The effect of seeding rate and fungicide applications on lentil cultivars

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By

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ABSTRACT

Recent research has shown that yield potential of lentil can be increased. Lentil (*Lens culinaris* Medik.) is one of the least competitive crops grown on the Canadian prairies. Additional drawbacks to lentil production include susceptibility to disease and lack of knowledge for optimal seeding rates. Yield-density studies elucidate a specific crop's ability to maximize field resources, and current literature suggests that lentil seeding rates should be increased to raise yield potential, notably under weedy conditions. However, disease pressure is of concern when lentil plant populations are elevated, so a balance is necessary. The objective of this research was to determine the effect of plant population on grain yield of different market classes of lentil, and to evaluate the interaction of disease control and seeding rate in different lentil market classes. To determine this, two field experiments were conducted in 2015 and 2016, in Saskatchewan, Canada. The first study involved six lentil cultivars and five seeding rates, while effects and interactions among them were examined. The second experiment involved the effect of four fungicide treatments, two seeding rates, and three red lentil cultivars. In the first experiment, the maximum yield was reached for all varieties at a seeding rate between 160 to 220 plants m^{-2}. In the second experiment, the highest yield was accomplished at a rate that achieved a target plant population of 240 plants m^{-2} paired with two fungicide applications (Headline® and Bravo®). This treatment yielded statistically higher than both treatments that included only a single fungicide application. The treatment with Headline plus Bravo also contributed to the lowest overall visual disease severity rating in 2016, compared to all other treatments. These results, reinforced by previous studies with similar conclusions, establish that the current recommendation in Saskatchewan of 130 lentil plants m^{-2} is likely insufficient for obtaining maximum seed yield.

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LIST OF ABBREVIATIONS

ANOVA - Analysis of Variance

LSMEANS - Least Squares Means

IWM - Integrated Weed Management

IPM – Integrated Pest Management

CPWC - Critical Period of Weed Control

CFY – Constant Final Yield

1.0 Introduction

Lentil production in Canada is protected by a politically stable nation and relatively constant climatic conditions during the growing season that result in secure production of an important protein. Lentil production is achievable in areas around the world but political instability in some areas dominates over lentil production in spite of vast areas of fertile land (Pavleska and Kerr, 2015). Thus, the Canadian Prairies have the means to provide responsibly sourced, protein rich food. Increasing lentil yield through agronomic research in Western Canada has the potential to feed more of the population without using excessive amounts of synthetic inputs.

Recent research has revealed the positive seed yield effect from increased lentil seeding rates. The target plant population currently recommended to producers in Saskatchewan is 130 plants m^{-2} (Saskatchewan Pulse Growers, 2018a); however, some literature has suggested to raise that figure. Redlick et. al (2017) demonstrated a yield increase from higher plant populations, although the trial included high populations of weeds sown within the lentil crop. Prior to that, Baird et al. (2009) conducted a trial under organically managed conditions that reported increased yield potential with a seeding rate that was nearly double that of the current recommendation in Saskatchewan. The previously mentioned studies however, occurred in conditions that incorporated weeds in natural populations or weeds seeded into the trial. Regardless, these results display different results than weed-free studies due to different types of competition present in the field. Lentil plant population studies conducted under conventionally (not organic) managed plots are uncommon however, results have been displayed under these conditions that show no yield increase beyond a seeding rate of 130 plants m^{-2} (Wall, 1994). Other studies have shown yield benefits from slight increases in the target plant population, where a seeding rate of 150 plants m^{-2} was found to provide the highest economic benefit (Siddique et al., 1998). Varying results in this area of study could be due to inconsistent environmental conditions or different responses from lentil cultivars of different seed sizes. An analysis of cultivar response to varying seeding rates is needed to recognize optimal agronomic conditions for lentil production under typical herbicide regimes on the Canadian Prairies.

Increased plant populations to increase yield potential would seem to be an obvious practice to follow; however, increased disease severity is likely at high population densities. This occurs because the plants have less distance separating them at higher seeding rates (Burdon and Chilvers, 1982). Lentil crops have had yield reductions of up to 60% from pathogens including Botrytis stem and pod rot (*Botrytis cinerea* Pers.:Fr.), Anthracnose (*Colletotrichum lentis* (Damm)) and Ascochyta blight (*Ascochyta lentis* Vassilievsky) (Morrall, 1997). Studies carried out on Ascochyta blight and Botrytis stem and pod rot have demonstrated that higher disease severity occurs on lentil crops as higher plant populations are achieved (Bailey et al., 2000a, Jurke and Fernando, 2008). However, additional studies have demonstrated that fungicide applications can preserve yield potential in high disease pressure situations that dense crop canopies produce; thus, ensuring lentil crop profitability (Bailey et al., 2000b).

This project focused on imperative lentil agronomics including seeding rate, fungicide applications and the effect of six different cultivars on seed yield. These management techniques, although only involving a fraction of agronomic techniques applied to a crop, will contribute to knowledge gaps that occur related to optimal seeding rates paired with fungicide application. In addition to research contribution, these objectives include methods that are easily adopted by producers, and therefore may result in substantial impact through minor changes to farming operations. The hypotheses of this research was that the highest plant population would result in the highest yield when two fungicide applications were applied, and that lentil market classes would differ in optimum weed free densities and optimal fungicide control practices. To test these hypotheses, the objective of the first study was to distinguish differences in the yield density response among representatives of six different lentil market classes. In the second study, the objective was to determine the interaction of seeding rate in three red lentil cultivars with fungicide application in regard to plant disease control and seed yield. The revised recommendations arising from this research will allow for improvement of yield of specific cultivars.

2.0 Literature Review

2.1 History of Lentil

The lentil plant (*Lens culinaris* Medik.) was domesticated as far back as 7,500-6,500 BC and, as archaeological evidence has suggested, originated from South Western Asia and the Mediterranean region (Cokkizgin and Shtaya, 2013). In Western Canada specifically, lentil production started in 1970 with only about 1500 acres and in 40 years, production in Saskatchewan grew to 2.4 million acres (Saskatchewan Pulse Growers, 2000a). From 2008 to 2012, Canada produced 37% of the world's lentil followed by India (23%) and Turkey (8%) (Janzen et al., 2014). Saskatchewan is the main lentil producer across Canada, contributing to 96% of Canada's lentils in 2014 (Saskatchewan Pulse Growers, 2016b).

2.2 Lentil Consumption

Lentil seeds are grain legumes that have a high protein, lysine and tryptophan content. These characteristics contribute to a balanced diet with essential amino acids when lentils are served with rice or wheat (Erskine and Sarker, 2006). Protein content in lentil is one of the highest in field crops, at approximately 25% (dry matter basis) and of that protein, 93% of it is digestible, allowing for optimal availability to the body (Bhattacharya et al., 2005). Anti-nutritional factors are of little concern in humans as lentil causes low flatulence and has a low postprandial glycemic index, making it suitable for consumption by diabetics (Bhatty, 1988). While lentil is low in fat, it is high in vitamins, minerals, fibre and complex carbohydrates which can result in lowering blood cholesterol. In addition, lentil is an inexpensive form of protein that offers a low number of calories (Yadav et al., 2007), making it a good addition to the diet of North Americans.

2.3 The Lentil Plant

Lentil is a cool-season annual legume, belonging to the Leguminosae family. Lentil is grown in Canada as a pulse crop and is harvested for the dry seed (Balasubramanian, 2015). With assistance from *Rhizobium* bacteria that initiate root nodule growth and nitrogenase activity (Chanway et al., 1989). Pulse crops work symbiotically to convert nitrogen that is drawn from the atmosphere into plant available nitrogen. With this process, the addition of the macronutrient chemical fertilizer, nitrogen, is not required (Janzen et al., 2014). In comparison to other field crops grown on the Canadian Prairies, lentil plants are short in stature, ranging from approximately 15 to 45 cm with many long branches. Leaves are arranged in an alternate pattern along the stem with six pairs of oblong leaflets running along them and tucked into the axils of the leaves are pale blue flowers that become a pod, usually housing 2 disc-shaped lentil seeds in each legume pod (Yadav et al., 2007). Lentil exhibits an indeterminate growth habit that requires some form of stress to progress from the vegetative stage into the reproductive phase. However, when available soil moisture is low and nitrogen availability is not high around the time of flowering, indeterminacy may not be a problem that affects seed set (Saskatchewan Pulse Growers, 2018b). Lentil plants possess a taproot with a mass of fibrous lateral roots that support nodules on healthy plants that will appear anywhere from 15 days after emergence until flowering or longer (Saxena, 2009).

Lentil is placed within two different categories based on seed size; the Chilean type that has larger seeds averaging 50 grams or more per 1000 seeds, and smaller seeds or the Persian type, averaging 40 grams or less per 1000 seeds (Saskatchewan Pulse Growers, 2000a). All lentil seeds are disc-shaped with a wide range of color variation from yellow, red or green cotyledons with clear, green, brown, gray, black or blotched purple seed coats (Saskatchewan Pulse Growers, 2000a).

2.4 Management of Lentil

2.4.1 Weed Control

Successful field crops in Western Canada often reflect many herbicide options or one broad spectrum herbicide available to control a variety of weeds. This is not true however, for lentil production in Western Canada as few herbicides are registered for efficacious, selective post-emergence control of broadleaved weeds that leave the crop unharmed. The group 3 pre-plant herbicides trifluralin and ethalfluralin can be incorporated into the soil in the fall prior to seeding a spring lentil crop. These herbicides control some broadleaved and some grassy weeds (Government of Saskatchewan, 2018). Prior to 2007, the only registered broadleaf herbicide for use after seeding, was metribuzin (Sencor®) and this group 5 herbicide commonly results in injury to lentil plants, especially in wet environmental conditions (Elkoca et al., 2005). Metribuzin is only registered for control of broadleaved weeds including *Sinapsis arvensis* L. (wild mustard), *Thlaspi arvense* L. (stinkweed) and *Chenopodium album* L. (lamb's quarters). This leaves some economically important weeds like *Kochia scoparia* (L.) Schrad. (kochia), *Capsella bursa-pastoris* (shepherd's purse), *Amaranthus retroflexus* L. (redroot pigweed) and perennial weeds uncontrolled in a lentil crop (Government of Saskatchewan, 2018). In addition to these shortcomings, metribuzin can antagonize many graminicides, lowering their efficacy on weeds such as *Avena fatua* L. (wild oat) and; therefore, two herbicide applications are generally required in one season (Kirkland et. al., 1989).

Clearfield® technology was commercially introduced for lentil producers in Western Canada in 2007. This technology was developed at the University of Saskatchewan (Slinkard et al., 2002) and it targets the plant and microorganism specific enzyme acetolactate synthase (ALS or AHAS). ALS is the target site for sulfonylurea, imidazolinone and triazolopyrimidine herbicides (Devine, 2000). These herbicides provide control of previously hard to target broadleaved weeds and additionally, some grassy weeds, without harming crop plants. In addition to ease of use, other reasons for the quick adoption of ALS or AHAS inhibitors include the control of diverse weed species at low herbicide rates (Devine, 2000) and low risk of toxicity to animals including mammals, fish and amphibians, as these individuals lack the ALS enzyme (Zhou et al., 2007).

Crop and weed stage for efficacy of herbicide application is critical for effective weed control. The desired crop stage for metribuzin application in a lentil crop is one to four above ground nodes, while the desired crop stage for an imidazoline application in a lentil crop is one to nine above ground nodes. The timing of herbicide application should coincide with the desired time of weed removal from a crop for maximum yield and economic benefit. This timing is known as the critical period for weed control (CPWC) and in lentil, this crucial timing is between the 5th and 10th node stage. This stage is approximately between the time when weeds begin to build up a considerable amount of biomass and when the crop canopy closes (Fedoruk, et. al., 2011). During this critical and relatively short period of time, it is important to keep weed stress at a minimum in a lentil crop, to ensure that yield potential is not affected (Knezevic et al., 2002).

2.4.2 Integrated Weed Management and Herbicide Resistance

Lentil is one of the least competitive crops grown on the Canadian Prairies (Blackshaw et. al., 2002) as a consequence of slow growth in the beginning of the season and short crop height (McDonald, et al., 2007). In addition, there are a minimal number of post emergence herbicides to use for control of broadleaved weeds and because of this, research in this area is necessary to allow for a more aggressive lentil regime with higher yield potential. An integrated weed management approach should be taken to decrease weed populations in lentil crops.

Herbicides for post emergence control in lentil include; group 1 herbicides clethodim, sethoxodim and quizalofop to control grassy weeds; group 5 metribuzin (controls broadleaf weeds) and; group 2 imazamox and imazethypyr (control a number of diverse weed species including grassy and broadleaf weeds) (Government of Saskatchewan, 2018). The use of group 2 herbicides has become a concern as data collected on herbicide use from 2006-2010 showed high dependency on group 2 ALS inhibiting herbicides in pulse crops (Beckie et al., 2013). Clearfield® technology in lentil has gained popularity among producers however, overuse and subsequent resistance has been threatening lentil production in Western Canada. On a global scale, ALS inhibitor-resistant weeds cover a large fraction of resistant cases with 133 out of 404 positive events (Heap, 2014). This phenomenon is due to a number of factors including the widespread use of group 2 herbicides, the resistance mechanism itself (generally an altered ALS enzyme) and strong selection pressure exerted in fields with group 2 herbicides applied, alongside the characteristic of persistent soil residual activity for group 2 herbicides (Tranel and Wright, 2002). Random samples collected throughout Saskatchewan in 2009 showed that 15% of all weeds were group 2 herbicide resistant (Beckie et al., 2013). As this technology is most prone to resistance, there is a dire need for an integrated weed management system to be put in place by producers on the Canadian Prairies.

Wild oat, false cleavers (*Galium spurium* L.) and wild mustard (*Sinapsis arvensis* L.) populations all have potential to threaten a lentil crop being grown on the Canadian Prairies and these species were found to possess resistance to group 1, group 2 or both group 1 and group 2 herbicides in Saskatchewan in 2009 (Beckie et. al., 2013). Reliance on chemical weed control methods has dwindled as 30 years have passed since the last site of action was added to the market and environmental regulations for herbicide registration has heightened, (Heap, 2014) making the timely approval of new herbicide technology even less likely. Saskatchewan field surveys in 2014 and 2015 revealed that 57% of surveyed fields contained herbicide resistant weeds. Specifically, 32% and 43% of the fields sampled in Saskatchewan and Manitoba respectively, had wild oat populations resistant to group 2 herbicides (Hugh Beckie, personal communication). This phenomenon is more problematic when considering that 25% of the sampled fields have group 1 and group 2 resistant (multiple group resistance) wild oat populations in Saskatchewan.

Integrated weed management (IWM) is an important part of lentil production due to limited herbicide options. Since the reliance on herbicides as the sole form of weed control is not an effective means of IWM, (Saskatchewan Pulse Growers, 2017) other tools and methods for weed control should be investigated, including the effect of increased seeding rates. Group 2 weed resistance is a growing concern on the Canadian Prairies, which leaves little options available for weed control in lentil if group 2 herbicides continue to lose efficacy. Tools including sowing crops with varying lifecycles, utilizing different seeding dates, the use of clean seed and equipment and exhibiting proper crop rotation, (Saskatchewan Pulse Growers, 2018a) will lessen reliance on herbicides. Increasing crop seeding rates and practicing the act of diminishing the weed seed bank (Walsh, 2007) are additional practices that will extend the continued use of herbicides and allow producers to pursue high yielding crops in Western Canada.

2.4.3 Integrated Weed Management and Increased Seeding Rates

The IWM strategy of increasing seeding rate to increase crop competition is one that shows promise without applying extensive change to a farming operation. In an Italian study done by Paolini et al. (2003), the increased competitive ability of lentil and weed suppression that took place at crop densities of 177 to 250 plants m^{-2} were sufficient in restricting a significant amount of yield loss. These findings are supported by those found by Ball et. al (1997) who discovered that weed density and dry weight were reduced as seeding rates increased. Additionally, when seeding rate was increased alongside the use of herbicides in conditions with high precipitation, yield losses from weed infestation did not affect lentil crop yield as dramatically as yield losses at lower seeding rates. In West Asia, the optimal seeding density with the greatest economic return for lentil was found to be from 280 seeds m^{-2} to 320 seeds m^{-2} (Silim et al., 1989). An organic study conducted in Canada had a similar outcome resulting in a recommendation for organic production systems of sowing at 375 seeds m^{-2} to maximize economic return while providing weed suppression as well (Baird et al., 2009). The need to further study on increased lentil seeding rates is apparent when considering results from this research alongside the growing concern of herbicide resistance in lentil crops.

2.4.4 Plant Population

Constant final yield (CFY) is an observed and measured phenomenon that pertains to total biomass production. The maximum attainable stand of biomass is reached at different densities after a period of plant growth. At low plant populations, biomass and density increase linearly however, at higher plant populations, density increases faster than biomass, due to plant competition. When final biomass is reached (CFY), escalation in density is the equivalent to decreases in mean plant weight, therefore, the curve levels off into a flat line (Weiner and Freckleton, 2010). When considering the yield or biomass response resulting from seeding rate studies, differences are noted between studies that include natural or seeded weed populations and weed free studies. Weed free studies engage in one type of competition (intraspecific competition) whereas studies involving weeds exhibit intraspecific and interspecific competition between plants. Seeding rate or plant population studies conducted under weedy conditions including Baird et al. (2009) and Kirkland et al. (2000), include the measurement and occurrence of weed populations in analyses. Therefore, the yield and biomass response resulted in outcomes that did not reach CFY. In studies including those done by Wall (1994) and another by Siddique et al. (1998) that were conducted under weed free conditions, yield response resulted in an asymptote and a decrease in yield thereafter. Through these varying responses, it is evident that intraspecific and interspecific competition between plants allow for diversified results.

2.4.5 Seeding Rate in Lentil

The target plant population for lentil production is currently 130 plants m^{-2} (Saskatchewan Pulse Growers, 2018a); however, recent research contradicts this value. Several studies have taken place that advocate increasing seeding rates to maximize yield, including research conducted by Wall (1994) that showed a positive relationship between increased seeding rates and seed yield. A Canadian organic lentil study reported increases in yield up to a seeding rate of 375 plants m^{-2} (Baird et al., 2009). Although this seeding rate was to maximize weed suppression in weedy conditions, these results gave rise to continued research on the topic of lentil seeding rate optimization. Most recently, a seeding rate study conducted on small red lentil demonstrated that increased seeding rates raised lentil seed yield and yield was not affected by increased plant populations in wet years (Redlick et al., 2017). Different weed treatments were included alongside applicable seeding rates however, some treatments showed increases in yield up to 520 plants m^{-2} whereas all treatments resulted in higher seed yields at 260 plants m^{-2}

versus 130 plants m^{-2}. Most seeding rate recommendations come from the assumption that the crop is weed-free (Mohler, 2001); however, this is rarely the case in lentil crops throughout Western Canada. Increasing the seeding rates of lentil has the potential to not only provide economic benefit but, from an agronomic point of view it could increase competitiveness and in turn, yield (Baird, et. al., 2009).

2.5 Lentil Disease

2.5.1 Microenvironment and Plant Population Density

In 1891 Berlin, Robert Koch developed the foundation of infectious disease (Koch, 1891) and the same concept of Koch's postulate applies to plant epidemiology as they are centered around the fact that organisms are the cause of disease. About 80 years later, Zadoks (1972) noted that Koch's postulate was also applicable to the quality disease characteristics, but they were lacking a quantitative aspect that is necessary for describing disease in plant communities or plant populations. The science of disease in populations, or epidemiology, involves important methodology and host frequency is a necessary part of this (Zadoks, 1972), especially where field crops are concerned.

Host frequency or crop density has a direct effect on disease and it has been established that dense plant stands contribute to a higher likelihood of disease epidemics. The direct effect of this circumstance is centered around the fact that in a given area, a higher number of host plants increases the probability of disease inoculum interception (Burdon and Chilvers, 1982). Lentil seeding rate studies by Bailey et al. (2000a) and Redlick et al. (2017) demonstrated that as plant population increased, disease severity increased as well. Although increases in host frequency has potential to increased disease severity, yield has also been shown to increase with higher seeding rates (Nerson, 1980, Uzun and Açikgöz, 1998). Taking this into account, defining the optimal balance to maximize seed yield and minimize disease infection with increased seeding rates is challenging.

Plant disease and the effect that plant density has on populations of single or mixed host genotypes is a topic that is understudied (Garrett and Mundt, 2000). It is recognized that host frequency influences pathogen development, but little documentation has been published on the actual quantity (whether monetary or percentage of yield loss) of the effect. A study done on chickpea demonstrated that plant population and incidence of ascochyta blight increased simultaneously; however, seed yield increased with seeding rate as well, regardless of the considerable disease found in the trial specimen (Gan et. al., 2007). Results involving the optimal plant population in regard to seed yield varied among chickpea cultivars as well, establishing the need for variety specific seeding rates. Similar results have been shown with upright beans (*Phaseolus vulgaris* L.) inoculated with *Sclerotinia sclerotiorum* (Lib.) de Bary or white mould. Higher planting densities resulted in a higher measure of white mould alongside higher yields (Saindon et. al., 1995). The impact of Mycosphaerella blight (*Mycosphaerella pinodes* (Berk. & Blox.) Vestergr) on field pea (*Pisum sativum*) was also studied, where Mycosphaerella blight severity was highest at increased seeding rates and disease severity was lowest at lower seeding rates. However, again in the study, it was found that the lower plant populations compromised yield potential and, in addition, one foliar fungicide application of chlorothalonil increased pea yield by 20% (Hwang et. al., 2006). On a field scale, the dominant dispersal method of infectious propagules is by a passive means (Mazzi & Dorn, 2012) through vectors such as air, water or soil. Through these types of dispersal, it is evident that in circumstances where plants are closer together, the pace of disease spread will accelerate. Results of the previously mentioned studies demonstrate that plant disease severity is often a function of geographical distance and density of host plants and is therefore a factor that requires further study.

2.5.2 Disease Management and Plant Population

For plant disease to develop, an interaction must take place requiring a susceptible host, a causal agent or pathogen and environmental circumstances that are advantageous for that pathogen. These factors also determine the severity of that specific disease (Krupinsky et al., 2002). When considering disease control, any type of management practice that disrupts the disease triangle has potential to lessen pathogen impact on the host. The interaction of six different factors was shown to determine the economic importance of pathogen levels after disease exposure under favourable conditions. The factors included the environment (temperature or precipitation), the length of the infection period, how widespread the pathogen was, the pathogen virulence (the degree of damage the pathogen can exert on the host), the age of the host and the hosts' susceptibility to the specific disease (Scholthof, 2007).

Practices to disrupt the pathogen infection process have potential to lessen or forestall disease epidemics in field crops. These practices include crop residue management, using resistant cultivars or even altering characteristics in the microenvironment of the crop

(Krupinsky et al., 2002). The impact of most lentil diseases can be lessened in part with foliar fungicide treatments, but an integrated disease management approach should be practiced. (Government of Saskatchewan, 2011). The continual reliance on chemical fungicide is not an ideal routine as excessive utilization increases pathogen selection pressure and allows resistance an antagonistic advantage (Avenot and Michailides, 2010). Fungicides are not the only control measure that is susceptible to inefficacy due to pathogen adaptation, as disease resistant crop varieties can be vulnerable to diseases that break down the genetic host resistance as well (McDonald and Linde, 2002). Microenvironment modifications have previously been shown to be efficacious through research in common bean (Blad et al., 1978), carrot (Smith, 1988) and rye (Giesler et al., 1996). In these studies, dense plant populations often increased the severity of disease as compared to those with thinner canopy densities or those with slighter plant stands, as the microenvironment can be more stimulating for pathogen development. In saying this, seeding rate adjustments have the potential to produce different yield outcomes when managed accordingly.

2.5.3 Lentil Disease in Saskatchewan

In approximately ten years from the introduction of lentil to Saskatchewan, diseases that reduce lentil yield became apparent with the detection of ascochyta blight (*A. lentis* Vassilievsky) in 1978, anthracnose (*Colletotrichum lentis* Damm) in 1987 and the increasing severity of botrytis grey mould (*Botrytis cinerea* (Pers.: Fr.) (*Helotiales, Sclerotiniaceae*) and *Botrytis fabae* (Sard)) in the early 1990's (Morrall, 1997). It did not take long for scientists and growers to discover that the most devastating biotic stress to lentil yield potential was disease (Erskine and Sarker, 2006). A wide range of microorganisms can infect lentil, but the most devastating are diseases caused by fungal pathogens (Yadav et al., 2007). The two most detrimental lentil diseases in Saskatchewan are anthracnose and ascochyta. Other major lentil diseases on the Canadian Prairies include; botrytis grey mould, sclerotinia white mould (*Sclerotinia sclerotiorum* (Lib.) de Bary) and stemphylium blight (*Stemphylium botryosum* Wallr.) (Government of Saskatchewan, 2011).

2.6 Lentil Pathogens

2.6.1 Seed Rot, Damping-off, Seedling Blight and Root Rot

Soil-borne micro-organisms including rhizoctonia, pythium, fusarium and botrytis grey mould are common fungal pathogens of lentil in Saskatchewan that can cause disease including seed rot, seedling blight, damping-off, wire stem and root rot. These pathogens can infect and decimate lentil during any point of its lifecycle; however, the incidence of these pathogens is generally irregular, so economic loss is not commonly noted (Saskatchewan Pulse Growers, 2016a). Severe damage can occur however, when seedling blight and damping off are caused by botrytis grey mould (Bailey et al., 2003). The newly reported Aphanomyces root rot (*Aphanomyces euteiches*) was discovered in Canada in 2012 (Banniza et al., 2013) and has caused destruction in lentil regions across Saskatchewan and Alberta, notably in 2013 (Armstrong-Cho et al., 2014). Research on Aphanomyces root rot continues, to minimize the impact on legume crops in Canada.

In recent years, root rots have become more problematic in lentil producing areas in North America. Root pathogens were likely overlooked previously, due to a lack of root examination capabilities. Increased incidence could be due to shortened crop rotations that embody high numbers of pulse crops (Gossen et al., 2016). As production continues to expand into areas that are not as suited to lentil production (like the wetter black soil zone), the concern and significance of root rot is likely to increase (Hwang et al., 2000).

2.6.2 Anthracnose

Anthracnose (*Colletotrichum lentis* Damm) is widespread in Saskatchewan and in all locations in North America that grow lentil. This devastating fungus survives northern winters on plant debris as microsclerotia. The microsclerotia that overwinter on debris are the primary source of inoculum (Buchwaldt et al., 1996) and they can remain animate on lentil debris for up to four years (Bailey et al., 2003). Besides the movement of infected debris, infected lentil seeds can effectively move the pathogen from field to field and even internationally (Kaiser, 1997) however in North America, seedborne infection is of little concern as wind dispersed inoculum is the main means of disease spread (Buchwaldt et al., 2018). Persistent and hardy microsclerotia are splashed onto uninfected lentil plants by rain, where infection then occurs on lower parts of the plant (Bailey et al., 2003). Disease development in lentil is optimized when a growth stage of four to six weeks is reached and when temperature is from 20 to 24ºC (Chongo and Bernier, 2000).

Anthracnose in lentil is recognized symptomatically by oval lesions that are white to grey in colour that develop on the bottom plant leaflets. Stem lesions appear darker in colour and concave, sometimes girdling the stem (Bailey et al., 2003). In the centre of lesions is where acervuli are formed (Sutton, 1992). Acervuli give rise to conidia, which are responsible for secondary infection when carried in water droplets by blowing rain or splashes (Buchwaldt, 2011).

Control of anthracnose is essentially limited to fungicides and crop rotation; however, crop rotation is not always effective as anthracnose inoculum spreads by wind (Chongo and Bernier, 1999). Utilization of lentil cultivars with some resistance alongside fungicide use and crop rotation is recommended for the control of anthracnose; however, cultivars with partial resistance are all that is available (Chongo & Bernier, 1999). In addition, the use of disease-free seed and elimination of hosts (including volunteer lentil and wild vetch) are key to preventing the introduction of anthracnose to uninfected areas (Bailey et al., 2003). Although the effect of seeding rate on anthracnose development has not been extensively studied, higher seeding rates produce field conditions that increase humidity in the crop canopy. The high humidity conditions are likely suited to increase anthracnose development in a lentil crop.

2.6.3 Ascochyta Blight

When a lentil plant has been exposed to ascochyta blight (*Ascochyta lentis* Vassilievsky), it takes six hours from inoculation for conidia to germinate. After ten hours, appressoria have developed and therefore, a pathogen has successfully invaded the plant. Physical symptoms appear seven to nine days after the process begins and within 14 days, the pycnidia are visible (Cole et. al., 1995). Lentil plants are susceptible to ascochyta blight from the seedling stage until maturity and symptoms are displayed on leaflets, petioles, stems, peduncles or pods. Infected lentil seeds become discoloured and appear purple to brown, while other infected plant parts will exhibit light grey, round lesions that gradually grow darker to develop a dark brown margin with scattered pycnidia in the centre, a distinguishing feature of ascochyta blight. Cool and wet weather will increase infection rates as rain splash is the leading vector for disease spread (Bailey et al., 2003). Pycnidia are created during the anamorph (asexual stage) of the lifecycle (Yadav et al., 2007). Pycnidiospores arise from pycnidia and are spread by rain splash

(Davidson and Kimber, 2007). *A. lentis* has a teleomorph (sexual) as well and, this stage is critical to the survival and persistence of the pathogen. *Didymella* sp., the teleomorph, could be attributable for this pathogen's genetic diversity, making the breeding of resistant cultivars a difficult task. In addition, the teleomorph has potential to produce airborne ascospores that result in primary inoculum and, this would help the pathogen overwinter on plant debris (Kaiser et al., 1997). Ascospores burst from pseudothecia, which are formed during the sexual reproduction phase. Wind and rain are capable of spreading ascospores, making ascochyta blight infection a threat over long distances as well (Davidson and Kimber, 2007).

Lentil cultivars are available in Canada with partial resistance to ascochyta blight (Saskatchewan Pulse Growers, 2016a). In addition to the utilization of genetically resistant cultivars, a four-year break between lentil crops should be practiced, as it is a seed and stubble-borne disease. The location of adjacent lentil fields should also be monitored to lessen the likelihood of infection. The causal fungal agent is specific to lentil so other pulses could be included in rotation. The management of crop residue to bury viable pycnidia, combined with the use of disease-free seed to reduce the risk of seed to seedling transmission, are important practices to reduce impact of ascochyta blight (Davidson and Kimber, 2007). Foliar fungicide can be applied during flowering to reduce pod abortion and seed infection, (Bailey et al., 2003) as this is the mechanism that can cause the greatest amount of yield loss (Morrall, 1997). Fungicide application was shown to increase yield in chickpea, as a result of protection from ascochyta blight. However, it has been demonstrated that at least two applications of foliar fungicide are needed to increase yield under high disease pressure conditions (Chongo et al., 2003). Sowing pulses at a later seeding date can also be an effective means of reducing ascochyta blight disease pressure. This is true when ascospores are not the main source of pathogen inoculum or if the seeding date does not coincide with the time that the ascospore structure releases more inoculum. This later seeding date simply lowers the incidence of pycnidiospore cycles that occur when the lentil plant is at the highest level of risk (Davidson and Kimber, 2007). In saying this, sowing lentil late in Western Canada is not likely a viable option due to a short growing season.

The effect of plant population on ascochyta blight levels in chickpea has been studied and results indicated that higher plant populations resulted in more disease (Gan, 2007). This circumstance is likely due to microenvironment modifications reducing air movement and evaporation. Reduced air movement leads to longer periods of leaf wetness and decreased amounts of direct sunlight (Siddique and Bultynck, 2004). Ascochyta blight infection has been shown to increase when wetness periods occurred beyond six hours during the infection period (Trapero-Casas and Kaiser, 1992). In the same study, disease suppression was also displayed when temperature rose above 30 °C and this gives justification to the mechanism behind higher ascochyta blight severity that corresponds to higher plant population densities.

2.6.4 Botrytis Stem and Pod Rot (Grey Mould)

Botrytis grey mould has been present in Canada since lentil was first grown in 1969 but the pathogen remained of little interest at first (Morrall, 1997). It is known that both causative agents *Botrytis cinerea* (Pers.: Fr.) (*Helotiales, Sclerotiniaceae*) and *Botrytis fabae* (Sard) can infect lentil (Davidson & Krysinska-Kaczmarek, 2007).

Botrytis grey mould in lentil acts as a stem and pod rot in thick crop canopies that close later in the season. Thick, closed canopies occur more commonly in dark brown or black soil zones (Morrall, 1997) so, this specific disease pressure is more common in central to northern Saskatchewan. Symptoms of *Botrytis* spp. can be found on lentil shoots as small, dark green lesions that turn light brown and eventually take on a cream color as they coalesce over the whole leaflet (Yadav et al., 2007). When lentil plants are infected as seedlings, this seedling blight is characterized by grey mycelial growth displayed at the soil line (Morrall, 1997).

Although foliar fungicide and other control measures are utilized in other lentil producing regions around the world (including Australia), control of *Botrytis* spp. is not common on the Canadian Prairies (Davidson & Krysinska-Kaczmarek, 2007). A wide variety of active ingredients can sufficiently control botrytis grey mould but, foliar fungicide application for this type of disease can be uneconomic (Taylor et al., 2007). As seedling blight can develop from diseased seed, the use of disease-free seed will stop this occurrence (Martens et al., 1988). Practices that increase the flow of air through the crop canopy have the potential to decrease disease severity of botrytis stem and pod rot (Bailey et al., 2003). Practices that postpone the development of a dense canopy consist of the adjustment of seeding dates, lower seeding rates, wider row spacing and providing optimal fertilizer levels for the crop while avoiding the addition of high amounts of nitrogen.

2.6.5 Sclerotinia White Mould

Sclerotinia white mould (causative agent *Sclerotinia sclerotiorum* (Lib.) de Bary) can infect a wide range of host plants (including lentil) and this necrotrophic pathogen is identified by the production of sclerotia that remain viable for many years in soil. Apothecia form after these sclerotia are imbibed, resulting in the release of a substantial amount of ascospores (Li et al., 2004). Infection in lentil crops is primarily initiated by lodged plants that come in immediate contact with the small black sclerotia bodies (Bailey et al., 2003).

Although infection of lentil is not a common concern for producers on the Canadian Prairies, lentil is susceptible from early flower to pod filling, when the climate is wet and cool (Yadav et al., 2007). Symptoms caused by sclerotinia generally appear as watery soft-rot on tissue that is infected, primarily the leaves and stems (Boland and Hall, 1994) although, the presence of white mould and black sclerotia can be noticed as well. An increase in canopy ventilation and foliar fungicides may control this disease, as crop rotation has little effect, (Bailey et al., 2003) due to characteristics of wide host range and extended soil viability.

2.6.6 Stemphylium Blight

Stemphylium blight of lentil results from infection of *Stemphylium botryosum* Wallr. (*Pleosporales, Pleosporaceae*) and the degree of destruction of this pathogen is highly dependent on environmental conditions (Saha et al., 2010). This disease has established in North America in recent years (Mwakutuya & Banniza, 2010); however, samples reported from Saskatchewan commercial seed tests have revealed high levels of stemphylium blight infection (Morrall, 2006). A wide range of geographical locations and host plants are favorable for this pathogen and plant death can occur as rapid as two to three days in environments with ideal conditions (Saha et. al., 2010).

Airborne conidia are responsible for transmission of stemphylium blight, giving rise to symptoms that are prominent in the upper canopy of the plant including leaf drop, a sharp decrease in biomass and finally, decreased seed yield in some circumstances (Bailey et al., 2003). Due to the nature of airborne conidia, it is likely that higher plant populations have a higher likelihood of infection due to an increased number of plant hosts. Symptoms of stemphylium can occur anytime throughout the season (Bailey et al., 2003) and more specifically, symptoms involve small, tan coloured lesions on leaves, stems, flowers or pedicels that expand and merge, oftentimes blighting the entire plant. Lentil seeds that are infected take on a stained appearance and germination rate is decreased (Mwakutuya & Banniza, 2010).

Due to the saprophytic nature of this pathogen, crop rotation likely has a small effect on severity and the potential for effective chemical control is uncharted. However, residue management and clean seed are good practices to follow as this fungus will survive in seed and on plant residue (Bailey et al., 2003). In Canada, there are no fungicides registered for control of stemphylium blight as there is limited information on fungicide efficacy to control the disease (Podder et al., 2013).

2.7 Foliar Fungicides in Lentil Production

Different pathogens including viruses can infect lentil plants, however, fungal pathogens are the most significant. Fungi infect different parts of the plant, ultimately leading to low seed yield or seed that cannot be sold (Taylor et al., 2007). Foliar fungicides can be used as part of an integrated disease management system with other means, including the utilization of resistant cultivars and seed treatments, crop rotation, the management of crop residue and microenvironment modifications (Krupinsky et al., 2002).

To aid producers, the Fungicide Resistance Action Committee (FRAC) was organized to oversee and monitor fungicide resistance. When a fungicide is registered, it is assigned a FRAC code that categorizes each active ingredient in the product by the target sites within each mode of action (Jackson-Ziems et al., 2017). The group or mode of action of fungicide applied to an area should be alternated, to extend the longevity of the fungicide efficacy (Government of Saskatchewan, 2018). Depending on the pathogen, several different groups are registered for protection against the same disease.

BLAD polypeptide is sold commercially as Fracture® Fungicide and is registered on several horticultural crops including grapes, tomatoes and strawberries. Application for horticulture is recommended to be repeated throughout the season and treated as a preventative means to control disease (FMC Corporation, 2016). The BLAD polypeptide accumulates in the cotyledons of Lupinus plants after germination. It has been recognized that this intermediary product of β-conglutin catabolism possesses antifungal characteristics that can be used in agriculture due to high efficacy against fungal organisms (Monteiro, 2015). The adequacy and affordability of Fracture® for use on field crops including lentil, is currently unknown.

Pyraclostrobin is a synthetic strobilurin fungicide that acts in the mitochondria (Balba, 2007). Also known as quinone outside inhibitors (QoIs), they account for 22% of the fungicide market share (Hirooka and Ishii, 2013) as they are widely used and adopted by producers. Fungicides in the strobilurin family act rapidly as the spore stage is targeted through this specific mode of action (Balba, 2007). In the cell of fungi and the quinol oxidation site specifically, electron transfer between cytochrome b and cytochrome c is halted. Through this process, the synthesis of adenosine triphosphate (ATP) and the oxidation of nicotinamide adenine dinucleotide (NADH) is interrupted and since these products are necessary for energy production, fungal death is inevitable (Balba, 2007). Strobilurin fungicides and pycraclostrobin specifically, are active at one site in the fungal cell and because of this, pathogen resistance to this mode of action is easily developed (Balba, 2007). Pyraclostrobin insensitivity has been found to be predominantly qualitative (Ypema et al., 1999). With this mechanism, the pathogen has become completely unresponsive to the fungicide in comparison to quantitative insensitivity, where higher rates can still be effective for pathogen control (Bowness et al., 2016). Pyraclostrobin is registered on a very wide range of crops and diseases including the lentil diseases, anthracnose and ascochyta blight (Saskatchewan Ministry of Agriculture, 2018).

The development of pathogen insensitivity is much less likely for chlorothalonil than for a pyraclostrobin herbicide due to the specific site of action (Von Jagow et al., 1986). The mode of action that chlorothalonil possesses is reducing certain enzymes that contain glutathione as their reaction centers (Yang, 2011). Chlorothalonil translocation within the plant is very limited (Chang, 2007); but, fungicides with multisite activity are widely used in field crops because they provide a broad spectrum of disease control (Yang, 2011). Chlorothalonil is deemed low risk for fungicide resistance and is registered for use on several crops including wheat, peas, lentil and potatoes for protection against a broad spectrum of diseases including fusarium head blight, mycosphaerella blight, anthracnose and late blight (Government of Saskatchewan, 2018).

3.0 The Effect of Lentil Market Class on Optimum Seeding Rate

3.1 Introduction

In 1976 when Dr. Slinkard developed a lentil seeding rate, the optimum plant stand was recorded at 100 plants m^{-2} (Slinkard, 1976). Two years after this, the Laird lentil was introduced as the first lentil that originated specifically for the prairie provinces and, the first lentil cultivar licensed in Canada (Slinkard and Bhatty, 1979). Forty years later, the Saskatchewan Government now reports a recommended target of 130 lentil plants m^{-2} (Saskatchewan Pulse Growers, 2018a); however, this recommendation is likely still based on the standard for large green Laird lentil. While large green lentil is still grown in Canada, the production of other lentil market classes, within the now dominant small red lentil class, was at 1.6Mt while large green lentil production was less than a third of that in 2017 (Agriculture and Agri-Food Canada, 2017). Crop seeding rates depend on the size of seed and there is an extensive range in seed size amidst cultivars produced on the Canadian Prairies (Ali-Khan and Kiehn, 1989) and this calls for a need for cultivar specific seeding rates.

Yield density studies are fundamental to field crop research to achieve the precise balance between the competition for resources when seeds are planted at densities that are elevated and, between the waste of valuable resources, when crops are seeded at densities that are too low (Deng et al., 2012). Recent work has been done in Saskatchewan on lentil yield density studies that involve weedy conditions including work done by Redlick et al. (2017) that examined optimal seeding rates under weedy conditions with different weed control treatments. Additionally, a small red lentil study completed in the Pacific Northwest dryland cropping region on seeding rate with cross seeded treatments and herbicides for weed control, involved weedy conditions as well (Ball et al., 1997). Baird et al., (2009) discovered that under organically managed conditions, increased profitability in lentil could be achieved if seeding rate was increased to 229 plants m^{-2}. An additional lentil study that occurred under weedy conditions also examined the effect of reduced herbicide rates in exchange with higher lentil seeding rates; however, results did not show sufficient control when herbicide use was omitted and the seeding rate response for lentil was inconsistent (Kirkland et al., 2000). The previously mentioned studies sought different means of weed control besides that of herbicide; however, plant population studies involving weed free conditions are of smaller prevalence. Weed free (studies with herbicides used for weed control) field crop yield density studies generally produce different responses than those that have weeds planted or have high existing populations in them.

In 1994, Wall conducted a pre-emergent herbicide study on lentil that involved weed free conditions and a number of different seeding rates. It was discovered that seed yield did not increase substantially beyond 30 kg ha^{-1} and from 30 to 90 kg ha^{-1}, there was only a slight further increase in seed yield. Studies that include weed populations produce results like the organic lentil experiment produced by Baird et al. (2009) where a maximum yield asymptote was not reached and at the highest sowing rate, yield was continually increasing. In contrast, weed-free studies including an Australian study by Siddique et al. (1998) often have produced results where yield reached an asymptote and declined thereafter, as the maximum field capacity was being reached. The aforementioned Australian study proposed targeting a plant population of 150 plants m^{-2} however, this recommendation was based on the mean from 96 to 228 plants m^{-2} as this range was the resulting economic optimum between all sites.

There is a lack of weed free studies conducted on lentil yield responses to seeding rates and due to this scarcity in research, lentil yield potential is not likely reached. In a study involving pea cultivars, the effect of seeding rate was examined and it was found that the highest seeding rates resulted in the highest seed yields (Uzun and Açikgöz, 1998). Results from more recent studies including one done by Gan and Shirtliffe et al., (unpublished) displayed the seeding rate effect on lentil yield and that current recommendations of 130 plants m^{-2} (Saskatchewan Pulse Growers, 2018a) are not sufficient in the utilization of all field resources under weed free conditions in small and extra small red lentil. In saying this, the highest possible economic return for lentil producers is not being accomplished. Different market classes of lentil reflect varying seed sizes, effecting the cost of seed and therefore the economic consequence of using increased seeding rates in larger seeded varieties will result in higher costs. In addition, response to disease pressure is varied throughout different lentil cultivars. As a result, I hypothesize in this experiment that all lentil market classes will differ in optimum seeding rate. The objective of this study was to determine if there were differences in the yield density response among representatives of six different lentil market classes.

3.2 Materials and Methods

3.2.1 Site Description

Field experiments were conducted on conventional cropland in central Saskatchewan in 2015 and 2016 near the Kernen Crop Research Farm (Nasser) (52°08'45.5"N 106°31'50.9"W) located near Saskatoon, Saskatchewan. In 2015, two trials were conducted with one located near

Osler, Saskatchewan (52°24"27.6"N 106°38'35.0"W), and the second at the Goodale Research Farm (GRF) (52°03'28.8"N 106°29'32.7"W). In 2016, one trial was grown on irrigated land near Outlook, Saskatchewan (52°31'48.6"N 106°53"15.4"W), and the second was on the Kernen Crop Research Farm (Nasser), for a total of four site-years. All locations were selected in the black or dark brown soil zones (Table 3.1) and were fertilized according to lentil crop requirements (Table 3.2).

Table 3.1 Soil Characteristics and site description for Osler, Goodale Research Farm, Outlook and Nasser in 2015 and 2016.

Location	Soil Type	Soil Texture	Previous Crop
Osler	Hamlin Orthic Black Chernozem	55% sand 28% silt 17% clay	wheat
Goodale	Bradwell Orthic Dark Brown Chernozem	50% sand 32% silt 18% clay	fallow
Outlook	Asquith Orthic Dark Brown Chernozem	77% sand 13% silt 10% clay	canola
Nasser	Sutherland Orthic Dark Brown Chernozem	16% sand 44% silt 40% clay	wheat

Table 3.2 Available soil nutrient levels (ppm) and properties for each separate trial site in 2015 and 2016. Depth in cm.

	Goodale (2015)		Osler (2015)		Nasser (2016)		Outlook (2016)	
Depth (cm)	0 -15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
Available Nitrogen	25	11	9	35	12	24 (6-24")	22 (0-12")	NA
Available Phosphorus	>67	NA	50	NA	21	NA	67 (0-12")	NA
Available Potassium	>670	NA	>670	>146 (6-24")	>670	NA	>897	NA
Available Sulphur	45	NA	17	NA	9	90 (6-24")	50 (0-12")	NA
Organic Matter	3.3%	NA	NA	NA	NA	NA	NA	NA
pH	6.1	6.9	6.2 - 6.3	6.2 - 6.3	NA	NA	6.3	NA

3.2.2 Experimental Design

The experiment was a 5 x 6 factorial treatment, randomized complete block design with four replicates. The two factors assessed were seeding rate (target plant population) and lentil cultivar or market class. The five seeding rates involved were 60, 120, 180, 240 and 320 seeds m^{-2}, and the six market classes were extra small red (CDC Imperial), small red (CDC Maxim), large red (CDC KR-1), small green (CDC Imvincible), medium green (CDC Imigreen), and large green lentil (CDC 3339-3). Each lentil cultivar will be referred to as the corresponding lentil market class name for the remainder of the thesis.

All plots were seeded with a small plot, cone seeder with hoe openers, with each plot containing six rows of crop, spaced 30 cm apart, creating a 6 m by 1.8 m plot. At the time of seeding, granular Tag Team® inoculant (*Penicillium bilaii* and Rhizobium leguminosarum; Novozymes) and 11-52-0-0 fertilizer was placed with the seed. In 2015, all trials received 54 kg ha^{-1} of 11-52-0-0 while 2016 trial sites received 64 kg ha^{-1} of 11-52-0-0 at both sites.

In 2015 and 2016, lentils were first treated with Apron Maxx RTA® (fludioxonil and metalaxyl-M and S-isomer) at a rate of 325 mL 100 kg^{-1} of seed. The seed treatment is a group 4 and group 12 fungicide, to protect seeds from soil-borne diseases. Before seeding, all plots received a pre-seed herbicide application of glyphosate and after emergence, an in-crop herbicide treatment of Odyssey® (imazamox and imazethapyr) was applied at 30 g ai ha^{-1} before the 9-node stage was reached. If grassy weeds were abundant then Poast Ultra® (sethoxydim) was applied at 500.4 g ai ha^{-1}. Hand-weeding was carried out after that when needed, to attempt to maintain weed-free plots. In 2015 trials, a group 11 fungicide application of Headline® (pyraclostrobin) was applied when lentils began to flower, at a rate of 98.84 g ai ha^{-1}. Group 7 and 11 Priaxor® (fluxapyroxad and pyraclostrobin) was applied at the same timing in the 2016 trials for disease protection, at a rate of 75.15 g ai ha^{-1} and 149.85 g ai ha^{-1} of fluxapyroxad and pyraclostrobin, respectively. Additional disease protection was not needed in the 2015 field season due to a low amount of precipitation however, in 2016 humid conditions called for a second fungicide application at late flower and Bravo® (chlorothalonil), a group M5 fungicide, was applied to both locations at a rate of 1976.8 g ai ha^{-1}. When the majority of lentil plants turned brown in the bottom third of the plant and rattled when shaken (Saskatchewan Pulse Growers, 2000b), desiccation was carried out with Reglone® (diquat) at a rate of 415 g ai ha^{-1}. All pesticide rates, water volumes and timings followed label recommendations.

3.2.3 Data Collection

Crop emergence counts were conducted in two $0.25m^{-2}$ quadrats per plot (one in the front and one in the back) and these two values were averaged before analysis. Biomass collection was also carried out in two $0.25m^{-2}$ quadrats per plot when the crop had reached physiological maturity (pods rattled when shaken). Lentil plants were cut off at ground level, collected in paper bags and dried in oven driers for approximately 48 hours at 70°C to dry the samples at time of weighing. Lentil seed was harvested with a plot combine, collecting the inside rows (a total of four rows were harvested), allowing for a reduced edge effect with the removal of the two outside rows (Table 3.3). Harvested yield was weighed and cleaned after drying to a constant moisture content and the final lentil yield and seed weight were recorded.

Table 3.3 Field operation dates including seeding, crop counts, biomass collection and harvest in 2015 and 2016 at each site.

	2015		2016	
Measurement	Osler	Goodale	Outlook	Nasser
Seeding	09-May	14-May	16-May	03-May
Crop counts	08-Jun	03-Jun	02-Jun	18-May
Biomass	30-Jul	05-Aug	15-Aug	02-Aug
Harvest	18-Aug	18-Aug	25-Aug	17-Aug

3.2.4 Statistical Analysis

The MIXED procedure was used in SAS® software version 9.4 (SAS Institute, 2017) to analyze data for this study. The fixed effects for analysis were seeding rate (target plant population) and cultivar (market class). The site-year, replicate, and the interaction between the treatments with site-year were considered random effects. Significance of treatment effect was determined at $P<0.05$ although some results were closely significant and were therefore analyzed by site-year and with all sites joined simultaneously. The data was subject to an analysis of variance (ANOVA) and the means were compared using a protected least squared means (LS means) test. Results from the type 3 tests of fixed effects were used to distinguish differences between treatments.

A quadratic model was used to model seed yield by emergence, where plant counts were used as a continuous variable and a regression was utilized with plant counts (or emergence) as an independent variable for analysis. Through a polynomial quadratic analysis, it was possible to model a clear trend since a linear regression would not be sufficient at displaying the point in which maximum yield was reached and began to decline. Although an ANOVA could show this decline in yield, the variable seeding rate is continuous and therefore not best suited to an ANOVA or categorical analysis. For the quadratic model, separate quadratic curves were fitted for each cultivar. For the quadratic seed yield by seeding rate model, the seeding rate was used as the independent variable. The same process was used for biomass analysis. Thousand seed weight (TSW) was examined using mixed model analysis in SAS. Seeding rate, lentil cultivar and fungicide treatment were used as fixed effects while site-year and repetition nested in site-year were considered the random effects. LS means and differences in least squared means were used to determine treatment differences.

3.3 Results and Discussion

3.3.1 Environmental Conditions

The total field season precipitation averages were typical in 2015 and 2016 compared to the long-term normal however, late season moisture was higher in July and August (Table 3.4 and 3.5). At least one hail event at the Outlook site (on July 20, 2016) increased the likelihood of disease infection and damaged yields.

Table 3.4 Saskatoon and Outlook weather data with precipitation and temperature including 2015 and 2016 averages compared to the 30 year normals.

	Precipitation			Temperature		
Month	2015	2016	Normal ¥	2015	2016	Normal ¥
		(mm)			(°C)	
			Saskatoon			
May	0.4	41.6	43.0	10.1	13.7	11.8
June	13.6	49.7	65.8	17.2	17.4	16.1
July	84.3	58.6	60.3	19.4	18.7	19.0
August	45.2	70.2	42.6	17.4	16.9	18.2
			Outlook			
May	NA	55.7	42.6	NA	13.5	11.5
June	NA	45.8	63.9	NA	17.5	16.1
July	NA	194.6	56.1	NA	18.6	18.9
August	NA	69.9	42.8	NA	16.9	18.0

¥ 1981-2010 Canadian Climate normals obtained from Environment Canada (2018). (Government of Canada(a), 2018) (Government of Canada(b), 2018). NA = not applicable.

In the months of July and August in 2016, when full vegetative crop canopy occurred, precipitation levels were almost 1.5 times the average in Saskatoon and, the Outlook location received almost 4 times the amount of average precipitation in July. Alongside higher precipitation levels in 2015 and 2016, the average temperature in June, July and August was also marginally higher (Government of Canada, 2018b) allowing for conditions conducive to disease development in lentil.

3.3.2 Crop Emergence

Lentil crop emergence was explored at each location where each site showed a significant effect for seeding rate and for cultivar. At the Nasser location, a significant two-way interaction between the seeding rate and cultivar factors was noted (Table 3.5).

Table 3.5 The effect of seeding rate, cultivar and seeding rate by cultivar and the corresponding *P*-Values for ANOVA on crop emergence at separate site-years in 2015 and 2016.

	P-Value			
Effect	Goodale (2015)	Osler (2015)	Nasser (2016)	Outlook (2016)
Seeding Rate (SR)	<.0001	<.0001	<.0001	<.0001
Cultivar (C)	0.0012	0.0008	<.0001	0.0002
SR * C	0.1907	0.329	0.0384	0.3849

Significance (P<0.05) of the variable seeding rate in emergence at each site was expected because various seeding rates were a desired outcome in this experiment. The significant *P*-values for the cultivar effect at each site regarding emergence can be described by the typical genotype by location interaction. Different cultivars or genotypes are not consistent when grown in one environment compared to the next, as genotypes are adapted to one particular growing environment more than another (Baker, 1988). The significant interaction of seeding rate and cultivar at the Nasser location in 2016 will be examined.

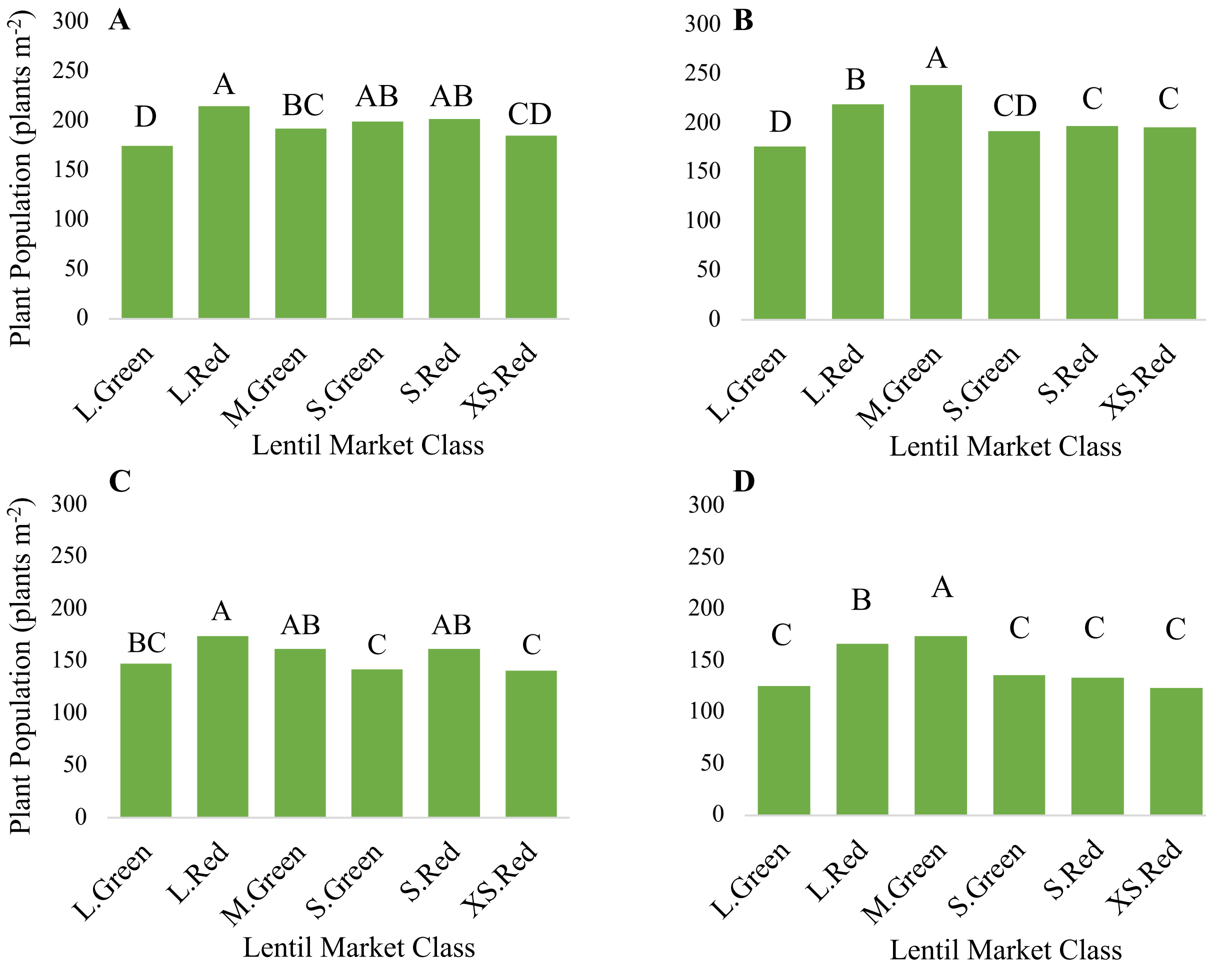

Figure 3.1 Crop emergence response by each cultivar for both seeding rates at separate locations Goodale 2015 (A), Nasser 2016 (B), Osler 2015 (C) and Outlook 2016 (D).

Averaged over all seeding rates, the emergence was 184 plants m^{-2}. At Goodale in 2015, large red, small green and small red lentil cultivars reached the highest plant populations with medium green and extra small red in the middle and large green contributing to the lowest plant population for all cultivars in this study. At the other three locations, large green lentil also showed the lowest emergence counts. At Nasser in 2016, medium green lentil had a significantly higher population followed by large red lentil. Small red and extra small red cultivars had mid-range emergence. At Osler in 2015, large red, medium green and small red lentil contributed to the highest populations with small green and extra small red in the middle. A similar trend was observed at Outlook in 2016 and although plant populations were lower overall, medium green lentil had the highest population followed by large red lentil, while the three other cultivars followed with the lowest emergence counts.

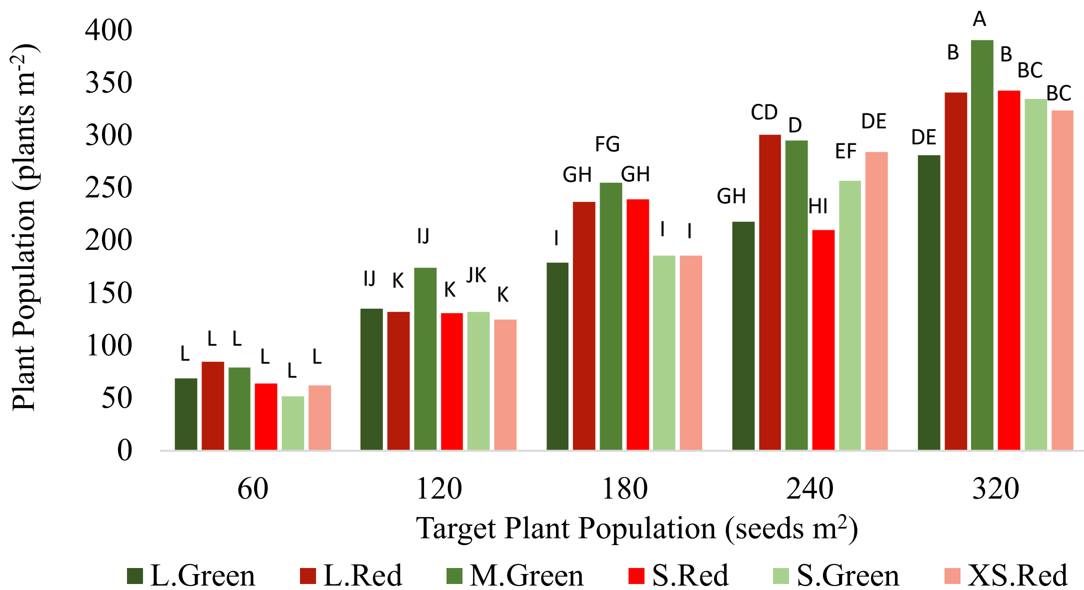

Figure 3.2 Plant emergence response of the interaction between the variables of seeding rate and cultivar (Table 3.5) at the Nasser location in 2016. Letters represent significant difference.

The interaction at the Nasser site in 2016 (Table 3.5 and Figure 3.2) displayed differences between plant population and the target population of each lentil cultivar. At the lowest target population, no specific cultivar had a significantly different amount of plants than the other however, as the seeding rate increased, significance in plant population became more apparent. Large differences were noted between 180 and 240 seeds m^{-2} and between medium green at 320 seeds m^{-2} and the rest of the cultivars seeded at the same rate. At the second lowest target population (120 plants m^{-2}), medium green and large green lentil had the highest population and had significantly higher numbers than the three red lentil cultivars in the trial. At 180 plants m^{-2}, a different result was displayed as large red, small red and medium green lentil had a significantly higher population than other market classes. As seeding rate increased to 240 plants m^{-2}, a different trend was shown as large red displayed the highest population, small red and large green lentil had the lowest and the rest of the cultivars were in the middle. The highest seeding rate (320 plants m^{-2}) showed a large range of population numbers with medium green having a significantly higher number of plants and large green having a significantly lower number of plants than the other target seeding rates. Large green lentil at the highest seeding rate was not significantly different from the majority of lentil cultivars at the seeding rate below (240 plants m^{-2}). Overall, medium green lentil had greater emergence at Nasser and this trend was supported by results at other locations, including Outlook in 2016.

3.3.3 Quadratic Modelling of Crop Biomass by Seeding Rate: Combined Analysis

Analysis of the biomass parameter determined that all sites could be combined as there was no significant site-year by treatment interaction (Table 3.6).

Table 3.6 ANOVA results for combined sites in 2015 and 2016 for quadratic biomass analysis by seeding rate with parameters site-year, seeding rate and cultivar.

Parameter	*P*-Value
Repetition(Site-Year)	0.0220
Site-Year	0.1137
Seeding Rate * Site-Year	0.1923
Site-Year * Cultivar	0.0685
Cultivar	0.4309
Seeding Rate * Cultivar	0.0177
Seeding Rate * Seeding Rate * Cultivar	0.0344

The significant ($P<0.05$) interaction of seeding rate and cultivar is demonstrated in Figure 3.3 (below) where large red lentil had the highest amount of biomass followed by small red lentil, large green, medium green, small green and finally, extra small red lentil with the production of the least amount of biomass.

When a significant two-way interaction arises, it becomes apparent that the effect of an independent variable on a dependent variable is determined by the other independent variable in the study. So, in reference to this current study, the effect of seeding rate on biomass depends on the impact that different cultivars display on biomass. In saying this, the interaction here is likely due to the reduction in biomass with large green lentil at the highest seeding rate, as the seeding rate response of large green lentil is displayed differently than the other cultivars (Fig. 3.3).

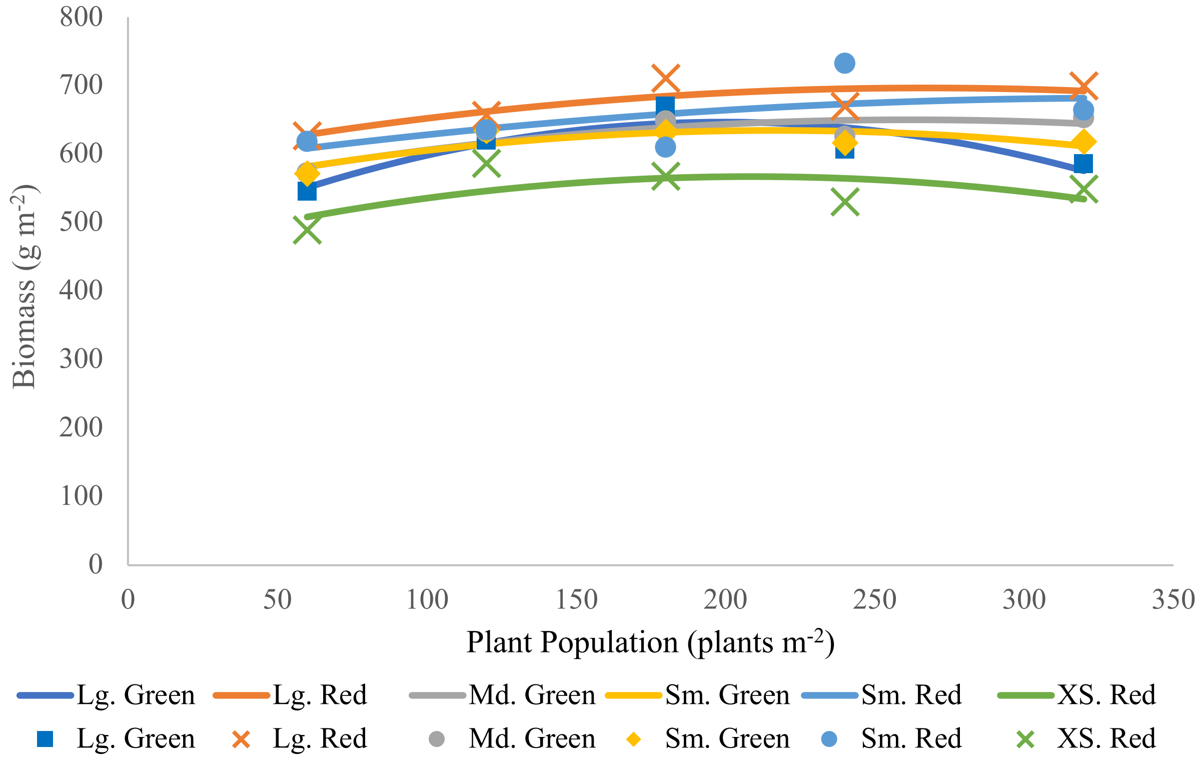

Figure 3.3 The regression (as the solid lines) for lentil biomass by the independent variable, seeding rate. Dots display the LS means of biomass for each market class distinguished by color. All sites combined for 2015 and 2016.

Table 3.7 Slope parameters for quadratic seeding rate analysis displaying intercept, linear and quadratic parameters with standard error on biomass for all lentil market classes. Data combined for 2015 and 2016.

Market Class	Intercept	Linear	Quadratic
Large Green	451.5823	1.9559	-0.0049
Large Red	583.14	0.8495	-0.00159
Medium Green	534.8049	0.8804	-0.00168
Small Green	533.572	0.9321	-0.00215
Small Red	572.95	0.6502	-0.00097
Extra Small Red	451.26	1.1108	-0.00266

Relatively flat curves (Figure 3.3) produced by modelling the biomass response displayed that plant population had a small effect on biomass production. Biomass response relative to seed yield response to the effect of plant population is compared in section 3.3.8.

3.3.4 Quadratic Modelling of Crop Biomass by Emergence: Combined Analysis

Biomass results were also analyzed by plant populations or emergence of lentils as well, to ensure that differences in plant emergence were accounted for. Results showed similarities to those of biomass with seeding rate as the independent variable.

Table 3.8 ANOVA results for quadratic analysis for biomass by emergence with the effects of site-year, plant counts and cultivar in 2015 and 2016. All sites were combined.

Parameter	*P*-Value
Repetition(Site-Year)	0.0224
Site-Year	0.1141
Plant Counts * Site-Year	0.1543
Site-Year * Cultivar	0.0988
Cultivar	0.5979
Plant Counts * Cultivar	0.045
Plant Counts * Plant Counts * Cultivar	0.0454

The quadratic trend displayed by biomass with emergence was not shown as results were complementary to the quadratic biomass by seeding rate trend. The significance found between plant counts and cultivar (Table 3.8) is likely attributed to the typical genotype by location interaction.

3.3.5 Combined Data of Analysis of Variance for Lentil Seed Yield

The site-year by cultivar (market class) effect (Table 3.9) demonstrates that the growing environment at each site determined how each lentil variety yielded. Thus, the seed yield of each lentil cultivar is dependent on growing environment; so, results were analyzed by site-years individually as well.

Table 3.9 ANOVA for the effect of seeding rate, site-year and market class on seed yield at all locations in 2015 and 2016.

Parameter	*P*-Value
Repetition	0.2954
Site-Year	0.1147
Site-Year * Seeding Rate	0.1197
Site-Year * Cultivar	0.0091
Residual	<.0001
Seeding Rate	0.0040
Cultivar	0.0029
Seeding Rate * Cultivar	0.7823

The location of the trial seemed to have no effect or an insignificant effect on the yield results at different seeding rates so, results were compiled and analyzed together. Although, due to variation, this phenomenon will be examined within site-years as well.

The seeding rate effect (Table 3.9 and Figure 3.4) suggests that a target plant population around 120 plants m^{-2} is sufficient for lentil production of any market class on the Canadian Prairies. However, it is likely that this relationship represents outcomes that can occur when excessive late season moisture impairs the potential of a lentil crop. Due to the nature of the variables applied in this study, a different approach was used to display results that are suited to the explanation of a quantitative variable including seeding rate. To elucidate the effects at individual sites, the seeding rate response at individual site-years was explored as well.

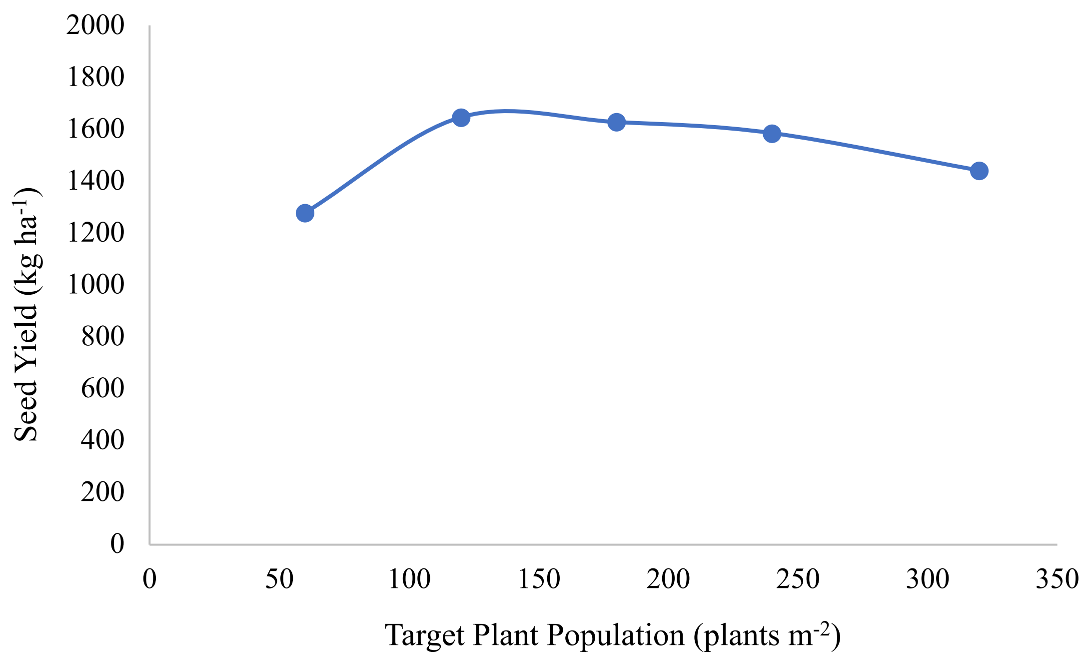

Figure 3.4 The seeding rate effect produced by the LS means of lentil seed yield results for all six cultivars with the 2015 and 2016 trials combined.

The seeding rate effect shows that at 120 plants m^{-2}, the maximum yield (1646 kg ha^{-1}) potential is reached when six different lentil market class yields were combined. These results contradict findings discovered in a lentil seeding rate study where seed yield showed a curvilinear yield response as yield increased between 15 to 375 seeds m^{-2} and the constant final yield had not yet been reached at that time (Baird et al., 2009). The difference in these results is likely due to the presence of weeds in the study by Baird et al. and explains why no asymptote is reached. Saleem et al. (2012) conducted a lentil seeding rate study that produced results showing an increasing grain yield up to a seeding rate of 43 kg ha^{-1} (approximately 170 plants m^{-2}). These results are similar to what was produced in this current study. In the aforementioned Pakistani study, over 50 mm of monthly annual precipitation was received, contributing to similar environmental conditions as lentil plots were subject to in this Canadian study.

3.3.6 Individual Site-Year Analysis of Variance for Seed Yield

Results from the combined analysis showed significance with site-year and the parameter of market class. Although this effect is common as cultivar performance differs with location, a site-year analysis was conducted. Through this analysis, differences in seeding rate response across different sites were noted.

Table 3.10 Individual location results for ANOVA for categorical seed yield analysis in 2015 and 2016 for the effect of seeding rate and cultivar.

	Goodale (2015)	Osler (2015)	Nasser (2016)	Outlook (2016)
Seeding Rate (SR)	0.0001*	0.0144*	0.0019*	0.0103*
Cultivar (C)	0.0003*	<.0001*	<.0001*	<.0001*
SR * C	0.8643	0.1154	0.0899*	0.1549

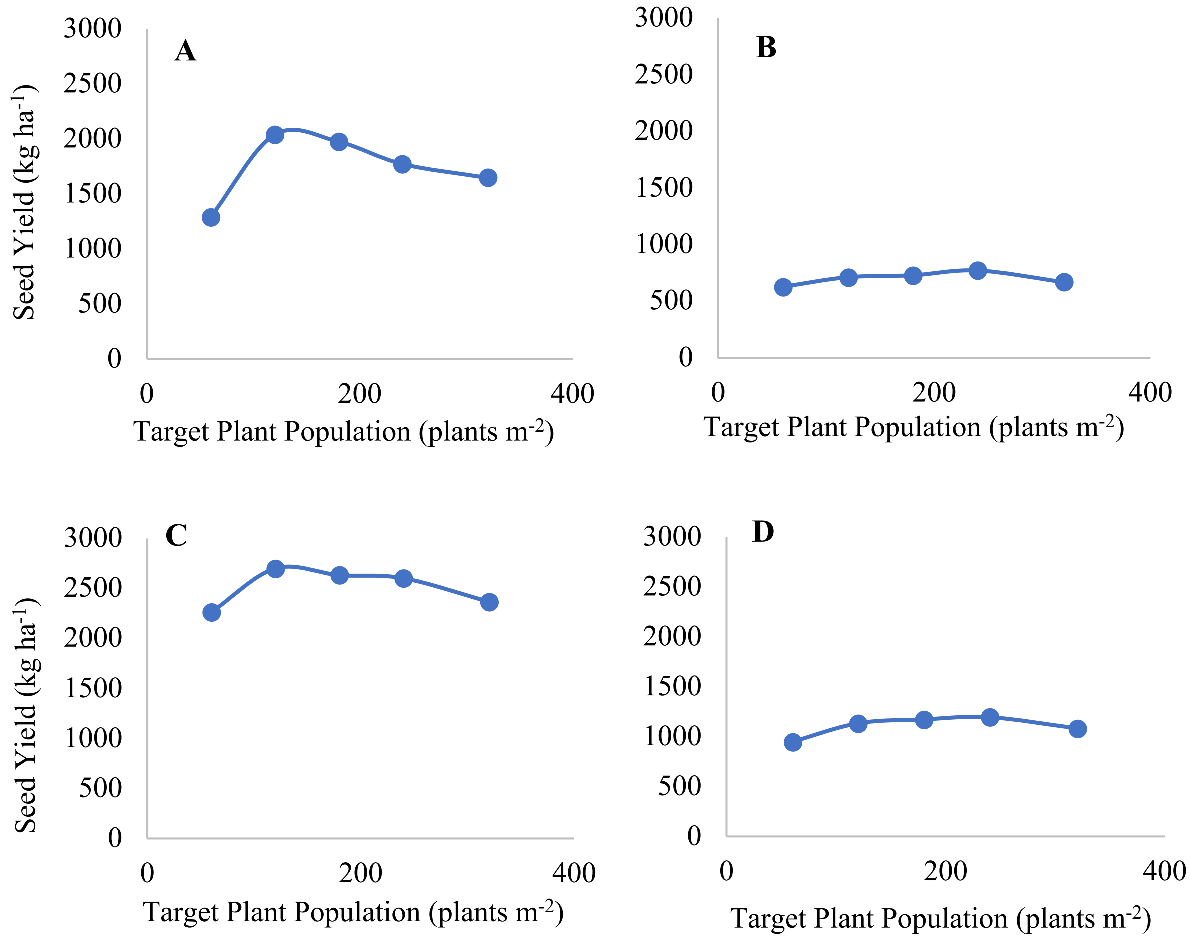

Figure 3.5 Seeding rate response is displayed as target plant population changes. Goodale 2015 (A), Outlook 2016 (B), Nasser 2016 (C) and Osler 2015 (D) locations displayed for ANOVA for seed yield analysis. The smoothed line connects the average yield response for each target plant population.

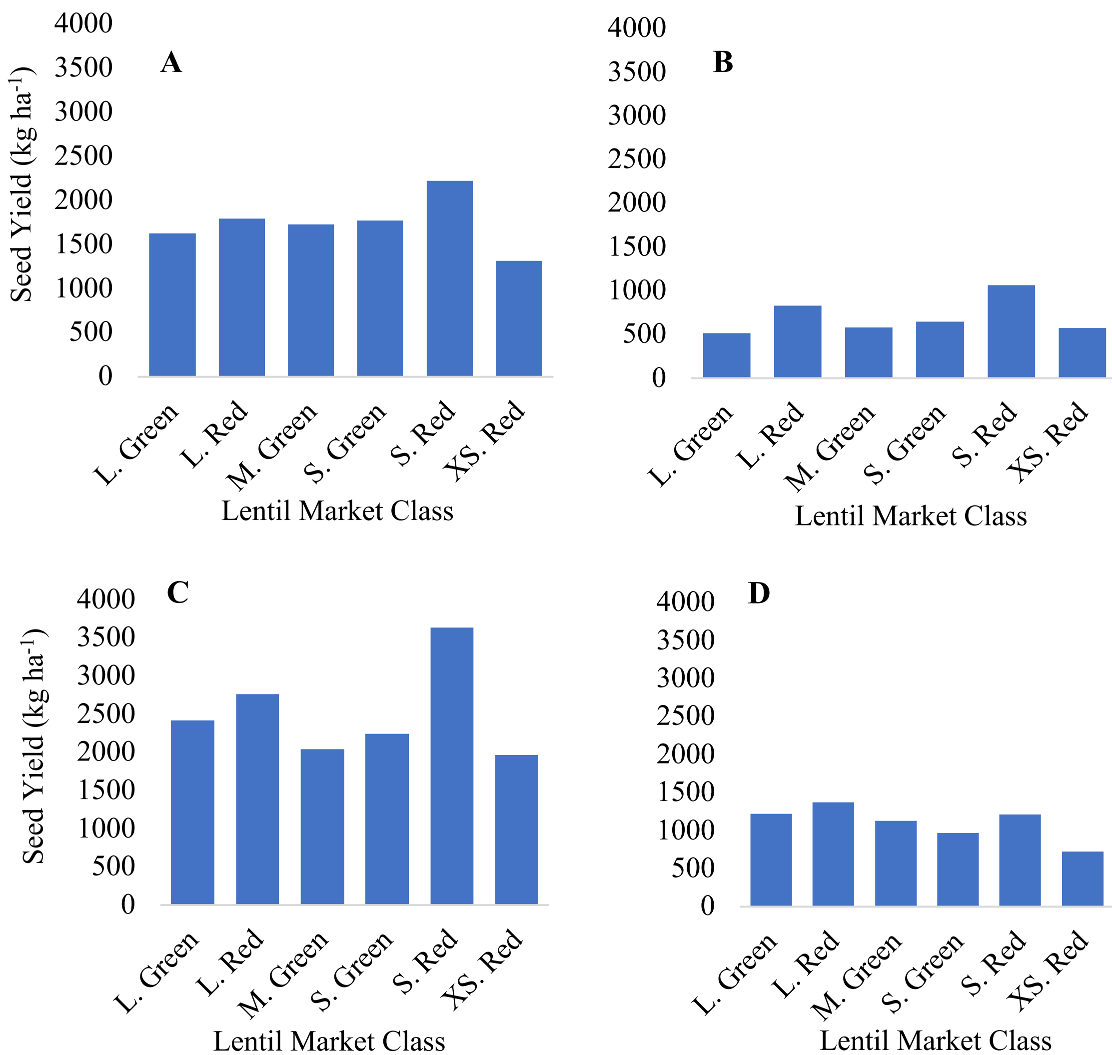

Figure 3.6 Yield response for cultivars at each site Goodale 2015 (A), Outlook 2016 (B), Nasser 2016 (C) and Osler 2015 (D).

3.3.6.1 Goodale Analysis of Variance for Seed Yield

The significant (*P*<0.05) effect that lentil market class had on yield at the Goodale location (Table 3.10) makes it evident that different cultivars allow for an array of yield outcomes. At the Goodale site in 2015, small red lentil yield was greater than large red, followed by small green, medium green and large green while extra small red lentil displayed the lowest yields (Figure 3.6A).

Goodale results were produced under relatively normal environmental conditions and soils with low clay content (18%). The maximum yield was reached at a plant population of 120 plants m^{-2} (Figure 3.5A). A study similar to this was conducted by Wall (1994) where the optimum seeding rate of the small green Eston lentil was examined and lentil seed yield increased up to a seeding rate of 30 kg ha^{-1} and after that, lentil yield did not increase enough for an economic advantage. This aforementioned seeding rate translates to approximately 80 plants m^{-2} which is notably lower than results found in recent studies suggesting to increase seeding rates past the current recommendations for optimal yield. However, the study done by Wall (1994) was conducted throughout wet growing seasons and on land with heavy clay (34%) content so differences in these results could be attributable to soils with high clay compared to those at GRF where soil type is more favorable for lentil production. Taking this into account, the trend produced at the Goodale location (Figure 3.5A) showed similar results to those of Wall (1994) however, the growing conditions that this current study and Wall et. al. (1994) were subject to, are not those typical or favorable for profitable lentil crops in Western Canada. Both studies resulted in typically produced trends to be found in field crop studies that are weed-free as a maximum yield generally reaches an asymptote and continues to decline thereafter.

A study done by Ali-Khan and Kiehn (1989) involved what are now considered low seeding rates and at the highest rate in the study, 100 plants m^{-2}, the constant final yield had not yet been reached. Results of the current study (Figure 3.5) confirm that the previously mentioned trial likely did not include seeding rates high enough to reach a constant final yield.

3.3.6.2 Outlook Analysis of Variance for Seed Yield

The Outlook site experienced extremely wet conditions compared to other sites but, small red lentil still yielded highest across all lentil market classes. Although plant stature and seed size may be thought to predetermine lentil yield in these conditions, it appears that seed size does not play a role in yield outcome. Lentil plants with larger seed do not produce higher overall seed yields than smaller seeded lentil varieties, as is seen in results at different sites. Yield differences across different cultivars could be attributed to varying responses to disease pressure, produced by varying lentil stature, growth habit or genetics.

An interesting trend was produced in reference to seed yield results and the seeding rate response at the Outlook site. Higher than normal precipitation presented challenging growing conditions and, this resulted in low overall yields; however, the seed yield results still show an intriguing trend (Figure 3.5B) as the highest yield was gained from a seeding rate of 240 plants m^{-2}. This result is substantiated by other studies to have shown that higher target plant populations in a lentil crop will gain higher yields. It has been demonstrated, that in an environment with high amounts of precipitation, increased seeding rate used alongside other measures including herbicides, can enhance weed suppression as well (Ball et al., 1997). Low weed populations were noted at the Outlook location suggesting that this phenomenon could have developed.

3.3.6.3 Nasser Analysis of Variance for Seed Yield

The yield trend produced by the Nasser location was nearly significant (P=0.0899) between the factors seeding rate and cultivar. The different ways in which each lentil variety responded to seeding rate at the Nasser site individually, is displayed in Figure 3.7. These trends suggest that the current seeding rate recommendation of 130 plants m^{-2} may be suitable for varieties including large red, small green, extra small red and medium green however, large green and small red lentil could yield higher at an increased seeding rate.

Considering the yield response of lentil at Nasser, (Figure 3.5C) the difference between the two highest yielding points on the y axis is diminutive while the yield at 120 plants m^{-2} is 2700 kg ha^{-1} and the yield at 240 plants m^{-2} is 2602 kg ha^{-1}. A review concerning lentil agronomy by Saxena (1981) demonstrated that increased lentil seeding rates display a higher positive yield response when soil moisture is ample and when the crop has been seeded later in the season. Results from this current study could validate results that Saxena (1981) produced if conditions were different, considering that the yield response between the low and high seeding rates were minimal. In saying that, these opposite outcomes between the studies could be attributed to lentil genotypes that are more suited to wet versus dry environmental conditions as lentil production in Canada versus that in the United Kingdom is largely different.

The significant seed class effect that lentil cultivars had on yield is similar to other trial sites (Figure 3.6). The lowest yielding variety was extra small red lentil while the highest yielding was small red lentil, similar to results at the Goodale and Outlook locations

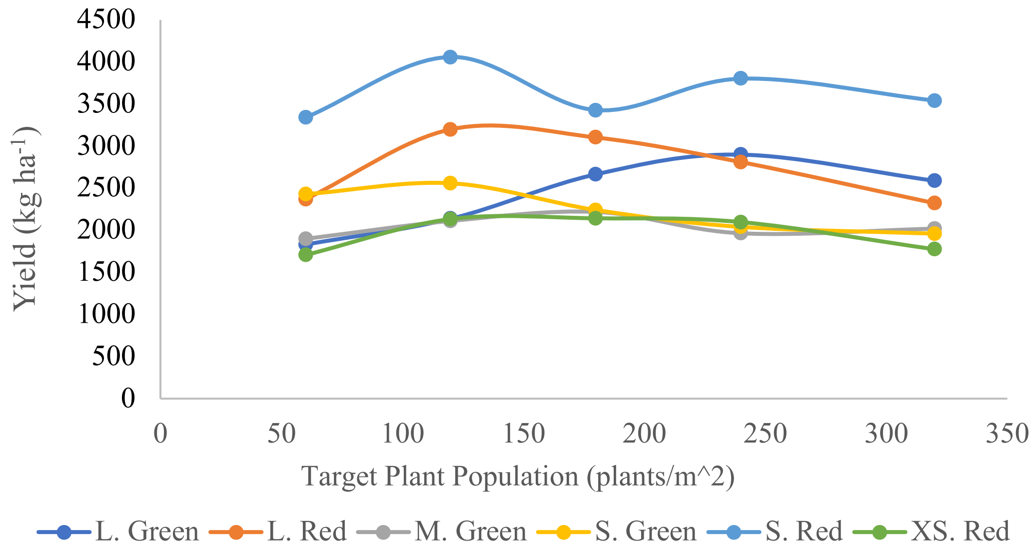

Figure 3.7 The interaction of cultivar and seeding rate is displayed for the Nasser location in 2016 where each color denotes a lentil market class and dots display the targeted plant populations in the trial.

3.3.6.4 Osler Analysis of Variance for Seed Yield

Yield results from the Osler site differ from other trial locations in this study, as the peak in yield was reached at a higher seeding rate (Figure 3.5D). This phenomenon could be attributed to a more typical lentil growing environment at Osler compared to the high amounts of moisture at other sites. Although climatically, the Osler site is located out of the typical lentil growing area (in the black soil zone), the soil type seemed to be suited to lentil growing conditions, with a higher sand content (55%). In addition, precipitation was likely lower at Osler than other sites, notably the 2016 sites. In reference to the cultivar effect at Osler, large red lentil produced the highest yield. Large red lentil and specifically CDC KR-2, reported to yield higher than the small red lentil, CDC Maxim, in lentil growing areas (Saskatchewan Pulse Growers, 2016a).

Although the Osler yield trend is different from other locations in this study, this trend produced similar results to those found in other lentil seeding rate studies. A Southern Australian investigation determined a desired seeding rate of 150 plants m^{-2} however, even higher rates were supported in unfavorable conditions where individual plant growth was limited (Siddique et al., 1998). The Osler trial site experienced the most typical lentil environmental growing conditions and this could call for these current results that are supported by other studies.

3.3.7 Quadratic Modelling of Seed Yield by Emergence: Combined Analysis

The differences in plant population displayed in Figure 3.1 gave rise to analysis of seed yield on a plant emergence basis. Some varying emergence rates were noted throughout the different cultivars in this study so, emergence was used in yield analysis as an independent variable.

Table 3.11 ANOVA for quadratic seed yield by the independent variable emergence or plant counts and corresponding *P*-values for the effect of site-year, cultivar. All sites combined for 2015 and 2016.

Parameter	*P*-Value
Repetition (Site-Year)	0.0227
Site-Year	0.1184
Plant Counts * Site-Year	0.2406
Site-Year * Cultivar	0.0085
Cultivar	0.242
Plant Counts * Cultivar	<.0001
Plant Counts * Plant Counts * Cultivar	<.0001

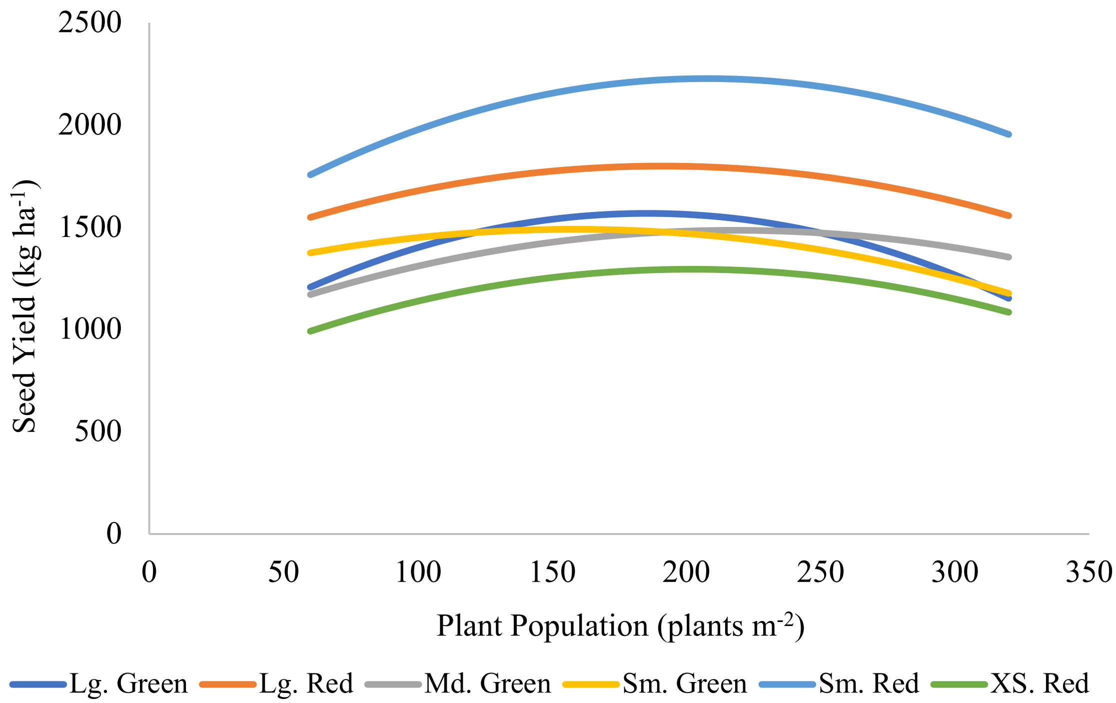

Figure 3.8 The regression of lentil seed yield by the independent variable emergence, with all sites combined in 2015 and 2016. Each colored line dictates a separate lentil market class.

Table 3.12 Slope parameters for seed yield by emergence showing intercept, linear and quadratic parameters with standard error for all sites combined and all lentil market classes in 2015 and 2016.

	Intercept	St. Error	Linear	St. Error	Quadratic	St. Error
Large Green	779.5597	336.79	8.5	3.1817	-0.02292	0.00962
Large Red	1265.91	331.89	5.5796	2.4382	-0.0146	0.00572
Medium Green	886.89	318.21	5.4879	2.2156	-0.01259	0.00517
Small Green	1190.23	317.7	3.7882	2.4949	-0.01197	0.00640
Small Red	1295.83	300.47	8.9684	1.9882	-0.0216	0.00477
Extra Small Red	681.15	NA	6.079	2.0791	-0.01506	0.0052

The quadratic curves displayed in Figure 3.8 show the range between optimal seeding rates for different lentil cultivars. From this figure, it is apparent that none of the cultivars used in this trial were capable of reaching a maximum yield by applying the seeding rate that is currently recommended. Although quadratic seed yield results are contradictory to results in the ANOVA (categorical) lentil seed yield analysis (Figure 3.4), the quadratic analysis describes the quantitative seeding rate data. Because the factor seeding rate is not categorical and is a continuous variable, the categorical analysis (ANOVA) provides less insight into the results.

The modelled curve that obtained the highest predicted seed yield (small red lentil) reaches the maximum yield at a target plant population of 210 plants m^{-2}, and at a higher optimal population, medium green lentil reached the maximum yield at 220 plants m^{-2}. Extra small red lentil reached the maximum yield at 200 plants m^{-2} followed closely by large red and large green at 190 plants m^{-2}. Finally, small green lentil reached the greatest yield at 160 plants m^{-2}. The high yield response of small red lentil is likely due to a higher yield potential as this was also displayed in research from Gan and Shirtliffe (unpublished). The small green lentil reached the maximum yield at the lowest population than any other cultivar however, this value is still significantly higher than 130 plants m^{-2}, the currently recommended target plant population in Saskatchewan.

Plant biomass and seed yield are typically expected to have a positively correlating relationship however, that trend is not displayed in the biomass data as the highest biomass was achieved by large red lentil (Figure 3.3). The highest seed yield was adversely achieved by small red lentil (Figure 3.8). To explain this phenomenon, corresponding studies have revealed that grain yield of lentil is positively associated with harvest index however, it is unassociated with plant dry matter while plant height and pod number can be a better measurement to predict lentil seed yield (Singh, 1977).

3.3.8 Quadratic Modelling of Seed Yield by Seeding Rate: Combined Analysis

Lentil seed yield was analyzed by seeding rate (target plant population) in addition to seed yield analysis by emergence (previously in section 3.3.7) and seeding rate was used as an independent variable for analysis to produce a regression similar to that which was produced by emergence counts. Due to the quantitative nature to the seeding rate variable in this study, analysis was completed by fitting quadratic curves instead of using a categorical means or an ANOVA to describe a quantitative variable.

Table 3.13 ANOVA for the effect of seeding rate, cultivar and site-year on quadratic seed yield by the independent variable, seeding rate. All sites included from 2015 and 2016.

Parameter	*P*-Value
Repetition(Site-Year)	0.0237
Site-Year	0.1163
Seeding Rate*Site-Year	.
Site-Year*Cultivar	0.0084
Cultivar	0.0183
Seeding Rate*Cultivar	<.0001
Seeding Rate*Seeding Rate*Cultivar	<.0001

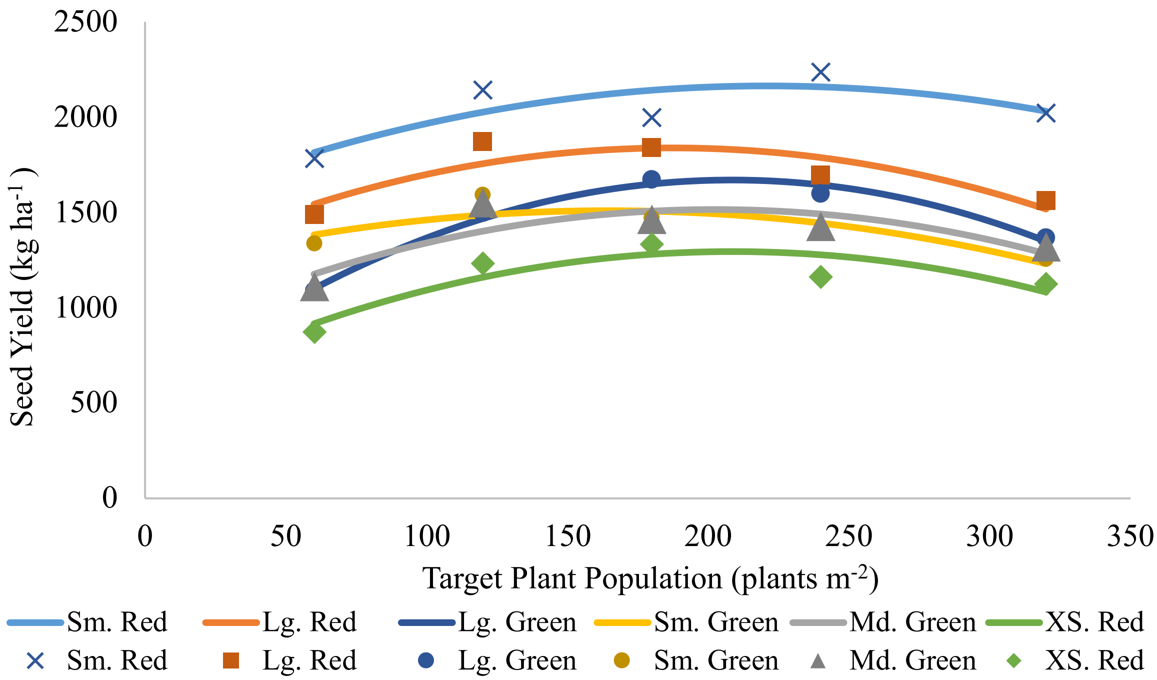

Figure 3.9 The regression (as the solid lines) for lentil seed yield by the independent variable, seeding rate or target plant population. Dots display the least squared means of yield. Each market class is distinguished by color and all sites were combined for analysis in 2015 and 2016.

Table 3.14 Quadratic seed yield by seeding rate for all sites in 2015 and 2016 for all market classes. Intercept, linear and quadratic slope parameters displayed with standard error.

	Intercept	St. Error	Linear	St. Error	Quadratic	St. Error
Large Green	547.5742	331	10.7704	2.4428	-0.02583	0.006252
Large Red	1199.39	328.24	6.8256	2.4192	-0.01822	0.006249
Medium Green	830.63	327.96	6.7673	2.4069	-0.01672	0.006189
Small Green	1195.75	327.96	3.8067	2.4069	-0.01155	0.006189
Small Red	1505.07	327.96	5.9647	2.4069	-0.0135	0.006189
Extra Small Red	548.86	NA	7.1492	2.4069	-0.01713	0.006189

Results from the yield analysis when seeding rate was used at the independent variable (Figure 3.9) were similar to results when emergence was used as the independent variable (Figure 3.8). Knowing this, emergence rates did not affect the seed yield analysis. The maximum yield of each cultivar was reached at a range between 160 and 240 plants m^{-2}. This result has the chance to change the current seeding rate recommendations that are currently used, and it suggests that 130 plants m^{-2} does not allow for maximum yield to be obtained in the field.

Similar results have been produced in lentil seeding rates studies that have suggested increased seeding rates of 150 plants m^{-2} and higher (Ball et al., 1997, Baird et al., 2009, Redlick et al., 2017). Although these studies included weed populations that were incorporated in comparison with the conventional means of weed control that was executed in this current study, the continual trend that has been produced is that increased seeding rates correspond with higher yields. Notably, these results have been produced in seasons with higher than average amounts of precipitation and this contradicts the notion that in years with high precipitation, seeding rates should be lowered to manage disease in pulses.

The cultivar response to biomass was displayed versus an overall response, to present biomass production in comparison to lentil seed yield (displayed below in section 3.3.8). The relatively flat curves (Figure 3.3) produced by the biomass response, suggest that plant population has a small effect on biomass production compared to the response displayed by seed yield. In saying this, results suggest that biomass production did not necessarily develop into seed yield.

3.3.9 Lentil Seed Weight

Table 3.15 ANOVA results for lentil seed weight (TSW) with parameters of site-year, seeding rate and cultivar at all sites in 2015 and 2016.

Parameter	*P*-Value
Repetition(Site-Year)	0.0279
Site-Year	0.237
Site-Year * Seeding Rate	0.1656
Site-Year * Cultivar	0.0087
Seeding Rate	0.5566
Cultivar	<.0001
Seeding Rate * Cultivar	0.6941

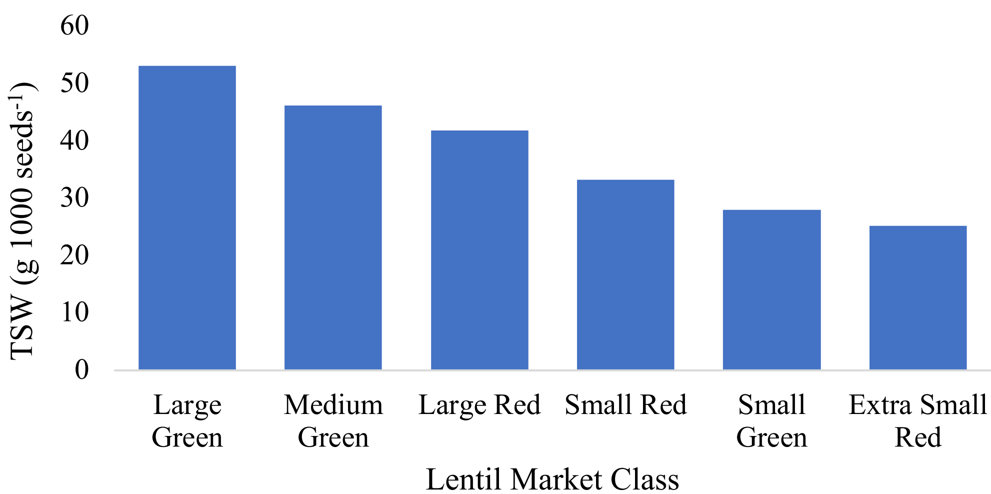

Figure 3.10 Least squared means for variable of cultivar (market class) for the thousand seed weight effect at all sites in 2015 and 2016.

There was a significant effect on seed weight for the parameter cultivar; however, this result was anticipated as the six different cultivars involved in the study possess different traits, including varied seed weights. It has been shown that higher plant population densities can negatively affect seed size due to smaller plants as a result of crowding. These smaller plants will often result in a smaller proportion of reproductive biomass production and therefore produce smaller seeds (Weiner, 1988). However, this was not the case in this study as seeding rate did not have an effect on the harvested seed weight results (Table 3.15).

3.4 Conclusion

Before implementation of this field study, it was hypothesized that different lentil cultivars or classes would require different seeding rates than that which is currently recommended. Through analysis of this trial, this hypothesis can be accepted. On the Saskatchewan Prairies, it is recommended to target a population of 130 plants m^{-2} in lentil field crops (Saskatchewan Pulse Growers, 2018a). In contrast, all cultivars in this trial reached a maximum yield from a target seeding rate of 160 to 220 plants m^{-2} (approximately 95 kg ha^{-1} for small red lentil) so, with further study and more site-years attributed to this cause, lentil seeding rate recommendations will likely increase. An economic analysis in an organic study displayed the highest economic return at a seeding rate of 375 viable seeds m^{-2}. This seeding rate also achieved the highest seed yield in the study and was therefore considered to be the highest optimal seeding rate for organic lentil production in Saskatchewan (Baird et al., 2009). For lentil production conducted under conventional means, some results have suggested that a higher economic return is achieved by increasing the seeding rate to 1.5x the recommended rate (120 kg/ha) (Kirkland et al., 2000). However, seed costs and commodity prices must be considered at that point in time. The results of this study could be suited to forecast the outcome of lentil seed yield amidst years with higher than average precipitation however, pulse disease can be one of the highest yield constraints in high precipitation years. Knowing this, further study will likely result in increased seeding rates in years with average amounts of precipitation as well.

4.0 The Effect of Seeding Rates on Disease Management in Three Red Lentil Market Classes

4.1 Introduction

Prior to the rapid growth of lentil production in Saskatchewan in 1969, disease pressure and protection was not a concern. However, in less than ten years after the introduction of lentil, it became apparent that there was a need for disease management practices on the prairies (Morrall, 1997). Currently, lentil producers in Saskatchewan often apply fungicide at early flower, regardless of conditions. However, producers are provided with a disease decision support checklist that takes plant stand, previous precipitation, the weather forecast and specific disease symptoms into account (Buchwaldt, et al., 2018). The resulting score should guide producers to a decision on whether to spray a foliar fungicide; however, this resource has not been widely adopted. Current seeding rate recommendations for conventional lentil crops that are not managed organically, are targeted at 130 plants m^{-2} (Saskatchewan Pulse Growers, 2016) as this population is believed to allow for substantial crop competition while minimizing disease pressure due to excessive plant crowding (Banniza et al., 2010). However, recent research has demonstrated that increasing lentil seeding rates could be agronomically beneficial and economically viable in comparison or alongside the use of herbicides, especially in certain cultivars like extra small red lentil (Redlick et al., 2017). Many methods exist to lessen the impact of disease on lentil production including the use of cultivars with genetic disease resistance, the use of disease-free seed and manipulation of sowing date (Davidson and Kimber, 2007). These methods used together, can provide disease containment and can ensure longevity of a control method if used as a complete system called Integrated Pest Management (IPM). IPM involves a number of principles to attempt to overcome organisms that are constantly evolving and exhausting control methods that are currently used in agriculture. Although IPM is a strategy that is critical to the future of global food production, modern agriculture often fails to devote research to issues that agricultural pests constantly produce (Owen et al., 2015). By looking at outcomes among interactions of different IPM strategies together, it is possible that the impact of pests will diminish without sole reliance on a chemical control approach.

In plant disease epidemiology, the pathogen is prominently studied rather than the host. In 1982, Burdon and Chilvers accredited this circumstance to agriculture science and the belief that a change in host density was implausible for something as insignificant as disease reduction

(Burdon and Chilvers, 1982). In the period from 1900-2012, trends have shown increased precipitation and temperatures in southern Canada while snowfall amounts have declined (Vincent et. al., 2015). This phenomenon could potentially increase the chance of pathogen survival and epidemics, modifying emphasis on disease management factors. In saying this, changes in host density to decrease disease in field crops or to increase yield, could be a viable option. There are direct and indirect effects that result from changes in plant density. The direct effects involve a change in the number of susceptible plants and changes in host spacing. These events alter the likelihood of the host intercepting inoculum (Burdon & Chilvers, 1982). The direct effects outline the axiom in plant pathology that the further away hosts remain, the slower the speed of an epidemic (Zadoks, 1972). Changes in plant population cause a number of indirect responses as well, including plant competition where host properties can be altered and microenvironment modification that can change the parameters that influence inoculum levels such as different light intensities, relative humidity and temperature (Burdon and Chilvers, 1982). The use of non-host plants as physical barriers to lessen disease spread has also been displayed as a non-chemical means of disease control. Ascochyta blight in chickpea has been significantly reduced compared to a sole chickpea crop, when intercropped with barley or wheat (Guar and Singh, 1996). Studies that involve plant population can lead to results that display at what point a crop is put at high risk of specific inoculum spread and therefore, what can be done to control this undesirable circumstance.

Many studies including those done by Bailey et. al, (2000a) on *Asochyta lentis* and *Botrytis cinerea* in lentil crops and also on *Sclerotinia sclerotiorum* in canola (Jurke and Fernando, 2008) have shown that plant disease severity increased as susceptible plant hosts increased, or as seeding rate was elevated. Bailey et al. (2000b) demonstrated that crop yield can increase by single or multiple fungicide applications in barley, wheat, canola and pea. Genetic resistance paired with foliar fungicide application is the most effective of treatments compared to resistance or fungicide protection alone, against the destructive lentil pathogen anthracnose (*Colletotrichum lentis* Damm) (Chongo et al., 1999). Regarding the aspect of cultivar or market class, the expected effect of seed size is generally thought to result in the best field performance with large seeded compared to smaller seeded cultivars of lentil (Ambika et al., 2014). This is why research has suggested that benefits including increased yield and reduced weed biomass will result from increasing the seeding rate in small seeded lentil (Ball et al., 1997, Redlick et al., 2017). While these studies have shown effects of plant population, fungicide application or other means of disease management, the interaction between seeding rate and fungicide application on lentil is an area that is understudied. The optimal agronomics for lentil involve an expanse of interactions between environment, seeding rate, soil type and health, field crop rotation history and disease management practices like fungicide application. This study will contribute to some of those knowledge gaps and through it, the interaction between seeding rate and fungicide control regime will be explored. The objective of this study is to ascertain the interaction of seeding rate in three red lentil cultivars with fungicide application, in regard to plant disease and yield. It is hypothesized that increased seeding rates will result in greater yields when disease is suppressed by fungicide application versus treatments without fungicide application. This experiment is also intended to challenge existing agronomic recommendations in reference to fungicide use and seeding rate and therefore increasing the potential lentil yield and decreasing production risk for producers on the Canadian Prairies.

4.2 Materials and Methods

4.2.1 Site Description

Field experiments were conducted in central Saskatchewan in 2015 and 2016. In 2015, Osler, Saskatchewan (52°24"27.6"N 106°38'35.0"W) and Goodale Research Farm, (GRF) (52°03'28.8"N 106°29'32.7"W) which is southeast of Saskatoon, were the two trial sites. One of the three trials in 2016 was located east of Outlook, Saskatchewan (52°31'48.6"N 106°53"15.4"W), one was on the Kernen Crop Research Farm (Nasser) (52°08'45.5"N 106°31'50.9"W) and another was at the University of Saskatchewan (USask), (52°08'26.9"N 106°38'10.9"W) along the South Saskatchewan River in Saskatoon.

Table 4.1 Soil characteristics and site description of Osler and GRF in 2015 and Outlook, Nasser and at USask in 2016.

Location	Soil Type	Breakdown	Previous Crop
Osler	Hamlin Orthic Black Chernozem	55% sand 28% silt 17% clay	wheat
GRF	Bradwell Orthic Dark Brown Chernozem	50% sand 32% silt 18% clay	fallow
Outlook	Asquith Orthic Dark Brown Chernozem	77% sand 13% silt 10% clay	canola
Nasser	Sutherland Orthic Dark Brown Chernozem	16% sand 44% silt 40% clay	wheat
University of Saskatchewan	Asquith Orthic Dark Brown Chernozem	77% sand 13% silt 10% clay	fallow

Table 4.2 Saskatoon and Outlook weather data with precipitation and temperature including 2015 and 2016 averages compared to the 30-year normals from May to August.

	Precipitation			Temperature		
Month	2015	2016	Normal ¥	2015	2016	Normal ¥
		(mm)			(°C)	
			Saskatoon			
May	0.4	41.6	43.0	10.1	13.7	11.8
June	13.6	49.7	65.8	17.2	17.4	16.1
July	84.3	58.6	60.3	19.4	18.7	19.0
August	45.2	70.2	42.6	17.4	16.9	18.2
			Outlook			
May	NA	55.7	42.6	NA	13.5	11.5
June	NA	45.8	63.9	NA	17.5	16.1
July	NA	194.6	56.1	NA	18.6	18.9
August	NA	69.9	42.8	NA	16.9	18.0

¥ 1981-2010 Canadian Climate normals obtained from Environment Canada (2018). (Government of Canada(a), 2018) (Government of Canada(b), 2018)

4.2.2 Experimental Procedures

The experiment was a 2 x 3 x 4 factorial treatment design with two levels of seeding rate, three levels of market class and four levels of fungicide treatments. The experimental design was a split-split plot design with four replicates.

The two seeding rates evaluated were 120 plants m^{-2} and 240 plants m^{-2}. The lentil cultivars included extra small red lentil (CDC Imperial), small red lentil (CDC Maxim) and, large red lentil (CDC KR-1) which were chosen due to their significance in the marketplace and similar genetic disease resistance (Table 4.4). For the remainder of the thesis, the cultivars will be referred to by their corresponding market class. The four foliar fungicide treatments included; 1) an untreated check, 2) Fracture® (Banda de Lupinus albus doce (BLAD protein)), a group BM01 biological fungicide with multiple modes of action applied once at 450 g ai ha^{-1}, 3) Headline EC® (pyraclostrobin), a group 11, systemic fungicide applied once at 98.84 g ai ha^{-1} and 4) another treatment consisting of 98.84 g ai ha^{-1} Headline EC® followed by a later season application of Bravo 500® (chlorothalonil) which is a group M5 fungicide with multi-site contact activity, applied at 1976.8 g ai ha^{-1}.

Table 4.3 Lentil cultivar resistance ratings for anthracnose and ascochyta for cultivars including CDC Imperial, CDC Maxim and CDC KR-2.

Size	Lentil Class	Anthracnose	Ascochyta	Seed Weight (g/1000 seeds)
XSR	CDC Imperial	MR	MR	30
SR	CDC Maxim	MR	MR	40
LR	CDC KR-2	MR	MR	55

S=Susceptible, MS=moderately susceptible, I= intermediate resistance, MR=moderately resistant, R=resistant. (Saskatchewan Pulse Growers, 2016)

All locations were seeded with a small plot, cone seeder with hoe openers and inoculated with granular Tag Team® (*Penicillium bilaii* and *Rhizobium leguminosarum*; Novozymes). Alongside inoculant, all plots received a seed placed fertilizer treatment of 11-52-0-0 (monoammonium phosphate). The soil test results between GRF and Osler locations were comparable so, both sites received 54 kg ha^{-1} of 11-52-0-0 in 2015. In 2016, another blanket fertilizer application was applied to all trial land as soil tests called for similar rates and 64 kg ha^{-1} of 11-52-0-0 was applied. All plots contained 6 rows of crop spaced 30 cm apart, allowing for a single plot to be 1.8 m by 6 m for a total area of 10.8 m^{-2}. Prior to seeding, Apron Maxx RTA® (fludioxonil and metalaxyl-M S-isomer), a group 4 and 12 fungicide seed treatment was used to protect lentil seeds from soil borne disease. Seed treatment was applied at a rate of 325 mL 100 kg^{-1} of seed. Glyphosate, a group 9 non-selective herbicide, at 900 g ai ha^{-1} was applied to the plots prior to seeding. After emergence and up until the 9 node above ground lentil stage, Odyssey® (imazamox and imazethapyr), a group 2 herbicide was applied to all plots at a rate of

14.9 g ai ha^{-1} of imazamox and 14.9 g ai ha^{-1} of imazethapyr. If additional grassy weed control was required, Poast Ultra® (sethoxydim) was applied at a rate of 500.4 g ai ha^{-1}. Herbicide treatments were followed with hand weeding applications throughout the season, to attempt to maintain weed-free plots.

4.2.3 Data Collection

Crop counts and biomass collection were carried out in two 0.25 m^{-2} quadrats per plot and these two values were averaged for analysis. Biomass collection took place when the crop had reached physiological maturity and pods rattled when shaken. Lentils were collected in paper bags, cut off at ground level and dried in oven driers for approximately 48 hours at 70°C. Before each fungicide application and after fungicide applications were completed, trials were visually assessed for disease severity. In each plot, five samples were assessed using a disease scale from zero to five (Table 4.4). Plots were visually rated three separate times throughout the growing season. Lentil seed was harvested with a plot combine which collected the inside four rows. This allowed for a reduced edge effect with the removal of the two outside rows. Harvested yield was dried to a constant moisture, cleaned and, final lentil yield and seed weight were recorded.

Table 4.4 Visual disease severity rating scale used for 2016 disease ratings.

Numeric Value	Amount of Visual Disease Observed
0	No disease
1	Trace to 5% infection
2	5 – 15%
3	15 – 35%
4	35 – 67.5%
5	67.5 – 100%

Table 4.5 Field operations including seeding, crop counts, fungicide applications, biomass and harvest timing for Osler, Goodale, Outlook, Nasser and the USask site in 2015 and 2016.

	2015		2016		
	Osler	Goodale	Outlook	Nasser	U of S
Seeding	May 9	May 14	May 16	May 3	May 4
Crop Counts	June 8	June 3	June 2	May 18	May 19
1st Fungicide	June 25	June 25	June 29	June 21	June 21
2nd Fungicide	July 15	July 15	July 19	July 8	July 8
Biomass	July 30	August 5	August 15	August 2	July 27
Harvest	August 18	August 18	August 25	August 17	August 16

4.2.4 Statistical Analysis

Statistical analyses on yield was conducted using the MIXED procedure in SAS® software version 9.4 (SAS Institute, 2017). The fixed effects in analysis were seeding rate (or target plant population), market class and fungicide, while the random effects included site-year and replicate nested in site-year as well as random interactions of site-year with the fixed effects. Prior to biomass analysis, an exploratory analysis was implemented and, this resulted in a square root transformation to satisfy the assumptions of ANOVA. The assumptions of ANOVA included checking the data for normality (the normal distribution of residuals) and ensuring that variances were homogenous. A value of $P<0.05$ was used to determine the significance of treatment effect however, concerning the fungicide by seeding rate interaction in the seed yield analysis, a value of $P=0.0513$ was considered significant. An analysis of variance (ANOVA) was administered on the data and the least squared means (LS means) were used to establish treatment differences and the differences in least squared means were used to determine significance at a value of $P<0.05$. Results from the type 3 tests of fixed effects were used to distinguish differences between treatments. The same process was used on emergence, biomass, TSW and disease rating data.

Visual inspection of the spatial effects on plant yield indicated that for some locations, there was spatial covariance in the yield that was not removed by blocking. Therefore, a spatial covariance model was used to determine if spatial variance could be modelled. If the Aikike Information Criteria (AIC) value was decreased by 2 or greater when the spatial covariance model was utilized, the spatial model was used as this indicates that it is more precise. For the Goodale location in 2015 and the USask trial site in 2016, this analysis was used as well as on the combined seed yield analysis where it was nested within site-year.

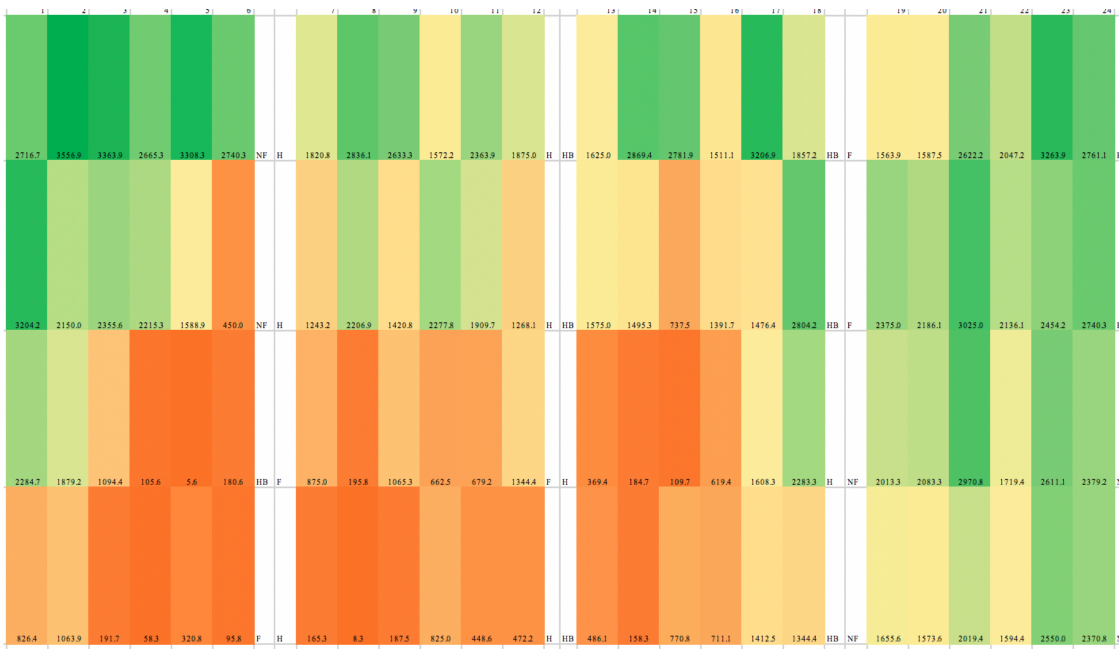

Figure 4.1 The spatial heat map of the Goodale trial location where color is based on seed yield. Below the trial (bottom left off of the figure) was a slough, creating the spatial variation shown in this trial. The color range from dark green to dark red indicates progressively declining yield as dark red represents the lowest yielding plots.

The spatial analysis was done to remove variation due to a poor randomization outcome. The untreated fungicide check plots were randomly placed in the highest yielding areas (green in Fig. 4.1) at the Goodale location, while the treatments that included Headline with a subsequent treatment of Bravo were placed in the low yielding red areas. Prior to the spatial analysis, results displayed the highest yields produced from plots that were not treated with a fungicide compared to those that were. A slough that was close to the bottom left (east) of the Goodale trial was responsible for the low yield result.

4.3 Results & Discussion

4.3.1 Lentil Emergence

Emergence rates were explored to determine whether differential emergence amount treatments contributed to final results of the experiment.

Table 4.6 ANOVA for lentil plant population (field emergence) with parameters of site-year, seeding rate, fungicide and cultivar. Results for all site-years combined in 2015 and 2016.

Parameter	*P*-Value
Repetition(Site-Year)	0.0205
Site-Year	0.1111
Repetition * Fungicide(Site-Year)	0.0104
Site-Year * Seeding Rate	0.0895
Site-Year * Cultivar	.
Seeding Rate	0.0008
Cultivar	<.0001
Seeding Rate*Cultivar	<.0001
Fungicide	0.3676
Fungicide * Seeding Rate	0.9519
Fungicide * Cultivar	0.4869
Fungicide * Seeding Rate * Cultivar	0.2867

The lentil emergence results show that the variables cultivar and seeding rate had a significant effect however, this result is expected due to the different seeding rates involved in the trial and due to different characteristics of plant genotypes. The emergence that was accomplished for this trial was satisfactory. The emergence achieved for targeted plant populations of 120 and 240 plants m^{-2} was 127 plants m^{-2} and 235 plants m^{-2} respectively.

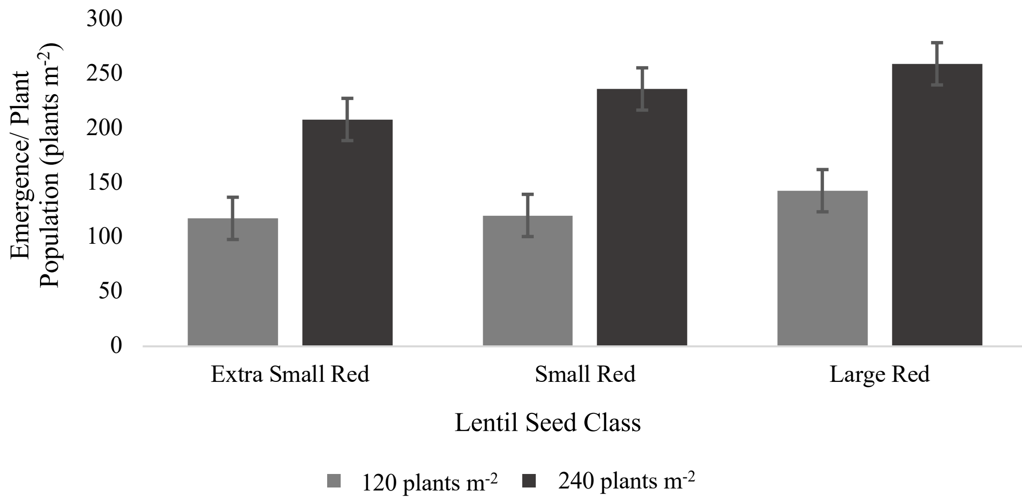

Figure 4.2 The effect of seeding rate and cultivar on emergence after approximately 2 weeks at all locations combined in 2015 and 2016. The light gray bar indicates the low seeding rate while the dark gray bar indicates the higher seeding rate. Pooled standard error is displayed.

Extra small red lentil had the lowest emergence with 118 plants m^{-2} at the low seeding rate and 208 plants m^{-2} at the high seeding rate, followed by small red lentil that resulted in 120 plants m^{-2} and 236 plants m^{-2}. Large red lentil showed the highest emergence counts with 143 plants m^{-2} at the lowest seeding rate and 260 plants m^{-2} at the high seeding rate. The large red lentil emergence values exceeded the value that was targeted in this study for a plant stand in the field.

Large red lentil (KR-2) showed higher emergence than other cultivars in the trial (Figure 4.1) however, this phenomenon is not uncommon. It has been demonstrated that in field conditions, seeds that are larger in size, present a higher emergence and survival rate compared to smaller seeded varieties (Leishman and Westoby, 1994). Imperial (extra small red) lentil had the lowest overall emergence counts in this trial, as is expected from smaller seeded varieties.

4.3.2 Lentil Biomass

From the ANOVA test on lentil biomass, there was a significant (P<0.05) effect of fungicide and site-year (Table 4.6) and due to these results, the effect of fungicide on biomass was also analyzed by site-year (Table 4.7). The significant cultivar effect is expected from different lentil varieties with distinct characteristics, including the production of different levels of dry matter.

Table 4.7 ANOVA for measurement of biomass with all sites combined in 2015 and 2016. Parameters in analysis included site-year, fungicide, seeding rate and cultivar.

Parameter	*P*-Value
Repetition(Site-Year)	0.0133
Site-Year	0.0903
Repetition * Fungicide(Site-Year)	0.0005
Site-Year * Seeding Rate	0.1442
Site-Year * Cultivar	0.0922
Seeding Rate	0.3896
Cultivar	0.0010
Seeding Rate * Cultivar	0.8815
Fungicide	0.0103
Fungicide * Seeding Rate	0.5859
Fungicide * Cultivar	0.8178
Fungicide * Seeding Rate * Cultivar	0.4857

Table 4.8 ANOVA results for site-year analysis on square root transformed biomass values for the effects of fungicide, seeding rate and cultivar at each separate location in 2015 and 2016. Numbers reflect *P*-values.

Effect	Goodale(2015)	Osler(2015)	USask(2016)	Outlook(2016)	Nasser(2016)
Fungicide (F)	0.1055	0.0098	0.5154	0.0695	0.0044
Seeding Rate (SR)	0.0985	<.0001	0.3169	0.0864	0.3604
Cultivar (C)	0.0943	0.001	<.0001	<.0001	<.0001
SR * F	0.3101	0.134	0.8078	0.7149	0.9826
C * F	0.8434	0.6039	0.873	0.836	0.3954
SR * C	0.7191	0.3005	0.3391	0.2122	0.7138
SR * C * F	0.9218	0.2061	0.2187	0.6297	0.5668

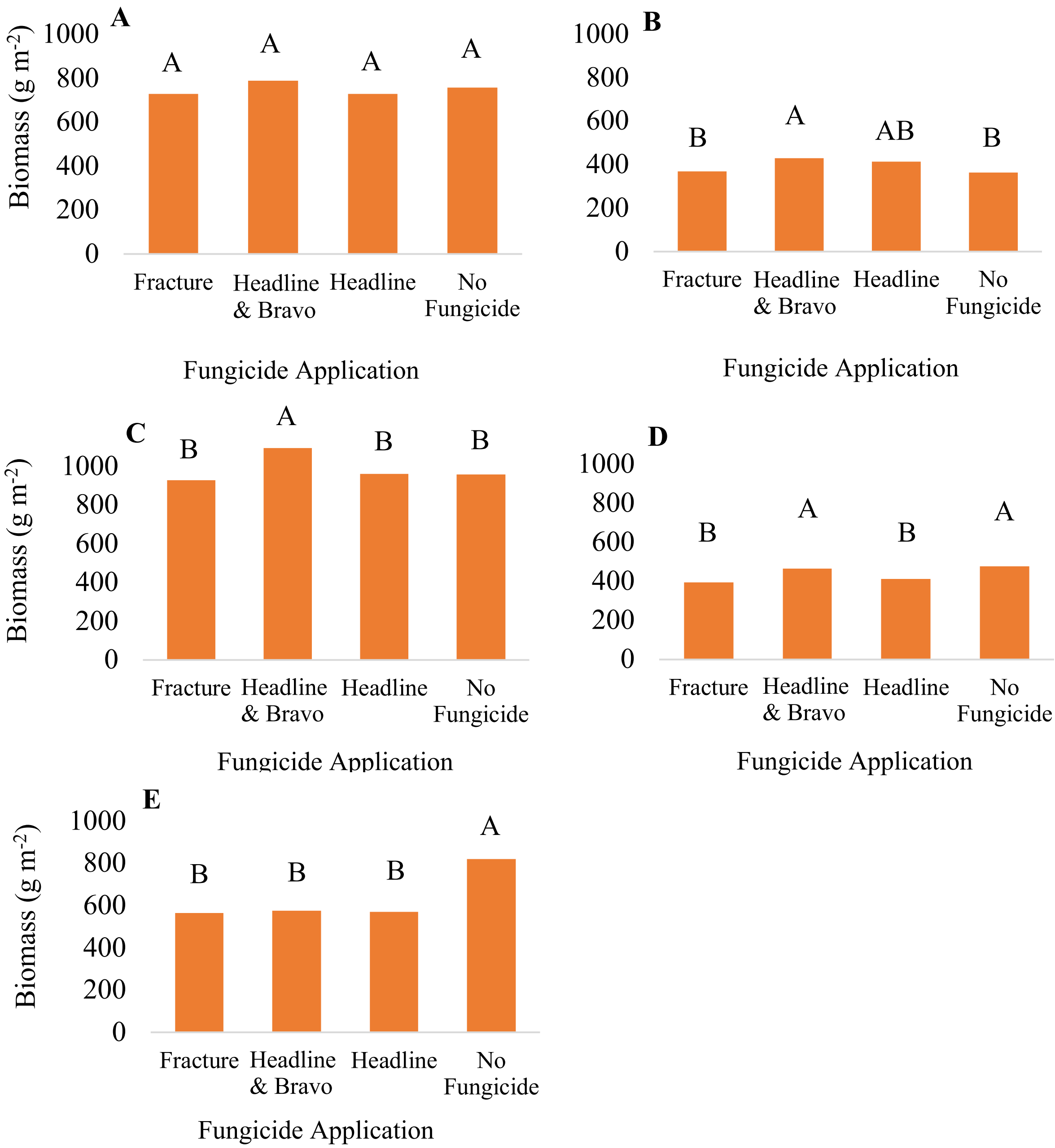

Figure 4.3 Biomass production by each separate fungicide treatment, at separate sites: USask site (A) in 2016, Outlook (B) in 2016, Nasser (C) in 2016, Osler (D) in 2015 and Goodale (E) in 2015, averaged across the two seeding rates.

Site-year analysis results demonstrated a significant effect from fungicide at the Osler and Nasser sites while near significance was noted at Goodale and Outlook. At the USask trial site, no significant differences for biomass production were noted among the four different fungicide treatments. At the Outlook site with high precipitation and high disease pressure present (Figure 4.6), the treatment involving two fungicide applications yielded a significantly higher amount of biomass than the treatment with Fracture and with no fungicide applied. At the highest yielding site (Nasser), the sequential Headline plus Bravo plots produced the highest amount of biomass compared to any other treatment. Concerning the 2015 trials (Goodale and Osler), the untreated check yielded significantly higher than any other treatment except Headline plus Bravo at Osler. In 2016, at sites where a significant fungicide effect was produced, treatments with the sequential Headline plus Bravo treatment produced the highest amount of biomass and the dry matter yield was significantly higher than every other treatment except the sole Headline application at one site.

4.3.3 Lentil Seed Yield

The site-year analysis determined that the fungicide effect should be evaluated by studying individual site results due to a significant fungicide by site-year effect in the non-spatial analysis (data not presented). Different yield outcomes were anticipated from different cultivars in this study at separate locations as there was an expected genotype by environment effect in the non-spatial analysis as well. The significant cultivar effect (Table 4.8) is common in field studies as different cultivars perform accordingly to the type of environment they are exposed too (Baker, 1988). Through the cultivar analysis in the previous study in this thesis, it was determined that different lentil cultivars result in varying yields depending on the site, so the same conclusion arose from this result. In the non-spatial analysis, the interaction of fungicide and seeding rate was regarded as significant at a value of P=0.0513. Due to a problem with spatial variation at Goodale in 2015 that caused the control to have a higher yield, a spatial analysis was conducted. Through the spatial analysis, two interactions were noted including fungicide and seeding rate as well as the interaction of seeding rate and cultivar, significant at the P<0.05 level. Both interactions from the spatial analysis are displayed below (Figure 4.3 and Figure 4.4).

Table 4.9 ANOVA results for spatial analysis parameters including site-year, fungicide, seeding rate and cultivar on the response of seed yield at all sites in 2015 and 2016.

Parameter	Spatial *P*-Values
Repetition(Site-Year)	0.0312
Site-Year	0.1145
Repetition * Fungicide(Site-Year)	0.4418
Site-Year * Seeding Rate	0.1810
Site-Year * Cultivar	0.0255
Seeding Rate	0.6304
Cultivar	0.0126
Seeding Rate * Cultivar	0.0018
Fungicide	0.0009
Fungicide * Seeding Rate	0.0513
Fungicide * Cultivar	0.5955
Fungicide * Seeding Rate * Cultivar	0.2782

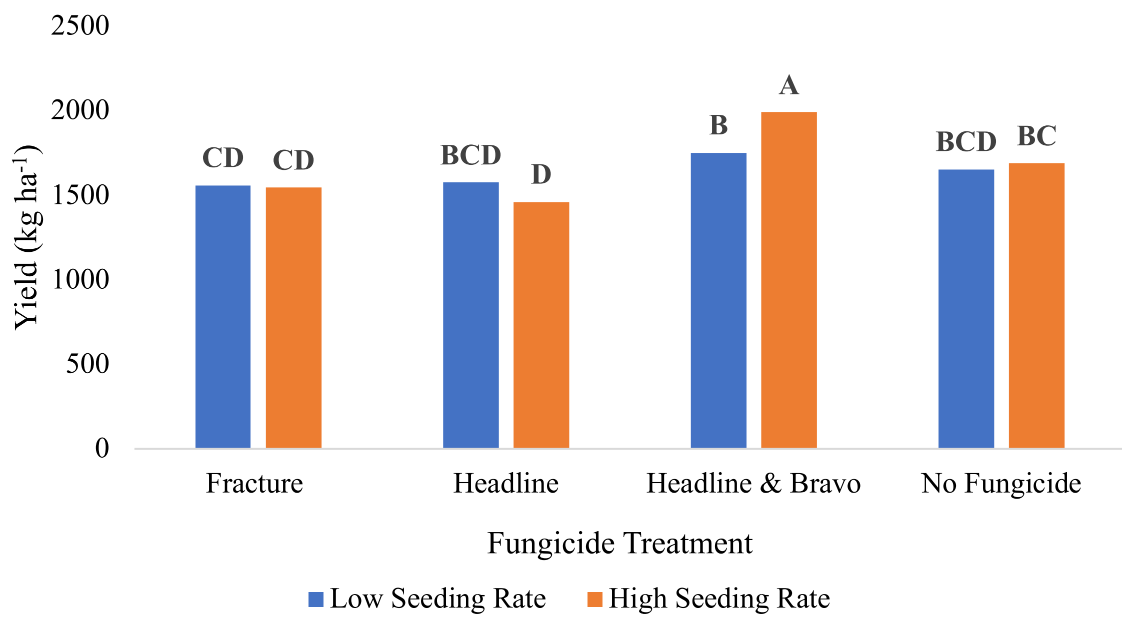

Figure 4.4 Interaction of fungicide and seeding rate for spatial analysis of seed yield results at all sites in 2015 and 2016. Blue represents the low seeding rate (120 plants m^{-2}) and orange represents high seeding rate (240 plants m^{-2}).

The highest seeding rate paired with two fungicide applications (Headline plus Bravo), achieved the highest yield, reaching a value of 1992 kg ha^{-1} (Figure 4.3). This result implies that increased seeding rates are effective when the practice is paired with two fungicide applications. The treatment with Headline alone at the highest seeding rate resulted in the lowest yield at 1461 kg ha^{-1}. All other treatments achieved similar yields to the untreated check.

Varietal genetic resistance is important in lentil production as it has been shown that genetic resistance can be more effective than a fungicide application of chlorothalonil alone, when high disease pressure is present (Chongo et al., 1999). In relation to the current study, (Figure 4.3) the treatment involving chlorothalonil (Headline EC® and Bravo 500® applied separately) resulted in the highest yield. In the study conducted by Chongo et al., (1999) lentil cultivars that were used included varieties with partial resistance versus susceptible varieties, compared to having all cultivars with moderate resistance included in this study. As seen in results displayed in Figure 4.3 and other research including a pulse crop study highlighting ascochyta blight, disease management should include the use of cultivars with partial resistance, as this will result in the highest lentil yields (Davidson and Kimber, 2007).

In the treatments that included Headline and Fracture applied once per season, the treatment with the higher seeding rate generally resulted in lower yields than to those with lower seeding rates. This event could have transpired from higher disease prevalence in denser plant stands. In addition to higher disease pressure, plants in dense stands will compete for resources and therefore, their disease resistance potential will not be reached, compared to the performance of the same cultivar in a thinner plant stand (Pennypacker and Risius, 1999). This response was not seen in the treatment that involved Headline plus Bravo and this is likely due to better disease control over a longer period of time that resulted from two separate fungicide applications spaced approximately 20 days apart.

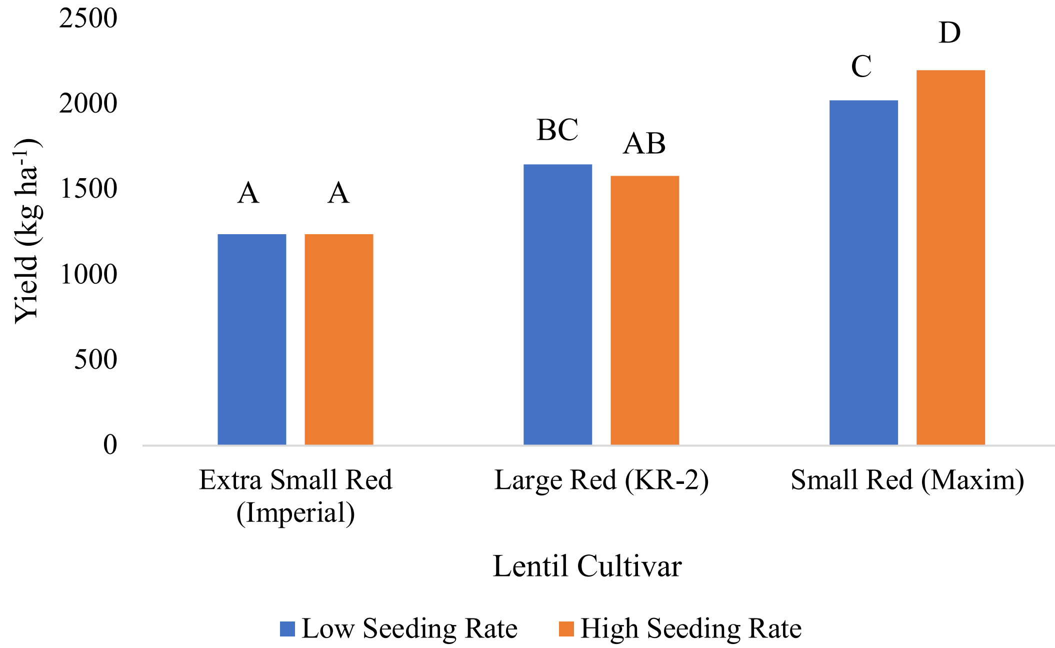

Figure 4.5 Interaction of seeding rate and cultivar for spatial analysis of seed yield results for all sites in 2015 and 2016. Blue represents the low seeding rate (120 plants m^{-2}) and orange represents high seeding rate (240 plants m^{-2}). Significance at P<0.1.

The interaction of seeding rate and cultivar that arose from the spatial analysis (Table 4.8) shows that small red lentil is the only market class tested to exhibit a significant (P<0.1) response to increased seeding rates (Figure 4.4). This suggests that CDC Maxim lentil (small red) yielded greater with the higher seeding rate, (240 plants m^{-2}) across all fungicide treatments. In contrast, large red lentil displayed a lower overall yield trend with the higher seeding rate compared to the low seeding rate. Extra small red lentil showed an insignificant difference in yield between the low and high seeding rates. Differences in yield trend between lentil cultivars could be attributed to varying degrees of disease resistance. Although all three lentil cultivars involved in the study were rated with moderate susceptibility to anthracnose and ascochyta blight (Table 4.3), varying levels of resistance are still likely and, other unnamed pathogens could have affected the outcome. Variation in genetic resistance of different lentil cultivars could arise from the infection test that was used to measure the reaction to the disease, during cultivar development. Variation in genetic resistance could also be attributed to how host resistance was quantified. Area under the disease progress curve, percent seed infection and, the measure of disease incubation period or latent period have all been used to test genetic resistance of lentil cultivars (Ye et al., 2002). Through these diversified methods, varying degrees of genetic resistance to a number of pathogens is evident.

4.3.3.1 Site-Year Analysis for Seed Yield

Individual site-year analysis was conducted to examine the significant fungicide effect on lentil seed yield (Table 4.9). This was performed in the absence of a site-year by fungicide interaction because the environment differed greatly between sites and presumably the conditions necessary for a disease epidemic. At the 2016 Outlook site (Figure 4.5B), differences between fungicide treatments were evident as the sequential Headline and Bravo fungicide application yielded significantly higher than any other treatment, while the untreated check and the Fracture fungicide application yielded the lowest. Results from the Outlook site were affected by high amounts of precipitation and hail, leading to increased disease pressure and therefore the highest yield was produced by the treatment with the most aggressive fungicide regime. The fungicide effect that was displayed at the Outlook location could be linked to hail damage. Successful pathogen infection likely increased after hail damage exhibited on plants, increasing disease and causing a notable fungicide effect. Similar results were displayed at Nasser in 2016, (Figure 4.5C) where the treatment with Headline plus Bravo yielded significantly higher than the treatment with no fungicide applied and also higher than the treatment with Fracture fungicide applied. Results at the USask site (Figure 4.5A) were comparable as well, as the sequential treatment with Headline and Bravo yielded the highest. The previously mentioned sites included the site with the highest disease pressure (Outlook) and the site with the highest overall yield and the lowest amount of visual disease presence (Nasser). At the Goodale trial site (Figure 4.5E), no significant differences in yield were displayed. At the other 2015 trial (Osler), no fungicide resulted in the highest yield from the untreated check followed by the Headline plus Bravo treatment, the treatment with one Headline application and finally, the Fracture fungicide application (Figure 4.5D).

Table 4.10 ANOVA for seed yield at separate sites including Goodale, Osler, Nasser, Outlook and the USask in 2015 and 2016. Parameters include fungicide, seeding rate and cultivar. Values reflect *P*-value. Goodale and USask from spatial analysis. Osler, Nasser and Outlook from non-spatial analysis.

Effect	Goodale(2015)	Osler(2015)	Nasser(2016)	Outlook(2016)	USask(2016)
Fungicide (F)	0.6616	0.0046	0.0607	0.0013	0.2047
Seeding Rate (SR)	0.6679	0.4684	0.0004	0.4602	0.0292
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001
SR * F	0.0663	0.0007	0.0265	0.3436	0.1520
C * F	0.1228	0.3307	0.7985	0.4641	0.8640
SR * C	0.2624	0.1805	0.025	0.1969	0.0029
SR * C * F	0.2521	0.8099	0.9385	0.3814	0.0450

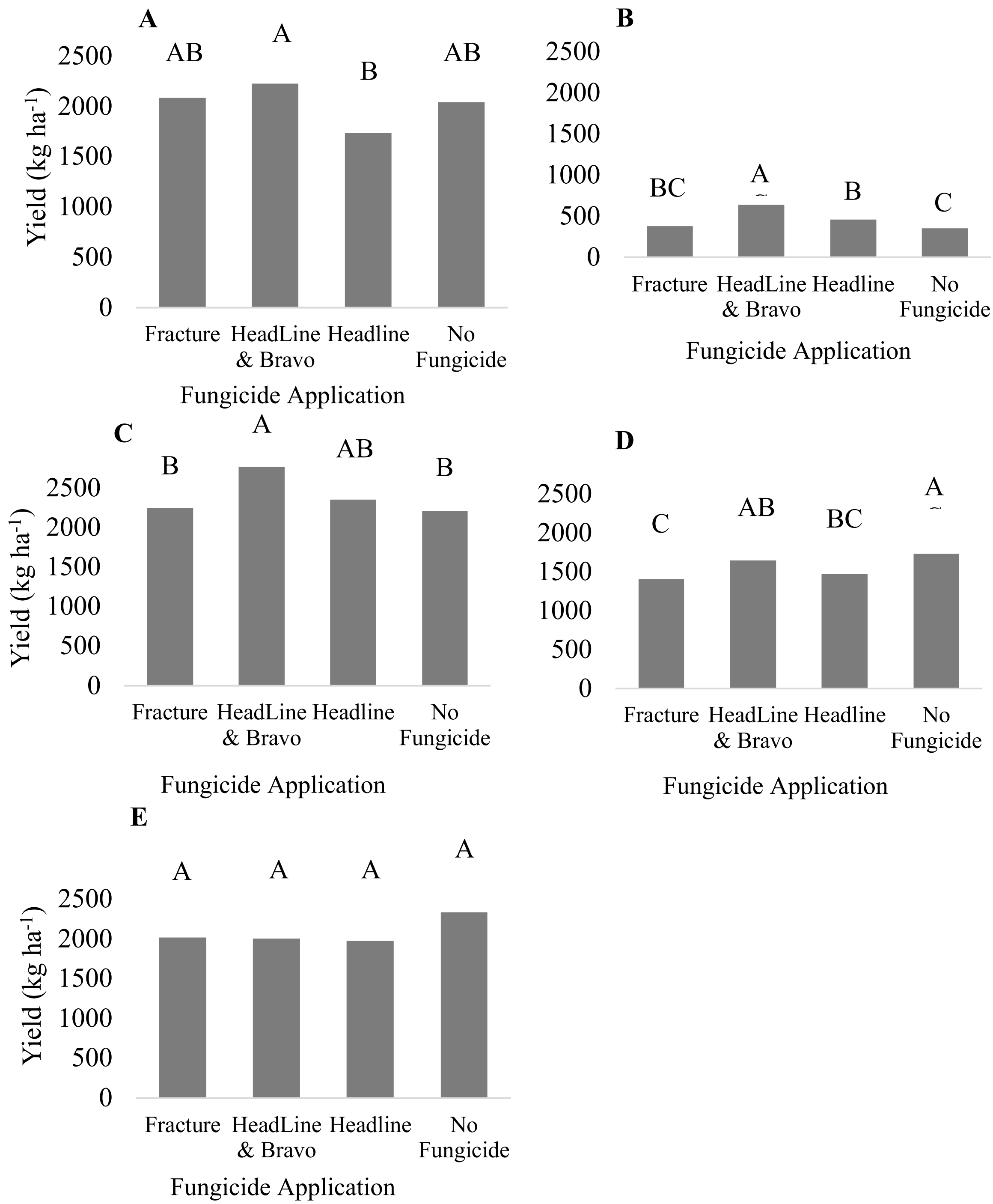

Figure 4.6 The effect that fungicide treatment expended on seed yield for both seeding rates combined is displayed at each separate site including; the USask site in 2016 (A), Outlook in 2016 (B), Nasser in 2016 (C), Osler in 2015 (D) and Goodale in 2015 (E). Goodale and USask values from spatial analysis while the Outlook, Nasser and Osler values are from non-spatial analysis.

An individual site-year analysis was performed on the fungicide by seeding rate interaction (Table 4.9) in addition to the fungicide by seeding rate interaction for all sites combined (Figure 4.3). For the individual site-year analysis (Figure 4.6), the greatest yields were produced by the sequential treatment with Headline plus Bravo at the Outlook, Nasser and Osler locations. For all sites except Nasser in 2016, the treatment of Headline and Bravo at the high seeding rate (240 plants m^{-2}) yielded more than the treatment of Headline at the low seeding rate. This is likely due to increased disease pressure and the inability of a sole fungicide application to control it.

Figure 4.7 Fungicide by seeding rate interaction for individual site-year analysis with both seeding rates. Locations include USask site in 2016 (A), Outlook in 2016 (B), Nasser in 2016 (C), Osler in 2015 (D) and Goodale in 2015 (E). Goodale and USask values from spatial analysis and Outlook, Nasser and Osler from non-spatial analysis.

The spatial site-year analysis revealed a three-way interaction at the USask site in 2016 between fungicide, seeding rate and cultivar (Table 4.9). The interaction showed a yield response at the low seeding rate (120 plants m^{-2}) from the treatment with both Headline plus Bravo in the small red and extra small red market classes (Figure 4.7). A similar yield response was displayed at the high seeding rate (240 plants m^{-2}) for the sequential Headline and Bravo treatment in large red and small red lentil (Figure 4.8). Small red lentil exhibited an increase in yield with the Headline plus Bravo treatment at both seeding rates whereas other market classes were not consistent in that response. Small red lentil had the highest yield potential, consistent with results in the previous chapter (Figure 3.8).

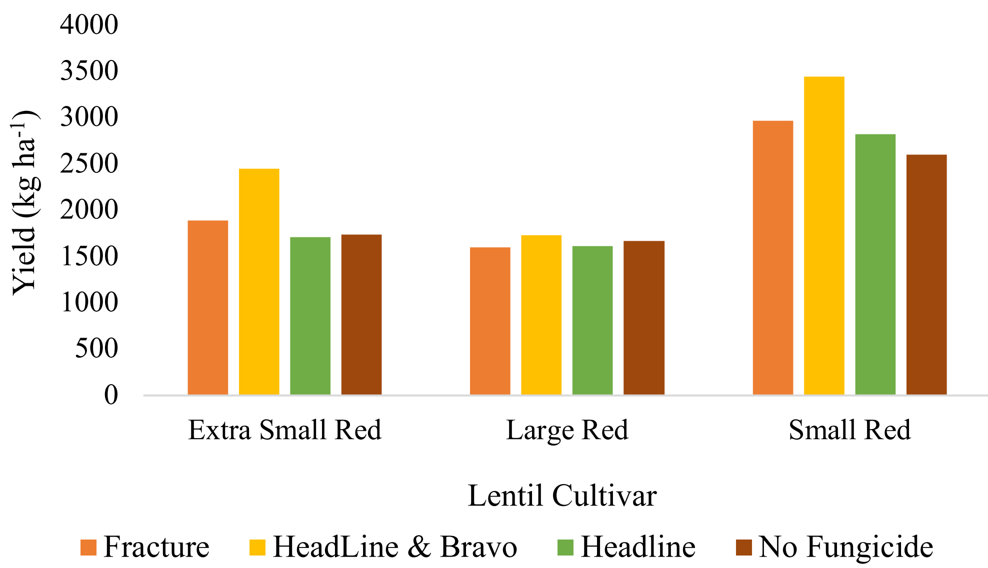

Figure 4.8 Three-way interaction from spatial analysis between fungicide, seeding rate and cultivar for 2016 USask data at the low seeding rate (120 plants m^{-2}).

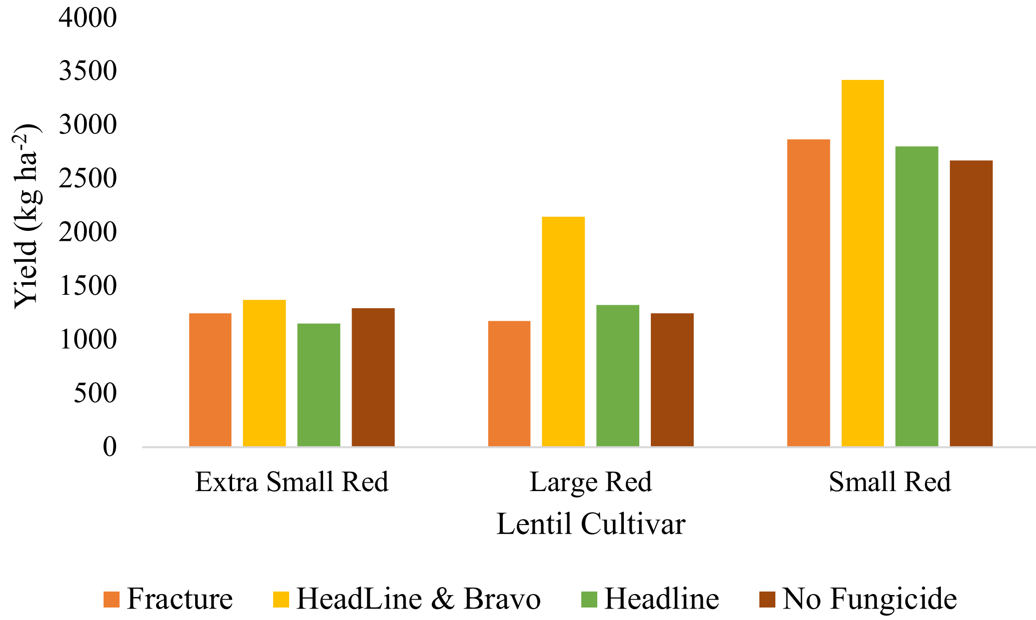

Figure 4.9 Three-way interaction from spatial analysis between fungicide, seeding rate and cultivar for 2016 USask data at the high seeding rate (240 plants m^{-2}).

4.3.4 Thousand Seed Weight

From the ANOVA performed on the thousand seed weight parameter, significance was displayed for each parameter in this trial; seeding rate, cultivar and fungicide. Differences in TSW for cultivar is evident as characteristically, different genotypes produce different sizes of seed. In reference to the effect of seeding rate, significance was also observed. Differences in seed weights from the different treatments of seeding rates may be due to plant crowding that manipulated the production of reproductive biomass. Finally, the effect of fungicide was examined as well and due to significance with site-year (Table 4.10), sites were investigated separately (Table 4.11).

Table 4.11 ANOVA for the effects of site-year, fungicide, seeding rate and cultivar on TSW with all sites combined in 2015 and 2016.

Parameter	P-Value
Repetition(Site-Year)	0.0967
Site-Year	0.1099
Repetition * Fungicide(Site-Year)	0.0191
Site-Year * Seeding Rate	N/A
Site-Year * Cultivar	0.0303
Seeding Rate	0.0044
Cultivar	<.0001
Seeding Rate * Cultivar	0.8526
Fungicide	0.0002
Fungicide * Seeding Rate	0.9496
Fungicide * Cultivar	0.5814
Fungicide * Seeding Rate * Cultivar	0.5516

4.3.4.1 TSW Site-Year Analysis

Through site-year analysis, it was determined that the Outlook site was the only location to show a significant fungicide effect for the TSW parameter (Figure 4.5). At Outlook, the treatment with two fungicide applications (Headline plus Bravo) proved to have significantly heavier seeds than the treatment with Fracture and the untreated check. This effect is likely due to the high disease pressure at the site and hail events that increased the likelihood of pathogen infection. The second fungicide application of Bravo protected lentil seeds at the end of the season when disease pressure was the highest due to late season precipitation. The same outcome has been demonstrated on pulses, as one or more applications of a strobilurin fungicide during flowering has been determined to increase the TSW of chickpea due to reduced seed-borne infection and seed discoloration caused by pathogen disruption (Chongo et al., 2003).

Table 4.12 ANOVA results for TSW for separate sites including Goodale, Osler, Nasser, Outlook, and the USask in 2015 and 2016 for the effects of fungicide, seeding rate and cultivar.

Effect	Goodale(2015)	Osler(2015)	Nasser(2016)	Outlook(2016)	USask(2016)
Fungicide (F)	0.1105	0.533	0.2925	0.0105	0.2468
Seeding Rate (SR)	0.4297	0.0654	0.1807	0.3257	0.1871
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001
SR * F	0.1937	0.1903	0.2064	0.8505	0.2218
C * F	0.4382	0.7348	0.66	0.507	0.648
SR * C	0.5735	0.959	0.0235	0.146	0.2
SR * C * F	0.5693	0.4203	0.7947	0.6328	0.9473

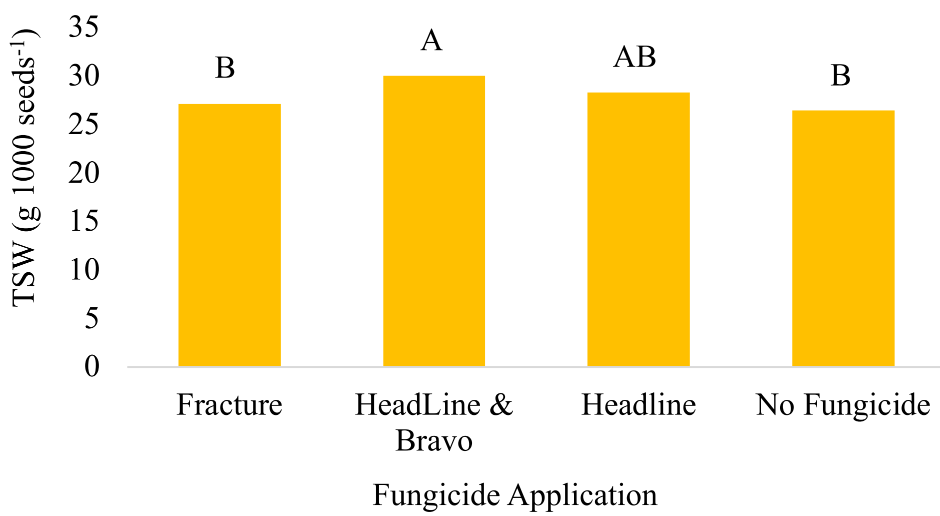

Figure 4.10 The effect that fungicide treatments exhibited on thousand seed weight at the Outlook site, where high disease pressure was present.

4.3.5 Lentil Disease Ratings

Disease ratings were taken throughout 2016, at the Outlook, USask and Nasser field locations. In 2015, disease pressure was low and ratings are not available. Ratings were conducted on a scale from zero to five with zero presenting no lesions on the plant and five resulting in total plant coverage with pathogenic lesions. Ratings were taken three times per season however, only the final rating was analyzed. The ANOVA results from the last disease rating of the season are presented in Table 4.12.

Table 4.13 ANOVA results for disease rating data for all 2016 trial sites combined including Outlook, the USask site and Nasser. Effects included site-year, fungicide, seeding rate and cultivar.

Parameter	*P*-Value
Repetition(Site-Year)	0.0381
Site-Year	0.1897
Repetition * Fungicide(Site-Year)	0.0015
Site-Year * Seeding Rate	0.1914
Site-Year * Cultivar	0.1133
Residual	<.0001
Seeding Rate	0.1275
Cultivar	0.0382
Seeding Rate * Cultivar	0.8878
Fungicide	0.0008
Fungicide * Seeding Rate	0.095
Fungicide * Cultivar	0.1289
Fungicide * Seeding Rate * Cultivar	0.1433

The effect of fungicide had a highly significant result regarding disease severity ratings and was analyzed by site-year. The effect of cultivar was also significant; however, this outcome is likely due to the varying levels of genetic resistance present in the genome of different lentil cultivars used in this study.

Table 4.14 ANOVA results for visual disease ratings for separate locations including Nasser, Outlook and the USask site in 2016. Effects included in analysis consist of fungicide, seeding rate and cultivar.

Effect	Nasser(2016)	Outlook(2016)	USask(2016)
Fungicide	<.0001	0.0465	0.1343
Seeding Rate	0.0748	<.0001	<.0001
Seeding Rate * Fungicide	0.0003	0.5864	0.3508
Cultivar	<.0001	0.0005	<.0001
Cultivar * Fungicide	0.0014	0.7818	0.6658
Seeding Rate * Cultivar	0.0342	0.5816	0.6288
Seeding Rate * Cultivar * Fungicide	0.2417	0.0634	0.2601

The site-year analysis conducted on disease severity ratings resulted in significant differences among fungicide treatments at the Nasser and Outlook locations in 2016 (Table 4.13). Additionally, at all three locations where disease severity ratings were taken, the treatment with the Headline plus Bravo fungicide application had the lowest overall ratings (including a tie with the sole Headline treatment at Nasser) and a significantly lower amount of disease than the untreated check. The lowest disease ratings at every site were produced by treatments that included an application of Headline. This could possibly highlight the importance of the utilization of a systemic fungicide in lentil production.

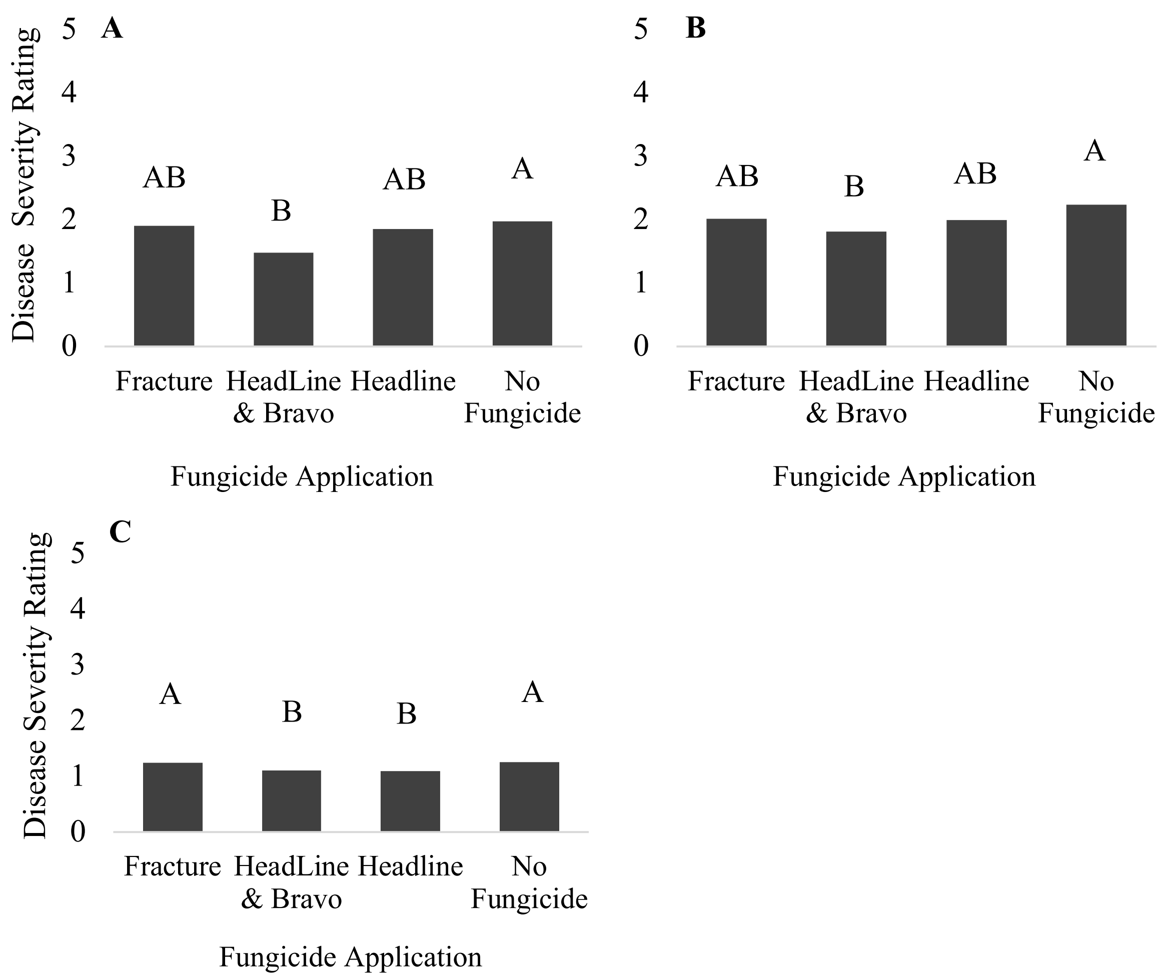

Figure 4.11 Visual disease severity ratings with corresponding fungicide treatment at separate sites including the USask (A), Outlook (B) and Nasser (C) in 2016. Higher disease severity rating corresponds with more disease visually present.

4.4 Conclusion

A similar fungicide treatment effect on biomass, seed yield, thousand seed weight and disease severity ratings was displayed throughout the results of this trial. Generally, the higher biomass, yield, TSW and lower disease ratings were achieved with the treatment that included two sequential fungicide applications of Headline and Bravo in the growing season. This result was also displayed in conditions with high disease pressure due to excessive precipitation. If economic conditions are suited to a scenario that allows for increased seeding rates paired with two fungicide applications, this is likely the most profitable outcome however, more research will substantiate this probability. When seeding lentil at higher rates, protection of investment by the timely application of more than one fungicide application during the growing season is a reasonable recommendation to maximize seed yield.

5.0 General Discussion

The objective of this research was to examine the effect of different lentil cultivars and note differences in yield response when field conditions are modified by varied seeding rates and fungicide treatments, specifically for lentil production. Principally, the research anticipated improved guidelines for producers when growing lentil, providing them with seeding rate recommendations and fungicide application guidelines that are based on plant populations, that will allow for the highest seed yield of the specific cultivar they decide to grow. Furthermore, it was specifically hypothesized that the treatment with the highest plant population would result in the highest yield when two fungicide applications were applied and that all lentil market classes would differ in optimum seeding rate, notably in larger seeded varieties. In addition, this experiment was expected to predict the effect of yield-density interactions so that the most advantageous seeding rate would be in place for lentil market classes in the future.

5.1 The Effect of Market Class on Optimum Seeding Rate in Lentil

The first study included example cultivars from three different lentil market classes for both red and green lentil cultivars grown on the Canadian Prairies. These were grown over a wide range of seeding rates in order to determine the agronomically optimum yield for each cultivar. It was expected that all lentil market classes would differ in agronomically optimum seeding rate, notably in larger seeded varieties. Although beyond the scope of this thesis, the search for the most profitable seeding rate would optimistically result in future recommendations for each lentil market class.

This research examined the yield response of six different lentil cultivars specifically, and it was shown that each genotype had a different yield response. Recommendations for seeding rates are based on seed size however, yield and biomass results in this study did not show trends associated with seed size. Although seed size plays a large economic role in lentil production due to seed costs based on weight, it seemed to play less of a role agronomically in this study, as seed size was not relative to plant size and yield outcome. This research suggests that seeding rates based on lentil seed size is likely not sufficient and that knowledge on yield density interactions of specific lentil market classes would be beneficial in predicting yield.

Seedling emergence is an important factor in the development of a field study trial and importance is crucial when the trial is a plant population study. Keeping this in mind, the investigation of yield for this study involved using crop emergence as an independent variable in analysis to ensure that emergence did not affect the study and invalidate results. Although emergence showed differences among site-years in this trial, when seed yield was regressed against emergence rates versus seed yield shown against seeding rates, the results were similar, and the same knowledge was derived from the results. The measurement of biomass production presented relatively flat curves as density increased, indicating constant final biomass. These plateau trends in the curves suggested that seeding rate did not have a large effect on biomass production in comparison to the effect that seeding rate had on final seed yield.

Yield results were relative to what was hypothesized as all market classes did differ in regard to agronomically optimum seeding rates for the greatest yield outcome. The maximum yield for large red lentil and large green lentil was reached at 200 plants m^{-2}, for medium green lentil it was reached at 220 plants m^{-2} and for small green lentil the maximum yield was reached at 160 plants m^{-2}, with other market classes falling in between these values. In saying this, an economic analysis may bring to light what was hypothesized in regard to large seeded varieties as higher seeding rates lead to higher seed costs and economically, the maximum yield may not be feasible with increased seeding rates.

5.2 The Effect of Seeding Rates on Disease Management in Three Red Lentil Market Classes

The second study focused on the yield outcome from using different fungicide treatments accompanying a low and a high plant population, with the interaction of three different lentil cultivars. Disease ratings were taken in 2016 to measure impact of disease management methods, alongside final seed yield.

The estimated outcome was confirmed in this study as the greatest yields were produced by the treatment with the highest seeding rate (240 plants m^{-2}) and two applications of fungicide in the growing season. This treatment with two applications yielded significantly higher than any other treatment involved in this trial. Alongside the previously mentioned estimation, it was hypothesized that results would contrast current agronomic recommendations commonly followed. This hypothesis was also accepted as increased seeding rates (almost double that which is recommended), would likely result in the most profitable outcome when protected by two separate applications of fungicide, spaced approximately 20 days apart.

The 2016 disease rating data and analysis determined that greater yields from increased seeding rates are attainable when protected against disease outbreak by two fungicide applications in the season, especially in conditions with high precipitation. This was confirmed at the Outlook site that received over 200 mm of precipitation in July and August and where the highest seeding rate with two fungicide applications, had a significantly lower amount of disease than the untreated check. Seed yield results also reflected this conclusion as the same treatment had a significant yield advantage. Another intriguing outcome that came from the Outlook site involved the thousand seed weight parameter. The sequential treatment of Headline and Bravo had significantly heavier seeds than the untreated check and the treatment with Fracture fungicide applied. In saying this, seed yield and lentil seed weight parameters both showed improvements when protected with a more vigorous fungicide regime, as lentil seed yield and seed weight are high correlated.

5.3 Final Conclusions and Recommendations

From this research, it is evident that lentil production in wet years should be paired with two fungicide applications. If seeding small red lentil (CDC Maxim), a currently prominent variety on the prairies, a seeding rate of 240 plants m^{-2} (96 kg ha^{-1}) is recommended to attain the highest yield. Some research has displayed a positive economic return at a seeding rate of 80 kg ha^{-1} (Kirkland et al., 2000) however, a current economic analysis is needed to decipher the financial viability. The optimal seeding rate range found in this study is particularly relevant in wet years and with more research, the effect of increased seeding rates in dry years will become more apparent. In saying this, producers should consider seed and fungicide prices relative to the selling prices of lentil at that point in time.

Adjustments in lentil seeding rates are important due to a number of factors including but not limited to; an increase in yield potential and economic profitability. In addition, increasing seeding rates can constitute a lessened reliance on group 2 herbicides if an integrated approach to weed control is taken. As inevitable group 2 herbicide resistance becomes more widespread, producers will be impacted as there are limited options left for weed control in lentil crops. On a positive note, producers can promptly adopt the practice of increased seeding rates, easily enhancing the competitive ability of their crop.

The overall hypothesis of this research was accepted as the treatment with the highest plant population resulted in the highest yield when two fungicide applications were applied. Additionally, all lentil market classes are diverse regarding the optimum seeding rate to attain the highest seed yield. Although concrete recommendations for seeding each market class cannot be made from this specific study, this research paired with similar results from additional studies, confirmed that current lentil seeding recommendations in Saskatchewan are not always sufficient to reach maximum seed yield.

5.4 Future Research

Elevated seeding rates in the field can show a number of advantages including improved weed control in lentil crops (Redlick et. al, 2017). With this proven advantage alongside a yield boost, lentil producers in Saskatchewan should certainly consider increasing the amount of lentil seed they sow. To produce more solid conclusions in reference to lentil seeding rate recommendations, it would be beneficial to add more genotypes to each lentil market class in a future study.

The common assumption that increased rates in pulses will lead to disease levels that outweigh any advantage, has been invalidated through this study, as high precipitation and doubled seeding rates still presented yield advantages when two fungicide applications were made. In the future, this line of study would benefit from a lentil seeding rate study that involves many site-years, additional target plant population levels and different fungicide chemistries, as Headline is highly susceptible to the progression of pathogen resistance.

Indeterminacy in lentil growth habits is a crop characteristic that presents producers with another obstacle. When conditions are conducive to vegetative production of biomass, it is continually being produced while less energy from the plant goes into pod filling and therefore, less seed is produced. However, results from this study seem to suggest that biomass does not absolutely translate into grain yield as large red lentil produced the highest amount of biomass overall however, small red lentil produced the highest amount of seed yield. In a search to increase lentil yields in Canada, maybe the maximization of biomass production does not hold the fundamental key and, more focus should be aimed at the end product of seed yield and therefore the advancement of harvest index.

5.5 Final Remarks

For many producers, pulse crop production and especially lentil production involves many challenges to undertake and therefore the addition of a pulse to their crop rotation is omitted. As researchers, the responsibility of making lentil crops more suitable for production on the Canadian Prairies, is a big one. Adding a pulse crop to a rotation not only presents producers with benefits, but all of humanity can benefit as well. Pulse crops including lentil, is a cheap form of protein from which is built with minimal amounts of added synthetic fertilizer. Fertilization and the overuse of synthetic fertilizers have negatively affected the planet by harming water bodies, however, without it, mankind would likely suffer from famine. These benefits are not to mention those that come from increased seeding rates as a step closer to less reliance on herbicides and while herbicide resistance becomes more of an obstacle every year on the Canadian Prairies, researchers and producers are always looking for methods to slow this inevitable process. Lentil is just one among many crops that could hold the key to a brighter future and it is the author's aspiration that this research has contributed to knowledge that will lead to more environmentally friendly and producer favorable options for future crop production.

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