CHICKPEA VARIETY EVALUATION AND INTERCROPPING FOR DISEASE MANAGEMENT

AND YIELD

by

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DEDICATION

My work is dedicated to my father, mother, sister, and my adviser who were always there for me and supported me every day. To my friends, Yuhuan Xie, Lin Shi, Muzhou Chen, Huiwen Zhang, Pragya Kiju, and Shreya Gautam, for their encouragement and support. Finally, my boyfriend, Hung-Kai Sun who has provided tireless patience and encouragement throughout my journey of graduate school, for that I will always be grateful.

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important food grain legume, but production is constrained by the soilborne pathogen complex, *Ascochyta rabiei*, and the development of fungicide resistance. Cultivar resistance is one of the most efficient strategies in disease management. However, chickpea cultivars with resistance to soilborne pathogens or complete resistance to *A. rabiei* have not been developed. Intercropping chickpea-flax has the potential for Ascochyta blight management. To minimize the impact of soilborne disease and Ascochyta blight on chickpea production, refining of integrated pest management practices is essential. To evaluate the effect of cultivar selection in combination with seed treatment on soilborne disease control, twenty-five cultivars/lines were planted with or without fluxapyroxad, pyraclostrobin, and metalaxyl under field conditions. The area under disease progress curve (AUDPC), seed yield, and protein content were assessed. *Fusarium solani* was isolated and identified in the late season, and the disease severity of root rot was evaluated. The results showed that seed treatment effectively suppressed damping-off and improved chickpea yield but only slightly reduced late-season root rot. The AUDPC of NDC160166 and NDC 160236 was not significantly reduced by seed treatment, which could be a future resource of resistance. To assess the effects of configurations and resistant cultivar on yield and Ascochyta blight management in intercropping chickpea-flax, two chickpea cultivars (CDC Leader and Royal) were planted with flax under six configurations (monocrop chickpea, 70% chickpea-30% flax in mixture, 50% chickpea-50% flax in mixture, 50% chickpea-50% flax in alternate rows, 30% chickpea-70 flax in mixture, monocrop flax). Yield and nutrient content of component crops and Ascochyta blight infection were evaluated. Chickpea yield decreased as flax proportion increased in the mixture. Chickpea yielded higher in the alternate row design than in the mixture at the same seeding rate due to less interspecies competition in the alternate rows. Intercrop increased 2%-23% land productivity. Chickpea-flax intercrop effectively reduced Ascochyta blight under higher disease pressure. The configuration of 50% chickpea and 50% flax in the mixture was more effective in suppressing Ascochyta blight than in the alternate row configuration. Integrated resistant cultivar and intercropping configuration was most effective in disease suppression.

CHAPTER ONE

GENERAL INTRODUCTION

Chickpea (*Cicer arietinum* L.) plays an important nutritional role in human diet worldwide. Chickpea production has been increasing in the Northern Great Plain of the United States since 1990s. Soilborne diseases are a concern in chickpea production, which can cause considerable yield losses due to reduced stand establishment. *Pythium spp.*, *Fusarium spp.*, and *Rhizoctonia solani* are the most damaging disease agents, causing damping-off and root rot on chickpea. Fungicide seed treatment and resistant cultivars are the most effective and economical strategies to control soilborne disease. However, chickpea cultivars resistant soilborne disease has not been developed. Ascochyta blight is a foliar disease, caused by *Ascochyta rabiei,* that has been a major constraint in chickpea production in all chickpea growing areas across the continents, which resulted in reduced seed quality and yield losses. Partially resistant cultivars, foliar fungicide, crop rotation, and the use of disease-free seeds are the most common strategies in Ascochyta blight management. However, lack of cultivars utterly resistant to Ascochyta and development of resistance to fungicide has increased the risks of using fungicides in chickpea production. Intercropping is to grow two or more species of crops simultaneously in the same field. The benefits of intercropping have been realized, including greater land productivity, higher resource use efficiency, and reduced pest pressure. Intercropping chickpea-flax has potential to control Ascochyta blight of chickpea. The goal of this project is to evaluate and provide more effective and sustainable disease management strategies to improve chickpea production in the Northern Great Plains.

My thesis explores integrated disease management strategies for controlling both soilborne diseases and Ascochyta blight on chickpea. Chapter two focused on evaluating

cultivars/lines in combination with seed treatment for soilborne disease suppression, yield, and protein content. The work presented in Chapter three demonstrates the potential of intercropping chickpea-flax in conjunction with cultivar resistance under different seeding ratios and configurations for yield, competition of companion crops, and Ascochyta blight management.

<div align="center">Chickpea Distribution and Production</div>

Chickpea is a drought-tolerant, cool-season annual grain legume that ranks among the world's three most important pulses. It is one of the eight founder crops of the origins of agriculture (Abbo et al., 2003). The earliest records of chickpea used as food occurred about 7500 years ago in Turkey (Redden & Berger, 2007). Nowadays, chickpea is grown in over 50 countries, and 13,718,980 hectares are harvested annually worldwide. India is the world's leading chickpea producer with 9,547,030 hectares harvested in 2019 (FAOSTAT, 2019). Chickpea production has increased significantly in the U.S. since 2001, and the U.S. ranked in seventh among the top chickpea production countries at an estimated 163,490 hectares of chickpeas harvested in 2019 (FAOSTAT, 2019). Montana accounts for a large percentage of the U.S. increase, where chickpea acreage increased from 5,400 hectares in 2001 to 161,000 hectares in 2018 (USDA-NASS, 2020). Furthermore, USDA-NASS (2020) reported that Montana had the largest certified organic acreage among U.S. states in 2019 (70500 hectares), and Montana led the nation in organic chickpea production with 700 hectares in 2019 (USDA-NASS, 2020). With the domestic and export market demand increasing, Montana can develop a larger conventional and organic chickpea market in the future (Merga & Haji, 2019).

The two main types of chickpeas in the market are kabuli and desi chickpeas. Kabuli-type chickpea has large, cream-colored, round seeds with average seed weight about 1000 seeds per

pound. Plants are 2-3 feet tall with white flowers. It is used as a whole seed or in hummus primarily in the Mediterranean basin, the Near East, Central Asia, and America (Leport et al., 2006; Margheim et al., 2004). The desi type generally has shorter plants than kabuli, smaller leaves, and the seeds (about 2,300 seeds per pound) come in various colors. It is mainly grown in South Asia, and seeds are usually dehulled and split before cooking (Leport et al., 2006).

Chickpea generally contains 20~22 % protein and 60 % carbohydrates, which can provide a good source of protein for humans and livestock (Gossen et al., 2016; Roy et al., 2001). Chickpea seed also contains many minerals and vitamins (Jukanti et al., 2012). Furthermore, as a cool-season pulse crop, chickpea can play a critical role in a sustainable cropping system due to the ability of biological nitrogen fixation. When it is grown as a rotation crop with cereal or other crops, chickpea can help disrupt the cycle of pests (Leisso, 2008).

<u>Chickpea Soilborne Diseases</u>

<u>Impact of Soilborne Diseases on Chickpea</u>

Some soilborne diseases are persistent problems in chickpea production in broad geographical areas, including Pythium damping-off, Fusarium wilt and root rot, and Rhizoctonia seed, seedling and root rot (Chen et al., 2011). They can cause yield loss due to reduced plant establishment and seedling vigor (Chang et al., 2004; Hwang et al., 2000; Leisso et al., 2009). Several soilborne pathogens have been reported in the Northern Great Plains to be responsible for chickpea damping-off, root and stem rot disease, including *Pythium spp.*, *Fusarium spp*., and *Rhizoctonia solani* (Hwang et al., 2003; Kaiser & Hannan, 1983; Kaiser et al., 1989; Leisso et al., 2009). Kaiser and Hannan (1983) reported that *F. solani* and *P. ultimum* were frequently isolated from decayed seeds caused by damping-off in the Palouse region of eastern Washington.

Furthermore, Leisso et al. (2009) identified the isolates from Kabuli chickpea seeds affected by damping-off in field trials at three locations in Montana in 2007. They found that *F. oxysporum* f sp. *ciceris*, *F. avenaceaum*, *F. equiseti*, and *F. solani* are related to chickpea damping-off. Hwang et al. (2003) reported that root rot caused by *R. solani* can cause significant stand loss and decrease the nodulation of chickpea in the prairie region of western Canada. About 50% to 70% yield losses caused by these threes pathogens has been reported (Chang et al., 2004; Gossen et al., 2016; Hwang et al., 2000).

Life Cycle of Chickpea Soilborne Diseases

The soilborne pathogens causing damping-off and root rot have similar life cycles with a great diversity of host plants (Gossen et al., 2016). They can survive as resistant resting spores, sclerotia and mycelia in soil and root residue over years, serving as primary inoculum. Under favorable conditions, survival structures can germinate, penetrate plant cells, and infect seedling roots. Pathogens can grow and produce secondary spores or mycelium in host tissue to spread from plant to plant in soil (Gossen et al., 2016; Herr, 1976; Singh & Pavgi, 1978). *Pythium* spp. overwinter as hyphae or thick-walled sporangia and survive in the soil as oospores over a decade (Hendrix & Campbell, 1973; Kaiser & Hannan, 1983). It can germinate on the seeds or root and infect seeds and embryos by hyphae or zoospores produced in sporangia. *Fusarium* spp. exists in the soil or root residue as chlamydospore or mycelium (Nash et al., 1961). It can infect plants through invading root tissues. Macroconidia and microconidia are produced asexually, which can spread to nearby plants through the dispersal of infected soil or organic debris by water, wind, farm equipment, and human activity (Leslie et al., 2008). *R. solani* can exist as sclerotia in the soil, as a saprophyte or hyphae on crop residues (Porter et al., 2011; Papavizas et al., 1975).

Symptoms of Chickpea Soilborne Diseases

The symptoms of damping-off are classified as either pre-and post-emergence depending on the time of disease occurrence and timing of disease cycle components (Lamichhane et al., 2017). Pre-emergence damping-off occurs before seed germination with seed decay, brown/black lesions on the root, and emergence failure. Post-emergence damping-off symptoms appear when seedlings decay, wilt, and die after emergence with root rot, lesion on root and seedling, and stunted plants (Horst, 2013). *Pythium spp*. can cause pre-emergence damping-off by infecting seeds or seedlings before emergence or cause post-emergence via invading the rot system and hypocotyl (Hendrix & Campbell, 1973). In chickpea, the major symptoms of damping-off are pre-emergence seed decay and cotyledon degradation, as well as discoloration and necrosis of roots (Leisso et al., 2009). Fusarium root rot can cause blackening of the taproot, discoloration on lateral roots, and lack of secondary roots. Reddish-brown to blackish-brown lesions can be observed on roots, and vascular discoloration extends above the soil line (Gossen et al., 2016; Westerlund et al., 1974). The symptoms of Rhizoctonia root rot or dark root rot are reddish to dark brown lesions on roots, stunted and brown-to-dark stems with sunken lesions around the soil line, and yellowing lower leaves (Chang et al., 2005; Sunder & Kataria, 2012).

Management of Chickpea Soilborne Diseases

The management practices of chickpea soilborne diseases include partially resistant cultivars, early seeding date, crop rotation, and fungicide seed treatment. Previous research suggested that Desi-type chickpea is less susceptible to damping-off and root rot than Kabuli-type chickpea (Hwang et al., 2003; Kaiser & Hannan, 1983). Chang et al. (2004) reported that Rhizoctonia root rot decreased the emergence rate of late seeding twice as much as early seeding. Emergence of field pea was 10 to 15% lower and seed yield was 20 to 50% lower when plant

was seeded into warm soil infected with *Pythium* sp. in late May and Early June (Hwang et al. 2000). Therefore, early seeding effectively reduced in reducing stand and yield losses caused by soilborne disease. Crop rotation is effective in chickpea soilborne disease management. To minimize the risks of soilborne disease, chickpea should not be planted in the field that has been planted to pulse crops for at least two years (McKay et al., 2002). Seed treatments with fungicide are effective in controlling chickpea soilborne diseases. Previous study has found that fungicide seed treatments, thiram + carbathiin (Vitaflo 280) and carbathiin + thiabendazole (Crown), are effective in reducing the damage of *R. solani* and improving seedling emergence and seed yield (Hwang et al., 2003). Additionally, researchers suggested that producers should use fungicide seed treatments containing metalaxyl or mefanoxam to control Pythium and another fungicide to control Fusarium, Rhizoctonia. (Leisso, 2008; Leisso et al., 2009).

Ascochyta Blight of Chickpea

Impact of Ascochyta Blight on Chickpea

Ascochyta blight is caused by the fungal pathogen *Ascochyta rabiei*, which causes seed quality degradation and yield losses. *A. rabiei* can attack all the above-ground parts of the chickpea like leaves, stems, and pods, resulting in tissues death as well as small and shriveled seeds (Harveson et al. 2011). About 5% to 100% yield losses caused by Ascochyta blight in the Northern Great plains have been reported by Chongo et al. (2003). They also found that chickpea seed yield and disease severity of Ascochyta blight were strongly and negatively correlated.

Symptoms and Life Cycle of *A. rabiei*

A. rabiei can cause round light brown lesions on leaves, stems, and pods with dark submerged pycnidia irregularly scattered on infected tissue (Haware & Nene, 1981). Pycnidia is

present as concentric rings within the center of dark brown lesions on pods and leaves. It can produce masses of conidia that serve as inoculum for secondary infections. Asexual spores are released from pycnidia during humid weather and are spread to nearby plants by rain splash and winds (Reddy & Singh, 1990). When spores contact plant tissues, germination occurs after 12 hours and penetration starts within 24 hours (Pandey et al., 1987). The appearance of symptoms varies between 5 and 7 days depending on temperatures provided (Trapero-Casas & Kaiser, 1992). At the sexual phase, pseudothecia can produce ascospores at low temperatures on senescent chickpea debris, contributing to long-distance traveling of Ascochyta blight (Gamliel-Atinsky et al., 2005). It is also an important source of primary inoculum (Kaiser, 1997).

Management of *A. rabiei*

As described by McKay et al. (2002), in the various varieties, the symptoms initiate differently. For example, symptoms sometimes begin with leaf lesions and sometimes with stem lesions. Furthermore, symptoms are frequently observed in the areas with the most moisture and from the edges that are closest to the previous field with infected debris. Additionally, Ascochyta does not spread significantly until the flowering stage, when the canopy begins to close and trap humidity in moderately resistant varieties (Chongo & Gossen, 2001). Therefore, applying one or more foliar fungicides during the flowering or early podding can reduce the damage of Ascochyta blight (Chongo et al., 2003). *A. rabiei* can survive in infected tissues as mycelium and/or pycnidia at 10-35°C and 0-3% relative humidity at the soil surface over two years, and the pathogen loses little viability in infected seeds after 5 years (Kaiser, 1973). Therefore, disease-free seeds and 4-year rotation are recommended for Ascochyta blight management (Gossen & Miller, 2004; McKay et al., 2002). Seed treatment is another strategy to reduce the impact of seed-borne inoculum. Wise et al. (2009) found that a combination of fungicide seed treatments

(thiabendazole, ipconazole, azoxystrobin, and metalaxyl) can increase seedling emergence and suppress Ascochyta blight development. However, the most important management of Ascochyta blight is to integrate several disease management strategies that involve disease-free seeds, crop rotation, resistant cultivar, foliar fungicide, and seed treatment (Gan et al., 2006).

<div align="center">Intercropping Chickpea-Flax</div>

Definition and Benefits of Intercropping System

Intercropping is a system of growing two or more crops on the same land simultaneously (Ofori & Stern, 1987). It can provide the greatest opportunity to use soil and environmental resources efficiently compared to monoculture. Chapagain and Riseman (2014) reported that intercropping barley and pea have 12–32% higher land productivity compared to monoculture plots. Additionally, intercropping pea developed more nodules than the monocrop pea, resulting in increased N fixation by 9%-17%. The barley component fixed a higher proportion of CO_2 in intercrop plots than monoculture barley, and increased biomass N of barley within pea-barley intercrops was also reported (Chapagain & Riseman, 2014).

Moreover, an intercropping system has a greater tolerance to pest and disease stress which provides the system insurance against total crop failure. Fenández-Aparicio et al. (2007) found that faba bean-oat intercrops and pea-oat intercrops reduced the *Orobanche crenata* infection on faba bean and pea in the field. With an increased proportion of oats in the intercrops, the number of *O. crenata* infected plants decreased. Fernández-Aparicio et al. (2010) reported that the pea-triticale and pea-faba pea intercropping reduced *Didymella pinodes* severity under high disease pressure by 63.8-73.6% and 64.8-82.3%, respectively. Disease severity was reduced when pea was intercropped with oat (14.1%-45.2%), barley (10.2-41.1%), and wheat (59.3%),

respectively. Additionally, Schoeny et al. (2010) found that intercropping pea-wheat reduced the Ascochyta blight severity on pea pods and stems, which was attributed firstly to the change of the microclimate. Compared to monocrops, intercropping had higher air temperature and shorter leaf wetness period, creating a less favorable environment for disease development. Secondly, intercropping displayed a barrier role at the 5-7 leaves stage and a relay role at the 8-10 leaves stage. At the 5-7 leaves stage, the intercropped pea showed a higher reduction of lesion number than the reduced density pea monocrop. However, the lower density pea monocrop had more reduction of disease development than intercrop at the 8-10 leaves stage. It indicates intercropping can decrease disease through dilution effects and physical barrier role effects of non-host crops.

Crop Compatibility and Competition

The component crops in an intercropping system compete for available resources during growth. For a successful intercropping system, the competition between crop species should be reduced to a minimum (Fukai & Trenbath, 1993). The competition is determined by selected crop species, plant population, row configuration, and sowing date (Andrade et al., 2012; Chalmers, 2014; Chen et al., 2004; Echarte et al., 2011). Wahla et al. (2009) assessed the competition function of 5 barley-based intercropping systems (barley+lentil, barley+gram, barley+methra, barley+linseed, and barley+canola) under different nutrient levels. The competitive ratio proved that canola is more competitive than other intercrops in barley-based intercropping systems. Rao and Willey (1983) examined two millet genotypes and four sorghum genotypes compatible with four pigeon peas in a semiarid region of India. They found that the earlier maturity and taller cereal genotype had less competitive effects on the pigeon pea. Previous studies have found that competitions between component crops can be manipulated by

altering sowing ratio and row configurations. Adeniyan et al. (2014) suggested that increased

plant population density of maize in a cassava/maize intercropping system has negative effects

on cassava growth due to above-ground competition for light. Chen et al. (2004) reported that

pea produced lower biomass in mixed barely-pea intercrop than in separated row arrangement.

However, barely-pea in mixture showed greater total biomass yield and higher land productivity.

Andrade et al. (2012) found that sunflower yield was increased by 30 days delay sowing of

soybean in a sunflower-soybean intercropping system.

Several indices have been developed to describe competition of intercrops including land

equivalent ratio (LER), competitive ratio, aggressivity, relative crowding coefficient, actual yield

loss, monetary advantage, and intercropping advantage (Dhima et al., 2007). In particular, LER

is used to assess the combined yield of two or more intercropped crops compared with the yields

of the species grown in monocrop setting (Ofori & Stern, 1987). The LER can be used to

compare the land use efficiencies between intercrop systems versus monocrop systems, between

different cultivars, as well as among multiple intercropping configurations.

Intercropping Chickpea-Flax for Potential Ascochyta Blight Management
and Yield Benefit

Ascochyta is a major threat to chickpea production worldwide. As the largest chickpea

producer in the U.S., Montana producers need information on effective and sustainable

Ascochyta blight management (USDA-NASS, 2020). It has been extensively investigated in

terms of host resistance, fungicide seed treatment, crop rotation, and foliar fungicide (Crutcher et

al., 2022; Gossen & Miller, 2004; Sharma & Ghosh, 2016; Wise et al., 2009). However, highly

resistant cultivars to *A. rabiei* have not been developed and the resistance of the moderate

resistant cultivars declines as the plant matures (Sharma & Ghosh, 2016; Sharma et al., 2010). *A.*

rabiei resistance to fungicide has been reported in the Northern Great Plains (Wise et al., 2009). Little research focused on the effect of chickpea-flax intercropping on disease management and yield potential. Ascochyta blight is a host-specific disease and flax is tolerant to this pathogen. Intercropping flax and chickpea could reduce chickpea density and function as a barrier. Thus, fewer ascospores released from pycnidia could reach the nearby chickpea plants and reduce infection. Similar to other intercropping systems, intercropping chickpea-flax has potential benefits like higher land-use efficiency, less N fertilizer input, and lower pest damage. Integration of intercropping, cultivars resistance, sowing rate, and row configuration could improve disease management efficiency. Potential benefits of chickpea-flax intercropping for Ascochyta blight management will promote the intercropping chickpea-flax application in Montana. As a part of the sustainable cropping system, this application could improve resource use efficiency and disease management, which has practical benefits for chickpea production in Montana. However, growing chickpea and flax tighter will cause each species competition for growth resources resulting in yield decrease. It is not well known how flax affects chickpea yield.

Summary

Two research projects were carried out during this Master degree project. The first project was combination of seed treatment and cultivar selection for effective control of soilborne diseases in chickpea, and the second study was intercropping chickpea-flax for yield and Ascochyta foliar disease management. The details of the studies are reported in Chapter 2 and Chapter 3 of this thesis. The goals of the research were 1) to evaluate seed treatment and cultivars for chickpea soilborne disease management; 2) to investigate effect of intercropping

chickpea-flax with different configurations on control of chickpea Ascochyta blight and chickpea

seed yield.

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CHAPTER TWO

COMBINATION OF SEED TREATMENT AND CULTIVAR SELECTION FOR EFFECTIVE CONTROL OF SOILBORNE DISEASES IN CHICKPEA

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ABSTRACT

Damping-off and root rot are diseases of concern in chickpea (*Cicer arietinum* L.) production and can cause seedling loss, increase weed populations, and reduce yield. A complex of soilborne pathogens is responsible for damping-off and root rot in the Northern Great Plains. Species within this complex include *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani*. Currently, partially resistant cultivars and fungicide seed treatments are the most effective for control of damping-off and root rot. This study examined 25 chickpea cultivars and breeding lines with and without a fungicide seed treatment (fluxapyroxad, pyraclostrobin, and metalaxyl) for stand establishment and grain yield in field conditions. Experimental plots were located in dryland and irrigated fields at the Eastern Agriculture Research Center (EARC) in Sidney MT in 2020 and 2021, respectively. Stand counts were recorded three times to calculate area under disease progress curve (AUDPC). Yield, and protein content was assessed after harvest. In 2021, pathogens were isolated from chickpea roots showing signs of root rot and identified as *Fusarium solani*. The results showed that the seed treatments effectively decreased soilborne disease development (13.8% to 37.9%) and improved chickpea yield (75.5% to 88.0%) in 2020 and 2021, and increased protein content (0.69 %) in 2021. Different cultivars did not have a consistent impact on disease development and yield protection. NDC160166 and NDC160236 displayed consistently lower disease and higher yield and might be a future source of disease resistance. Yield of untreated seeds decreased and AUDPC increased for all cultivars/lines in both years. A negative correlation was also observed between protein content and chickpea yield.

Introduction

Chickpea (*Cicer arietinum* L.) is a high-value pulse crop. In the U.S., chickpeas are grown primarily in Montana, Washington, Idaho, and North Dakota (Leisso et al., 2011). The semi-arid climate of Montana provides a well-suited environment for chickpea production, leading to Montana becoming the largest chickpea producer (35% of total U.S. production) in 2020 (Miller et al., 2002; USDA-NASS, 2020).

Soilborne diseases of chickpea, including damping-off and root rot, cause considerable yield losses. *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani* are considered the most common causal agents of root rot and damping-off of chickpea in the Northern Great Plains (Gossen et al., 2016; Hwang et al., 2003; Kaiser & Hannan, 1983; Leisso et al., 2009). Yield loss of 50% by *Pythium* spp., 60% by *Fusarium* spp., and 70% by *R. solani* has been reported (Chang et al., 2004; Gossen et al., 2016; Hwang et al., 2000b).

Pathogens causing soilborne diseases have a large host range and survive as resting spores or sclerotia in soil and residues over many years (Gossen et al., 2016; Herr, 1976; Singh & Pavgi, 1978). Environmental conditions can affect population densities and the community composition of soilborne pathogens, which may affect the aggressiveness of different species under the given conditions (Liu et al., 2020). *Pythium* spp. are most damaging in a wet or moist environment, increasing the zoospore production and enhancing root symptoms (Li et al., 2015). *Fusarium* spp. can be more aggressive under extreme conditions, for example, drought or flooding (Tu, 1994). Wet soil and higher organic matter in soil can promote *Rhizoctonia* spp. development and increase stand loss (Porter et al., 2011).

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The symptoms of damping-off are classified to pre- and post-emergence. Pre-emergence damping-off causes seed decay and the death of seedlings before they emerge from the soil. The symptoms of post-emergence damping-off include wilting, stunting, root rot, root lesions, and seedling death (Horst, 2013). Symptoms of root rot can appear from seedling emergence to maturity (Chen et al., 2011). Discolored crown and hypocotyl tissue, reddish to black lesions on roots, discolored root vascular system, and stunted and yellowing aerial parts can be observed on roots attacked by *Pythium* spp., *Fusarium* spp., and *R. solani* (Nene et al., 2012; Gossen et al., 2016; Chang et al., 2004).

Several strategies are currently used to control chickpea soilborne diseases, including partially resistant cultivars, early planting dates, fungicide seed treatment, and biological control (Kaiser & Hannan, 1983; Landa et al., 2004; Leisso et al., 2009). Resistant cultivars and fungicide seed treatments are the most effective management strategies for control of soilborne disease. Breeding efforts on improving chickpea yield, seed quality, environmental adaption, and disease resistance have contributed to improved chickpea cultivars and germplasm (Bandillo et al., 2021; Muehlbauer et al., 2004; Vandemark et al., 2015; Warkentin et al., 2005). In addition, the application and combination of several fungicide seed treatments are effective to control chickpea damping-off and root rot, such as fludioxonil, mefenoxam, thiram, and thiabendazole. (Artiaga et al., 2012; Hwang et al., 2003; Leisso et al., 2009).

Chickpea lines with partial or even high levels of resistance to soilborne pathogens have been identified in India (Gupta & Babbar, 2006; Zope et al., 2014). In the Northern Great Plains, research related to chickpea cultivar resistance to soilborne pathogens reported that desi-type chickpeas are more resistant than the kabuli-type (Hwang et al., 2003; Leisso, 2008). However,

kabuli-type chickpeas dominate U.S. chickpea production. A report in Saskatchewan indicated

that there is no resistance to *F. avenaceum* in current chickpea cultivars widely grown in Canada

(Banniza et al., 2020). *F. solani* can significantly decrease the emergence rate of CDC Consul,

CDC Cory, CDC Leader, and CDC Orion. (Safarieskandari et al., 2021). Therefore, evaluating

well-adapted chickpea cultivars and breeding lines in the Northern Great Plains and the

combination of cultivars and fungicide seed treatment in soilborne pathogens control will help

growers make better decisions in disease management. This study evaluated 25 chickpea cultivars

and breeding lines in combination with a seed treatment that includes the active ingredients

fluxapyroxad, pyraclostrobin, and metalaxyl (Obvius® fungicide) for stand loss, root rot, yield,

and protein content.

Materials and Methods

Chickpea Cultivars and Breeding Lines Seed Health Test

Twenty-five chickpea cultivars and breeding lines were selected for this study (Table 1.1).

Five are commercial cultivars that are widely grown and the remainder are unreleased breeding

lines. Seed health tests were conducted for each chickpea entry, in which 500 seeds were

sterilized in 1% sodium hypochlorite for 10 min (Mathur & Kongsdal, 2003). After being rinsed

with sterilized water, the seeds were air-dried in the biological safety cabinet for 30 min.

Sterilized seed (10 per plate) were plated on potato dextrose agar (PDA; BD Difco™) and

incubated at room temperature for 7 to 10 days. The presence of seedborne pathogens that could

affect germination (i.e., *Ascochyta rabiei*, *Botrytis* spp.) were confirmed by viewing the colonies

and fruiting bodies with microscope (Motic® SD digital microscope) at 40X magnification.

Finally, the percentage seeds infected with fungal contaminant was calculated as $n/N \times 100$,

where n is the number of seeds contaminated with a particular fungal and N is the total number of

seeds tested.

Table 1.1. Description of chickpea cultivars/line evaluated.

Cultivar/Line	Breeder†	Reference
CDC Frontier	CDC, Canada	(Warkentin et al., 2005)
CDC Leader	CDC, Canada	(Ashokkumar et al., 2014)
CDC Palmer	CDC, Canada	(Tar'an, 2014)
MT043	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT044	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT072	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT074	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT075	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT111	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT251	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT252	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT394	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT484	USDA-ARS	(McPhee, personal communication, January 25, 2022)
MT485	USDA-ARS	(McPhee, personal communication, January 25, 2022)
NDC150001	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160049	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160078	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160133	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160138	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160146	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160166	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160186	NDSU, USA	(McPhee, personal communication, January 25, 2022)
NDC160236	NDSU, USA	(McPhee, personal communication, January 25, 2022)
Royal	USDA-ARS	(Vandemark et al., 2019)
Sierra	USDA-ARS	(Muehlbauer et al., 2004)

† CDC, Crop Development Centre, University of Saskatchewan; USDA-ARS, U.S. Department of Agriculture-Agricultural Research Service; NDSU, North Dakota State University.

Field Seed Treatment and Seedling Emergence Study

Trial Sites.Field trials were conducted at the Eastern Agricultural Research Center (EARC) dryland farm (47∘46′ N, 104∘14′ W; 670 m asl) and the EARC irrigated farm (47∘73′ N, 104∘15′ W; 594 m asl) in 2020 and 2021, respectively. The soils are classified as a William clay loam at the dryland farm and as a Savage silty clay loam at the irrigated farm. Monthly temperature and precipitation from April to August for each year were are summarized in Table 1.2. The field trial in 2021 was conducted under irrigation due to persistent drought conditions entering the growing season and received 106 mm irrigation.

Table 1.2. Maximum, minimum, and mean temperature and total precipitation ($\pm$ SE) from April–September for Sidney, MT in 2020 and 2021.

Month	Precipitation	Temperature			Precipitation	Temperature		
	2020				2021			
	—mm—	———————C°———————			—mm—	———————C°———————		
		Max Air Temp	Min Air Temp	Avg Air Temp		Max Air Temp	Min Air Temp	Avg Air Temp
April	0.51(±0.012)	11.52(±1.68)	-3.67(±1.20)	3.92(±1.40)	4.32(±0.003)	14.32(±1.49)	-2.61(±0.69)	5.85(±0.93)
May	40.67(±0.64)	19.63(±1.07)	5.18(±0.85)	12.40(±0.89)	41.66(±0.02)	18.79(±1.06)	4.52(±0.66)	11.65(±0.74)
June	26.67(±0.49)	25.87(±0.64)	11.24(±0.63)	18.56(±0.65)	52.60(±0.04)	28.50(±0.72)	11.91(±0.57)	20.21(±0.56)
July	45.21(±0.64)	29.16(±0.76)	13.52(±0.47)	21.34(±0.49)	0.51(±0.00)	32.22(±0.67)	15.76(±0.47)	24.08(±0.47)
August	23.14(±0.57)	31.33(±0.76)	7.12(±11.72)	22.52(±0.58)	27.18(±0.018)	28.20(±1.03)	11.89(±0.55)	20.05(±0.64)
September	8.38(±0.24)	22.82(±0.96)	7.12(±0.69)	14.97(±0.76)	10.62(±0.01)	26.01(±0.93)	7.05(±0.51)	16.53(±0.57)
Total	144.58				136.89			

Experimental Design.Twenty-five breeding lines and check cultivars were planted on April 23rd, and May 5th in 2020 and 2021, and the crops were harvested on Aug 31st, and Aug 30th in 2020 and 2021, respectively. The experiment included twenty-five chickpea entries with or without seed treatments. Seeds without fungicide treatment were only treated with thiamethoxam (Cruiser 5FS) insecticide at a rate of 0.5 g a.i. kg^{-1} seeds. Seeds with fungicide seed treatment were treated with fluxapyroxad, pyraclostrobin, and metalaxyl (Obvius® fungicide) at a rate of 0.18 g a.i. kg^{-1}

seeds prior to planting. Rhizobial inoculant (Primo GX2, Verdesian Life Sciences, Cary, NC) was mixed with seeds at planting. The chickpea seeds were planted at a rate of 40 seeds m^{-1} seeding rate and a depth of 5 cm. The experiment was arranged in a randomized complete block design with four replications and each plot was 1.52 m $\times$ 3.05 m. The twenty-five entries with and without seed treatment were randomly assigned into each block. Miravis® Neo (Syngenta Crop Protection, Greensboro, NC) was applied as a banded treatment at a rate of 2.8 kg a.i. ha^{-1} to prevent Ascochyta blight on June 13th, 2020 and June 25th, 2021

Data Collection

Two 0.5 $\times$ 0.5 m squares were marked at the front and end of each plot with flags after chickpea emergence. Seedlings were counted three times for each square at 3-7d, 14-28d, 35-42d after emergence. The entire plot was counted when the squares were not representative due to poor seedling emergence (i.e., when the number of plants < 5). The area under disease progress curve (AUDPC) was calculated by considering the seedling loss rate as a measurement for seedling damping-off incidence following Campbell and Madden (1990): $\text{AUDPC} = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$, where t_i is the sample time point, y_i is the seedling loss rate at the i_{th} observation. The seedling loss rate is the number of seedlings lost as a percentage of the live seeds planted. Chickpea seeds were harvested using a Wintersteiger Classic plot combine (Wintersteiger) on August 31st 2020 and August 30th 2021. Seed moisture and protein content were determined using an automatic grain analyser Infratec NOVA (FOSS, Denmark). It uses NIR transmission spectroscopy to measure the mean protein content of bulk grain samples (Engstrøm et al., 2021).

Root Disease Identification

Fungal Isolation.Three cultivars (MT044, MT074, MT075) displaying the lowest

emergence rate and three cultivars (NDC160146, NDC160186, NDC160166) showing the highest

emergence rate in 2020 were sampled from treated and untreated plots in 2021 for disease

identification. On July 21st, 2021, 10 plants of the six selected cultivars were collected from the

end of each plot at the podding stage. The roots were washed in tap water and dried on a paper

towel. Disease severity was evaluated using the 0-10 scale (Figure 1.1), which is designed by the

percent of lesion coverage on the hypocotyl, epicotyl, and root system where: 0 = no disease

symptoms; 1= percent of lesion coverage <10%; 10= >90% or plant dead. Root sections adjacent

to areas of rot from plants with disease severity ratings of 5-7 were cut into 0.5 cm pieces and

surface sterilized using 70% ethanol for 2 min. After rinsing with sterilized water for 2 min, these

pieces were transferred aseptically to sterilized PDA supplemented with antibiotics (0.01 mg ml^{-1}

chloramphenicol, 0.005 mg ml^{-1} tetracycline). Plates were incubated at room temperature for 14 d

with a 12-hour light-dark cycle. Thereafter, isolates were transferred to 15% water agar. After 14

d, sporodochia were plated on Spezieller Nahrstoffarmer agar (SNA) for single spore isolation.

Purified isolates were stored in 30% glycerol at -80°C. Isolates were maintained on SNA.

Figure 1.1. Chickpea root rot rating scale for disease severity evaluation in the field trial. 1 through 10 are designated by the percent of lesion coverage on the hypocotyl, epicotyl, and root system as follows: 1 = < 10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, 10 = > 90% or a dead plant.

Morphological Characterization.All isolates were cultured on both PDA and carnation leaf agar (CLA) to characterize isolate morphology and formation of reproductive structures, respectively (Leslie et al., 2008). Isolates grown on PDA were incubated at 25°C for 14 d and examined for colony color, growth, and mycelium amount and color. All isolates were plated on CLA and incubated at 25°C for 14 d for morphological evaluation. The formation of reproductive structures including macroconidia, microconidia, and chlamydospores were evaluated under microscope (Motic® SD digital microscope) at 40X magnification and photographed.

DNA Sequencing.The purified isolates were grown in 25% potato dextrose broth (PDB; BD Difco^TM) for 5 d by inoculating the culture with three 10 mm^2 agar plugs cut from the actively

growing edge of 5 to 7 d-old-cultures. Fungal tissue was collected by straining through miracloth (Millipore®). Approximately 0.2-0.3 g of mycelium was mixed with 0.5 ml of extraction buffer (50 mM Tris-HCl, 50mM EDTA, 2% SDS, pH 8.0) and incubated at a 68°C in heat block for 30 min. The supernatant was transferred following centrifugation and DNA was purified by the phenol-chloroform method (Xu et al., 1996). The translation elongation factor (TEF)-1α sequence was amplified with the primer EF1 (5'-ATG GGT AAG GAR GAC AAG AC-3') and EF2 (5'-GGA RGT ACC AGT SAT CAT-3') by PCR (Donnell et al., 2000). The PCR reaction was carried out in a 50 µl final volume containing 25 µl One Taq® 2X Master Mix (New England Biolabs), 1 µl of each of 10 µM forward and reverse primers, 21 µl of nuclease-free water, and 2 µl of DNA (approximate 200 ng/µl). The PCR amplification was performed under the following thermal cycling conditions: initial denaturation at 94°C for 5min; 35 cycles of denaturation at 94°C for 30s, annealing at 51°C for 30s, and extension at 68 °C for 1min; and a final extension at 68°C for 5 min, followed by a hold at 4°C. The PCR products were electrophoresed in 1% agarose pre-stained with ethidium bromide and sized with a DNA size standard (Invitrogen, Thermo Fisher Scientific, Waltham, MA). The DNA bands were visualized under ultraviolet light in Gel Doc E.Z. Imager (Bio-Rad Laboratories, Inc., Hercules, CA). The PCR products were cleaned and Sanger sequenced with primer pairs used for their amplification in both direction via ABI 3730XL Genetic Analyzer (GATC Company) at the Molecular Cloning Laboratories (San Francisco, CA). Assembly of DNA sequences for each gene was carried out in Sequencher 3.0 (Applied Biosystems, Foster City, CA). The consensus sequences (EF-1α) were compared with *Fusarium* sequences in *Fusarium*-ID database (fusariumdb.org).

Koch's Postulates Test.After the pathogens were isolated and identified, twelve isolates were tested for pathogenicity in the greenhouse. The NDC160146 chickpea seeds were sterilized in a 1% sodium hypochlorite for 10 min. After rinsing with sterilized water, the seeds were air-dried for 30 min and placed in 15 mm petri dishes (83 mm diameter) with slightly damp paper towels for 5 days to germinate. Pregerminated chickpea seeds were sown at 4 seeds per pot into 10 cm $\times$ 10 cm pot filled with sterilized perlite. Inoculum at 1×10^5 conidia/ml was pipetted on each planted seed with 100 µl. Pots were fertilized at planting and the following week with 50 ml Peters Professional® STEM at 0.6 g L^{-1} and 0.3 g L^{-1} and 50 ml Peters Professional® 20-20-20 at 0.16 g L^{-1}. Plants were watered daily and maintained for 14 days on the greenhouse bench at 21°C with 16-h light and 8-h dark cycles. Plants were rated using a 0-10 (Figure 1.2) based on the percent area of the lesion and aggressiveness scales were determined based on the percentage of disease severity. The infected plants with 5-7 disease severity scores were collected to re-isolate and confirm the pathogen from root tissue as described above.

Figure 1.2. Chickpea root rot rating scale for disease severity evaluation in Koch's postulates test. 1 through 10 are designated by the percent of lesion coverage on the hypocotyl, epicotyl, and root system as follows: 1 = < 10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, 10 = > 90% or a dead plant.

Data Analysis

The scoring of disease severity for each plant was converted to a percentage value. The percentage scores of the severity ratings were used for statistical analysis. The proportion data bounded at 0 and 1, like disease severity and protein content, and was not normally distributed or homoscedastic (Kieschnick & McCullough, 2003). However, the numerator can be considered a count variable. Thus, they were analyzed using a beta regression model, which can be used to account for the fact that responses are bounded by (0,1) (Geissinger et al., 2022). An ANOVA-like table for factor terms was produced with the joint_tests function in the *emmeans* package, which was based on linear functions of predictors in a beta regression model (Lenth et al., 2019). When appropriate, the mean comparisons were conducted by Šidák-corrected (or Dunn–Šidák) multiple comparisons to control for familywise error rates, which is an appropriate pairwise multiple comparison for nonparametric data (Cribari-Neto & Zeileis, 2010; Dinno, 2015). Other

data collected were subjected to analysis of variance (ANOVA) to test the effects of the seed treatments and varieties. ANOVA was performed on chickpea seed yield and AUDPC for the twenty-five chickpea varieties, and the effects were considered statistically significant at P<0.05. Fisher's protected LSD (P≤0.05) based on student's t-test was used to compare the means of different treatments and varieties. Correlation analysis was conducted to determine the correlation of protein and AUDPC with chickpea grain yield. Correlation coefficients between pairs were determined using Pearson's correlation coefficient. Normality of all dependent variables was assessed using the Shapiro-Wilk test. Outliers were detected through a bivariate approach. All the statistical analyses were conducted in R studio (Version 4.0.3, R Studio Team, 2020).

Results

Chickpea Cultivars and Breeding Lines Seed Health Test

The seed health test results of 2020 showed eight chickpea lines (MT074, MT485, MT252, MT484, MT394, MT251, MT044, MT072, MT075) were infested with *A. rabiei* with more than 2% infection (Table 1.3). Other pathogens were not observed. The seed health test results for 2021 suggested that all the seeds were free of seedborne pathogens.

Table 1.3. Percentage of seeds infected with *A. rabiei* for 25 chickpea cultivars and lines in 2020 and 2021

Cultivar/Lines	2020	2021
	Infection (%)	
CDC Frontier	0	0
CDC Leader	0	0
CDC Palmer	0.2	0
MT043	1.6	0
MT044	2.6	0
MT072	3.2	0
MT074	4.8	0
MT075	4.8	0
MT111	1.4	0
MT251	7.2	0
MT252	2.4	0
MT394	5.6	0
MT484	2	0
MT485	5.6	0
NDC150001	0	0
NDC160049	0	0
NDC160078	0	0
NDC160133	0	0
NDC160138	0	0
NDC160146	0	0
NDC160166	0	0
NDC160186	0	0
NDC160236	0	0
Royal	0	0
Sierra	0	0
Mean	1.66	0

Chickpea Damping-Off and Root Rot Development

AUDPC was analyzed by year due to the interaction between year with variety and/or treatment ($P<0.01$; $P<0.01$; $P<0.01$). AUDPC was affected by seed treatment ($P<0.01$; $P<0.01$) and cultivar ($P<0.01$, $P=0.07$) in both years, but the effect of seed treatment x cultivar interaction was only observed in 2020 ($P<0.01$). Seed treatment significantly reduced AUDPC by 88.6% and

75.5% in 2020 and 2021, respectively (Table 1.4). A significant difference in AUDPC occurred among the 25 cultivars in 2020 (P<0.01). NDC160049, NDC 160133, NDC160146, NDC 160236, and Royal had significantly lower AUDPC than other commercial cultivars (Table 1.5). In 2021, MT074 had the lowest AUDPC, followed by NDC160138, and CDC Frontier (Table 1.5). Furthermore, the seed treatments did not have a consistent effect on stand establishment over both years of the study. In 2020, the AUDPC of NDC150001, NDC160049, NDC160078, NDC160133, NDC16046, NDC160166, NDC160236, and Royal was not decreased by seed treatment significantly (Figure 1.3). In 2021, seed treatment had little effect on the AUDPC of CDC Leader, CDC Frontier, MT072, MT074, MT111, MT252, MT394, MT485, NDC160186, NDC 160166, and NDC160236 (Figure 1.3). There was no difference in AUPDC of NDC160166 and NDC160236 for two years.

Figure 1.3. Interactive effects of seed treatment and cultivar on area under the disease progress curve (AUDPC) in 2020 (A) and 2021 (B). Dark gray bars represent AUDPC of chickpea with a seed treatment (fluxapyroxad, pyraclostrobin, metalaxyl, and thiamethoxam); light gray bars represent AUDPC of chickpea without seed treatment. *Represents a significant difference between the AUDPC of treated and untreated chickpea at $P < 0.05$. Vertical bars represent the standard error (SE).

<u>Disease Identification</u>

Disease evaluation of selected plants showing root rot symptoms revealed that only seed treatment had a significant effect on disease severity (P=0.03; Table 1.4). The disease severity was decreased from 4.9 to 4.3 by seed treatment. Twelve isolates were obtained and plated on PDA and SNA for morphology characterization. The color of the colony varied from white to light brown (Figure 1.4A). The isolates produced abundant sporodochia (Figure 1.4B), with three isolates green in color, and the rest cream colored. Slightly curved macroconidia with 3 to 4 septa were observed and formed in false heads on very long monophialides (Figure 1.4C). The length of macroconidia ranged from 32 to 42 µm. Oval and kidney-shaped, 0 to 2 septate microconidia were 8.5 to 11 µm length (Figure 1.4D). Chlamydospores appeared after 4 weeks (Figure 1.4E). The TEF-1α sequences of twelve isolates were 98.9% to 100% homologous with the TEF-1α sequence of *F. solani* isolates. All isolates were identified as *F. solani*.

When evaluated in the greenhouse, all twelve isolates were found to be pathogenic on chickpea with symptoms that resembled those observed in the field. Control seeds which were not inoculated did not display symptoms. The disease severity of twelve isolates ranged from 3.3 to 7.1. Under greenhouse conditions, all *F. solani* isolates demonstrated high to moderate aggressiveness on chickpea.

Figure 1.4. Colony growth of isolates on PDA after 15 days (A), sporodochia on carnation leaves (CLA) (B), false-heads on CLA (C), macroconidia and microconidia on CLA (D), and chlamydospores on CLA (E).

Seed Treatment and Cultivar Effects on Chickpea Grain Yield

The yield data was analyzed separately for each individual year due to the interaction of variety, treatment, and year (P=0.03). Cultivar had a significant effect on yield in 2020, but only a moderate effect in 2021 (P<0.01, P=0.07). There were significant seed treatment x cultivar interactions in 2020 but not in 2021 (P<0.01; P=0.65). Yield was significantly affected by seed treatment in both years (P<0.01; P<0.01). Seed treatment increased yield by 37.9% and 13.7% for 2020 and 2021 (Table 1.4). In 2020, Chickpea yield ranged from 1297 to 3028 kg ha^{-1} for NDC160236 and MT111, respectively (Table 1.5). Yields of six NDC lines (NDC160236, NDC160133, NDC160049, NDC160186, NDC160146, and NDC160166) and Royal were significantly higher than the other cultivars in 2020. With irrigation, the chickpea grain yields in 2021 were much higher than in 2020; the grain yield ranged from 3269 to 4978 kg ha-^{1} (Table 1.5). Similar to 2020, the highest yield was obtained with NDC160236 in 2021. Sierra, a large kabuli chickpea, produced the lowest grain yield in 2021. Furthermore, the grain yields of CDC

Palmer, NDC150001, NDC160078, NDC160166, NDC160186, and Royal were not significantly affected by seed treatment for 2020 (Figure 1.5). In contrast to the 2020 results, the effects of seed treatment on all cultivars were similar except for NDC160236 in 2021. Further study is needed to investigate the effect of seed treatment x cultivar interaction on yield.

Figure 1.5. Interactive effects of seed treatment and cultivar on yield (kg ha^{-1}) in 2020 (A) and 2021 (B). Dark gray bars represent chickpea yield with a seed treatment (fluxapyroxad, pyraclostrobin, metalaxyl, and thiamethoxam); light gray bars represent chickpea yield without seed treatment. *Represents a significant difference between untreated chickpea yield and treated chickpea yield at P< 0.05. Vertical bars represent the standard error (SE).

Seed Treatment and Cultivar Effects on Chickpea Protein Content

Chickpea protein content was only measured in 2021 because of upgraded NIR instrumentation capable of chickpea protein measurements procured that year. Seed treatment and cultivar had significant effects on chickpea protein content (P<0.01, P<0.01). However, the effect of interaction between seed treatment and cultivars was not observed (P=0.78). In 2021, seed treatment improved protein content of chickpea by 0.69% (P<0.01; Table 1.4). Cultivars showed variations in protein content, ranging from 17.1% to 21.0% (P<0.01, Table 1.5). NDC160049 produced the highest protein content followed by NDC160146 and NDC160138.

Table 1.4. The effect of fungicide seed treatment on AUDPC, yield, disease severity, and protein of chickpea in 2020 and 2021

Cultivar	2020		2021			
	AUDPC	Yield	AUDPC	Yield	Disease Severity	Protein
		— kg ha^{-1} —		— kg ha^{-1} —	(%)	(%)
Treated	233b[a]	2270a	390b	4202a	4.25b	18.94a
Untreated	1951a	1646b	1587a	3694b	4.86a	18.25b
P-value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.03**	**<0.01**

[a]Within columns, means followed by the same letter are not significantly different according to a Student's t-test (P<0.05) or Šidák-corrected multiple comparisons (P<0.05)

Table 1.5. The effect of 25 cultivars on AUDPC, yield and protein content of chickpea in 2020 and 2021

Cultivar	2020		2021		
	AUDPC	Yield	AUDPC	Yield	Protein
		-kg ha^{-1} -		-kg ha^{-1} -	(%)
CDC Frontier	1257efg	2181b-e	526c	4700ab	18.8b-f
CDC Leader	918ghi	1773d-h	541c	4200a-e	18.0c-g
CDC Palmer	1074fgh	1840 d-g	1230a	4194a-e	18.4c-g
MT043	1898a-d	1431gh	630bc	3723b-e	18.5c-g
MT044	1487b-g	1441gh	649bc	3356.de	19.1bcd
MT072	1913abc	1489fgh	1302a	4023a-e	18.4c-g
MT074	1679b-e	1600fgh	491c	3382de	18.8b-f
MT075	1358c-g	1772d-h	1046abc	3461c-e	19.2bcd
MT111	1999ab	1298h	826abc	3821b-e	17.8d-g
MT251	1886a-d	1447gh	853abc	3844b-e	18.0c-g
MT252	2300a	1587fgh	867abc	3484cde	17.2fg
MT394	1619b-f	1715e-h	801abc	3826b-e	18.8b-e
MT484	1423b-g	1901d-g	1158ab	3695c-e	17.4efg
MT485	1252efg	1492fgh	578c	4293a-d	17.1g
NDC150001	480ij	1967c-f	821abc	3450c-e	18.2c-g
NDC160049	281j	2553ab	1303a	4214a-e	21.0a
NDC160078	389ij	2261bcd	966abc	4233a-e	18.1c-g
NDC160133	218j	2636ab	610c	4415abc	19.2bcd
NDC160138	607hij	1835d-g	525c	4244a-e	20.2ab
NDC160146	331.j	2460b	950abc	3717cde	20.3ab
NDC160166	397ij	2401bc	669bc	3878b-e	19.2bcd
NDC160186	582hij	2542ab	684bc	4222a-e	19.4bc
NDC160236	302j	3028a	830abc	4978a	18.4c-g
Royal	331j	2499b	852abc	4081a-e	18.3c-g
Sierra	1324d-g	1804d-g	806abc	3269e	17.3efg
P-value	**<0.01**	**<0.01**	**0.07**	**0.07**	**<0.01**

[a]Within columns, means followed by the same letter are not significantly different according to a Student's t-tes (P<0.05) or Šidák-corrected multiple comparisons (P<0.05)

Correlation between Protein and Yield

A significant and negative correlation was observed between protein content and seed yield (R=-0.46; P<0.01) in 2021 (Figure 1.6). Protein content decreased with increased chickpea grain yield, but the data scattered widely.

Figure 1.6. Correlation coefficient of protein content with yield (kg ha^{-1}) in 2021. r represents the correlation coefficient.

Correlation between AUDPC and Yield

Grain yield and AUDPC of treated seeds did not correlate strongly in 2020 (R=-0.2, P=0.05) and 2021 (R=0.054, P=0.6). However, significant and negative correlations were observed between AUDPC and the grain yield of untreated seeds in 2020 and 2021 (Figure 1.7A, B). With the increase of AUDPC, the grain yield of untreated seeds decreased, indicating that yield losses might be attributed to chickpea damping-off and root rot in this study. The yield decline is steeper in 2021 than in 2020 with increased AUDPC.

Figure 1.7. Correlation coefficient of AUDPC of untreated chickpea seeds with yield (kg ha^{-1}) in 2020 (A) and 2021 (B). r represents the correlation coefficient.

Discussion

Results of this study indicated that seed treatment was effective in chickpea soilborne disease control. Previous studies suggested that metalaxyl reduces stand and yield loss in chickpea infected by *P. ultimum* (Kaiser & Hannan, 1983; Leisso et al., 2009). Fluxapyroxad and pyraclostrobin are broadly used to control root diseases attributed with *Rhizoctonia* spp. and *Fusarium* spp., as well as to protect chickpea from seedborne *A. rabiei* (Luo et al., 2020; Sharma et al., 2021; Wise et al., 2009). However, a single fungicide is not sufficient to control soilborne disease under field conditions due to the presence of various soilborne pathogens or the short window of efficacy for fungicidal seed treatments (Oyarzun et al., 1990; Xue, 2003). Therefore, seed treatments formulated with different fungicide active ingredients are recommended in chickpea soilborne disease management (Leisso, 2008).

In this study, all the isolates obtained from field study in 2021 were identified as *F. solani*,

and virulent on chickpea. This pathogen is considered as one of the most aggressive soilborne pathogens of chickpea in the Northern Great Plains, causing severe disease symptoms on chickpea and other pulse crops (Chang et al., 2003; Leisso et al., 2011). Application of fluopyram, penthiopyrad and prothioconazole in-furrow have been previously shown to be effective in field to control dry pea root rot caused by *F. solani* (Modderman et al., 2018). Previous studies have indicated that seed treatment is a sufficient management strategy for soilborne disease. Hwang et al. (2000a) reported that seed treatment with carbathiin and thiabendazole consistently improved seedling survival, reduced root rot severity, and increased seed yield of *F. avenaceum* inoculated lentil. Leisso et al. (2009, 2011) found that seed treatment with mefenoxam was effective in increasing seedling emergence and yield of kabuli chickpea at three field locations in Montana, where *Fusarium* spp. and *Pythium* spp. were recovered from kabuli chickpea seeds affected by damping-off. Therefore, fungicide seed treatments are sufficient to protect stand establishment and yield from chickpea soilborne disease. In this study, the disease severity of plants selected for disease identification was slightly reduced by seed treatment. Unlike the damping-off results, cultivars displayed similar levels of disease severity of Fusarium root rot. Safarieskandari et al. (2021) reported that chickpea cultivar and plant age had interactive effects on aggresiveness of *F. solani* f. sp. *pisi*., which was not observed in this study. Previous studies indicated that fungicide seed treatments are effective in limiting the reduction in emergence caused by soilborne pathogens (Chang, 2014; Gossen et al., 2016). Nevertheless, fungicide seed treatment may not be sufficient to manage late-season root rot (Willsey, 2018).

Genetic resistance of chickpeas to pathogens is critical for cultivar's defense against pathogens. Banniza et al. (2020) reported that CDC Leader, CDC Palmer, and CDC Frontier are

susceptible to *F. avenaceum*. *F. solani* can significantly decrease the emergence rate of CDC

Leader without producing symptoms on emerged plants (Safarieskandari et al., 2021). *Fusarium*

spp. and *Pythium* spp and *R. solani* can cause severe disease and yield loss in Sierra. (Agarwal et

al., 2021; Crutcher et al., 2021; Leisso et al., 2009). Above cultivars displayed higher

susceptibility than NDC160049, NDC160133, NDC160146, NDC160236, and Royal regardless

of seed treatment in 2020. These NDC lines and Royal yielded better than other susceptible

cultivars except for CDC Frontier. Thus, the above NDC lines and Royal may be more tolerant to

chickpea soilborne pathogens. However, this result was not observed in 2021. The main reason

could be that the root rot disease complex is different between the two locations. Moreover, the

AUDPC of NDC160166 and NDC160236 was not decreased by seed treatments for both years,

indicating that those two breeding lines are possibly more resistant than other cultivars to

chickpea soilborne disease.

In this study, seed treatment and genetic resistance have demonstrated significant effects

on chickpea yields. In both years, the yield reduction of untreated seeds was associated with

chickpea soilborne disease development. In contrast, AUDPC of treated seeds was not strongly

correlated with yield. Thus, correlation indicated that the AUDPC is a strong indicator for

chickpea yield when a seed treatment is not used.

This study suggests that the fungicide seed treatment can increase the grain yield of

chickpea (Chang, 2014; Hwang et al., 2000a, 2000b). Yield losses decreased by 12.1% to 27.5%

when a seed treatment was used. The 25 kabuli chickpeas genotypes evaluated in this study

yielded differently in 2020 and 2021. The breeding line NDC160236 achieved the highest yield

regardless of seed treatment for both years in this study. Compared to Sierra, the yield of

NDC160236 increased 52.3% to 67.8%. Previous yield trials conducted in Bozeman, MT, and

Moccasin; MT showed that NDC160236 is a high yielding line at these locations (McPhee,

personal communication, January 25, 2022). The NDC breeding lines yielded better than MT

lines in 2020 likely due to a negative effect of seedborne Ascochyta blight on seed germination

rate. Chickpea germination can be decreased by almost 50% when infested with *A. rabiei* (Wise et

al., 2009). These results support current recommendations for use of disease-free seed to reduce

disease development and improve chickpea yield. The Montana State University Regional Pulse

Crop Diagnostic Laboratory regularly performs seed health tests and recommends that seeds with

Ascochyta contamination more than 0.3% should not be used for planting (McKay et al., 2002).

Additionally, the seed health test results summarized here suggest that seed source and quality is

critical for effective experimental design. In 2020, the NDC160236, a high yield breeding line,

had a significantly higher yield of untreated seeds than treated seeds. However, it was not

observed in 2021, and the applied fungicide was not reported to have phytotoxicity at the used

rate on chickpea (Kaiser & Hannan, 1983). Therefore, it is most likely not statistically

representative.

Precipitation was significantly lower than average (< 200 mm) in 2020 and 2021. Thus,

the study was moved to an irrigated field in 2021. The previous crops of the 2020 and 2021 field

trials were wheat and sugar beets, respectively. Therefore, the root rot disease complex in those

fields might be affected by irrigation and different rotations (Liu et al., 2020; Moparthi et al.,

2021). Liu et al. (2020) reported that irrigation can affect the population and community of three

pathogens in soybean production. The population density of *Pythium* spp. and *Rhizoctonia* spp.

were higher under irrigation versus non-irrigation. In contrast, the population density of *Fusarium*

spp. in dryland conditions was higher than in irrigated. Pankhurst et al. (1995) suggested that pasture-wheat rotation reduced the *Pythium* spp. infection on wheat due to an increase of particulate organic material. Rupe et al. (1997) found that sorghum and wheat effectively reduce the soil population density of *F. solani* and control soybean sudden death syndrome. Pulse crops rotated with cereals for three years can suppress the *R. solani* population on the Northern Great Plains, where cereals are the only nonhost planted on large acreage (Gossen et al., 2016).

Seed treatment was effective in increasing protein content in our study. Previous research in soybean indicated that soybean inoculants can increase seeds protein content whereas seed treatment has little effect (Schulz & Thelen, 2008). However, seed treatment reduced the protein content when it was applied with an inoculant. One of the main reasons for this variation is the decrease in the viability of *Bradyrhizobium japonicum* on soybean seed caused by fungicide, which can reduce soybean nodulation (Revellin et al., 1993). Similar to soybean, the fungicide treatment can decrease the number of viable *Rhizobia* on the chickpea seeds, nodules, and N fixation (Kyei-Boahen et al., 2001). However, the decrease of nodulation caused by seed treatment is not a generalized response (Schulz & Thelen, 2008). Artificial *R. solani* inoculum resulted in little effect from seed treatment on faba bean nodulation (Chang, 2014). Seed treatment with the fungicide Thiram^{TM} can enhance root nodule production and reduce damping-off of pea and lentil, compare to untreated seeds (Huang & Erickson, 2007). Therefore, our study indicates the synthetic seed treatment might protect root growth and nodulation from soilborne pathogens to increase N fixation and protein content of chickpea. Therefore, our study indicates the combination of synthetic seed treatment and inoculant might protect root growth and nodulation from soilborne pathogens to increase N fixation and protein content of chickpea.

Twenty-five varieties had variable levels of protein contents, which is consistent with other studies (Kaur & Singh, 2005; Mafakheri et al., 2011; Zia-Ul-Haq et al., 2007). Although the negative correlation between protein content and seed yield are not considerably strong, previous research reported that an increment in protein content has a negative effect on chickpea seed yield (Gaur et al., 2016).

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CHAPTER THREE

INTERCROPPING CHICKPEA-FLAX FOR YIELD AND DISEASE MANAGEMENT

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ABSTRACT

Ascochyta blight (caused by *Ascochyta rabiei*) is a primary concern of chickpea production worldwide. Intercropping chickpea with a non-host crop has the potential to suppress this disease and increase the use of resources and crop yield. This study aimed to evaluate the effects of the seeding rate and row configuration in intercropping on 1) yield and seed quality and 2) the disease incidence and severity of Ascochyta blight. Field trials were conducted at the Eastern Agricultural Research Center (EARC), Sidney, MT**,** and the Southern Agricultural Research Center (SARC), Huntley, MT**,** in 2020 and 2021. Chickpea was planted with flax in 4 intercropping configurations (70% chickpea – 30% flax in mixture, 50% chickpea – 50% flax in alternate rows, 50% chickpea – 50% flax in mixture, and 30% chickpea – 70% flax in mixture). The seeding rates of monocrop chickpea and flax were 40 seeds m^{-2} and 730 seeds m^{-2}, respectively. In contrast to the flax yield, chickpea yield decreased with increased flax proportion in the mixed intercrops at both locations. The alternate row configuration with 50% chickpea – 50% flax seeding rate produced higher chickpea yield and lower flax yield than in the mixture configuration with the same chickpea and flax seeding rates in Sidney. Land equivalent ratio (LER) of intercropping was greater than 1, showing improved land productivity (2% -23% greater than monocropping). Flax was benefited from the intercropping in terms of nitrogen uptake; it produced 0.2% to 1.3% higher protein content in the intercrop than in the sole crop. The disease severity of Ascochyta blight in the intercropped chickpea decreased by 2.3% to 7.5% in Sidney and 4.8% to 9.4% in Huntley. These results indicated that seed ratio and configurations of intercropping chickpea-flax are critical in increasing resource efficiency and improving Ascochyta blight management.

Introduction

Intercropping is defined as an agronomic practice of simultaneously growing two or more crops in the same field (Ofori & Stern, 1987). The reported benefits of intercropping involve increased productivity, improved resource use efficiency, and enhanced pest control (Adeniyan et al., 2014; Chapagain & Riseman, 2014; Fenández-Aparicio et al., 2007). Yield benefits of intercropping two crops have been reported. Land Equivalent Ratio (LER) is the key metric for measuring the relative land area a crop requires as a monoculture to produce the same yield as under intercropping, which can display the yield advantage and land use efficiency of an intercropping system (Fletcher et al., 2016). A LER greater than one indicates a yield advantage of the intercropping over monoculture. The results form Echarte et al. (2011) suggested that intercropping sunflower-soybean with an optimal seeding ratio can improve the total yield and LER compared to the solo soybean. Chapagain and Riseman (2014) found that intercropping barley-pea with 2:1 and 1:1 planting ratio showed 12-32% higher LER than monocrop. Compared to solo barely, intercropped barley displayed higher biomass N, grain protein and sequestered higher C in the soil. Intercropping also increased nodulation and symbiotic N_2 fixation of intercropped pea. Seeding rate and row configuration affected the competition of companion crops, and thereby yield and land use efficiency. Chen et al. (2004) reported that pea produced lower biomass in mixed barely-pea intercrop than in separated row arrangement. However, barely-pea in mixture showed greater total biomass yield and higher LER than in a separated row arrangement. In an oat-pea intercropping system, land productivity based on grain yield increased with the sowing ratio of oat increased due to higher competition of oat than intercropped pea (Neugschwandtner & Kaul, 2014).

Chickpea (*Cicer arietinum* L.) was grown on approximately 69,000 hectares of land in Montana during 2020 (USDA-NASS, 2020). Montana is also the leading producer of organic chickpea in the US, where 700 ha were produced in 2019 (USDA-NASS, 2020). Ascochyta blight is the most threatening disease affecting chickpea production resulting in reduced seed quality and yield (Pande et al., 2005). Ascochyta blight is a host-specific disease caused by the fungal pathogen *Ascochyta rabiei*, which can cause black round lesions on all above-ground parts of chickpea plants (Nene, 1982). At the asexual stage, conidia are released from infected tissue and disperse to nearby plants via wind and rain splash. In the sexual stage, pseudothecia play an important role in the long-distance dispersal of ascospores, serving as primary inoculum to infect distant chickpea fields (Kaiser, 1997). The pathogen can survive in the field for at least five years under favorable conditions (Gossen & Miller, 2004). Therefore, rotation with non-host plants and the use of disease-free seeds are critical strategies to prevent pathogen introduction.

Integration of several different strategies is critical in Ascochyta blight management and include the use of resistant cultivars, planting disease-free seed, fungicide seed treatment, foliar fungicide application, and crop rotation (Chongo et al., 2003; Gan et al., 2006; Wise et al., 2009b). However, lack of durable resistance of chickpea cultivars to the disease and reported resistance to fungicide makes Ascochyta blight a major concern in conventional chickpea production (Chongo & Gossen, 2001; Wise et al., 2009a). In organic farming systems, disease management is based mainly on maintaining biological diversity and soil health using balanced crop rotations, including nitrogen-fixing and cover crops, intercrops, addition of manure and compost, and reductions in soil tillage (van Bruggen et al., 2016). There is no biological fungicide registered for use on organic chickpea for control of Ascochyta blight (Gan et al., 2006).

Intercropping may offer significant assistance in pest management due to variations in host physiology, direct pathogen inhibition, altered canopy microclimates, reduction in host density, and barrier effects of intercrops (Boudreau, 2013; Schoeny et al., 2010). Previous studies have reported that two intercropped species can effectively control foliar disease in pulse crops (Fernández-Aparicio et al., 2010; Schoeny et al., 2010). Fernández-Aparicio et al. (2010) suggested that when pea was intercropped with faba bean, barley, oat, triticale, or wheat, the disease severity of pea Ascochyta blight and vertical progress of lesions were significantly decreased. Similar results were observed by Schoeny et al. (2010) that intercropping pea-wheat decreased the splash dispersal of conidia in a controlled environment and reduced disease severity of Ascochyta blight in pea in the field with moderate to severe disease pressure. The disease reduction was associated with reduction of host density altered microclimate and physical barrier to spore dispersal. It is not well documented if chickpea intercropped with flax will reduce Ascochyta infection and spread. Although some growers have tested intercropping chickpea-flax in the Northern Great Plains, research needs to be conducted to investigate the effect on disease management and seed yield potential (Reid et al., 2020).

Previous legume-based intercropping research initially focused on general performance metrics, such as land productivity, nutrient uptake indices, disease pressure, and crop competition (Njira et al., 2021). Since chickpea is the major cash crop, it is important to know how intercropping affecting chickpea yield in addition to the total combined yield and LER. The effect of configuration and spatial arrangement in intercropping chickpea-flax on protein content of intercrops, oil content of flax, land use efficiency, and Ascochyta blight management has not been investigated. The objectives of this study are to (i) evaluate the effects of seeding rate and row

configuration on yield, protein, and LER, and (ii) investigate the seeding rate and row configuration effect on Ascochyta blight infection and severity.

Materials and Methods

Trial Sites

An intercropping chickpea-flax study was conducted at the Eastern Agricultural Research Center (EARC) in Sidney, MT and the Southern Agriculture Research Center (SARC) in Huntley, MT. In Sidney, the field trials were located at the EARC dryland farm (47∘46′ N, 104∘14′ W; 670 m asl) and the EARC irrigated farm (47∘73′ N, 104∘15′ W; 594 m asl) in 2020 (April 21st to August 25th) and in 2021 (May 12th to August 16th), respectively. In Huntley, the field trails were located at the SARC irrigated farm (45.92′ N, 108∘24′ W, 594 m asl) in 2020 (April 27th to August 24th) and 2021 (April 28th to August 31st). The soil and weather conditions varied among locations. The soils are William clay loam (fine-loamy, mixed, superactive, frigid Vertic Argiustolls) at the dryland farm in Sidney, Savage clay loam (fine, smectitic, frigid Vertic Argiustolls) at the irrigated farm in Sidney, and Lohmiller silty clay loam (fine, smectitic, calcareous, mesic Torrertic Ustifluvents) in Huntley. A composite soil sample was collected prior to planting the intercrop. The soil test results of nitrate and phosphate are listed in Table 2.1. Monthly average precipitation and air temperature from April to August at each location in 2020 and 2021 are summarized in Table 2.2. Field trials were conducted under irrigation during the 2021 season due to extreme drought conditions in Sidney and received 100 mm irrigation. The intercrop trials conducted in Huntley during 2020 and 2021 seasons received 120 mm and 180 mm irrigation, respectively.

<u>Experiment Design and Field Management</u>

During the 2020 and 2021 season, two chickpea cultivars, CDC Leader (moderately

resistant to *A. rabiei*) and Royal (susceptible to *A. rabiei*) and a flax cultivar, Glas, were selected

for the intercropping study. The experiment was a randomized complete block design. Treatments

included 1) chickpea grown as monocrop with a sowing rate of 40 seeds m^{-2}, 2) as a mixture with

30% monocrop chickpea and 70% monocrop flax (30C/70F in mixture), 3) as a mixture with 50%

monocrop chickpea and 50% monocrop flax (50C/50F in mixture), 4) with 50%-50% ratio of

monocrop chickpea and monocrop flax in alternate rows (50C/50F in alternate rows), 5) as a

mixture with 70% monocrop chickpea and 30% monocrop flax (70C/30F in mixture), 6) flax

planted as monocrop with 730 seeds m^{-2}. Two chickpea cultivars with the six seeding rates and

row configurations were randomly assigned to each plot within the block and replicated 4 times.

Plot size and row spacing varied between locations based on planter specifications. Plot sizes

were 1.5 m $\times$ 6 m in Sidney and 1.5 m $\times$ 7 m in Huntley. In Sidney, each plot contained six rows

of intercrops at 23 cm row spacing (Figure 2.1A). In Huntley, plots were four rows for both

monocrop and intercrops in the mixture at 37 cm row spacing (Figure 2.1B). For plots with 50%

chickpea and 50% flax in alternate rows, each plot contained four rows of flax and three rows of

chickpea planted alternatively with a row spacing of 18 cm. In Sidney, chickpea and flax were

planted at 2.5 cm depth to accommodate the flax on April 21st, 2020 and May 20th, 2021. In

Huntley, the sowing depth of component crops was adjusted for chickpea at 3.8 cm. The planting

dates were April 27th, 2020 and April 28th, 2021 in Huntley.

Chickpea seeds were treated with thiamethoxam (Cruiser® 5FS, BASF Corporation,

Research Triangle Park, NC) insecticide at a rate of 0.5 g a.i. kg^{-1} seed and thiamethoxam and

fluxapyroxad, pyraclostrobin, and metalaxyl (Obvius® fungicide, BASF Corporation, Research

Triangle Park, NC) fungicide at a rate of 0.18 g a.i. kg^{-1} seed prior to planting. At planting, it was

inoculated with a commercial rhizobial inoculant (Primo GX2 Verdesian Life Sciences, Cary,

NC).

Figure 2.1 Diagrams of monocrop and intercrops row configurations in Sidney (A) and in Huntley (B). Continuous lines represent chickpea rows and dash lines correspond to flax rows. C: chickpea; F: flax. 70C/30F represents 70% chickpea and 30% flax. 50C/50F represents 50% chickpea and 50% flax. 30C/70F represents 30% chickpea and 70% flax.

Table 2.1. Initial soil test results of field trials in Sidney and Huntley for 2020 and 2021

Field trial	Year	Depth	NO$_3$-N	P-Olsen
		cm	ppm	
Sidney-Dryland Farm	2020	0-61	11	21
Sidney-Irrigated Farm	2021	0-61	5	23
Huntley-Irrigated Farm	2020	0-61	23	17
Huntley-Irrigated Farm	2021	0-61	26	20

Table 2.2. Maximum, minimum, and mean temperature and total precipitation (± SE) from April–August for Sidney, MT and Huntley, MT in 2020 and 2021.

Month	Precipitation	Temperature			Precipitation	Temperature		
		2020				2021		
	-mm-	————C°————			-mm-	————C°————		
		Maximum Air Temperature	Minimum Air Temperature	Average Air Temperature		Maximum Air Temperature	Minimum Air Temperature	Average Air Temperature
Sidney								
April	0.51(±0.012)	11.52(±1.68)	-3.67(±1.20)	3.92(±1.40)	4.32(±0.003)	14.32(±1.49)	2.61(±0.69)	5.85(±0.93)
May	40.67(±0.64)	19.63(±1.07)	5.18(±0.85)	12.40(±0.89)	41.66(±0.02)	18.79(±1.06)	4.52(±0.66)	11.65(±0.74)
June	26.67(±0.49)	25.87(±0.64)	11.24(±0.63)	18.56(±0.65)	52.60(±0.04)	28.50(±0.72)	11.91(±0.57)	20.21(±0.56)
July	45.21(±0.64)	29.16(±0.76)	13.52(±0.47)	21.34(±0.49)	0.51(±0.00)	32.22(±0.67)	15.76(±0.47)	24.08(±0.47)
August	23.14(±0.57)	31.33(±0.76)	7.12(±11.72)	22.52(±0.58)	27.18(±0.02)	28.20(±1.03)	11.89(±0.55)	20.05(±0.64)
September	8.38(±0.24)	22.82(±0.96)	7.12(±0.69)	14.97(±0.76)	10.62(±0.01)	26.01(±0.93)	7.05(±0.51)	16.53(±0.57)
Total	144.58				136.89			
Huntley								
April	20.07(±0.38)	12.61(±1.57)	-3.56(±1.01)	4.56(±1.23)	30.23(±0.47)	14.5(±1.27)	-2.11(±0.66)	6.17(±0.86)
May	40.13(±0.37)	19.83(±1.02)	5.56(±0.79)	12.67(±0.78)	49.02(±0.58	19.89(±1.01)	4.39(±0.54)	12.11(±0.72
June	120.65(±1.67)	26.22(±1.04)	10.72(±0.55)	18.5(±0.71)	9.65(±0.15)	31.27(±0.77)	10.88(±0.59)	21.06(±0.62)
July	26.67(±0.53)	30.83(±0.56)	11.78(±0.52)	21.33(±0.50)	3.3(±0.06)	34.89(±0.64)	13.94(±0.57)	24.44(±0.52)
August	18.29(±0.36)	32.06(±1.18)	11.67(±0.59)	21.89(±0.85)	37.85(±0.56)	29.05(±1.12)	10.89(±0.57)	19.94(±0.77)
September	13.97(±0.42)	25.72(±1.43)	5.67(±0.49)	15.72(±0.90)	1.78(±0.05)	27.05(±0.95)	5.78(±0.72)	16.44(±0.75)
Total	239.78				131.83			

Measurements

Grain Yield, Protein, and Oil Concentration.Chickpea and flax were harvested on August 25, 2020 and August 16, 2021 in Sidney, and August 24, 2020, and August 31, 2021 in Huntley using a plot combine harvester (Wintersteiger, Salt Lake City, Utah). After harvesting, the total grain weight was determined from each plot. The flax and chickpea were then separated using a screen to determine the yield of each companion crop under intercropping. The chickpea and flax yield data are reported on drymass basis.

Fifty grams of chickpea and five grams of flax seed samples were homogenized into a fine powder (<6 mm) using a the UDY cyclone sample mill (UDY Corporation, Fort Collins, CO). Meanwhile, the moisture content of the ground sample was measured by weighing 1.5 g samples prior to and following a 48-hr oven-drying at 65 °C (Jones Jr. & Case, 1990). Seed nitrogen (N) concentrations of chickpea and flax were determined by the Pregl-Dumas method on a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer Inc., Waltham, MA). Seed protein concentration was calculated by multiplying the total grain N concentration to a N-to-protein conversion factor of 6.25. The protein concentration reported in this paper is on drymass basis.

Flax oil content was measured by a nuclear magnetic resonance analyzer (NMR) (Oxford Instruments Industrial Analysis Group, Abingdon, Oxon.). Four grams of flax seed were weighed in 40 ml sample tubes and calibrated using a sample of pure flaxseed oil. The readings are expressed as the percentage oil content on drymass basis.

Disease Evaluation.Ten plants were selected from each plot to evaluate the disease severity (DS) and disease incidence (DI) when symptoms were observed in the field. DS was

determined by a percentage scale developed by Gowen et al. (1989) (Table 2.3). The infected

plants were counted to calculate DI by the following formula.

$$DI(\%) = \frac{number\ of\ infected\ plants}{10 \times 100\%}$$

Table 2.3. Rating scale for disease severity applied to each plant.

% Infection	Symptoms
0-10	No infection - small lesions
11-20	Some stem lesions - minor stem breakage in upper foliage
21-30	1-2 branches broken - several girdling stem lesions low down on some branches
31-40	Large basal stem lesions or several branches broken near to main stem
41-50	Half foliage dead or partly severed
51-60	> Half foliage dead or dying, young shoots still actively growing from base
61-70	Most foliage dead - some healthy stem tissue with lateral buds
71-80	Most foliage dead, no healthy lateral buds in leaf axils
81-99	Most foliage dead, decreasing areas of living stem tissue
100	Plants completely dead

Data Analysis

LER was calculated to compare the yields of companion crops obtained from intercropping

chickpea-flax to the yields obtained from monocultured crops. The LER was calculated (Mead &

Willey, 1980) as follows:

$$LER = LERc + LERf = \frac{intercrop\ yield_{chickpea}}{monocrop\ yield_{chickpea}} + \frac{intercrop\ yield_{flax}}{monocrop\ yield_{flax}}$$

Data were analyzed separately by location due to different configurations among locations. Yield, LER, protein, oil, DI, and DS were analyzed using R studio (Version 4.0.3, R Studio Team, 2020). The beta regression model was used to analyze the percentage data (DS, DI, protein content, and oil content) by the *betareg* package because they are nature bound at 0 and 1, and not normally distributed or homoscedastic. (Cribari-Neto & Zeileis, 2010, Kieschnick & McCullough, 2003). An ANOVA-like table for factor terms was produced with the *joint_tests* function in the *emmeans* package, which was based on linear functions of predictors in a beta regression model (Lenth et al., 2019). The posthoc mean comparisons were conducted by Šidák-corrected multiple comparisons to control for familywise error rates using *emmeans* and *multcomp* package (Hothorn et al., 2008). Šidák-correction or Dunn–Šidák correction is an appropriate nonparametric pairwise multiple-comparison procedure (Dinno, 2015). Analysis of variance (ANOVA) was performed on yield and LER for data from Sidney and Huntley. The effects were considered statistically significant at $p < 0.05$. Tukey's HSD test was conducted for mean comparisons with 5% significance levels.

Results

Seed Yield

The chickpea and flax seed yields in both monocrop and intercrop were assessed for each field trial. Since the growing environments are very different at the Sidney and Huntley sites, data were analyzed separately for the two locations.

Sidney Site:The ANOVA results showed that chickpea yield was significantly affected by year, cultivar, configuration, and configuration x year interactions in Sidney (Table 2.4).

Chickpea yielded greater in 2021 (3297 kg ha^{-1}) than 2020 (1850 kg ha^{-1}) due to irrigation (Figure 2.2B). Royal produced a higher yield (1450 kg ha^{-1}) than CDC Leader (1272 kg ha^{-1}) (Figure 2.2A). Although ANOVA analysis showed significant year x configuration interactions, the effects of configurations showed the similar trend in 2020 and 2021 (Figure 2.2B). The highest chickpea yield was observed for monocrop chickpea, and chickpea yield decreased with reduced chickpea seeding rate and increased flax proportions in mixed intercrop. Configurations with 50C/50F in alternate row produced similar chickpea yield as 70C/30F in mixture, and significantly higher than 50C/50F in mixture (Figure 2.2B).

Flax yield was significantly affected by year, configuration, and configuration $\times$ year interaction (Table 2.4). Like chickpea yield, flax produced a higher grain yield in 2021 than in 2020 (Figure 2.2C). Monocrop flax had the highest yield for both years (Figure 2.2C). Flax yield decreased with increased chickpea proportion in the mixed intercrop for both years. However, the significant decrease in flax yield in intercrop was only observed for mixed 70C/30F in 2020. The alternate row configuration with 50C/50F displayed a significantly lower flax yield than that in mixture for 2021, but not in 2020.

Significant differences among years, cultivars, and configurations were observed in chickpea and flax combined yield. Effect of configuration $\times$ year interaction also was detected for combined yield. Chickpea and flax had a greater combined yield in 2021 than in 2020 (Figure 2.2E). The combined yield demonstrated a similar trend in both years, i.e., the yield decreased with the increased flax proportion in the mixed intercrops, ranging from 939 to 1228 kg ha^{-1} in 2020 and 1885 to 2584 kg ha^{-1} in 2021. The alternate row configuration with 50C/50F yielded higher than 50C/50F in mixture in 2021. However, there was no significant difference of

combined yield between 70C/30F in mixture, 50C/50F in alternate rows, and 50C/50F ratio in

mixture for 2020.

Table 2.4. Analysis of variance (ANOVA) table showing the effects of year, cultivar, and configuration on chickpea yield, flax yield, and combined yield of chickpea and flax in Sidney.

Source of Variance	df	Chickpea Yield $P>F$	df	Flax Yield $P>F$	df	Combined Yield $P>F$
		kg ha^{-1}				
Year	1	**<0.01**	1	**<0.01**	1	**<0.01**
Cultivar	1	**<0.01**	1	0.56	1	**0.01**
Configuration	4	**<0.01**	4	**<0.01**	5	**<0.01**
Cultivar × Year	1	0.18	1	0.54	1	0.14
Configuration × Year	4	**<0.01**	4	**<0.01**	5	**<0.01**
Cultivar × Configuration	4	0.2	4	0.92	5	0.18
Cultivar × Configuration × Year	4	0.29	4	0.91	5	0.41

Figure 2.2. Effect of cultivar on chickpea yield in Sidney (A); interactive effects of configuration and year on chickpea yield in Sidney (B); interactive effects of configuration and year on flax yield in Sidney (C); effect of cultivar on chickpea and flax combined yield in Sidney (D); interactive effects of configuration and year on chickpea and flax combined yield in Sidney (E). C: chickpea, F: flax. C+F yield represents chickpea and flax combined yield. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Tukey's HSD test. Vertical bars represent the standard error (SE).

Huntley Site:In Huntley, chickpea yield was significantly affected by cultivar and configuration (Table 2.5). There were significant cultivar x configuration interactions (Table 2.5). Two chickpea cultivars yielded differently among configurations, i.e., CDC Leader produced higher seed yield than Royal, which was contrary to the Sidney site (Figure 2.3A). The yield of CDC Leader varied among five configurations, ranging from 1534 to 3941 kg ha^{-1}. The yield decreased with increased flax proportion in the intercrop. In contrast, there was no significant difference between monocrop and other intercropping configurations in chickpea yield of Royal except for 30C/70F. Chickpea yield in 30C/70F was lower compared to monocrop chickpea. The chickpea yield of Royal ranged from 788 to 1729 kg ha^{-1}.

Flax yield varied among configurations (Table 2.5). There were significant configurations × year interaction on flax yield. The mixture with 30C/70F and 50/C/50F produced similar flax yield to monocrop flax in 2020 (Figure 2.3B). However, monocrop flax demonstrated highest flax yield in 2021, which was significantly higher than all intercropping configuration.

There were significant differences among cultivars and configurations in chickpea and flax combined yield (Table 2.5). An interaction between cultivar and configuration was observed (Table 2.5). CDC Leader and flax in monocrop chickpea produced the highest combined yield, which was not significantly different from CDC Leader and flax in 70C/30F mixture (Figure 2.3C). Other intercropping configurations for CDC Leader and flax showed lower combined yield than in the monocrop. There were no differences in the combined yield between 50C/50F mixture and alternate rows. The combined yield for CDC Leader and flax ranged from 2456 to 3985 kg ha^{-1}. However, Royal in chickpea-flax intercropping system produced similar combined yield as

in the monocrop chickpea and flax (Figure 2.3C). The combined yield of Royal and flax ranged

from 1745 to 2213 kg ha^{-1}.

Table 2.5. ANOVA table showing the effects of year, cultivar, and configuration on chickpea yield, flax yield, and combined yield of chickpea and flax in Huntley.

Source of Variance	df	Chickpea Yield P>F	df	Flax Yield P>F	df	Combined Yield P>F
				kg ha^{-1}		
Year	1	0.09	1	0.18	1	0.09
Cultivar	1	**<0.01**	1	0.36	1	**<0.01**
Configuration	4	**<0.01**	4	**<0.01**	5	**<0.01**
Cultivar × Year	1	0.57	1	0.99	1	0.75
Configuration × Year	4	0.08	4	**<0.01**	5	0.14
Cultivar × Configuration	4	**<0.01**	4	0.9	5	**<0.01**
Cultivar × Configuration × Year	4	0.55	4	0.95	5	0.72

Figure 2.3. Interactive effect of cultivar and configuration on chickpea yield in Huntley (A); interactive effects of configuration and year on flax yield in Huntley (B); interactive effects of cultivar and configuration on chickpea and flax combined yield in Huntley (C). C: chickpea, F: flax. C+F yield represents chickpea and flax combined yield. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Tukey's HSD test. Vertical bars represent the standard error (SE).

Land Equivalent Ratio

Sidney site:The ANOVA for combined data of Sidney site across years showed significant effects of year, cultivar, and configuration on LER and LERc (Table 2.6). LERc is higher in 2021 than in 2020 (Figure 2.4A). LERc of Royal was higher than that of CDC Leader by 0.03 (Figure 2.4B). The LERc value of intercropping chickpea-flax ranged from 0.24 to 0.48 (Figure 2.4C). LERc was decreased as the flax proportion increased in mixed seed configurations. The LERc of alternate rows with a 50C/50F seeding rate was higher than in the mixture.

LERf varied among configurations (Table 2.6). It ranged from 0.54 to 0.78 in different configurations of intercropping chickpea-flax (Figure 2.4D). LERf of 50C/50F in mixture was similar to 30C/70F in mixture, but higher than 50C/50F in alternate rows.

LER was not affected by cultivar, but was significantly affected by configurations (Table 2.6). Alternate rows with a 50C/50F seeding rate had the highest LER, improving LER by 13% when compared with the monocrop (Figure 2.4E).

Table 2.6. ANOVA table showing the effects of year, cultivar, and configuration on Land equivalent ratio of chickpea (LERc), Land equivalent ratio of flax (LERf), and Land equivalent ratio (LER) in Sidney.

Source of Variance	df	LERc $P>F$	df	LERf $P>F$	df	LER $P>F$
Year	1	**<0.01**	1	0.3	1	0.84
Cultivar	1	**0.047**	1	0.06	1	0.92
Configuration	4	**<0.01**	4	**<0.01**	5	**<0.01**
Cultivar × Year	1	0.14	1	0.71	1	0.69
Configuration × Year	4	0.33	4	0.06	5	0.33
Cultivar × Configuration	4	0.15	4	0.48	5	0.95
Cultivar × Configuration × Year	4	0.55	4	0.59	5	1

Figure 2.4. Effect of year (A), cultivar (B), and configuration (C) on chickpea land equivalent ratio (LERc) in Sidney; effects of configuration on land equivalent ratio of flax (LERf) (D) in Sidney; and effects of configuration on combined land equivalent ratio (LER) in Sidney (E). C: chickpea, F: flax. LERc represents land equivalent ratio of chickpea. LERf represents land equivalent ratio of flax. LER represents combined land equivalent ratio of chickpea and flax. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Tukey's HSD test. Vertical bars represent the standard error (SE).

 Huntley Site:In Huntley, ANOVA was performed for LERc, LERf, and LER of two years of combined data.

Cultivar and row configuration had significant effects on LERc (Table 2.7). The cultivar performs in LERc under different configurations were presented due to the moderate (p=0.07) cultivar ✕ configuration interactions. Similar to chickpea yield, LERc of Royal was not significantly decreased by intercropping chickpea-flax compared to monocrop chickpea, excluding 30C/70F in the mixture, which was decreased by 54% (Figure 2.5A). In contrast, all intercropping configurations reduced the LERc of CDC Leader. For both cultivars, the value of LERc was greater than 0.5 except for 30C/70F in the mixture. The value of LERc in 30C/70F configuration was 0.46 and 0.40 for Royal and CDC Leader, respectively.

There is a significant interaction of year and configuration on LERf in Huntley (Table 2.7). Configurations of 50C/50F and 30C/70F in the mixture showed similar levels of LERf to monocrop flax in 2020. The lowest LERf (0.62) was observed in the mixture with 30C/70F in 2020. The value of LERf decreased with increased chickpea proportion in the mixture for 2021, ranging from 0.34 to 0.62 (Figure 2.5B).

The value of LER was significantly affected by year, configuration, and year ✕ configuration interaction in Huntley. In 2020, all intercropping significantly improved LER by 30% to 67%. Alternate rows with 50C/50F demonstrated lower LER than the mixture. In 2021, the value of LER was significantly increased by the mixture configuration with 50C/50F (23%) compared to monocropping.

Table 2.7. ANOVA table showing the effects of year, cultivar, and configuration on LERc, LERf, and LER in Huntley.

Source of Variance	df	LERc $P>F$	df	LERf $P>F$	df	LER $P>F$
Year	1	0.09	-	-	-	-
Cultivar	1	**<0.01**	1	0.17	1	**0.04**
Configuration	4	**<0.01**	4	**<0.01**	5	**0.05**
Cultivar × Year	1	0.77	-	-	-	-
Configuration × Year	4	0.25	-	-	-	-
Cultivar × Configuration	4	0.07	4	0.34	5	0.37
Cultivar × Configuration × Year	4	0.84	-	-	-	-

Figure 2.5. Interactive effect of cultivar × configuration on land equivalent ratio of chickpea (LERc) in Huntley (A); interactive effects of year × configuration on land equivalent ratio of flax (LERf) in Huntley (B); interactive effects of year × configuration on combined land equivalent ratio (LER) in Huntley (C). C: chickpea, F: flax. LERc represents Land Equivalent Ratio of chickpea. LERf represents Land Equivalent Ratio of flax. LER represents Land Equivalent Ratio of chickpea and flax. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Tukey's HSD test. Vertical bars represent the standard error (SE).

Chickpea Protein Content, Flax Protein Content, and Flax Oil Content

Sidney Site:In Sidney, chickpea protein content was affected by year, cultivar, and row configuration (Table 2.8). The cultivar × year, configuration × year, and cultivar × configuration interactions were also detected (Table 2.8). Royal displayed a higher protein content than CDC Leader in 2020, but not in 2021 (Figure 2.6A). There was no significant difference in chickpea protein between monocrop and intercrop in 2020 (19.3 to 19.7%) (Figure 2.6B). In 2021, however, the chickpea protein content under five configurations ranged from 16.9 to 18.6%, and the 50C/50F mixture and 30C/70F mixture of chickpea and flax had lower protein contents compared to monocrop for CDC Leader chickpea (Figure 2.6C). The protein content of CDC Leader ranged from 17.5 to 18.8%. Royal in four intercropping configurations showed similar protein levels as in the monocropping. The protein content of Royal ranged from 18.7 to 19.4%.

Flax protein content varied among years and configurations in Sidney (Table 2.8). There were also interactions between configuration and year (Table 2.8). Flax protein significantly increased in intercropping in 2020, except the 30C/70F in mixture (Figure 2.6D). In 2021, however, only the alternate row arrangement had an improved flax protein.

Year, row configuration, and year × configurations interactions had significant effects on flax oil content (Table 2.8). The highest flax oil content was observed in monocrop flax for both years (45.7% in 2020, 44.8% in 2021) (Figure 2.6E). Lower oil concentration was observed in all intercropping configurations in 2020, and in 50C/50F alternate rows and mixture configurations in 2021.

Table 2.8. ANOVA table showing the effects of year, cultivar, and configuration on chickpea protein content, flax protein content, and flax oil content in Sidney.

Source of Variance	df	Chickpea Protein Content	df	Flax Protein Content	df	Flax Oil Content
		%		%		%
		P>F		*P>F*		*P>F*
Year	1	**<0.01**	1	0.53	1	**<0.01**
Cultivar	1	**<0.01**	1	0.87	1	0.23
Configuration	4	**<0.01**	4	**<0.01**	4	**<0.01**
Cultivar × Year	1	**<0.01**	1	0.09	1	0.42
Configuration × Year	4	**<0.01**	4	**<0.01**	4	**0.05**
Cultivar × Configuration	4	**<0.01**	4	0.14	4	0.12
Cultivar × Configuration × Year	4	0.09	4	0.34	4	0.73

Figure 2.6. Interactive effects of cultivar and year on chickpea protein content in Sidney (A); interactive effects of configuration and year on chickpea protein content in Sidney (B); interactive effects of cultivar and configuration on chickpea protein content in Sidney (C); interactive effects of configuration and year on flax protein content in Sidney (D); interactive effects of configuration and year on flax oil content in Sidney (E). 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Šidák-corrected multiple comparisons. Vertical bars represent the standard error (SE).

Huntley Site:Chickpea protein content was affected by year and cultivar in Huntley (Table 2.9). The interaction of configuration × year and cultivar × configuration was observed. The chickpea protein content under monocrop chickpea and four intercropping configurations ranged from 20.7 to 22.4% in 2020 and 24.8 to 26.2% in 2021(Figure 2.7A). Royal (22.6 to 24.9%) produced higher protein than CDC Leader (22.6 to 23.8%) (Figure 2.7B). The configuration of 30C/70F significantly decreased chickpea protein content in 2020 (Figure 2.7A). The significant reduction of chickpea protein content was also detected Royal in mixed 70C/30F (Figure 2.7B).

Configuration and the configuration × year interactions had significant effects on flax protein content in Huntley. The highest flax protein was observed for 70C/30F in the mixture (27.8%) in 2020, which was significantly higher than 50C/50F in alternate rows (Figure 2.7C). Flax in intercrop and monocrop displayed a similar level of protein. In 2021, there was no difference in flax protein content between intercropping configurations and monocropping.

Flax oil content varied among configurations, ranging from 39.9 to 44.0% (Table 2.9, Figure S2D). A mixture of 70C/30F and 50C/50F increased flax oil content by 3.8%. Other configurations produced the same level of flax oil content as in monocropping.

Table 2.9. ANOVA table showing the effects of year, cultivar, and configuration on chickpea protein content, flax protein content, and flax oil content in Huntley.

Source of Variance	df	Chickpea Protein Content	df	Flax Protein Content	df	Flax Oil Content
		%		%		%
		$P>F$		$P>F$		$P>F$
Year	1	**<0.01**	1	<0.01	1	**0.1**
Cultivar	1	**<0.01**	1	0.21	1	0.7
Configuration	4	**0.06**	4	**0.04**	4	**<0.01**
Cultivar × Year	1	**0.051**	1	0.21	1	0.3
Configuration × Year	4	**0.03**	4	**0.03**	4	**0.2**
Cultivar × Configuration	4	**0.02**	4	0.75	4	1
Cultivar × Configuration × Year	4	0.82	4	0.34	4	0.6

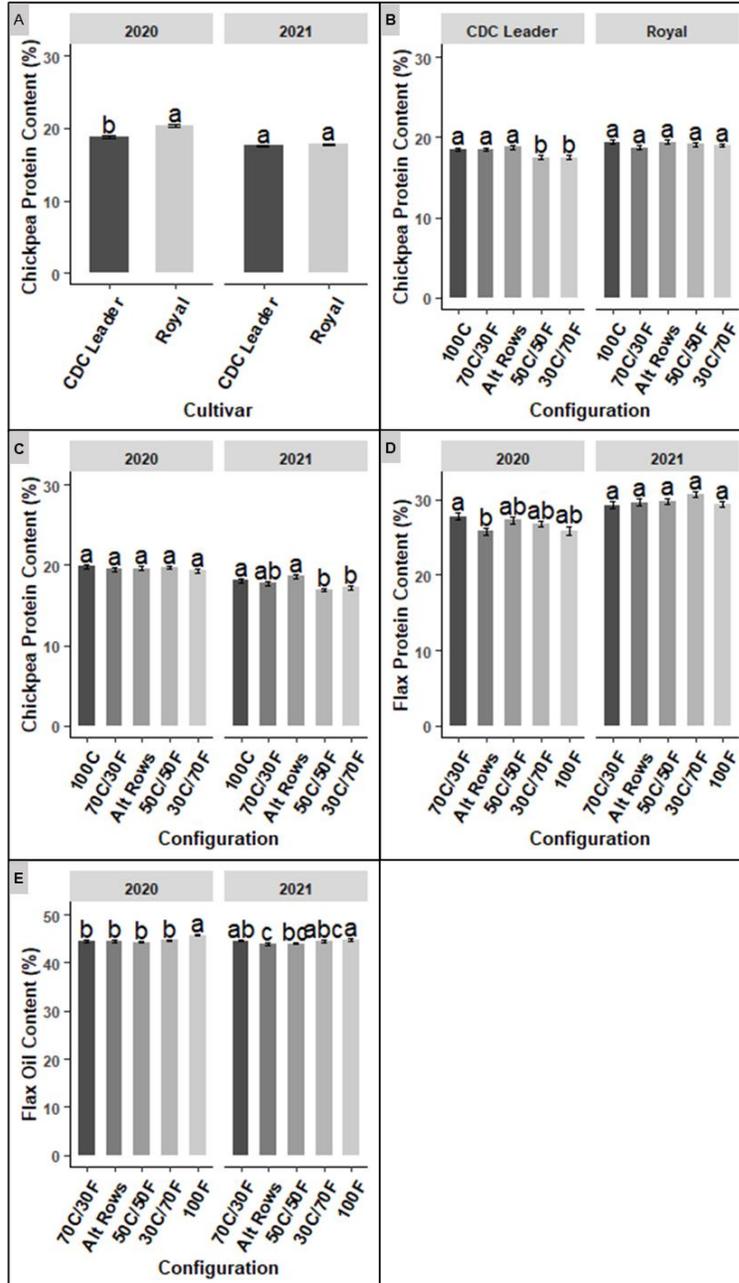**Figure 2.7.** Interactive effects of configuration and year on chickpea protein content in Huntley (A); interactive effects of cultivar and configuration on chickpea protein content in Huntley (B); interactive effects of configuration and year on flax protein content in Huntley (C); effects of configuration on flax protein content in Huntley (D). C: chickpea; F: flax. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. 100F represents monocrop flax. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Šidák-corrected multiple comparisons. Vertical bars represent the standard error (SE).

Disease Assessment

Sidney Site:In Sidney, the DS and DI were analyzed separately for each year due to the

cultivar × configuration × year interactions (Table 2.10). Effect of cultivar on DS and DI was only

observed in 2020. DS and DI were significantly affected by configuration and variety ×

configuration interactions in both years. DS of Royal was higher than CDC Leader in 2020,

ranging from 2.18% to 5.45% (Figure 2.8A). For Royal, all intercropping configurations showed

similar DS to monocrop chickpea in 2020 except for 50C/50F in mixture. CDC Leader in

70C/30F mixture and 30C/70F mixture significantly reduced DS compared to monocrop. Similar

to DS, Royal had significant higher DI than CDC Leader, ranging from 34% to 87% (Figure

2.8B). For Royal, the highest DI was observed for 70C/30F mixture and the lowest DI in the

50C/50F mixture. All intercropping configurations showed a similar level of DI to monocropped

Royal chickpea in 2020. For CDC Leader, a significant DI reduction in 70C/30F mixture and

30C/70F mixture than in monocropping was observed in 2020.

In 2021, intercropping decreased DS of chickpea in all intercropping configurations

compared to the monocropping (Figure 2.8C). All intercropping configurations had similar effects

on DS reduction in Royal. In contrast, CDC Leader in 30C/70F mixture and 50C/50F mixture has

a significantly greater reduction of DS than that in 70C/30F mixture. The DS of Royal ranged

from 4.8 to 10.2%; and the DS of CDC Leader ranged from 2.4 to 12.7%. The DI showed a

similar trend to DS (Figure 2.8D). The comparable reduction of DI from monocropping to

intercropping was also observed in Royal. All intercropping configurations had similar effects on

DI reduction of Royal. The reduction of DI was not observed for CDC Leader in 50C/50F in

alternate rows (Figure 2.8D). Although intercropping chickpea-flax in mixture decreased DI of

CDC Leader, there is no difference in DI reduction among three mixed intercropping

configurations.

Table 2.10. ANOVA table showing the effects of year, cultivar, and configuration on disease severity (DS) and disease incidence (DI) in Sidney.

Source of Variance	df	Disease Severity (%)	Disease Incidence (%)
		$P>F$	$P>F$
Year	1	**<0.01**	**<0.01**
Cultivar	1	**<0.01**	**<0.01**
Configuration	4	**<0.01**	**<0.01**
Cultivar × Year	1	**<0.01**	**<0.01**
Configuration × Year	4	**<0.01**	0.23
Cultivar × Configuration	4	0.06	**<0.01**
Cultivar × Configuration × Year	4	**<0.01**	**<0.01**
2020			
Cultivar	1	**<0.01**	**<0.01**
Configuration	4	**<0.01**	**<0.01**
Cultivar × Configuration	4	**0.02**	**<0.01**
2021			
Cultivar	1	0.92	0.25
Configuration	4	**<0.01**	**<0.01**
Cultivar × Configuration	4	**0.02**	**<0.01**

Figure 2.8. Interactive effects of cultivar and configuration on disease severity in 2020 in Sidney (A); interactive effects of cultivar and configuration on disease incidence in 2020 in Sidney (B); interactive effects of cultivar and configuration on disease severity in 2021 in Sidney (C); interactive effects of cultivar and configuration on disease incidence in 2021 in Sidney (D). C: chickpea; F: flax. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Šidák-corrected multiple comparisons. Vertical bars represent the standard error (SE).

Huntley Site:Only DS was evaluated in Huntley in 2020. DS was significantly affected by cultivar and cultivar × configuration interactions (Table 2.11). CDC Leader had significantly lower DS than Royal (Figure 2.9). There is no significant difference in DS between monocrop chickpea and intercropping chickpea-flax of CDC Leader (Figure 2.9). In contrast, all mixed intercropping configurations decreased DS of Royal by 13.9 to 19.4%. The DS of Royal in 50C/50F in alternate rows did not differ from the monocrop chickpea.

Table 2.11. ANOVA table showing the effects of cultivar and configuration on DS in Huntley.

Source	df	Disease Severity (%)
		$P>F$
Cultivar	1	**<0.01**
Configuration	4	0.07
Cultivar × Configuration	4	**0.048**

Figure 2.9. Interactive effects of cultivar and configuration on disease severity for 2020 in Huntley. C: chickpea; F: flax. 100C represents monocrop chickpea. 70C/30F represents 70% chickpea and 30% flax in mixture. Alt Rows represents 50% chickpea and 50% flax in alternate rows. 50C/50F represents 50% chickpea and 50% flax in mixture. 30C/70F represents 30% chickpea and 70% flax in mixture. Different letters at top each bar within a crop component represent a significant difference at the 0.05 probability level according to the Šidák-corrected multiple comparisons. Vertical bars represent the standard error (SE).

Discussion

Effectiveness of Intercropping Chickpea-Flax in Managing Ascochyta Blight

The advantage of intercropping in controlling crop diseases has been reported in numerous intercropping systems (Cao et al., 2015; Fernández-Aparicio et al., 2010; Fininsa & Yuen, 2002; Schoeny et al., 2010). The results of our study suggested that intercropping chickpea-flax can be an effective strategy to reduce Ascochyta blight in chickpea. Meanwhile, cultivars selection and intercropping configurations are also critical for disease management. In Sidney, for example, resistant cultivar CDC Leader had lower Ascochyta blight than susceptible cultivar Royal in

2020. Although not all intercropping configurations significantly reduced disease in 2020, integration of resistant cultivars (CDC Leader) with 70C/30F and 30C/70F in mixture was most efficient for disease suppression. All intercropping configurations reduced Ascochyta blight in 2021 (Fig. 2.8A, C). While the cultivar effect was not detected, the efficiency of integrated resistant cultivar and appropriate configurations in Ascochyta management was also observed in 2021. The reason of intercropping configurations demonstrating different control abilities in 2020 and 2021 was likely due to diverse environmental conditions generating distinct disease pressure. Although the precipitation in 2020 and 2021 was similar, the irrigation created a favorable environment for Ascochyta development and promoted the dispersion of secondary inoculum in 2021 similar to what has been observed in other studies (Kaiser, 1997). Therefore, the disease pressure is slightly higher in 2021 than in 2020, even though the disease pressure was generally low in both years.

In Huntley, the disease severity of monocropped CDC Leader was significantly lower than that of Royal. CDC Leader is considered moderately resistant to Ascochyta blight, whereas Royal is considered susceptible. When the susceptible cultivars combined with considerable higher humidity in Huntley created a relatively higher disease pressure, the intercrop showed highly effective in disease management on Royal, except in the alternate row configuration where intercrop in alternate row showed similar DS as in monocrop. These results are consistent with previous research conducted by Schoeny et al. (2010), where a 50% pea - 50% cereal reduced disease severity of pea Ascochyta blight in mixture under moderate and severe epidemics, but not under slight epidemic pressure. The plating of resistant cultivars is another efficient strategy in disease management, which is supported by the results of this study. In Sidney, the integration of

CDC Leader and flax intercrop provided the most efficient Ascochyta blight management for both years. Although the DS of intercropped CDC Leader was not significantly lower than monocrop, it showed a lower DS than Royal in intercrop. Previous studies suggested that integrating several strategies is the key in Ascochyta blight management of chickpea (Gan et al., 2006; Pande et al., 2005)

The mechanism of intercropping for disease management has been investigated, which includes the reduced density of host plants, barrier effects of non-host plants to spore dispersion, alteration of microclimate, and morphological and physiological changes in the host (Schoeny et al., 2010; Villegas-Fernández et al., 2021). In this study, the disease severity of intercrop did not decrease with increasing flax proportion. However, intercrop mixtures with a lower density of host plant showed lower disease severity under higher disease pressure environment. Although, it was not consistent at each location in each year, the dilution effects can still be observed. Moreover, Royal in 50C/50F mixture was more effective in limiting Ascochyta blight than in alternate rows in most cases. Fininsa and Yuen (2002) reported that separate row arrangement was less effective than mixture in controlling common bacterial blight in bean-cereal intercropping system. These results might be explained by the barrier role of non-host plants and/or alteration of microclimate. In contrast to the chickpea and flax were planted in separate rows apart from each other with row spacing in alternate row configuration of this study, the chickpea was grown next to flax in the mixed configuration, the dispersal interference and modification of microclimate effects were higher than alternate row configuration.

The results of the disease assessment corresponded to chickpea yield. In Huntley, CDC Leader yielded higher than Royal, which is opposite to the results from Sidney. The better

performance of CDC Leader in Huntley was attributed to higher Ascochyta blight resistance than Royal, and the disease pressure of the trial in Huntley was much higher than in Sidney. Light disease pressure did not cause damage to the yield in Sidney. That could explain why 50C/50F in mixture produced same or slightly higher chickpea yield than in alternate rows at Huntley, but not at Sidney. Higher disease dispersal interference and microclimate modification effects in mixture leaded to more effective yield protection than alternate row arrangement. These results suggested that intercropping can effectively control chickpea Ascochyta blight and protect seeds yield formation, and this protection is more obvious for the susceptible cultivar.

This conclusion is supported by evidence in other intercropping systems (Cao et al., 2015; Guo et al., 2021; Lithourgidis et al., 2011; Villegas-Fernández et al., 2021). Cao et al. (2015) reported that intercropping wheat-maize reduced wheat stripe rust by 16.7%-45.7%, and wheat powdery mildew by 14.7-27.0% with 4:4 and 4:2 seeding ratio of wheat and maize, compared to the wheat monocrop. The yield was increased by 52.4-140.0%. Similarly, Guo et al. (2021) found that faba bean rust occurrence was decreased in faba bean-wheat intercropping by 22.3-54.7% and was regulated by canopy microclimate and nitrogen nutrition. Intercropping was most effective in disease control with a 40-90 kg ha $^{-1}$ application of nitrogen fertilizer and contributed to a 34.4%-40.7% yield increase.

Competition of Component Crops in Intercropping

In Sidney, the proportion of flax was the critical variable for yield in the intercrop. Chickpea yield and LERc decreased while flax proportion is increasing in the mixed intercrop. Similar results were also observed in sunflower-soybean intercrops, where increased sunflower density results in decreased soybean yield (Echarte et al., 2011). Competitiveness of one

companion crop increased as the proportion of that crop increased, resulting in a yield increase of this crop. The competition could be more intense due to limited environmental resources since flax and chickpea share similar root zone and root growth rates as monocrop (Liu et al., 2011). The LERc value ranged only from 0.24 to 0.52 in Sidney. In Huntley, the value of LERc in other intercropping configurations was greater than 0.5 except for 30C/70F mixture. This is largely due to less water availability in Sidney than in Huntley. Andrade et al. (2012) found that intercropped sunflower with soybean was as productive as sole sunflower in an extremely dry cropping season due to its higher ability to capture water and radiation. Soybean became more competitive for light with less limiting water due to better vegetative growth (Dowling et al., 2021; Gan et al., 2009). As observed in the field, flax (average 43cm) grew taller than chickpea (average 33 cm) in Sidney, but chickpea (average 56 cm) had similar plant height as flax (average 57 cm) in Huntley (data not shown). Thus, sufficient environmental resources may be critical for stable yield production in chickpea-flax intercrop.

The alternate rows configuration with 50C/50F demonstrated a higher chickpea yield and LERc in Sidney for 2020 than in the mixture. Although they were planted with the same seeding rate, chickpea and flax were planted in the same rows for mixture and separate rows for alternate row design. In the mixture configuration, chickpea and flax were planted closer than in alternate rows. For a less competitive species, it performed better when planted in alternate rows. In a pea-barley intercropping system, the suppression of barley on pea biomass production was greater in mixture than separated arrangement (Chen et al., 2004). Similar results were reported by Chalmers (2014), who described that pea produced 21% higher yield in alternate rows than in mixture in a pea-canola intercrop, while canola yielded 49% higher in mixture than in alternate

rows. These results indicate that the alternate row arrangement has less interspecies competition

than the mixture at the same seeing rate. The alternate row configuration might be able to improve

competition of subordinate plants (e.g., chickpea, pea) with dominant plants (e.g., flax, canola,

barely) (Dowling et al., 2021). In contrast, 50C/50F in alternate row design showed similar or

lower productivity as chickpea in 50C/50F mixture in Huntley. One of the main reasons could be

that the closer row spacing between alternate rows in Huntley than in Sidney reduced the benefits

of alternate rows on chickpea (subordinate plants). This result is in accordant with Dedio (1994),

who described that the LER of pea in a sunflower-pea intercrop decreased with increased row

spacing between alternate rows.

Intercropping Effect on Land Productivity

Although the yield of chickpea and flax decreased in intercrop, the combined land

productivity improved based on the LER in this study. When the value of LER is higher than one

in the intercrop, intercropping produces a yield advantage and increased land productivity

compared to the monocrop. The improved efficiency of land utilization in a legume-oilseed

intercropping system has been reported (Andersen et al., 2005; Jo et al., 2022; Roberts et al.,

2019). A study conducted in South Australia found that legumes (pea, vetch, lentil) intercropped

with canola at 2:1 ratio over-yielded by 12-80% (Roberts et al., 2019). Jo et al. (2022) reported

that soybean and flax planted in ridge-furrow intercropping systems increased land productivity

by 103.5%.

In this study, 50C/50F displayed the highest LER in most cases at both locations, which

indicates that a 50%-50% seeding rate of chickpea and flax might be most efficient in improving

land productivity. Previous work has found that the crop component ratio can be critical to

improve intercropping advantage. In a sunflower-soybean intercrop, LER increased as the ratio of sunflower and soybean decreased from 8:30 to 3:30 (Echarte et al., 2011). Further reductions in sunflower plant ratio did not increase LER. These results suggest that the intercrop component ratio can be manipulated to improve land productivity and interspecific competition in the intercrop. The seeding rates may be manipulated to increase the LER for individual crops based on the market values of the individual crops.

The configurations at the same seeding rate were performed differently at the two locations. In Huntley, the LER of the 50C/50F mixture was higher or similar to alternate rows. However, the alternate rows with 50C/50F were more effective in improving land productivity than the mixture in Sidney. Different environmental conditions and row spacing could explain these results among locations. Higher water availability and wider row spacing in the mixture reduced competition of chickpea and flax in the intercropping but increased disease pressure in Huntley. Therefore, mixture configuration with more significant disease suppression produced a higher yield advantage than the arrangement of the alternate rows in Huntley. In contrast, light disease pressure and higher competition in Sidney were attributed to drought conditions and smaller row spacing in the mixture. Thus, the alternate rows with lower interspecies competition created a greater yield benefit than the mixture in Sidney.

Nutrient Content in Intercropping Chickpea-Flax

Chickpea protein content decreased as flax seeding rates increased in mixed intercrop when planted in low-N soil, suggesting flax might be more vigorous and competitive than chickpea in N uptake. Andersen et al. (2005) reported that the uptake of soil N by pea was suppressed by canola and barley when pea was intercropped with canola and/or barley. In this

study, all the 50% chickpea and 50% flax in alternate rows displayed a similar level of chickpea protein to monocrop, indicating that the competition for soil N between chickpea and flax in alternate rows was not intensive. Flax protein was improved by intercrop, which indicates that the nitrogen assimilation in flax may have benefited from the nitrogen fixation of chickpea (Gan et al., 2010). Xie et al. (2020) has reported that flax oil content was increased by higher soil phosphorus. The effects of intercrop varied among different locations. Intercrop reduced the flax oil content for 2020 in Sidney, and the reduction was observed when chickpea and flax were planted in a 50%-50% ratio for 2021. However, the oil content of intercropped flax was similar to monocrop in Huntley and even increased in 70C/30F mixture and 50C/50F alternate rows. These results could be attributed to the interaction of environment and intercropping configuration. Further studies need to be conducted to investigate the effects of interaction between environment and intercrop on flax oil content.

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CHAPTER FOUR

SUMMARY

Soilborne diseases are common in all fields regardless of cropping history, which is a concern in chickpea production on the Northern Great Plains. Cultivar resistance and fungicide seed treatment are the most effective soilborne disease control strategies. Tolerant kabuli chickpea cultivars have not been developed in the Northern Great Plains, where kabuli chickpea are dominant in chickpea production. In this study, a fungicide seed treatment containing fluxapyroxad, pyraclostrobin, metalaxyl, and thiamethoxam effectively reduced the damping-off development (75.6% to 88.0%). *F. solani* was isolated from roots with late season root rot symptoms in 2021 and showed moderate to high aggressiveness on chickpea. However, seed treatment only slightly decreased the disease severity of late season root rot (6.1%). The seed treatment improved the chickpea seeds yield by 14% to 38% and increased protein content by 0.96%. A significant negative correlation between the yield of untreated seeds and AUDPC was observed. NDC160049, NDC 160133, NDC160146, NDC 160236, and Royal had the lower disease and higher yield than known susceptible cultivars in 2020, which may indicate enhanced resistance or tolerance to soilborne pathogens. Disease development in two chickpea breeding lines (NDC160166 and NDC160236) was not affected by seed treatment and thus shows promise as future sources of disease resistance. However, different cultivars and breeding lines did not have a consistently significant impact on yield most likely due to differing seed quality, field conditions, and environmental conditions between years.

Previous research reported that legume-based intercropping systems were effective in improving resource use efficiency and controlling foliar disease of legumes. The sowing ratio of

component crops and row configuration can alter the competition and productivity of the intercrops. In this study, the yield of chickpea increased with the decreased proportion of flax in the mixed intercrop. Chickpea yielded higher in the alternate row design than in the mixture at the same seeding rate under light disease pressure due to less interspecies competition of intercrops in alternate rows. However, under high disease pressure environment with susceptible cultivar, intercropping with mixture configuration yielded higher than alternative rows due to more effective disease suppression in the mixture configuration.

Intercropping improved land productivity by 2% to 23%, depending on the seeding rates and row configurations. Seeding rate of 50%-50% chickpea and flax had the highest LER value at Sidney and Huntley in most cases, suggesting it might be the optimal seeding ratio for improving land productivity. Flax displayed higher competitiveness than chickpea, resulting in decreased yield and protein concentration in chickpea but increased yield and protein content in flax.

As the most threatening disease in chickpea production, Ascochyta blight was mainly controlled by planting disease-free seed, crop rotation, resistant cultivars, foliar fungicide, and seed treatment. However, only moderately resistant cultivars have been developed, and resistance declines as plants grow older. Additionally, resistance to fungicides has been reported in the Northern Great Plains. From our study, the suppression of Ascochyta blight when intercropping chickpea-flax was measured. Intercropping chickpea-flax decreased disease severity by 2.3% to 7.5% in Sidney and 4.8% to 9.4% in Huntley. Under relatively higher disease pressure, 50% chickpea and 50% flax in the mixture was more effective in suppressing Ascochyta blight than in the alternate rows especially for the susceptible cultivar. CDC Leader yielded better than Royal in Huntley, which is the opposite in Sidney due to less disease pressure in Sidney because Royal is

more susceptible to Ascochyta than CDC Leader. Integration of resistant cultivars and intercropping displayed the lowest disease severity at Sidney and Huntley. These results suggested that intercropping configuration in conjunction with resistant chickpea cultivar selection effectively managed Ascochyta blight on chickpea.

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