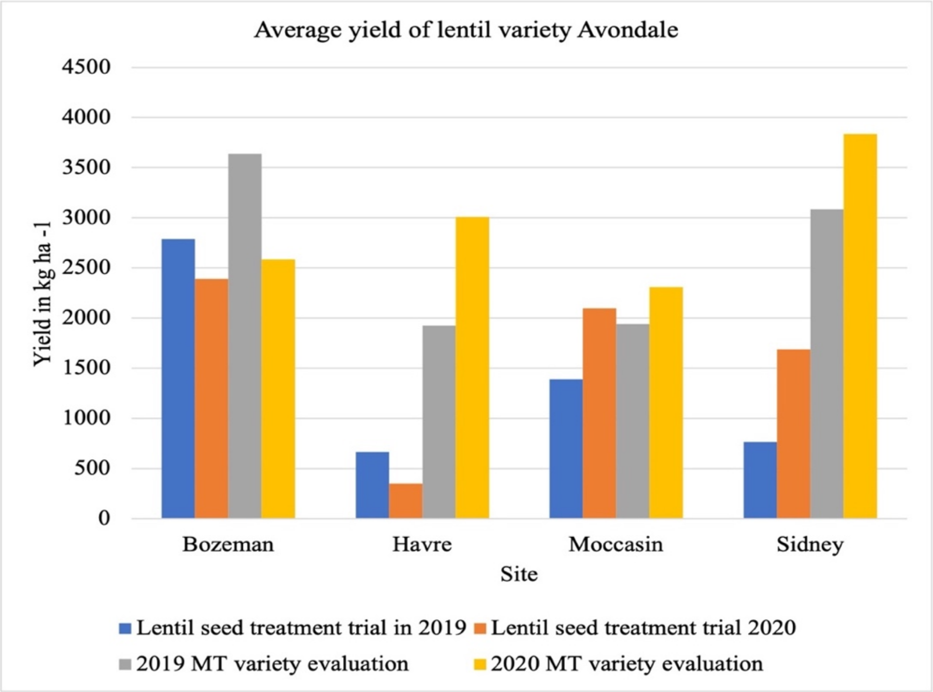

Figure 3-7. Average lentil yield of Avondale in all site-years. Data from 2019 and 2020 variety evaluations were from reports at the same research sites (Franck et al., 2019; Frank et al., 2020).

CHAPTER FOUR

CONCLUSION

This general goal of this research was to expand our understanding of lentil crop fertility for the semi-arid northern Great Plains and explore mitigation strategies for predominant root rot pathogens found in this region via two objectives. The first objective was to study lentil emergence, growth, disease severity, and grain yield and protein as affected by inoculant type, potassium, sulfur, and micronutrients. The second objective explored fungicidal seed treatments for the management of Fusarium root rot of lentil.

Response to inoculation was not consistent across site years in this two-year study. Rhizobial inoculant yield compared to the untreated control at only two of eight site-years; one without a history of pulses and the other with a history of pulse crops. Some factors that may impact inoculant efficacy include high soil nitrate-N, and otherwise low soil fertility which may limit biological N fixation. For example, at Havre in 2020 soil nitrate-N was high, so we may not expect an inoculant response. Soil-applied peat granular vs peat powder seed-coat inoculant improved lentil plant stand, growth, and yield remarkably at Sidney in 2019, likely reflecting inoculant failure with the seed-coat inoculant. Otherwise, the effects of inoculant formulation were not noteworthy. In Montana, soils are generally above the critical soil K level needed for K fertilization, which may explain why there was little response to K in this study. Only one site-year, Sidney 2019, was below the critical soil limit for K and there was a small yield response to K fertilizer of (84 kg ha^{-1}). Sulfur fertilizer application elicited many positive effects on lentil, especially yield. Soil test sulfate-S levels did not predict consistently when a response occurred. Three of eight-site-years exhibited a positive response to S fertilizer, and remarkably so at one site (Sidney 2019). If this response rate (37.5%) corresponds with farm fields in Montana, this demands much needed action on improved ways to predict lentil S needs.

Fusarium root rot disease severity was low at all research sites despite application of fusarium inoculum at six of eight site-years, and in 2020 replanting lentil following 2019 lentil at all sites. Despite these low infection levels, in three of eight site-years, seed treatments reduced disease severity, though inconsistently so. In 2019 at Bozeman, CruiserMaxx Vibrance Pulse, Obvius, and Rizolex+Valent had a lower DSI compared to the uninoculated control. In 2020 at Bozeman, six treatments, CruiserMaxx Vibrance Pulse, Essential oils+Apron XL, Rancona Summit, Rizolex+Apron XL, and Trilex 2000 had lower DSI than the inoculated control. At Havre in 2020, treatments CruiserMaxx Vibrance Pulse, EverGol Energy, and Obvius had lower DSI than the uninoculated control. However, plant and yield did not correspond closely with DSI, with the untreated control often appearing in the highest yield grouping. Yield results were also inconsistent. For example, in 2019 at Moccasin, the treatment Obvius had the lowest yield, whereas in 2020, Obvius was the highest yielding treatment. In 2019 and 2020, isolates from lentil roots in control plots were characterized and assessed for pathogenicity (see Fonseka, North Dakota State University, unpub). Interestingly, *F. graminearum* isolates were recovered at a high frequency on lentil roots in these studies in 2019, followed by *F. oxysporum*. *F. graminearum* was not introduced into plots, rather was naturally occurring inoculum. Both *F. oxysporum* and *F. graminearum* isolates were pathogenic on lentil.

In 2019, field surveys in Montana, North Dakota, Idaho, and Washington found that *F. graminearum* was the most frequent species isolated from lentil roots followed by *F. oxysporum* (Fonseka & Bugingo, Montana State University, unpub). This was an unexpected discovery since *F. avenaceum* and *F. oxysporum* are the most reported species found in pea and lentil fields in Canada (Chatterton et al., 2019) and dry pea in North Dakota (Chittem et al., 2015). In eastern Saskatchewan, *F. graminearum* was the least prevalent species isolated from lentil and pea (Fernandez, 2007). These differences in species prevalence can likely be explained by site differences. Previous research from Moparthi found that *F. avenaceum* and *F. acuminatium* were most frequently isolated from chickpea, dry pea and lentil.

However, the aforementioned 2019 surveys that took place in Montana, North Dakota, Idaho and Washington suggest that *F. graminearum* is also predominately found in the northern Great Plains.

Having four different locations and collecting data for three studies was a challenge for me during the first year of this study both physically and logistically. By the second year I had become more efficient in my field data collection methods. With some distance now between the end of my research and the present, I can pinpoint some key areas of improvement. One would be in relation to my use of the *Canopeo* application. A co-worker and I created a method of averaging three pictures from the middle, back and front perpendicular to the plot, this allowed me to achieve a reading unbiased by vantage point. Although this method worked, I believe that more accurate measurements could have been achieved by utilizing aerial imaging from drone using *Canopeo*.

Regarding statistics, I encourage other research to explore such analyses if they get high disease in the field. I also encourage other researchers with a categorical response variable such as disease severity, to explore models such as the cumulative link mixed model in the *ordinal* package (Christensen, 2019). Other options would be ordered logistic regression models and probit regression in the *MASS* package (Venables & Ripley, 2002). The cumulative link mixed model is a relatively new model that allows for nested and block random effects, that is great for multi-site and multi-block data. There is some statistics research on these newer models for categorical data. However, the interpretation of such models can be scarce. It takes practice with these models, and I wish I had more time to explore the interpretations at a greater depth and to implement them in this research. I hope this insight on model information will be useful for others when working with a categorical response.

For the lentil fertility study, I would have liked to collected data on nodulation for all blocks and site-years, however, with the workload that would have been impossible. I believe the work that Kaleb Baber is doing will be great supplementary data for the lentil fertility treatments on N-fixation. For the lentil seed treatment study, the addition of an uninoculated-untreated control (without Fusarium and without Fusarium seed treatments) would have been a great addition to the study. Even cutting back two treatments to increase the amount of replication. Another beneficial change would be to culture Fusarium longer on millet grain. The culturing process takes quite a bit of time, so it is important to screen early for pathogenicity and then take the time necessary to culture Fusarium longer for more colonization.

For the lentil fertility study, I encourage future researchers to further explore the environmental conditions that directly impact a S response for lentil. Maybe implementing studies at sites with different soil texture and rainfall. This would be great to implement leaf, soil moisture and temperature sensors. In this study, even though there was little response positive response to S fertilization on protein, more site-years of data may suggest otherwise. For the lentil seed treatment study, I encourage researchers to explore field inoculum methods, whether it is culturing on seed, or ground seed. Work by Chang et al. (2014) explored disease severity at different rates of inoculum that would be great to add to a split-plot, split block design. It would also be beneficial to study the competition between Fusarium species that cause Fusarium root rot of lentil. Implementing a trial inoculated with a single species predominantly found in the region would also be of beneficial researcher. A final thing I would inspire researchers to do, is to explore the economic side of their research. I think it is important to always relate the work back to the producers, for whom we are doing this research for. For example, S fertilizer cost approximately \$10 ha^{-1}. For the lentil fertility study, S fertilizer of 5.6 kg ha^{-1} increased yield in three of eight site-years by 300 kg ha^{-1}. If more farmers applied fertilizer S to lentil, the increase in yield would have easily exceeded their return on investment. For the lentil seed treatment study, newer seed treatments on the market may likely be more expensive containing multiple active ingredients for root rot control. It would be beneficial to determine which treatment achieved maximum yield potential under high disease pressure and calculate the return on investment based on seed treatment cost.

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APPENDICES

APPENDIX A

SUMMARY OF STATISTICAL ANALYSES FOR CHAPTER 2

Table A-1. Emergence, vigor, canopy cover, protein conc. and yield for lentil seed treatment trials in Bozeman, Havre, Moccasin and Sidney, MT in 2019 and 2020.

		2019 Analysis of Variance				2020 Analysis of Variance			
Site	Response Variable	NumDF[a]	DenDF[b]	F Value	p-value	NumDF[a]	DenDF[b]	F value	p-value
Bozeman	Emergence (plants m^{-2})	7	28	2.36	0.051	7	28	0.72	0.659
	Percent vigor rating	7	28	4.78	0.001	7	28	1.45	0.225
	FGCC[b]	7	26	0.98	0.636	7	28	11.3	<0.001
	Final yield (kg ha^{-1})	7	28	3.8	<0.001	7	28	18.78	<0.001
	Protein conc.	7	28	7.11	0.027	7	28	1.05	0.422
Havre	Emergence (plants m^{-2})	-	-	-	-	7	28	3.33	0.011
	Percent vigor rating	7	21	1.33	0.192	7	28	0.38	0.904
	FGCC[b]	7	21	9.97	<0.001	7	26	0.84	0.565
	Final yield (kg ha^{-1})	7	21	14.86	<0.001	7	27	0.96	0.482
	Protein conc.	7	21	11.47	<0.001	-	-	-	-
Moccasin	Emergence (plants m^{-2})	7	28	0.83	0.537	7	28	1.55	0.191
	Percent vigor rating	7	28	0.53	0.741	7	27	1.39	0.251
	FGCC[b]	7	28	0.88	0.525	7	26	13.61	<0.001
	Final yield (kg ha^{-1})	7	28	1.64	0.083	7	27	4.53	0.002
	Protein conc.	7	28	4.4	0.54	-	-	-	-
Sidney	Emergence (plants m^{-2})	7	28	2.18	0.058	7	28	0.64	0.717
	Percent vigor rating	7	28	1.44	0.288	7	27	1.91	0.107
	FGCC[b]	7	28	7.63	<0.001	7	27	1.18	0.344
	Final yield (kg ha^{-1})	7	28	278.6	<0.001	7	27	1.46	0.225
	Protein conc.	7	28	24.38	<0.001	7	28	0.34	0.929

Note. Significance of treatment effects were evaluated at $\alpha = 0.1$. Mixed model fit using *lme* in *nlme* package (Pinheiro et al. 2021).

[a] NumDF, numerator degrees of freedom; DenDF, denominator degrees of freedom.

[b] Fraction green percent canopy cover using Canopeo application (Oklahoma State University).

Table A-2. Model information used in 2019 and 2020 lentil fertility trials.

Site-Year		2019			2020		
	Response Variable	Obs[b]	σ^2 Bock	σ^2 Residuals	Obs[b]	σ^2 Block	σ^2 Residuals
Bozeman	Emergence (plants m^{-2})	35	20.1	92.4	40	0	146.3
	Percent vigor rating	35	60	9.1	40	1.7	3.3
	FGCC[c]	34	0	24.5	40	38.7	8.7
	Final yield (kg ha^{-1})	35	4480.4	27253.1	40	11331.1	5467.5
	Protein conc.	35	1.4	10.7	40	10.6	74
Havre	Emergence (plants m^{-2})	-	-	-	40	0	39.4
	Percent vigor rating	28[a]	1.2	4.5	40	32.3	63.1
	FGCC[c]	28[a]	1.3	9.9	38	0	14.8
	Final yield (kg ha^{-1})	28[a]	5555.7	4423.1	39	20093.9	8036.7
	Protein conc.	28[a]	46.3	41.3	-	-	-
Moccasin	Emergence (plants m^{-2})	35	5.1	110.7	40	22.1	231.3
	Percent vigor rating	35	14.1	94.4	39	0	3.2
	FGCC[c]	35	0.2	1	38	0.3	6.4
	Final yield (kg ha^{-1})	35	2267.2	18448.6	39	138.9	33071.5
	Protein conc.	35	0	12.9	-	-	-
Sidney	Emergence (plants m^{-2})	35	0	201.7	40	9.2	274
	Percent vigor rating	35	0.2	3.9	39	3	11.2
	FGCC[c]	35	2.1	6.9	39	11.6	16.2
	Final yield (kg ha^{-1})	35	580.9	6270.2	39	53233.1	23921.8
	Protein conc.	35	16.9	19.2	40	9.2	14.6

[a] Block five was taken out of dataset in 2019 at Havre, due to high variability and was in a low spot in the field causing a possible water gradient.

[b] Obs, is number of observations.

[c] Fraction green percent canopy cover using Canopeo application (Oklahoma State University).

APPENDIX B

SUMMARY OF STATISTICAL ANALYSES AND ADDITIONAL SITE INFORMATION FOR CHAPTER 2

Table B-1. ANOVA of greenhouse pathogenicity screening of Fusarium isolates from 2019 field surveys in Montana. First model was fit with trial as a fixed effect to test differences in trial to combine data.

ANOVA with trial as a fixed effect.					
Response: DSI	DF	Sum Sq	Mean Sq	F value	p-value
Trt number	24	17358.0	723.25	6.7	<0.001
Trial	1	0.2	0.22	0.0020	0.964
Residuals	123	13369.5	108.7		

Note. No significant difference between trials. Therefore, the data was combined using a linear mixed effects model with block variable nested in trial.

Table B-2. ANOVA of greenhouse pathogenicity screening of Fusarium isolates using a mixed effects model.

ANOVA table with combined data from Trial 1 and 2				
Independent Var.	numDF	denDF	F-value	P – value
Trt number	24	114	6.88	<0.001

Mixed model fit using *lme* in *nlme* package with trial nested before block (Pinheiro et al. 2021).

Table B-3. Mixed model information with trial nested before block.

Model info: Mixed effects linear regression	
Observations:	149
Pseuo-R2 (fixed effect):	0.52
Pseuo-R2 (total):	0.53
σ^2 trial:	0.0
σ^2 block:	7.8
Residuals:	11032.4

Table B-4. Virulence of Fusarium spp. isolates on field inoculum used in greenhouse trials in 2019 and 2020.

	ANOVA			
Independent Var.	numDF	denDF	F-value	P - value
Treatment 2019	5	25	70.99	<0.001
Treatment 2020	7	35	5.99	<0.001

B-5. Model summary of virulence trials of field inoculum used in greenhouse trials in 2019 and 2020

Model info: Mixed effects linear regression	2019	2020
Observations:	36	48
Pseuo-R2 (fixed effect):	0.91	0.47
Pseuo-R2 (total):	0.91	0.47
σ^2 block:	0.0	0.0
Residuals:	25	101.4

B-6. Emergence, vigor, canopy cover, disease severity index and yield for lentil seed treatment trials in Bozeman, Moccasin, Havre and Sidney, MT in 2019 and 2020.

	Year	2019 Analysis of Variance				2020 Analysis of Variance			
Site	Response Variable	Num DF[a]	Den DF[a]	F Value	p-value	Num DF[a]	Den DF[a]	F value	p-value
Bozeman	Emergence (plants m^{-2})	9	26	1.27	0.301	9	27	1.23	0.318
	Percent vigor rating	9	26	1.55	0.183	9	26	1.30	0.285
	FGCC[b]	9	26	2.88	0.017	9	26	0.34	0.952
	DSI%	9	27	2.44	0.035	9	27	3.45	0.006
	Final yield (kg ha^{-1})	9	26	1.29	0.291	9	27	1.01	0.455
Moccasin	Emergence (plants m^{-2})	9	27	0.59	0.792	9	27	2.12	0.063
	Percent vigor rating	9	26	2.01	0.079	9	27	0.77	0.646
	FGCC[b]	9	26	1.92	0.093	9	27	0.98	0.478
	DSI%	9	27	0.68	0.719	9	27	0.71	0.692
	Final yield (kg ha^{-1})	9	27	1.96	0.086	9	27	3.24	0.009
Havre	Emergence (plants m^{-2})	-	-	-	-	9	27	3.38	0.007
	Percent vigor rating	9	27	2.83	0.018	9	25	0.60	0.787
	FGCC[b]	9	27	1.00	0.465	9	25	1.05	0.432
	DSI%	9	27	0.65	0.742	9	27	3.80	0.003
	Final yield (kg ha^{-1})	9	27	0.92	0.525	9	27	0.92	0.52
Sidney	Emergence (plants m^{-2})	9	27	1.45	0.217	9	27	0.70	0.704
	Percent vigor rating	9	18	0.60	0.784	9	18	1.51	0.218
	FGCC[b]	9	18	0.37	0.933	9	18	1.84	0.13
	DSI%	9	27	1.04	0.438	9	27	1.36	0.256
	Final yield (kg ha^{-1})	9	27	1.06	0.424	9	27	0.84	0.589

Note. Significance of treatment effects was evaluated at $\alpha = 0.1$. Mixed model fit using *lme* in *nlme* package (Pinheiro et al. 2021).

[a] NumDF, numerator degrees of freedom; DenDF, denominator degrees of freedom.

[b] Fraction green percent canopy cover using Canopeo application (Oklahoma State University).

B-7. Notable Tukey’s HSD contrast results from lentil seed treatment trials in 2019 and 2020

Ind. Var. & Site-Year	Trt1	Trt2	p - value	Est. Diff.[b]	L.CI[b]	U.CI[b]
Plant stand Moccasin 2020	Obvius	Essential oils	0.034	32.7	-18.7	39.3
Plant stand Havre 2020	Rizolex+ApronXL	Apron Maxx	0.002	-16.8	-29.1	-4.4
	Rizolex+ApronXL	CruiserM. Vib. P.[a]	0.015	-14.9	-27.3	-2.6
	Rizolex+ApronXL	Essential Oils	0.006	-15.8	-28.2	-3.5
	Rizolex+ ApronXL	Rancona Summit	0.005	-16.2	-28.5	-3.8
	Rizolex + Apron XL	EverGol Energy	0.061	-13.1	-25.4	-0.7
	Trilex2000	Rizolex+ApronXL	0.049	13.4	1.1	25.8
FGCC Bozeman 2020	EverGol Energy	CruiserM. Vib. P.[a]	0.026	-8.6	-16.1	-1.5
	Obvius	Evergol Energy	<0.01	9.3	2.4	16.2
	Valent1	Evergol Energy	0.046	7.5	-12.4	1.4
Vigor Havre 2020	Valent1	Rizolex+Valent1	0.014	6.0	1.1	10.9
	Rizolex+Valent1	Apron Maxx	0.058	0.8	-4.2	5.7
	Rizolex+Valent1	Arysta Biological	0.037	-5.5	-10.4	-0.6
	Rizolex+Apron XL	Control	0.088	-5.0	-9.9	-0.1
DSI Bozeman 2019	CruiserM. Vib. P.[a]	Control	0.002	-8.4	-14.5	-2.3
	Obvius	Control	0.027	-7.0	-13.1	-0.9
	Rizolex+Valent1	Control	0.083	-6.2	-12.3	-0.1
DSI Bozeman 2020	CruiserM. Vib. P.[a]	Control	0.023	-14.6	-27.1	-2.1
	Essential oils + ApronXL	Control	<0.01	-17.0	-29.5	-4.5
	Rancona summit	Control	<0.01	-16.4	-28.9	-3.9
	Rizolex+Apron XL	Control	<0.01	-16.6	-29.1	-3.9
	Trilex2000	Control	0.012	-15.4	-27.3	-2.9
DSI Havre 2020	CruiserM. Vib. P.[a]	Control	<0.01	-5.8	-10.3	-1.3
	EverGol Energy	Control	<0.01	-6.4	-10.9	-1.9
	Obvius	Control	<0.01	-7.0	-11.5	-2.5
Yield Moccasin 2019	Obvius	Control	0.072	-254.5	-498.8	-10.2
Yield Moccasin 2020	Essential oils	Apron Maxx	0.040	-332.3	-632.1	-32.5
	EverGol Energy	Essential oils	0.023	350.3	50.5	650.1
	Obvius	Essential oils	0.013	367.7	67.9	667.5

[a] CruiserM. Vib. P., CruiserMaxx Vibrnace Pulse (Syngenta, Crop Protection, Inc., Greensboro, NC). Tukey’s HSD used in *multcomp* packaged (Hothorn et al.2008)

[b] Est. Diff., Estimated difference between treatment means; L.CI, lower confidence interval; U.CI., upper confidence interval.

B-8. Model information from 2019 and 2020 lentil seed treatment trials.

Site-Year		2019			2020		
	Response Variable	Obs[b]	σ^2 Bock	σ^2 Residuals	Obs[b]	σ^2 Block	σ^2 Residuals
Bozeman	Emergence (plants m^{-2})	39	17.1	114.6	40	38.0	127.1
	Percent vigor rating	39	1.2	5.9	39	2.3	7.3
	FGCC[c]	39	7.3	11.1	39	0.0	3.7
	DSI	40	0.0	8.7	40	18.9	36.8
	Final yield (kg ha^{-1})	40	2471.7	21687.7	40	896.8	20401.0
Havre	Emergence (plants m^{-2})	-	-	-	40	20.4	35.8
	Percent vigor rating	40	3.2	5.7	38	0.0	159.6
	FGCC[c]	40	19.6	22.1	38	0.0	66.1
	DSI	40	10.6	36.5	40	0.3	4.8
	Final yield (kg ha^{-1})	40	2211.6	12606.0	40	154.6	2350.4
Moccasin	Emergence (plants m^{-2})	40	49.5	174.4	40	0.0	197.3
	Percent vigor rating	39	15.5	48.2	40	1.1	4.3
	FGCC[c]	39	0.0	0.1	40	0.0	6.8
	DSI	40	9.0	41.9	40	1.1	19.8
	Final yield (kg ha^{-1})	40	0.0	14028.2	40	3351.0	21094.7
Sidney	Emergence (plants m^{-2})	40	0.0	230.4	40	30.0	157.2
	Percent vigor rating[b]	30	24.7	10.8	30	0.0	5.7
	FGCC[bc]	30	6.7	13.0	30	2.3	7.4
	DSI	40	30.6	49.2	40	8.1	39.1
	Final yield (kg ha^{-1})	40	2336.0	66160.1	40	21984.6	42876.8

Note. The data were fit using a linear mixed effects model with a random effect of block. Data was cross-referenced with field notes and notes from collaborators at research sites to clean up the dataset.

[a] Obs, is number of observations.

[b] Dead zone in front of plots influencing vigor and FGCC in Rep 1, at Sidney in 2019 and 2020. Possibly due to a chemical spill (*personal communication*, B. Frank). Final yield was adjusted by plot length.

[c] Fraction green percent canopy cover using Canopeo application (Oklahoma State University).

B-9. Field operations and data collection dates at Bozeman, Moccasin, Havre, and Sidney 2019 and 2020.

Site	Bozeman		Havre		Moccasin		Sidney	
Year	2019	2020	2019	2020	2019	2020	2019	2020
Planting	4 May	27 Apr	3 May	24 Apr	25 Apr	28 Apr	26 Apr	22 Apr
Date of emergence	19 May	8 May	10 May	6 May	11 May	10 May	1 May	2 May
Stand count	3 June	19 May	-	26 May	23 May	19 May	14 May	15 May
Vigor rating	1 July	6 July	25 June	22 June	11 June	14 July	17 June	28 June
Percent canopy cover	1 July	6 July	25 June	22 June	11 June	14 July	17 June	28 June
Root rot dig	1 July	7 July	25 June	22 June	12-13 June	14 July	18 June	28 June
Flower date	4 July	6-7 July	28 June	18 June	12 July	28 June	2 June	16 June
Days to flower[a]	61	71-22	56	55	78	61	59	55
Harvest	26 Aug	14 Aug	20 Aug	31 July	28 Aug	6 Aug	16 Aug	8 Aug

[a] Days to flower calculated from planting date to start of flowering (50% of plants with open flowers) in calendar days.

B-10. Pre and in-season pesticide applications by site and year.

Site-Year	Application	Date	CO	Brand	Active Ingredient	Product rate mL ha^{-1}	ai rate mL ha^{-1}
Bozeman 2019	Pre-plant	23 Apr	Loveland	Makaze	Glyphosate	2339	959
	Pre-plant	23 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	23 Apr	BASF	Sharpen	Saflufenacil	73	22
	In-crop	3 May	Syngenta	Warrior II	Lambda-cyhalothrin	143	33
Bozeman 2020	Pre-plant	28 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	28 Apr	BASF	Sharpen	Saflufenacil	73	22
Moccasin 2019	Pre-plant	25 Apr	Bayer	RT3	Glyphosate	2339	1141
Moccasin 2020	Pre-plant	29 Apr	Bayer	RT3	Glyphosate	2339	1141
Havre 2019	-	-	-	-	-	-	-
Havre 2020	Pre-plant	2 Apr	BASF	Prowl H2O	Pendimethalin	1754	679
	Pre-plant	2 Apr	BASF	Sharpen	Saflufenacil	73	22
Sidney 2019	Pre-plant	25 Apr	Corteva	Durango	Glyphosate	1754	881
	Pre-plant	25 Apr	BASF	Outlook	Dimethenamid-P	877	560
Sidney 2020	Pre-emergence	22 Apr	Bayer	PowerMax	Glyphosate	1754	854
	Pre-emergence	22 Apr	BASF	Outlook	Dimethenamid-P	877	560

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