The effects of winter rye cover crop on corn seedling disease, corn growth and development in respects to winter rye seeding spacing

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

The United States is the largest producer of corn (*Zea mays* L.) and Iowa contributes to approximately18 percent of corn in the U.S. In the corn-soybean (*Glycine maxx* L.) crop rotation system, erosion and nitrate leaching are problems and encouragement for farmers to produce crops sustainably is increasing. Cover cropping is a sustainable option to enhance agricultural production systems by improving soil and water quality. In Iowa, approximately 2 million acres were planted to cover crops (CCs) in 2018, which is far less than the 12.5 million acres goal called for by Iowa's Nutrient Reduction Strategy. Winter rye (*Secale cereale*) is the most commonly planted CC in Iowa due to its good establishment in the fall and winter hardiness. However, many studies and farmers have reported reduced corn yield after a winter rye CC, which discourages farmers from planting a winter rye CC.

Proposed reasons for corn yield loss after a winter rye CC include nitrogen immobilization, reduced water availability, allelopathy, and seedling disease. Corn and winter rye can be infected by some of the same pathogens including *Pythium* spp. Studies have shown that *Pythium* spp. overwinter in winter rye roots thereby creating a 'green bridge' effect. Moreover, seedling disease in corn increased when corn was planted into winter rye residue compared to corn planted into no CC. Seedling disease can cause damping-off and delay emergence, resulting in uneven stands and reduced plant vigor that negatively affects yield.

Previous research recommends terminating a winter rye CC 10 to 14 days before planting corn; however, this may be challenging in some years due to weather constraints. Cold, wet weather in the spring can delay termination of the rye CC and/or planting corn. This research focuses on spatially separating winter rye residue from corn to reduce seedling disease. We hypothesize planting corn at a distance from winter rye residue would decrease seedling disease

and benefit corn growth and development. A growth chamber study, experimental field plot study, and an on-farm study were conducted to test our hypothesis.

In the growth chamber study (Ch. 2) winter rye was planted on one side of 23 cm diameter pots. After winter rye was terminated, corn was planted into the winter rye residue and 8 to 10 cm away from the winter rye residue. Seedling disease and growth parameters of the corn were collected at corn growth stage V1 to V2. Root rot severity was numerically greater in corn planted in the winter rye residue compared to corn planted 8 to 10 cm away from rye residue but not significantly different (Run 1, $P = 0.1177$; Run 2, $P = 0.1939$). However, there were greater *Pythium* clade B populations in the roots of corn planted into winter rye residue compared to corn planted away from winter rye residue (Run 1, $P = 0.0233$; Run 2, $P = 0.004$). Additionally, corn shoot dry weight, radicle length and shoot length were greater in corn planted 8 to 10 cm away from winter rye residue compared to corn planted into winter rye residue.

The experimental field plot study (Ch. 3) consisted of four treatments; no CC control, broadcast planted winter rye, 3 rows of rye drilled 19 cm apart in the corn interrow, and one row of rye drilled 76 cm apart in the corn interrow. Thus, corn was planted within the winter rye residue, 19 cm and 38 cm away from winter rye residue. Corn root rot ratings at growth stage V3, growth parameters, and yield were collected. Additionally, soil samples were collected to understand populations of *Pythium* spp. in the soil spatially and temporally. Radicle and seminal rot severity differed among treatments in 2020 ($P = 0.0898$; $P = 0.0565$, respectively). And effect of winter rye treatments on *Pythium* clade B populations in the radicles was detected (2019, $P = 0.0455$; 2020, $P = 0.0209$). Furthermore, winter rye treatments affected corn yield (2019, $P = 0.0002$; 2020, $P = < 0.0001$). In general, as distance between winter rye and corn increased root rot decreased and corn growth parameters and yield improved. Results from the soil samples

demonstrated that *Pythium* clade B populations differed temporally but not spatially when winter rye is planted at different distances from the corn row.

The on-farm study consisted of three treatments; no CC control, 38 cm drilled winter rye, and 76 cm drilled winter rye. Corn root rot ratings and growth parameters at growth stage V3, and yield were collected. Soil samples were collected to understand spatial distribution of *Pythium* spp. populations in the soil; however, difference among treatments and sample location were not detected. In both years, radicle rot severity was highest in corn planted in 38 cm drilled winter rye compared to corn planted in 76 cm drilled winter rye and no CC (2019, $P = 0.0514$; 2020, $P = 0.0016$). Growth parameters and yield of corn did not differ among treatments in both years.

There are likely many factors that contribute to corn yield reduction after a winter rye CC. Further research is needed to fully understand the factors that play a role in reducing corn yield after a winter rye CC. This research demonstrated that spatially separating a winter rye CC from corn reduced seedling disease and benefited corn growth, development, and yield. An additional recommendation for incorporating a winter rye CC into the corn-soybean production system may include planting winter rye in the interrows to create a distance between the winter rye and corn.

CHAPTER 1. INTRODUCTION/ LITERATURE REVIEW

Thesis Organization

This thesis is organized into four chapters. Chapter one is the general introduction and literature review. Chapter two is a paper that was published in Plant Disease, entitled "Seedling disease of corn caused by *Pythium* increases with proximity of rye." Chapter three is a paper to be submitted to Plant Disease, entitled "Influence of spatial planting arrangement of winter rye cover crop on corn seedling disease and corn productivity." Chapter four is the general conclusion of this project.

General Introduction

In the United States, including cover crops (CC) in field crop production has been increasing in interest from an agronomic standpoint as they are known to reduce erosion and nitrate leaching, leading to improved water and soil quality (Snapp et al. 2005). In 2017, CC acres in the U.S. were doubled from that planted in 2012 (Zulauf and Brown 2019). In the Midwest, winter rye (*Secale cereale*) is the most commonly planted CC in field crop production because it establishes well in the fall and is able to survive the harsh winters and continue growth in spring. Although there is extensive research about the environmental and weed management benefits of a winter rye CC, research on the interactions of a winter rye CC with plant pathogens, is still fairly new.

In corn (*Zea mays* L.) production systems, a pathogen of particular interest is *Pythium*, which causes root rots. The objective of this study is to compare how winter rye spacing affects seedling disease, and growth and development of corn. To address this objective, a growth chamber study, and two years of experimental field plot and on-farm trials in Iowa were conducted.

Literature Review

Introduction. The United States is the largest corn producer in the world with over one third of the global corn production (Shahbandeh 2020). Additionally, corn is the most produced crop in the United States with 91.7 million acres planted in 2019 (Capehart and Proper 2019). In the Midwest, there are over 127 million acres of agricultural land and 75% of that area is planted to corn and soybean (*Glycine maxx* L.) (USDA 2017). Corn is used for livestock feed, starch, sweeteners, corn oil, beverage and industrial alcohol, ethanol fuel and is of high value for several countries ("Feedgrains Sector at a Glance" 2020). Due to high corn demand, it is important for farmers to use best management practices to ensure higher yields especially when incorporating a winter rye CC.

CCs are being encouraged for use by farmers to improve soil health and reduce the negative impacts of farming on the environment. There are several CCs that reduce soil nitrate leaching and improve soil health, but winter rye is the best (Bader 2020). It is important to understand how a winter rye CC influences corn growth and development, and corn seedling disease. Additionally, assessing how different planting arrangements of winter rye could affect seedling disease might provide better management tactics.

Cover Crops. Concerns about environmental contamination from agricultural practices have encouraged a transition to more sustainable crop production systems. CC are one of the many options to incorporate into a sustainable production system (Lu et al. 2000). A CC is a non-cash crop that is not intended for harvest but maintained to improve soil fertility, water quality, and manage pests (Nair et al. 2015). In recent years, cover cropping has become more prevalent in field crop production throughout the United States. In 2017, approximately 15.4 million acres were planted to a CC in the U.S., which was a 50% increase from 2012 (Zulauf and Brown

2019). The benefits of CCs can be both short and long-term including erosion control, nitrogen recycling, weed control, and soil moisture control (Lu et al. 2000; Snapp et al. 2005).

Soil erosion is gradual removal of soil by water or wind that causes the soil to deteriorate and is a severe problem worldwide (Al-Kaisi 2000). CCs are used to control soil erosion in areas where land would typically be bare and subject to erosion. CCs provide surface residue and a soil-stabilizing root system that protects the soil from wind and water runoff (Lu et al. 2000; Snapp et al. 2005; Kaspar et al. 2001). The residue and root system aid in nitrogen recycling by acquiring excess nitrogen in the soil and releasing it when the CC breaks down (Malpassi et al. 2000). This prevents the excess soil nitrogen from leaching into groundwater (Lu et al. 2000; Snapp et al. 2005). Another benefit of CC residue is weed control (Williams et al. 1998). A dense CC canopy and allelopathic compounds produced by some CCs can inhibit germination and growth of weeds (Lu et al. 2000; Snapp et al. 2005). Soil moisture is also controlled by CC residue in that it cools down soils and slows evaporation (Lu et al. 2000). Over time CCs build soil organic matter that increase water holding capacity of the soil (Lu et al. 2000). These benefits encourage incorporating CCs into agriculture production systems.

Grasses, legumes, and non-legume broadleaves are the three categories of CCs (Magdoff and Es 2000) and are either warm or cool season crops (Nair et al. 2015). Throughout the Midwest, CCs are typically cool season crops used in rotation with row crops (corn and soybean) that are planted in the fall concurrently with harvest of the main cash crop. This eliminates a fallow period over the winter and adds diversity to the crop rotation. Those CCs that survive winter are terminated in the spring, usually with an herbicide and typically before the cash crop is planted (Kaspar et al. 2001).

Winter Rye Cover Crop. The most commonly planted CC in Iowa is winter cereal rye (Bader 2020). Winter rye is an exceptional CC due to its excellent establishment in the fall and winter hardiness (Bader 2020; Snapp et al. 2005). In the fall, winter rye grows fast and produces a good ground cover (Grubinger 2010). It can germinate and grow at temperatures as low as 0.5°C and can tolerate temperatures as low as -36°C (Grubinger 2010). In the spring, it resumes growth quickly with minimal winter kill (SARE 2012).

Like many other CCs, rye absorbs excess soil nitrogen, reduces erosion, and suppresses weeds. Because of winter rye's large fibrous root system, it is one of the best CCs for scavenging soil nitrogen (Kasper et al. 2007; SARE 2012; Staver and Brinsfield 1998). Winter rye produces large amounts of biomass that protects the soil from wind and water erosion, out competes weeds, and adds plenty organic matter to the soil that improves soil structure (SARE 2012). Winter rye also produces allelopathic chemicals that can inhibit weed growth (SARE 2012). These benefits are intriguing to farmers and are why winter rye is the most commonly planted CC in Iowa.

Corn germination and growth. Like all plants, germination and growth of corn can be affected by many factors (moisture, temperature, light, soil nutrients, pests and disease). Both adequate moisture and relatively warm temperatures (10 to 35 °C) are needed for seeds to germinate and grow to maturity (Ennen and Jeschke 2019). For germination to occur, corn seeds need to imbibe approximately 30 percent of their weight in water (Nielson 2019) and 65-80 growing degree units (GDU) are needed for soil emergence (Channel 2020).

The radicle root is the first to emerge from the seed followed by the coleoptile then seminal roots (Nielson 2019). The radicle and seminal roots absorb water and nutrients until nodal roots grow and take over water and nutrient absorption for the corn plant at the 6th leaf stage (V6)

(Nielson 2002). From emergence to 6th leaf stage (VE to V6) the seedling is vulnerable to desiccation, cold stress, insects, and soilborne diseases (Nielson 2002, 2019). If the seedling is affected by any of these factors, it may emerge late, become stunted, or even die (Nielson 2002). Delayed emergence and stunted seedlings result in uneven stands and reduced plant vigor (Lauer 2002). When crop stands are not uniform, smaller seedlings that are one to two growth stages behind their neighbors may become out competed by larger seedlings for light, water, and nutrients (Weiner 1990). These smaller outcompeted seedlings become less vigorous plants that usually have smaller ears due to late pollination or no ears (barren) at all, both of which contribute to lower yields (Nielson 2002). Thus, factors that affect early growth of corn seedlings can indirectly affect their ability to produce optimum yields.

Winter Rye Cover Crop and Corn. With all of the benefits that a winter rye CC offers, there is still hesitation to add a winter rye CC into corn-soybean production systems. In some cases, winter rye has had a negative impact on corn yield. Several studies reported reduced yield when using winter rye CC before corn and is concerning to farmers (Dinnes et al. 2002; Johnson et al. 1998; Kaspar and Bakker 2015; Miguez and Bollero 2005). Understanding the reasons for corn yield decreases following a winter rye CC is important for the development of management strategies that will reduce these negative effects of winter rye and possibly increase yield of corn. Potential reasons for the reduction of corn yield after winter rye include nitrogen immobilization (Qi et al. 2011), reduced water availability (Munawar et al. 1990), allelopathy (Raimbault et al. 1990) and seedling disease (Bakker et al. 2016; Acharya et al. 2017, 2020b).

Seedling Disease in Corn. Seed rots and seedling diseases of corn are economically important in the United States and Ontario, Canada. An estimated 6.1 billion bushels of corn were lost between 2012 and 2015 due to diseases in corn (Mueller et al. 2016). Approximately

14 percent of the total disease loss was attributed to root rots, seedling blights, and nematodes (Mueller et al. 2016). Iowa had the most yield loss due to disease compared to all other states with an estimated 1.8 billion bushels lost between 2012 and 2015 (Mueller et al. 2016). Seedling disease contributed to 12 percent of the corn yield loss in Iowa (Mueller et al. 2016).

Seed rots and seedling diseases of corn can be caused by several species of fungi and bacteria. These organisms are divided into two categories; pathogens that are on or in the seed (seed-borne) and pathogens that are in the soil (soil-borne) (Smith and White 1988). The majority of corn seeds with seed-borne pathogens are removed from the seed lot with machines that discard seed with discoloration, small size, and low weight caused by these pathogens (Munkvold and White 2016; Smith and White 1988). Since pathogens inside the seed are harder to detect, germination tests are conducted to ensure the seed lots have minimal infection and are acceptable to plant (Munkvold and White 2016; Smith and White 1988). This means that seed rots and seedling diseases of corn will most likely not be caused by seed-borne pathogens after being planted in the field. Soilborne pathogens are naturally occurring in the soil and are typically what causes seedling disease and seed rots that occur after the corn is planted (Schumann and D'Arcy 2010). The most common soilborne pathogens that cause seed rots and seedling diseases on corn are *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani* (Munkvold and White 2016).

Corn seeds are most vulnerable during germination and seedling stages. Planting corn into cool, wet soils can cause chilling injury that in turn contributes to seedling disease susceptibility (Munkvold and White 2016). Symptoms of seedling disease include rotted seed, root rot, delayed emergence, chlorotic leaves, stunted plants, and damping off (plant death) (Munkvold and White

2016). Seedling diseases reduce stand density and plant vigor resulting in yield loss (Munkvold and White 2016; Broders et al. 2007).

Winter Rye and Corn Seedling Disease. Winter rye and corn are phylogenetically similar because they belong to the same plant family, Poaceae, and is possible that they share many pathogens (Gilbert et al. 2015). In fact, studies have shown that winter rye is a host of the three most common soilborne pathogens that infect corn; *Fusarium* spp. (Acharya et al. 2017, 2018; Bakker et al. 2016; Chango et al. 2001), *Pythium* spp. (Acharya et al. 2017, 2018, 2020b; Bakker et al. 2016; Vestberg 1990), and *Rhizoctonia* spp. (Neate 1989). When a winter rye CC is added to the production system the root residue of the CC remains in the soil where the corn crop is planted. Therefore, pathogen-infected CC roots may come in contact with corn roots and pathogen transfer may occur. When a winter rye CC is present the risk of corn seedling infection by these soilborne pathogens increases (Bakker et al. 2016).

Since winter rye overwinters well and is terminated in the spring seedling pathogens have living winter rye roots to inhabit in the fall, winter, and spring. The roots act as a 'green bridge' for the pathogens and ensure their survival (Bakker et al. 2016; Acharya et al. 2017). Bakker et al. (2016) found that *Fusarium graminearum*, *F. oxysporum*, *Pythium sylvaticum*, and *P. torulosum* were the most commonly isolated pathogens from rye roots that also caused infection in corn roots. Acharya et al. (2017) determined that corn radicle root rot caused by *Pythium* spp. increased when corn was planted following a terminated winter rye CC; however, radicle root rot caused by *Fusarium* spp. did not differ between terminated winter rye and no winter rye treatments. *Pythium* spp. belonging to clade B (*Pythium arrhenomanes*, *P. torulosum* (Acharya et al. 2017), and *P. volutum* (Bakker et al. 2017) were most frequently isolated from corn roots

suggesting that *Pythium* spp. from this clade are likely the causal agents of seedling disease observed on corn planted after a winter rye CC.

Some researchers have proposed that allelopathic chemicals produced by winter rye impact corn by inhibiting corn germination and growth (Pantoja et al. 2015; Raimbault et al. 1991). Recently, an in-vitro study by Acharya et al. (2020a) reported corn radicles were stunted when corn seeds were grown in agar media amended with the winter rye allelochemicals, 6-Methoxy-2-benzoxazolinone (MBOA). Additionally, they observed more severe root rot of corn grown in the presence of MBOA and some *Pythium* species. Acharya et al. (2020a) suggested winter rye allelochemicals and some *Pythium* species may interact synergistically, and increase pathogen infection in corn roots. Allelochemicals are released from the winter rye as it decomposes and can take 10 to 12 days to breakdown after rye is killed (Yenish et al. 1995) or longer (Otte et al. 2020). If corn is planted shortly after winter rye is killed, it is possible that allelochemicals and pathogens from rye will be present in the spermosphere and rhizosphere.

Pythium Disease Cycle. *Pythium* is a genus of oomycetes which appear similar to fungi by producing hyphae and causing like disease symptoms (Schumann and D'Arcy 2010). However, oomycetes have cell walls composed of cellulose, the diploid phase of their life cycle is dominant, and they have motile spores which are not characteristics of true fungi (Schumann and D'Arcy 2010). Oomycetes are very widespread and are typically found in soil and surface waters (Schumann and D'Arcy 2010). There are over two hundred species of *Pythium* (Fry et al. 2010) and many of these species are important soilborne pathogens that infect many plant species (Martin and Loper 1999). Symptoms of *Pythium* typically consist of seed rots, root rots, and pre and post-emergence damping-off (plant death) and are found in plants planted in poorly drained, low lying areas of the field (Fry et al. 2010).

Pythium spp. reproduce both sexually and asexually. Oospores are round sexual spores produced when the antheridium (male gamete) fertilizes the oogonium (female gamete) (Fry et al. 2010). The thick-walled oospore is the primary survival structure and can survive in the soil for long periods of time and withstand unfavorable conditions (Martin and Loper 1999). Oospores germinate during flooded conditions and produce mycelia that in turn produce sporangiophores and sporangia (Robertson 2008). A sporangiophore is a specialized hyphal structure that produces a sporangium, which are asexual reproductive spore-like structures (Heffer Link et al. 2002). Sporangia can survive long periods in the soil or be short-lived depending on their shape (Martin and Loper 1999). Oospores and sporangia need a chemical stimulant from either root and seed exudates, plant debris, or organic matter to germinate (Martin and Loper 1999). However not all oospores and sporangia germinate in ideal conditions (Martin and Loper 1999). Instead of germinating directly, sporangia may produce asexual spores called zoospores (Heffer Link et al. 2002) in the presence of certain nutrients or high moisture (Martin and Loper 1999). Zoospores are motile by way of two flagella; a tinsel flagellum and a whiplash flagellum (Heffer Link et al. 2002). These flagella assist in movement through liquid which is why *Pythium* spp. thrive in wet conditions (Fry et al. 2010; Heffer Link et al. 2002) however, zoospores are short-lived in the soil and will not survive in dry conditions (Martin and Loper 1999).

Pythium Management for Corn. There are several management practices that can be used to help control *Pythium* root rot such as cultural practices and chemical control products. For example, improved soil drainage is important to prevent favorable environments for oospore, sporangia, and zoospore production (Schumann and D'Arcy 2010). Avoiding compaction by tillage methods increase soil temperature and reduce soil moisture, which can reduce soilborne

pathogen levels (Broders et al. 2007; Munkvold and White 2016). Unfortunately, resistant corn varieties to *Pythium* are not available (Munkvold and White 2016).

A common disease management practice for *Pythium* root rot is the use of a fungicide seed treatment (Broders et al. 2007; Munkvold and White 2016; Kleczewski 2020). Metalaxyl, mefenoxam, azoxystrobin, trifloxystrobin, and pyraclostrobin are the active ingredients used in seed treatments that can fight against *Pythium* spp (Broders et al. 2007; Robertson et al. 2013). Although they are not entirely effective at controlling disease, they provide some form of protection during the first few weeks after planting corn (Kleczewski 2020).

Some recent studies have shown that termination time of the winter rye CC has an effect on corn seedling disease and yield (Acharya et al 2017; Duiker and Curran 2005). When there is more time between winter rye termination and corn planting the negative effects of rye are reduced. Farmers should plant corn 10 to 14 days after the winter rye is terminated (Hartzler 2014) as a means of reducing corn seedling disease in Iowa (Acharya et al. 2017). This allows more time for the winter rye to decompose, and is postulated, more time for allelopathic chemicals to dissipate, and pathogen numbers to decline in the soil.

Row spacing for disease control. A few studies have experimented with planting seed at a distance from pathogen inoculum. *Gaeumannomyces graminis* var. *tritici* is an important pathogen that causes take-all disease in wheat (Kabbage and Bockus 2002). Kabbage and Bockus (2002) demonstrated in a growth chamber study that planting wheat 10-15 cm away from the oat seeds inoculated with *Gaeumannomyces graminis* var. *tritici* decreased disease in wheat. Additionally, Garrett et al. (2004) estimated that planting wheat parallel to the previous year's row of wheat (potential inoculum) would reduce yield loss from *Gaeumannomyces graminis* var. *tritici* by 50% compared to planting the wheat at an angle from the previous year's row of wheat.

However, when wheat was planted at a distance from the previous year's wheat residue, Rhizoctonia root rot was similar to wheat planted in the previous year's wheat residue (Davis et al. 2008). Planting crops at a distance from previous crops with pathogen inoculum may be a beneficial management practice for some diseases.

Precision agriculture is becoming more widely used because it allows farmers to accurately plant their crops so spacing of each row is uniform. This gives farmers more control for crop management. Winter rye is planted by broadcast or drilled (Grubinger 2010). In the Midwest, corn and soybean are usually planted in 76 cm rows. As precision planting with GPS-controlled planting machinery becomes more common on Iowa farms, it is possible farmers could seed a winter rye CC in the interrows to physically separate the winter rye CC from the corn rows planted the following season. This may provide adequate distance between winter rye and corn to reduce the disease severity in corn radicles caused by *Pythium*, which was harbored in rye roots.

Justification for Research and Objectives. Winter rye CC is of increasing interest to improve soil quality and the environment, however the reason for its negative impact on corn is not completely understood. With the data that has been presented thus far more research is needed to understand how winter rye CC impacts seedling disease in corn. In some studies, planting seed at a distance from the source of pathogen inoculum has reduced seedling disease. This research was done to determine if separating the future corn row from the winter rye CC residue would reduce seedling disease and improve crop growth and development thereby mitigating reduced yields of corn.

The objectives of this research were to compare how planting winter rye at a distance from the corn affected (i) seedling disease, and (ii) growth and development of corn. To address these

objectives, a growth chamber study, and two years of experimental field plot and on-farm trials in Iowa were conducted.

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CHAPTER 2. SEEDLING DISEASE OF CORN CAUSED BY *PYTHIUM* INCREASES WITH PROXIMITY OF RYE

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Abstract

Yield loss of corn following a winter rye cover crop (CC) has been associated with increases seedling disease caused by *Pythium* spp. We hypothesized that physical separation between the CC and corn could reduce the risk of seedling disease, and benefit corn growth and development. In a growth chamber experiment, corn seedlings were planted at 0 cm and 8-10 cm, from terminated winter rye plants. Root rot severity was assessed at crop development stage V2, and quantitative PCR was used to estimate the abundance of *Pythium* clade B and clade F members present in corn roots. Radicle and seminal root rot severity was numerically greater when seedlings were planted 0 cm from terminated winter rye plants compared to seedlings planted 8-10 cm away. Moreover, a greater abundance of *Pythium* clade B was detected in corn grown within the terminated winter rye compared to corn planted further away ($P = 0.0003$). No effect of distance between corn and winter rye was detected for *Pythium* clade F. These data contribute to our understanding of the effect of a winter rye cover crop on corn and will inform field trial

management practices for farmers to reduce occasional yield loss of corn following a winter rye cover crop

Introduction

In Iowa, cover crops (CC) are planted just prior to or immediately after harvest of the cash crop in the fall and terminated in the spring, from a few days to weeks before planting the cash crop. Cover crops eliminate a fallow period over the winter and add diversity to the predominant corn (*Zea mays* L.) and soybean (*Glycine maxx* L.) crop rotation used in Iowa. Consequently, the cash crop planted germinates within the decomposing residue of the CC.

Winter rye (*Secale cereale*) is the most commonly used CC in Iowa because it establishes well in the fall, is hardy, and has numerous environmental benefits including improved soil and water quality (Snapp et al. 2005). However, reduction in grain yield of corn planted after a winter rye CC has been reported (Miguez and Bollero, 2005; Kaspar and Bakker, 2015), which has made farmers reluctant to incorporate winter rye into their corn-soybean production system. Proposed reasons for this corn yield reductions after winter rye include nitrogen immobilization (Qi et al. 2011), reduced water availability (Munawar et al. 1990), allelopathy (Raimbault et al. 1990) and seedling disease (Bakker et al. 2016).

There are a number of reports of CC residue affecting beneficial microorganisms in the rhizosphere, altering microbial interactions, or increasing disease pressure to the subsequent crop (Brennan & Acosta-Martinez, 2017; Smiley et al. 1992; Rothrock et al. 1995). Recently, Acharya et al (2017) and Bakker et al (2016) reported that roots of winter rye terminated with herbicides were a host of corn seedling disease pathogens, specifically *Pythium* and *Fusarium* spp. Acharya et al. (2017) showed, however, that the incidence of corn radicle root rot caused by *Fusarium* spp. did not differ between winter rye and no winter rye treatments while radicle root rot caused

by *Pythium* spp. increased when corn was planted following a terminated winter rye cover crop. Additionally, the majority of *Pythium* spp. isolated from corn roots belonged to *Pythium* clade B and clade F suggesting *Pythium* spp. from these two clades are likely the causal agents of the increase in seedling disease observed on corn planted following a winter rye cover crop (Acharya et al. 2017).

Lower corn yields were partially attributed to greater seedling disease particularly when corn was planted within one week of terminating the winter rye CC (Acharya et al. 2017). When corn was planted more than ten days after the winter rye was terminated less seedling disease occurred presumably because the longer time interval allowed more winter rye decomposition and a decrease in *Pythium* inoculum numbers in the soil. Similarly, Smiley et al. (1992) reported that a longer time interval between termination of wheat and planting of a spring barley crop allowed more time for competing soil microorganisms to reduce pathogen inoculum levels in the dead volunteer wheat roots. In some years however, terminating a winter rye CC approximately two weeks before planting corn is difficult in Iowa because of wet conditions in the spring that may delay termination of the CC. Therefore, multiple management strategies are needed to reduce the potential negative effect of winter rye on corn.

Recently, on-farm research conducted by Practical Farmers of Iowa showed improved corn yield in some fields in which the cover crop was strip-tilled in the previous fall or spring (Gailans 2019). Strip-tillage removes the cover crop residue by killing or removing plants and roots from the portion of the soil in a row to which the cash crop is seeded and leaves previous cash crop or cover crop residue in the interrow (Licht and Al-Kaisi 2005). Similarly, Bottenberg et al. (1999) reported higher yield in snap beans planted into a strip-tilled winter rye cover crop

compared to no-till winter rye cover crop. These reports suggest that removing the CC plants and residue from the future crop row may mitigate the negative effect of a CC on a cash crop.

In the past two decades, precision agriculture technology has revolutionized farm management, in particular, GPS-based automatic guidance allows for more precise planting (Zhang et al. 2002). Thus, it is conceivable that a winter rye CC could be seeded into inter-rows so the CC is physically separated from the rows of corn planted the following spring. We hypothesized that physical separation between the CC and corn could reduce the risk of seedling disease, and benefit corn growth and development. In a proof-of-concept study, we conducted growth chamber experiments and compared seedling growth and seedling disease development of corn planted within terminated winter rye plants and corn planted at a distance from the terminated winter rye plants.

Materials and Methods

A Canisteo silty clay loam field soil was collected from a continuous corn field (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx). This soil was naturally infested with *Pythium*, which is endemic in Iowa soils; however, the soil was not tested prior to use. The soil was sieved through a 6 mm sieve and mixed with ground corn residue (2,000 g soil: 50 g corn residue) to reduce soil consolidation and increase the availability of pathogen inoculum. The soil/corn residue mixture was used to fill 23 cm diameter by 20 cm tall pots. Twenty-five to thirty rye seeds (variety Elbon) were planted on one side of each pot, forming a half circle of rye from the edge of the pot to approximately 7.5 cm from the edge. All pots were placed in a growth chamber at 20℃ with 16 h of light and 10℃ with 8 h of darkness. After four weeks, the winter rye plants in all pots were terminated by spraying with glyphosate [(*N*-(phosphonomethyl) glycine; 6.6 g active ingredient/liter] with a pump spray bottle to result in an

application rate that approximated that used in the field (2.92 L/ha). The area in each pot not planted to winter rye was also sprayed with glyphosate. Three days after winter rye termination, fungicide-treated corn seeds (Hybrid P1151R, DuPont-Pioneer Hybrid International, Johnston, IA) that include fungicide treatment metalaxyl (Alliance; Bayer CropScience, 291 µl a.i./kg), pyraclostrobin (Stamina; BASF, 467 µl a.i./kg), fludioxonil (Maxim 4FS; Sygenta, 95 ml a.i./kg), ipconazole (Rancona; Valent, 30 µl a.i./kg), and sedaxane (Vibrance 500; Sygenta, 189 ml a.i./kg) were sown into the pots directly within (0 cm) or 8-10 cm away from terminated winter rye plants. Fungicide seed treatments are common on commercial corn in Iowa; thus, the fungicide seed treatment was used to be representative of corn production practices in Iowa. Four seeds, spaced 2 cm apart, were sown at each distance in each pot (Figure 1). A layer of perlite approximately 2 cm in depth was used to completely cover the top soil to reduce water evaporation. After planting corn, the growth chamber temperature was changed to 12℃ with 16 h of light and 10℃ with 8 h of dark for approximately 32 days. Winter rye shoots were collected for biomass 7 days after winter rye was sprayed with glyphosate so that only decomposing winter rye roots remained in the pots (data not shown). Plants were watered (150 to 200 ml) every 2 to 3 days and nutrient solution (Miracle-Gro®, Scotts Miracle-Gro Products, Marysville, OH; 2.22 mg/liter water) was applied once a week after corn emerged. Treatments included i) corn planted in decomposing winter rye roots (0 cm) and ii) corn planted away from decomposing winter rye roots (8-10cm). Pots within the growth chamber were arranged randomly with eight replications per treatment in run 1, and five per treatment in run 2. The experiment was repeated twice separated by a couple of months. No winter rye control pots were not included in this study because previous studies (Acharya et al. 2017, 2018, 2020; Schenck et

al. 2019) have consistently shown that corn grown in the absence of winter rye in pots in the growth chamber grew well with very little to no root rot.

Corn seedlings were sampled between 1st and 2nd vegetative leaf stages (V1-V2) (Abendroth et al. 2011) and carefully washed under running water to remove adhering soil. Seedling growth parameters (radicle length, shoot length, shoot dry weight (run 1 only) and emergence (run 2 only) were measured, and disease incidence and severity were assessed visually for the radicle and seminal roots. Disease incidence was calculated as the percentage of germinated seedlings in each pot with lesions on the radicle and seminal roots. Disease severity was visually assessed from all seedlings as the percentage of rotted root tissue.

After seedling growth parameters were measured and disease assessed, a composite corn root sample of 100mg from each treatment within each pot was used for DNA extraction to quantify *Pythium* densities of clade B and clade F populations. For DNA extraction, plants (N = 4) within each pot for each treatment were pooled together and sections of radicle root, approximately 1 cm in length from the zone of elongation and maturation zone, were cut from each plant and combined to weigh 100 mg. Samples were freeze dried for 24 hours. Dried tissue was ground at 3,000 rpm for two runs of 80 s each using a MO BIO laboratory, Inc PowerLyzer 24. Samples were incubated on ice for 5 minutes between runs to maintain the quality of the samples while grinding. A DNeasy Plant Mini Kit (Qiagen, USA) was used to extract DNA with some modifications that included: 750 µl of Buffer AW1 was added to all samples and only 50 µl of AE buffer was used at the end giving a total DNA solution volume of 50 µl. DNA samples were diluted with sterile molecular biology grade water to 10 ng/µl for qPCR reactions.

The density of *Pythium* spp. belonging to clade B and clade F present in corn seedling root tissue were measured using quantitative PCR (qPCR). *Pythium* clade B and clade F probes used

were based on the work of Acharya et al (2017) along with ITS6 and ITS7 primers, which are selective for oomycetes. Each reaction was 20 µl consisting of 10 µl of TaqMan Environmental Master Mix 2.0 (Life Technologies), 1 µl of 10 µM ITS6 forward primer, 1 µl of 10 µM ITS7 reverse primer, 1.5 µl of 3 µM clade probe (B or F), 1.5 µl of molecular grade water, and 5 µl of sample template. Three technical replicates of each samples were placed in a 96-well PCR plate. Samples were tested twice, with clade B and clade F probes. Quantification of *Pythium* clade B and clade F was done following (Acharya et al. 2017). Quantitative PCR reactions were done in a BioRad CFX96 thermocycler and consisted of 10 min at 95°C, followed by 40 cycles of denaturing for 10 s at 95°C, and annealing and extension for 1 min 20 s, before the plate was read. The annealing temperature was 60°C for clade B assay and 61.5°C for clade F assay. Synthesized DNA of the target sequence (clade B and clade F; Invitrogen) were used to make standard curves. The standard curve for all qPCR runs produced an R^2 >0.98, and PCR efficiencies were between 93 to 106%. Quantification was done only within the range of standard curve amplification. Measured pathogen density was expressed as pathogen ITS gene copies per synthesized DNA (Invitrogen GeneArt Strings, Invitrogen) in copy number.

Corn emergence, shoot and radicle length, shoot dry weight, root rot severity and incidence, and *Pythium* density for each treatment (0 and 8cm) were analyzed using PROC GLIMMX in SAS (version 9.4) for detection of treatment effects. Effect of runs and run x treatment interaction was significant for the majority of the measured parameters therefore data from individual runs were analyzed separately.

Results and Discussion

Seedling emergence was significantly affected by the distance between corn and winter rye roots (P = 0.0139) in run 2 (Table 1). Corn seedlings planted 8-10 cm away from decomposing

winter rye roots emerged sooner than corn grown in decomposing winter rye roots. Likewise, Kravchenko & Thelen (2007) and Kaspar et al. (2013) reported emergence of corn was delayed in the presence of winter rye in their controlled environment studies.

Corn shoot dry weight of seedlings planted 8-10 cm away from winter rye was greater than corn seedlings planted within decomposing winter rye roots in run 1 (P = 0.0004; Table 1). Similarly, Acharya et al. (2017) reported that shoot dry weight of corn at growth stage V2-V5 was reduced when planted directly into winter rye residue compared with that of corn seedlings planted into no winter rye in their field experiment.

Corn shoot and radicle length in both runs differed between treatments (P <.0001 and 0.0307, respectively; Table 1). Shoot lengths and radicle lengths of corn seedlings planted 8-10 cm away from winter rye were taller and longer, respectively, than those of corn seedlings planted within decomposing winter rye roots in both runs. Because the radicles were shorter in corn planted into decomposing winter rye roots compared to those planted 8-10 cm away from the decomposing winter rye roots, we suspect that radicles were infected sooner after germination than radicles of corn planted 8-10 cm away. Our data support other reported data that the presence of winter rye residue affected early growth of corn seedlings. Raimbult et al. (1991) reported shoots were shorter in corn planted into winter rye cover crop using no-till compared with strip-tilled winter rye. In their controlled environment and field studies, Acharya et al. (2018) reported the radicle length of corn seedlings planted into winter rye residue were shorter compared to the roots of corn planted into no winter rye.

Treatment effects were not detected for radicle rot incidence and severity in both runs; however, there was numerically greater rot severity in corn planted within the decomposing winter rye roots. Although not significant, an earlier infection of radicles of corn planted into

winter rye roots than radicles of corn planted 8-10 cm away would allow more time for infection severity to develop on the shorter roots. Seminal root rot incidence and severity differed between runs. Treatment effects were detected for seminal rot incidence in run 1, but not run 2. The incidence of seminal root rot was greater in corn planted into the decomposing winter rye roots compared to corn planted away from decomposing winter rye roots in run 1 ($P = 0.001$), but no difference in incidence between treatments in run 2 ($P = 0.3739$). Similarly, the incidence of pea root rot disease decreased with increasing distance from the inoculated source plants under controlled conditions and in the field (Willocquet et al. 2007). No difference in seminal rot severity from run 1was detected ($P = 1.0$), but more severe seminal rot was observed in corn planted within decomposing winter rye roots compared to corn planted 8-10 cm away from decomposing winter rye roots in run 2 ($P = 0.0038$).

Pythium clade B copy numbers were affected by treatment in both runs. Copy numbers were numerically greater in run 2 compared to run 1 likely due to different batches of soil used with varying levels of *Pythium*; however, the trends observed in both runs were similar. Clade B copy numbers were greater in corn roots grown within decomposing winter rye roots compared to clade B copy numbers from roots of corn grown 8-10 cm away from the decomposing winter rye roots in both runs (Table 2). *Pythium* spp. clade F populations did not differ between treatments in both runs (Table 2). Similarly, Acharya et al. (2017, 2020), Bakker (2016), and Schenck et al. (2019) reported *Pythium* spp. from clade B were more prevalent in corn planted following a winter rye CC compared to corn planted following no CC. We proposed that pathogen densities were lower 8-10 cm from decomposing winter rye roots and consequently there was less infection resulting in less severe root rot and lower numbers of *Pythium* clade B. In addition, it would take time for the roots of corn seedlings planted 8-10 cm away from winter rye to grow

and intercept the decomposing winter rye roots and their resident pathogen communities. Furthermore, the plants would be older and more tolerant to invasion of root pathogens (Mellano 1970).

Despite using fungicide treated seed, we still observed seedling disease on our corn, and detected *Pythium* infection in roots of the diseased seedlings. The metalaxyl present in seed treatments targets *Pythium* spp. Studies have shown that the sensitivity of *Pythium* spp. to seed treatment varies with temperature (Matthiesen et al. 2016). Another study by Rojas et al. (2019) showed that isolates belonging to clade B are less sensitive to mefenoxam, which is the more active isomer of metalaxyl. It is possible that the conditions in our study (cold and wet) were very conducive for disease development and consequently the efficacy of the seed treatment was reduced.

Data from this study support our previous studies reporting radicle and seminal root rot severity and incidence were greater when corn was planted into winter rye residue compared to corn planted into no winter rye residue (Acharya et al. 2017, 2020; Bakker 2016; Schenck et al. 2019). The data also support our hypothesis that physically separating the CC root residue from the corn seedlings could reduce the risk of seedling disease, and benefit corn growth and development. Seedling disease, which includes root rot, reduces emergence and affects plant vigor (Munkvold 1999). We suggest root rot that occurs during germination and early growth delays emergence by weakening or preventing the radicle and seminal roots from growing, thereby reducing water and nutrient uptake for seedling growth. Consequently, corn seedlings with root rot are smaller and less vigorous than seedlings with healthy roots. In the field, delayed emergence and reduced plant vigor, may result in lower yields. Corn yield is dependent on uniform crop stands (Nafziger et al. 1991). When plants are uneven in size, as often occurs with

seedling disease, smaller plants produce smaller ears and thus yields are reduced. While our data do not rule out nitrogen immobilization, reduced water availability, and allelopathy, they do support that spatial separation of corn from decomposing winter rye roots reduces negative effects on early corn growth including seedling disease. Precision planted cover crop and strip-tillage field trials are in progress in Iowa to advance our understanding of how distance from winter rye CC residue affects corn growth and seedling disease under field conditions.

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Figures

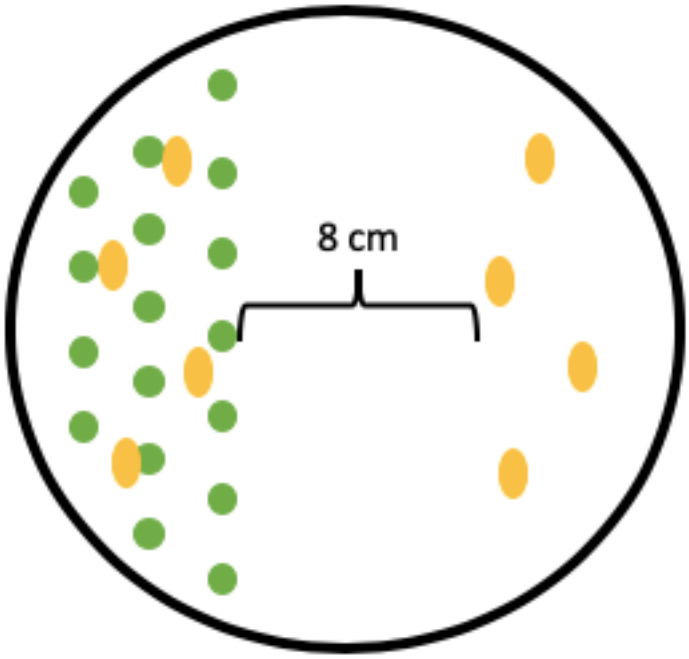

Figure 1. Layout of pots planted to rye (green circles), and corn (yellow ovals) planted within the rye or 8-10 cm away from rye.

Tables

Table 1. Emergence, shoot dry weight, radicle length, and shoot length means of corn seedlings grown in pots planted in decomposing winter rye roots or planted at a distance from the decomposing winter rye roots assessed at growth stage V1 to V2 in a growth chamber.

Treatments[w]	Emergence (DAP)[x]	Shoot dry weight (g)[y]	Radicle length (cm)	Shoot length (cm)[z]
Run 1				
0 cm	NA	1.29	6.5	11.21
8-10 cm	NA	1.83	9.79	13.10
P > *F*		0.0004	0.0182	<.0001
Run 2				
0 cm	20	NA	2.29	5.11
8-10 cm	16.6	NA	6.80	9.15
P > *F*	0.0139		0.0053	0.0094

[r] DAP = days after planting and Na = data not available.

[w] Treatments: Corn was planted with the decomposing rye roots (0 cm) and away from decomposing rye roots (8-10 cm) in run 1 and run 2. (N = 32 seedlings in run 1, N = 20 seedlings in run 2)

[x] Emergence was recorded as DAP when the first plant had emerged per treatment in each pot.

[y] Shoots were dried at 65ºC for seven days.

[z] Shoot length was measured from soil level to extended leaf.

Table 2. Radicle rot incidence, radicle rot severity, seminal rot incidence, seminal rot severity, and *Pythium* clade B and F copy number means of corn seedlings grown in pots planted in decomposing winter rye roots or planted at a distance from the decomposing winter rye roots assessed at growth stage V1 to V2 in a growth chamber.

Treatments[t]	Radicle rot incidence (%)[u]	Radicle rot severity (%)[v]	Seminal rot incidence (%)[w]	Seminal rot severity (%)[x]	Clade B (copies)[y]	Clade F (copies)[z]
Run 1						
0 cm	90.0	53.8	85.0 a	90.0	4539.1 a	0
8-10 cm	90.0	34.3	25.0 b	90.0	1374.7 b	0
P > *F*	1.0	0.1177	0.001	1.0	0.0233	0.1612
Run 2						
0 cm	100.0	35.4	100.0	67.0 a	339276.0 a	4162.3
8-10 cm	80.0	21.5	95.0	6.6 b	48576.0 b	3341.5
P > *F*	0.0993	0.1934	0.3739	0.0038	0.004	0.8211

[t] Treatments: Corn was planted with the decomposing rye roots (0 cm) and away from decomposing rye roots (8-10 cm) in run 1 and run 2. (N = 32 seedlings in run 1, N = 20 seedlings in run 2)

[u] Radicle rot incidence was calculated as the percentage of germinated seedlings with lesions on the radicle.

[v] Radicle rot severity was scored as the percentage of area of rotted radicle.

[w] Seminal rot incidence was calculated as the percentage of germinated seedlings with lesions on the seminal.

[x] Seminal rot severity was scored as the percentage of area of rotted seminal.

[y] Clade B was measured as target pathogen ITS gene copies per synthesized DNA, and is reported as copy number means of corn seedlings (N = 8 samples in run 1, N = 5 samples in run 2).

[z] Clade F was measured ad target pathogen ITS gene copies per synthesized DNA, and is reported as copy number means of corn seedlings (N = 8 samples in run 1, N = 5 samples in run 2).

CHAPTER 3. INFLUENCE OF SPATIAL PLANTING ARRANGEMENT OF WINTER RYE COVER CROP ON CORN SEEDLING DISEASE AND CORN PRODUCTIVITY

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Abstract

Despite numerous environmental benefits associated with cover crop (CC) use, some farmers are reluctant to include CCs in their production systems because of reported yield declines in corn. There are numerous potential reasons for this yield decline, including seedling disease. A winter rye CC serves as a 'green bridge' for corn seedling pathogens. We hypothesized that proximity of corn seedling roots to decaying rye CC roots contributes to corn seeding disease. An experimental field plot and on-farm study were done over two years to evaluate corn growth and development and disease severity of corn seedlings planted at various distances from decaying winter rye CC plants. The experimental field plot study was no-till corn-soybean rotation with five replications of a winter rye CC seeded as (i) no CC control (ii) broadcast, (iii) 19 cm drilled rows, and (iv) 76 cm drilled rows. The on-farm study was no-till corn-soybean rotation with four replications of a winter rye cover crop seeded as 38 cm drilled rows, 76 cm drilled rows and no CC control. The corn was planted on 76 cm rows shortly after rye was terminated. This seeding

arrangement of winter rye resulted in corn being planted at different distances from winter rye. Corn radicle root rot severity and incidence, shoot height, dry weight, corn height and chlorophyll at VT, ear parameters, and yield were collected. Soil samples were taken in the corn row and the interrow at winter rye termination, corn planting, and corn growth stage V3 to estimate the abundance of *Pythium* clade B members present in soil samples. Our results showed that increased distance between winter rye residue and corn reduced seedling disease and *Pythium* clade B populations in the radicles and soil, and increased shoot dry weight, leaf chlorophyll, plant height, and yield. This suggests that physically distancing the corn crop from the winter rye CC is one way to reduce the negative effects of a winter rye CC on corn.

Introduction

In recent years, cover cropping has become more prevalent in field crop production throughout the United States. In 2017, approximately 15.4 million acres were planted to a cover crop (CC) in the U.S., which is a 50% increase from 2012 (Zulauf and Brown 2019). CCs provide short and long-term benefits including reduced erosion and nitrate leaching, and consequently improved water and soil quality (Snapp et al. 2005). Throughout the Midwest, CCs are typically noncash crops that are planted in the fall concurrently with harvest of the main cash crop. This eliminates a fallow period over the winter and adds diversity to the crop rotation. Those CCs that survive winter are terminated in the spring, usually with an herbicide and typically before or when the cash crop is planted. As a result, the CC residue decomposes on the soil surface over the growing season providing additional benefits, such as weed suppression (Williams et al. 1998) and erosion control (Kaspar et al. 2001).

The most commonly planted CC in Iowa is winter cereal rye (*Secale cereal*) (Bader 2020). This CC establishes well (germinates with even emergence) in the fall and can withstand harsh

Iowa winters (Bader 2020; Snapp et al. 2005). However, some farmers remain hesitant to incorporate winter rye into corn (*Zea mays* L.) -soybean (*Glycine maxx* L.) production systems due to reports of reduced grain yield in corn when using a winter rye CC (Kaspar and Bakker 2015; Miguez and Bollero 2005). Numerous studies have suggested several reasons for the reduction of corn yield after winter rye, including nitrogen immobilization (Qi et al. 2011), reduced water availability (Munawar et al. 1990), allelopathy (Raimbault et al. 1990) and seedling disease (Acharya et al. 2017, 2020b; Bakker et al. 2016).

Seed rots and seedling diseases in corn are economically important in the United States with approximately 21.9 million tons of corn lost between 2012 and 2015 due to root rots, seedling diseases, and nematodes (Mueller et al. 2016). Planting corn into cool, wet soils can cause chilling injury that in turn contributes to seedling disease susceptibility (Munkvold and White 2016). Seedling disease in corn is most commonly caused by *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani*. Symptoms include seed rot, root rot, delayed emergence, chlorotic leaves, stunted plants, and damping off (plant death) that reduce stand density and plant vigor resulting in yield loss (Broders et al. 2007; Munkvold and White 2016).

Winter rye is a host of *Pythium* and *Fusarium* spp. that cause corn seedling disease (Acharya et al. 2017; Bakker et al. 2016). These pathogens overwinter in winter rye roots and infect the roots of corn seedlings growing in decomposing winter rye residue, resulting in a ‘green bridge’ effect (Acharya et al. 2017; Bakker et al. 2016). Acharya et al. (2017) determined that corn radicle root rot caused by *Pythium* spp. increased when corn was planted following a terminated winter rye CC; however, radicle root rot caused by *Fusarium* spp. did not differ between terminated winter rye and no winter rye treatments. *Pythium* spp. belonging to clade B (*Pythium arrhenomanes*, *P. torulosum* (Acharya et al. 2017), and *P. volutum* (Bakker et al. 2017)

were most frequently isolated from corn roots suggesting that *Pythium* spp. from this clade are likely the causal agents of seedling disease observed on corn planted after a winter rye CC.

A few studies have investigated practices to reduce the negative effects of winter rye on corn. Duiker and Curran (2005) observed no difference in yield when corn was planted 7 to 10 days after the winter rye was terminated compared with corn planted without winter rye. Additionally, Acharya et al (2017) measured a reduction in corn yield when corn was planted within one week of terminating a winter rye CC and observed an increase in seedling disease. As a means of reducing corn seedling disease in Iowa, farmers should plant corn 10 to 14 days after the winter rye is terminated (Acharya et al. 2017; Hartzler 2014). This allows more time for the winter rye to decompose, and is postulated, more time for allelopathic chemicals to dissipate, and pathogen numbers to decline in the soil.

In general, the distance between a pathogen and host influences the risk of pathogen infection (Real and McElhany 1996). One of the six principles for plant disease management is avoidance or exclusion, which recommends planting at a time or in a place where inoculum is low or not present (Maloy 2005). Therefore, physically separating a pathogen from a host may reduce the chance of host infection. The National Research Council (2004) reported physically distancing alternative hosts of *Xylella fastidiosa* from grapes controlled Pierce's Disease. Similarly, people worldwide have been instructed to keep their distance from others to avoid infection and spread of the coronavirus SARS-CoV-19 (WHO 2020).

A winter rye CC can be sown by broadcast seeding with a plane or ground equipment, or by drilling. Recently, a small number of farmers have used their cash crop planting machinery to seed winter rye in 76 cm rows (Theo Gunther personal communication). In the Midwest corn and soybean are usually planted in 76 cm rows. As precision planting becomes more common on

Iowa farms, it is possible farmers could seed a winter rye CC in the interrows to physically separate the winter rye CC from the corn rows planted the following season. It is possible this physical separation could reduce the impact of a winter rye CC on corn seedling disease and corn yield while continuing to provide environmental and soil health benefits. We hypothesized that physically separating corn from the terminated crowns of the winter rye CC would reduce the risk of seedling disease, and benefit corn growth and development. Recently, we demonstrated in a growth chamber study, that when corn was physically separated from winter rye residue, less root rot and improved growth of corn was observed (Kurtz et al. 2020). Thus, the objectives of this field study were to: (i) understand the impact of spatial planting arrangement of winter rye CC on corn seedling disease, growth, and development, and (ii) determine the *Pythium* spp. population density in the soil spatially and temporally. Data from this study would enable development of management practices for farmers when planting corn after winter rye CC.

Materials and Methods

Experimental plot field study. An experimental plot field trial was established during the years 2018 to 2020 at the Iowa State University Agriculture Engineering and Agronomy Farm, Boone, Iowa. This study was conducted in a field with a history of a corn-soybean rotation with soils Nicollet loam, Webster clay loam, and Clarion loam (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx). Each plot was 30 m long and ten rows wide of corn with 76 cm interrow spacing. Each plot had five corn rows for destructive sampling and five rows for non-destructive measurements and yield. The experimental design was a randomized complete block design with five replications.

A CC of Elbon (Green Cover Seed, Bladen, NE) winter rye was planted in the fall, immediately after soybean harvest, on 23 September 2018 and 18 September 2019. Treatments

included (i) a no CC control and three winter rye spatial planting arrangements (ii) broadcast, (iii) 19 cm, and (iv) 76 cm. For treatment 1, a cover crop was not planted to serve as the control. For treatment 2, rye was broadcasted and tilled in with a 33 cm cultipacker (International Harvester, Warrenville, IL). For treatment 3, a modified grain drill (19 cm opener spacing) was used to plant 3 rows of winter rye at a depth of 2.5 cm in the interrow between the future corn rows. The fourth opener was blocked, resulting in a 38 cm gap between each group of 3 rows of winter rye where the future corn row was planted. Thus, once emerged, the corn row was located 19 cm on either side from the terminated (sprayed) winter rye. For treatment 4, a modified grain drill was used to plant a single row of winter rye at a depth of 2.5 cm (three of four openers were blocked) into the center of the future corn interrow, resulting in a 76 cm gap between each row of winter rye where the future corn row was planted. Thus, once emerged, the corn row was located 38 cm on either side of the terminated winter rye. To ensure the winter rye seeding rate was equivalent across winter rye treatments, a seeding rate of 2,471,051 pure live seed (PLS) per treated ha, equivalent to approximately 111lbs/treated ha, was used. Consequently, in the broadcast treatment, where 100 percent of the area was treated with winter rye, 2,471,051 PLS/ha were sown. In the 19cm drilled treatment, 75 percent of the area (3 of 4 rows planted) was seeded with 1,853,289 PLS/ha (83.3lbs/treated ha). In the 76 cm drilled treatment, 617,763 PLS/ha (27.8lbs/ treated ha) were sown, since 25 percent (one of four rows) was planted. In the spring, the winter rye was terminated with a glyphosate application of 3 L/ha of RoundUp WeatherMax (Bayer Crop Science, St. Louis MO) using a custom dry boom sprayer with a centrifuge pump. The water volume was 150 liter/ha and the boom speed was 8 km/h with 10 Tee Jet 8004 flat-fan stainless steel nozzles placed 38 cm apart. Although termination time of the CC was targeted for three days before corn planting to potentially increase *Pythium* inoculum in

the soil, termination timing varied between the two years due to the influence of weather on field operations. In 2019, glyphosate was applied eight days before planting (26 April 2019). In 2020, glyphosate was applied three days before planting (26 April 2020). The no CC control plots were also sprayed with glyphosate at the same time the winter rye plots were sprayed for consistency of exposure to glyphosate.

Corn hybrid P1151R (Corteva Agriscience, Johnston, IA) seed treated with Cruiser 5FS (thiamethoxan; Syngenta, Wilmington, DE), Maxim Quattro (mefenoxam, azoxystrobin, thiabendazole, fludioxonil; Syngenta, Wilmington, DE), Raxil 2.6F (tebuconazole; Bayer CopScience, St. Louis, MO), Intego Solo (ethaboxam; Nufarm, Alsip, IL), and PPCT 2012 (*Bacillus amyloliquefaciens*; BASF, Florham Park, NJ) was planted on 4 May 2019 and 29 April 2020. Seed treatment rates used are proprietary. Corn planting rate was 88,960 seeds/ha in 76 cm rows using a five-row no-till planter (Kinze Manufacturing, Williamsburg Iowa, model 2600). Average monthly air temperatures and precipitation were obtained from a weather station located approximately 1 km from the experimental site (Iowa Environmental Mesonet).

An in-furrow (popup) 9-18-9 fertilizer was applied at a rate of 62.3 kg/ha at planting. This provided 5.6 kg each of N and K_2O, and 11.2 kg of P_2O_5 per hectare. A post-planting side-dress application of liquid urea-ammonium nitrate was applied 19 cm from the corn rows at a rate of 168 kg N/ha on 5 June in 2019 (31 DAP) and 15 June 2020 (47 DAP), when the corn was at growth stage V4 and V6 (Abendroth et al., 2011), respectively.

Winter rye sampling and processing. Winter rye shoot biomass samples were collected 1to 2 days prior to spraying glyphosate. All winter rye shoots were clipped close to the soil surface within two arbitrarily placed 33 cm radius circles in broadcast winter rye and one 30.5 cm by 76 cm quadrat, with the long side placed perpendicular to the row, for drilled winter rye plots.

Biomass samples were oven dried at 60ºC for seven days and weighed in grams. Biomass data were converted to kg per hectare for accurate comparison. In 2019 samples were finely ground for analysis of carbon and nitrogen content using the dry combustion-gas chromatography method (Schepers et al. 1989) with an EA1112 Flash NC Elemental analyzer (Thermo Electron Corp.) conducted by Dr. Amy Morrow's Lab at USDA-ARS Ames, Iowa.

Soil sampling and pathogen quantification. Soil samples were collected within the corn row and in the middle of the corn interrow in each treatment to determine the background *Pythium* spp. population density in the soil. Two soil samples were collected at each position (corn row or interrow) per treatment in three of the five replications. In 2019, the top 7.5 cm of soil from a 13 by 13 cm quadrat placed in the corn row or in the interrow was removed and 50 ml of soil was collected from 7.5 to 10 cm below the soil line. In 2020, soil samples were taken with a soil probe; two 15 cm soil cores were taken and the bottom 7.5 cm of the soil cores were placed in separate plastic bags with zip closures. Soil samples were collected one day before the winter rye was terminated, the day corn was planted, and at corn growth stage V2 to V3. Samples were placed in a cooler, taken to the laboratory, and placed in a -20ºC freezer.

Each soil sample was mixed well in the bag and a 100mg subsample of each soil sample was used for DNA extraction using the Dneasy Powerlyzer PowerSoil Kit (Qiagen, USA) with some modifications. Specifically, PowerLyzer Glass Bead Tubes were vortexed for 15 minutes and centrifuged for three minutes, and 50 µl of solution C6 was added. Each DNA sample was diluted to 15 ng/µl with sterile molecular biological grade water for qPCR reactions.

Quantification of *Pythium* clade B in the soil was done following methods described by Acharya et al. (2017) which consisted of using Probe arG190mod (FAM-AAACTTTCGTTCTCGGA-MGB·NFQ) with specificity for *Pythium* clade B, and ITS6

(GAAGGTGAAGTCGTAACAAGG) and ITS7 (AGCGTTCTTCATCGATGTGC) primers specific for oomycetes. Each reaction was 20 µl consisting of 10 µl of TaqMan Environmental Master Mix 2.0 (Life Technologies), 1 µl of 10 µM ITS6 forward primer, 1 µl of 10 µM ITS7 reverse primer, 1.5 µl of 3 µM clade B probe, 1.5 µl of molecular grade water, and 5 µl of sample template. Three technical replicates of each samples were placed in a 96-well PCR plate. Quantitative PCR reactions were done in a BioRad CFX96 thermocycler and consisted of 10 min at 95°C, followed by 40 cycles of denaturing for 10 s at 95°C, and annealing and extension for 1 min 20 s at 60°C, before the plate was read. Synthesized DNA of the target sequence (clade B; Invitrogen) was used to make standard curves spanning six orders of magnitude. The standard curve for all qPCR runs produced an R^2 >0.98, and qPCR efficiencies were between 93 to 106%. Quantification was done only within the range of standard curve amplification. Detected pathogen density was expressed as pathogen ITS region copies per synthesized DNA (Invitrogen GeneArt Strings, Invitrogen) in copy number.

Corn disease assessment and pathogen quantification in roots. Corn seedlings were sampled at growth stage V2 to V3 on 3 June 2019 and 1 June 2020. In rows three and four in the destructive rows of the plot, five corn seedlings, including the complete root system, from each row were arbitrarily dug up, placed in a labeled 4.5 L plastic bag and then placed in a cooler, taken to the laboratory, and stored in a 15°C large walk-in cooler for further processing. Within four days of sampling, seedlings were thoroughly washed with tap water and seedling roots were rated for disease. Growth stage of each seedling was determined as the number of fully collared leaves present (Abendroth et al. 2011). Shoot height was measured from the soil line of the shoot to the tip of the most fully extended leaf. Radicle root rot was assessed visually by estimating the percent area of rot and brown discoloration on the radicle root. Similarly, seminal root rot was

assessed visually by estimating the percent area of rot and brown discoloration on the seminal roots.

After seedling growth parameters were measured and disease assessed, corn shoots were cut from the roots at the soil line and dried at 60ºC for seven days and weighed in grams. Roots were stored in a -20ºC freezer for quantifying *Pythium* populations from root tissue. In 2019, dried shoot samples were processed for carbon and nitrogen content as described above for winter rye samples.

When quantifying *Pythium* populations from seedling roots, ten plant samples collected from each plot were combined and sections of radicle root, approximately 1 cm in length from the zone of elongation and maturation zone, regardless of symptomology, were cut from each plant and combined to weigh 100 mg for DNA extraction. Therefore, a composite corn root sample of 100mg from each treatment was used for DNA extraction to determine *Pythium* clade B population levels in the roots. Samples were freeze dried for 24 hours and then ground at 3,000 rpm for two runs of 80 s each run using a MO BIO laboratory, Inc PowerLyzer 24. Samples were incubated on ice for 5 minutes between runs to maintain the quality of the samples while grinding. A Dneasy Plant Mini Kit (Qiagen, USA) was used to extract DNA with some modifications to the protocol that included: 750 µl of Buffer AW1 was added to all samples and only 50 µl of AE buffer was used at the end giving a total DNA solution volume of 50 µl. DNA samples were diluted to 10 ng/µl with sterile molecular biology grade water for qPCR reactions.

Quantification of *Pythium* clade B present in corn seedling root tissue was done following methods described by Acharya et al. (2017) which consisted of using a clade B probe, and ITS6 and ITS7 primers specific for oomycetes. Quantitative PCR (qPCR) was completed as described above for soil samples in the soil sampling and pathogen quantification section.

Stalk rot ratings were taken at growth stage R6 in both years. From either row two or three of the destructive rows in the plot, the stalks of five consecutive plants were sliced longitudinally from directly below the primary ear to the crown of the plant. Stalk rot index was recorded as the number of nodes and internodes with rot divided by the total number of nodes and internodes and multiplied by 100.

Agronomic assessments. In both 2019 and 2020, stand counts were recorded approximately 21 days after planting (DAP) and again before harvest by counting the number of corn plants in a 15 m long staked area from row three in the non-destructive part of each plot. Plant height was measured at growth stage V18 (July 10, 2019) and VT (July 9, 2020). In each treatment ten plants in the eighth row of the non-destructive rows of the plot were measured from the soil to the tip of the highest leaf. Chlorophyll content was taken at growth stage VT in both years. Chlorophyll content was measured using a SPAD meter (Spectrum Technologies, Inc.) on 10 plants in rows eight and nine in the non-destructive rows of the plot. A reading was taken in the middle of the youngest fully expanded leaf on each plant. The ten readings were averaged to obtain a mean reading for each treatment per replication.

At growth stage R6, barren plants were determined by counting the plants with small ears (less than half the size of the majority of ears in the plot) or no ears within the same 15 m where stand counts were recorded.

Basal stalk and ear samples were taken at growth stage R6 in both years. Five consecutive plants were removed in row three of the destructive rows of each plot. The basal stalk was sampled by cutting a 20.3 cm piece of stalk 15 cm above the base of the plant and the ear removed. Basal stalk and ear samples were dried at 60^{o}C for seven days before processing. Basal stalk samples were ground to 1mm and analyzed for carbon and nitrogen content as previously

described for winter rye and corn shoot samples. Ears from each plant were measured for ear length, number of kernels, and 100 kernel weight data.

At the end of the season, corn yield data were collected from the five non-destructive rows of each plot. A modified combine (Colvin 1990) was used to harvest and measure corn yield (16 October 2019, 15 October 2020).

On-farm study. From 2018 to 2020, a field trial was established in coordination with the Iowa Soybean Association On-Farm Network® on a farm located in Hastings, Iowa. Each year the trials were in different fields with a soil type of Marshall silty clay loam in 2019 and Colo silty clay loam in 2020 (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx).

In the fall of 2018 and 2019, three treatments were planted in strips of 12 rows (9 m wide) and consisted of two winter rye treatments and a no CC control. There were 4 replications of each treatment. Winter rye variety Elbon was planted in 38 cm rows with a seeding rate of 58 kg/ha and 76 cm rows with a seeding rate of 29 kg/ha of Elbon rye in the fall of 2018 and 2019. Winter rye was terminated on 16 April 2019 with 59.3 oz Durango DMA (Corteva Agriscience, Johnston, IA) mixed with 140.3 L water per hectare and on 21 April 2020 with 2 L Accuron Flexi (Syngenta, Wilmington, DE) and 1.7 L Durango DMA mixed with 140.3 L water per hectare. The area without winter rye was also sprayed with glyphosate at the same time the winter rye treatments were sprayed for consistency of exposure to glyphosate.

In 2019, MY01C77 RA (Mycogen, Lost Nation, IA) corn seed was planted on 23 April with a seeding rate of 79,073 seeds/ha. In 2020, P1490AM (Corteva Agriscience, Johnston, IA) corn seed was planted on 30 April with a seeding rate of 76,603 seeds/ha. In each year there were 12 rows of corn, spaced 76 cm apart, planted in each treatment strip. A fertilizer application of 31.75 kg of nitrogen and 25 kg of sulfur in a 32% UAN and a 60/40 ratio of ammonium

thiosulfate, respectively, was applied before planting with open slot coulters on 20 April 2019 and 22 April 2020.

Two small plots were arbitrarily established in each treatment strip for sampling purposes. The small plots were aligned perpendicular to the replication strips. Each plot was two 15 m long rows in the center of each treatment (rows 6 and 7) and the two plots were spaced approximately 15 m apart.

Soil sampling and processing. Soil samples were taken when corn was at growth stage V2 to V3, on 30 May 2019 and 2 June 2020. Soil samples were taken with a soil probe. A 15 cm soil core was sampled and the bottom 7.5 cm of the soil core was collected. One soil sample was obtained within the corn row between two corn seedlings and one soil sample was obtained in the middle of the interrow of each plot. Samples were collected, placed into a cooler, taken to the lab, and placed into a -20ºC freezer. Soil samples were processed as previously described in the experimental plot trial, such that total DNA was extracted and qPCR used to quantify *Pythium* clade B population levels in the soil.

Corn sampling and assessment. Corn seedling samples were collected when corn was at growth stage V2 to V3, on 30 May 2019 and 2 June 2020. Five arbitrary corn seedlings were sampled from both rows six and seven in approximately the middle of each small plot. Samples were collected, placed in a cooler, taken to the lab, and placed in a large walk-in cooler at 15ºC.

Corn roots were washed, measured, and assessed for root rot within four days after collection. Leaf stage, shoot height, and radicle and seminal root rot were all assessed and processed as previously described in the corn assessment and pathogen quantification section for the experimental plot trial.

Stand counts, barren plants and yield. In both years stand counts were recorded at growth stage V2 to V3 and R6 by counting the number of emerged corn plants within the first row of each small plot. Barren ears were determined by counting the plants with small ears (less than half the size of the majority of ears in the plot) or no ears within the same 15 m row that stand counts were recorded at growth stage R6. Corn yield data were collected from each row in each strip on 26 September 2019 and 19 September 2020.

Data analysis. Analysis of variance (ANOVA) was performed on data for all measured parameters using PROC GLIMMX in SAS (version 9.4). Winter rye planting arrangement was treated as fixed factor and replication as a random factor. Since treatments varied between the experimental plot study and the on-farm study, data were analyzed separately. The interactions between year and treatments were significant for some parameters in both the experimental plot study and on-farm study; therefore, results were analyzed separately for each year. Data for pathogen quantification were not normally distributed therefore, data were square root transformed. For soil pathogen quantification, the interactions between year and time were significant in the experimental plot study; therefore, results were analyzed separately for time. The interaction between treatment and position for soil pathogen quantification was not significant, so position data were combined. If treatment effects were detected at 0.10 level of significance in the ANOVA, then treatment means were compared using Fisher's least significant difference at $P = 0.10$.

Results

Experimental plot study. *Air temperature and precipitation***.** Average air temperature and precipitation differed between years (Table 1). March was warmer and wetter in 2020 than in 2019 (Table 1). Between the time winter rye was sprayed with herbicide and corn was planted, it

was cooler with more precipitation in 2019 compared to 2020 (Fig 1). Similarly, in the week following corn planting, 2019 (day 124 to 130) was cooler and wetter while in 2020 (day 120 to 126) was warmer and drier (Fig 1). Additionally, in May, the month after planting, more precipitation occurred in 2020 (134.1 mm) compared to 2019 (100.8 mm) (Table 1).

Winter rye biomass. Winter rye treatments affected rye biomass in both years (2019, P = 0.0026; 2020, P = 0.0098; Table 2). Winter rye biomass was lower in 2019 compared with 2020 (Figure 2). The mean biomass values among treatments ranged from 551.9 to 1291.6 kg/ha and 1917.8 to 2634.7 kg/ha in 2019 and 2020, respectively (Table 2). In both years, broadcast winter rye produced more biomass compared to drilled winter rye treatments with 76 cm drilled winter rye producing the least amount of biomass. In general, winter rye biomass decreased as winter rye spacing increased in both years. In 2020, biomass of broadcast winter rye and 19 cm drilled winter rye was double, and 76 cm drilled winter rye biomass was more than triple compared to biomass of the same treatments in 2019 (Table 2).

Winter rye shoot nitrogen accumulation and C:N ratios differed between treatments (P = 0.006; P = 0.0005; respectively, Table 2). In 2019, winter rye shoot nitrogen accumulation was between 16.7 to 30.0 % and C:N ratios were between 13.6 and 17.8. For broadcast and 19 cm winter rye, nitrogen accumulation and C:N ratios were higher compared to 76 cm winter rye (Table 2).

Quantifying Pythium populations in soil. The effects of winter rye spacing treatments were significant for *Pythium* clade B ITS gene copy numbers in the soil at each sample time in at least one year (Figure 3). In 2019, *Pythium* clade B copy numbers in the soil differed among treatments when winter rye was terminated and corn was planted, but not when corn was sampled at V3 (P = 0.0008, P = 0.042, P = 0.3702; respectively, Table 3). In 2020, *Pythium*

clade B copy numbers in the soil differed among treatments at all three soil sampling times (rye termination, $P = 0.0086$; corn planting, $P = 0.0116$; V3, $P = 0.0215$; Table 3). In general, the greatest amount of *Pythium* clade B in the soil was detected in the broadcast winter rye treatments across all soil sampling times and years except at corn growth stage V3 in 2019. The second greatest amount of *Pythium* clade B copy numbers in the soil was found in the 19 cm winter rye across all soil sampling times and in each year except at corn planting in 2019. Soil from the no CC treatment had the least number of clade B copy numbers for all sampling times and in each year. Copy numbers of *Pythium* clade B in the soil were greatest when corn was planted in 2019 (3.1 to 72.1 copies) and when winter rye was terminated in 2020 (0 to 29.9 copies) (Table 3).

Corn seedling growth parameters. The effects of winter rye treatments on corn seedling parameters (corn shoot height, growth stage, and shoot dry weight) were significant in at least one year (Table 4). In 2019, corn seedlings were shortest in the broadcast winter rye treatment and tallest in the 76 cm drilled winter rye treatment. Treatment effects were not detected on corn shoot height in 2020 ($P = 0.2852$; Table 4). Treatment effects were not detected on growth stage in 2019 ($P = 0.2186$) but in 2020, corn planted in the no CC and 76 cm drilled winter rye treatments were approximately half a growth stage ahead of corn in the broadcast and 19 cm drilled winter rye treatments ($P = 0.0003$; Table 4). Treatment effects were detected on corn shoot dry weight in both years (2019, $P = 0.0092$; 2020, $P = < 0.0001$; Table 4). In general, corn shoot dry weight was greatest in no CC and 76 cm drilled winter rye treatments and least in the broadcast winter rye treatment.

Corn seedling root disease, Pythium population quantification in corn radicles, and stalk rot. The effects of winter rye treatments on corn seedling root disease and *Pythium* populations were

significant in radicle rot and seminal rot severity, and *Pythium* quantification in radicle roots in 2020 ($P = 0.0898$; $P = 0.0565$; $P = 0.0209$; respectively, Table 4). In general, incidence and severity of radicle rot and seminal rot were low and did not differ among treatments in 2019. In 2020, corn radicle and seminal rot severity of seedlings sampled from broadcast winter rye and 19 cm drilled winter rye treatments were almost double compared to that observed in seedlings from the no CC and 76 cm drilled winter rye treatments ($P = 0.0898$ and $P = 0.0565$; respectively, Table 4). Similarly, copy numbers of *Pythium* clade B from corn radicles were greater in corn from the broadcast winter rye and 19 cm drilled winter rye treatments compared to corn from the no CC and 76 cm drilled winter rye treatments in 2020 ($P = 0.0209$; Table 4). Unexpectedly, in 2019, the lowest number of copies of *Pythium* clade B were detected in corn radicles from the broadcast treatment compared to the other two winter rye treatments (19 cm and 76 cm drilled winter rye) (Table 4). Treatment effects were detected for stalk rot index in 2020 ($P = 0.078$; Table 5), but were not detected in 2020. Corn planted in broadcast winter rye had the least amount of stalk rot compared to the other treatments in 2020.

Agronomic assessments and corn yield. The effects of winter rye treatments on agronomic parameters were significant in at least one year on most of the parameters, including initial and final stand, chlorophyll reading, plant height at VT, stalk rot, basal stalk nitrate, barren plants, ear length, kernel number, kernel weight, and yield (Table 5). Initial corn stand at growth stage V1 was different among treatments in 2020 ($P = 0.0464$, Table 5). In both years initial corn stand was highest in the no CC treatment. Final corn stand at growth stage R6 was not different among treatments in both years. Corn stand increased by the end of the season in all treatments, except for 19 cm winter rye, in 2019 and no CC in 2020 (Table 5).

Treatment effects were detected on chlorophyll readings in both years ($P = 0.0008$; $P = < 0.0001$, respectively; Table 5). In general, chlorophyll content in corn was highest in the no CC treatment and decreased as winter rye spacing decreased. Similarly, corn plant height differed among treatments in both years ($P = < 0.0001$) and decreased as winter rye spacings decreased (Table 5). In 2019, corn basal stalk nitrate did not differ between treatments.

In 2020, treatment effects were detected for barren plants ($P = 0.0037$, Table 6). Corn planted in broadcast winter rye had more barren plants compared to the other treatments. In 2019, corn planted in 19 cm winter rye and 76 cm winter rye had a similar number of barren plants; however, in 2020, corn planted in 19 cm winter rye had more barren plants than corn planted in 76 cm winter rye. Treatment effects were not detected for ear length in both years. In 2019, kernel number was not different among treatments ($P = 0.8761$); however, numbers were different in 2020 ($P = 0.0226$, Table 6). The least number of kernels was found in corn planted into broadcast winter rye; whereas, the greatest number of kernels was found in corn planted into no CC. Treatment effects were detected for kernel weight per 100 seeds in both years (2019, P = 0.0424; 2020, P = 0.0035; Table 6). Although very similar, kernels weighed more in corn planted into no CC and weighed less in corn planted in 19 cm winter rye in 2019. In 2020, kernel weight was greater in corn planted into no CC and weight was lowest in corn planted in broadcast winter rye (Table 6).

The effects of winter rye treatments on corn yield were significant for both years (2019, $P = 0.0002$; 2020, $P = < 0.0001$; Table 6). Yield was highest when corn was planted into no CC. In 2019, there was no significant yield difference between the no CC and 76 cm winter rye treatments, but yields were different compared to the yields of broadcast and 19 cm winter rye

treatments (Table 6). In 2020, yield of all treatments were significantly different. In general, corn yield decreased as winter rye spacing decreased.

On-farm study. *Quantifying Pythium populations in soil.* The effects of winter rye treatments were not significant on copies of *Pythium* spp. belonging to clade B in soil for both years (P = 0.6145; P = 0.4189, Table 7). Similarly, location of soil samples (corn row and interrow) was not different among treatments for copies of *Pythium* clade B. In 2019, clade B copy numbers were numerically higher in soil sampled in the interrow compared to soil sampled in the corn row; conversely, in 2020, clade B copy numbers were higher in soil sampled in the corn row compared to soil sampled in the interrow.

Corn seedling growth parameters. The effects of winter rye treatments were not significant for both years on corn shoot length, shoot dry weight, and growth stage (Table 8), however these parameters were almost always numerically higher for corn planted in 76 cm winter rye compared to the corn planted in 38 cm winter rye and no CC treatments.

Corn seedling root disease and Pythium population quantification in roots. The effects of winter rye treatments on corn seedling root rot were significant in at least one year (Table 8). Corn radicle rot was more severe in corn planted in 38 cm winter rye compared to corn planted in 76 cm winter rye and no CC in both years ($P = 0.0514$; $P = 0.0016$ Table 8). In 2020, seminal rot of corn planted in 38 cm winter rye was most severe compared to corn planted in 76 cm winter rye and no CC (P = 0.0758; Table 8). Treatment effects were not detected on *Pythium* clade B copy numbers in corn radicles for both years. In 2019, *Pythium* populations belonging to clade B were greatest in radicles of corn planted in no CC and lowest in corn radicles planted in 38 cm winter rye; while in 2020, the reverse was true with the greatest of *Pythium* populations belonging to clade B in corn radicles planted in 38 cm rye.

Agronomic assessments and corn yield. Treatment effects were not detected on V3 and R6 stand counts, barren plants, and yield (Table 9). Corn stand increased by the end of the season for corn planted in 38 cm winter rye and 76 cm winter rye but decreased for corn planted in no CC in both years. Yield was greater in 2019 compared to 2020, and not different between treatments in both years.

Discussion

In Iowa, winter rye is a good option for a CC; however, there have been reports of reduced corn yield after a winter rye CC (Dinnes et al. 2002; Johnson et al. 1998; Kaspar and Bakker 2015; Miguez and Bollero 2005; Pantoja et al. 2015; Raimbault et al. 1990). Several studies have tried to understand under what conditions corn yield loss after a winter rye CC occurs. This research evaluated the effect of spatially separating the winter rye CC and corn cash crop on seedling disease, and corn growth and development. In our experimental plot study increased distance between winter rye residue and corn reduced seedling disease and *Pythium* clade B populations in the radicles and soil, and increased shoot dry weight, leaf chlorophyll, plant height, and yield. These data suggest that physically distancing the corn crop from the winter rye CC is one way to reduce the negative effects of a winter rye CC on corn.

In this study, winter rye was seeded at approximately the same date in each year yet CC biomass was more than doubled for all treatments in 2020 compared to 2019. The warmer temperatures in the fall of 2019 favored establishment and pre-winter growth, and a warmer spring of 2020 allowed for more winter rye growth in the second year of the trial. Similarly, Martinez-Feria et al (2016) reported warm springs favored CC establishment and growth. In addition, spatial planting of winter rye also affected the amount of winter rye biomass produced. In both years, broadcast winter rye had the most shoot biomass and 76 cm drilled winter rye had

the least shoot biomass per hectare. This is likely due to the unit area that was covered by winter rye. When considering the treatments, broadcast winter rye was planted on 100 percent of the unit area, 19 cm winter rye was planted on 75 percent of the unit area, and 76 cm winter rye was planted on 25 percent of the unit area. In 2020, winter rye in the 76 cm treatment was approximately 45 cm tall at termination compared to broadcast winter rye that was approximately 33 cm tall. Height differences among the winter rye treatments in 2019 were not noticed. This difference in height likely explains why there was less of a difference in biomass among treatments in 2020 than in 2019. These data have implications for management recommendations. A primary reason for growing CC is to protect soil and improve water quality (Dabney et al. 2001). Secondary reasons include weed management (Dabney et al. 2001). Since less ground is covered when winter rye is seeded in 76 cm rows, there is more bare ground that is subject to erosion and weed establishment. Moreover, less nitrates may be captured by the CC seeded in the 76 cm rows which could lead to more nitrate leaching and reduced water quality. Further studies to understand the effects of winter rye spacing arrangements on erosion, water quality, and weed control are needed.

Winter rye shoot nitrogen accumulation and C:N ratio was also affected by spatial planting of winter rye similar to that of winter rye shoot biomass. As spacing between winter rye and corn increased winter rye shoot nitrogen accumulation and C:N ratio decreased. The amount of winter rye biomass is correlated with the amount of nitrogen taken out of the soil system (Finney et al. 2016; Thorup-Kristensen 1994), which may partly explain better corn growth and higher yield in 2019 compared to 2020 in this study. Nitrogen plays an important role in photosynthesis, crop growth and development, and yield (Zhao 2003). One of the main purposes of a CC is to reduce soil nitrate leachates and winter rye is an excellent CC for this purpose (Kasper et al. 2007;

Staver and Brinsfield 1998). The winter rye CC pulls nitrogen from the soil, stores it in its roots and shoots that slowly decompose and release the nitrogen throughout the season (Malpassi et al. 2000). However, this may affect nitrogen availability for the corn crop in the spring (Thorup-Kristensen 1994). In 2020, nitrogen may have been less available to the corn crop because winter rye biomass was greater.

Population levels of *Pythium* spp. clade B in the soil were affected by treatments and changed over time in both years. The position of the soil samples (in corn row or interrow) did not affect soil pathogen levels. Although we did not separate rhizosphere soil from bulk soil, these data contrasts with Zhoa et al. (2014) reported greater pathogen populations in the rhizosphere of infected plants than in bulk soil. Winter rye roots are a host of *Pythium* spp. belonging to clade B (Acharya et al., 2017; Bakker et al., 2016, 2017), and we expected greater root biomass to contribute to greater pathogen populations in the soil. As expected, soil in the no CC treatment had the lowest copy numbers of *Pythium* clade B at all sampling times and in both years compared to soil sampled from the winter rye treatments. In general, *Pythium* clade B populations in the soil correlated with winter rye shoot biomass. Since Sheng and Hunt (1991) demonstrated that rye root biomass increased with shoot biomass, the broadcast winter rye treatment likely had more root biomass and consequently we detected greater populations of *Pythium* clade B than the two drilled winter rye treatments.

The trends for *Pythium* clade B numbers detected in the soil of the experimental plot study did however differ over time between years. In 2019, the greatest population of *Pythium* clade B in the soil was detected when corn was planted and was lower when winter rye was terminated and corn was at growth stage V3. In 2020, greater numbers of *Pythium* clade B in the soil were detected when winter rye was terminated and decreased at the later sampling times. These

differences in populations may be a function of when soil was sampled in relation to when the winter rye was terminated. In both years soil was sampled at winter rye termination, at corn planting, and at corn growth stage V3. In 2019, corn was planted eight days after winter rye was terminated (DAT) while in 2020, corn was planted three DAT. We speculate that pathogens harbored in the winter rye roots are released into the soil as the roots die and consequently populations increase before declining to background levels. Thus, we may have detected greater numbers of *Pythium* in 2020 if we had sampled soil eight DAT rather than three DAT when corn was planted. Also, weather conditions can affect how quickly winter rye dies, and consequently how quickly *Pythium* populations change. Depending on the air temperature, two to 14 days are required for complete kill of plants when using glyphosate (Kelly Products 2005).

Corn shoot height and growth stage between years was not consistent. Corn shoot height at growth stage V3 was lower in 2019 than in 2020, but corn growth stage was more advanced in 2019 than in 2020. Taller corn at V3 in 2020 may have been a result of corn competing with winter rye for light since there was more biomass in 2020, and therefore being etiolated in the earlier growth stages. Corn at V3 was shortest in the no CC treatment most likely because of no competition with winter rye residue. Corn planted in the 76 cm winter rye was tallest compared to the other winter rye treatments (broadcast and 19 cm rye). In both years, corn planted in no CC and 76 cm winter rye were at the same growth stage when sampled. In 2020, corn planted in broadcast winter rye and 19 cm winter rye was half of a growth stage behind the other treatments, which may be a result of more winter rye CC biomass maintaining cooler soil temperatures and delaying emergence. CC residue may keep soil temperatures 1 to 2 ^{o}C cooler than areas without cover (Teasdale and Mohler 1993). We did not collect data on date of corn

emergence but it is possible corn in the broadcast winter rye and 19 cm winter rye emerged later due to the cooler temperatures and consequently was slightly delayed in development.

Our field study corroborated our previous growth chamber study (Kurtz et al. 2020) in which we observed reduced seedling root rot and detected lower *Pythium* populations in the radicles when corn was planted 8 to 10 cm away from winter rye residue compared to corn planted within winter rye residue. Since winter rye roots are considered to be the main inoculum source of corn seedling disease pathogens (Acharya et al. 2017, 2018; Bakker et al. 2016), physically separating them from the corn seedling host likely reduced infection due to lower inoculum levels.

Spatially distancing the winter rye from the corn row did affect seedling disease. As expected, corn planted in broadcast winter rye and 19 cm drilled winter rye had the most severe radicle rot and corn planted in no CC and 76 cm drilled winter rye had less severe radicle rot. Additionally, the On-farm study supported the experimental plot study in that planting corn closer to the winter rye increased radicle rot severity observed. Both studies corroborate our growth chamber study (Kurtz et al. 2020).

The *Pythium* clade B copy numbers we detected in the soil did not correlate with the seedling root disease data in both years. More disease was observed in 2020 compared to 2019, despite lower populations of *Pythium* clade B at planting in 2020 compared to 2019. Similarly, Bithell et al. (2021) reported Phytophthora root rot of chickpeas did not correlate with population levels of *Phytophthora medicaginis* detected in soil. Since high moisture favors infection by *Pythium* spp. (Martin and Loper 1999), the frequent precipitation after planting in 2019 should have favored seedling disease. We speculate that development of the seedling may have played a role. Radicle rot was more severe in 2020 possibly because corn radicles were emerging when pathogen levels were higher in the soil whereas in 2019, which had a cooler start to the season that slowed

germination, the corn radicles emerged once pathogen levels declined in the soil. Since *Pythium* primarily infects roots (Martin and Loper 1999) our sampling times for *Pythium* may not have aligned with emergence of the radicle and seminal roots. Based on the required growing degree units for radicle emergence of corn (65 to 80 GDU, Channel 2020), in 2019, radicles would have emerged 10 to 11 DAP (14 to 15 May) or 18 to 19 DAT of winter rye, while in 2020, radicles would have emerged 7 to 10 DAP (6 to 9 May) or 10 to 13 DAT of winter rye. More sampling times after winter rye termination through corn emergence will be necessary to plot changes in *Pythium* populations after winter rye termination to support our above speculations. Although we focused on members of *Pythium* clade B, there are other soilborne pathogens that can cause root rot (Broders et al. 2007; Munkvold and White 2016). These pathogens may contribute to the variation in root rot that we see between years and treatments.

Corn stands at V1 and R6 were consistently higher in corn planted in no CC compared to corn planted in the rye treatments. Several studies saw similar reduced corn stands after a rye CC (Acharya et al. 2017; Eckert 1988; Kaspar and Bakker 2015; Pantoja et al. 2015) Additionally, corn stand from most treatments increased from the first count at growth stage V1 to the final count at growth stage R6. As previously mentioned, corn may have had delayed emergence due to lower soil temperatures in the rye CC treatments thus, showing increased corn stand counts from the initial count to the final count.

Spatially distancing the winter rye had an effect on both chlorophyll reading and corn height at growth stage VT and showed similar trends for both years. Chlorophyll content was greatest and corn was taller in corn planted in no CC and both measurements decreased as the distance between the winter rye residue and corn decreased. Chlorophyll measurements are proportional to the amount of nitrogen in the leaves (Evans 1989) and nitrogen is essential for plant growth.

Cereal rye is very efficient at removing nitrogen from soil (Finney et al. 2016). Although not measured, reduced available soil nitrogen for early corn growth and development could be a reason for the differences in chlorophyll measurements and plant height among the treatments.

More barren plants were observed in corn planted into broadcast winter rye and their number decreased as rye spacing increased. Previous studies have reported similar data after corn was planted into rye residue (Acharya et al. 2017, 2018; Kaspar and Bakker 2015). Corn yield is dependent on uniform crop stands (Nafziger et al. 1991). When plants are uneven in size due to uneven emergence (Ford and Hicks 1992) or seedling disease (Munkvold and White 2016), the smaller plants are outcompeted by neighboring plants and produce smaller ears or are barren thus reducing yields.

Spatially distancing the winter rye affected corn kernel number per ear only in 2020 and kernel weight. Kernel number and weight were higher in corn planted in no CC and decreased as winter rye spacing decreased. Kernel weight and number are two measurements that can be used to estimate corn yield (Nielsen 2018). In this study, these measurements correlated with measured yields. Moreover, they related well to our chlorophyll reading data. Similarly, Ghimire et al. (2015) reported chlorophyll readings had a significant correlation with kernel number, ear weight, and yield.

Yield was affected by spatial planting of rye in both years. In both years, yields were greatest for corn planted in no CC then decreased as spacing between the rye and corn decreased. In 2019, yield in 76 cm drilled winter rye was similar to the yield of corn planted in no CC and decreased by 12.3% and 11.6 % in the 19 cm winter rye and broadcast winter rye, respectively. In 2020, the effect of winter rye spacing on corn yield was more dramatic. Corn yield decreased by 8% in the 76 cm winter rye, 27.4% in the 19 cm winter rye, and 46.8% in the broadcast winter

rye compared to yield from corn planted in no CC. Since the 2020 growing season was considerably drier than the 2019 growing season, and therefore more stressful, the negative effect that winter rye may have on corn may have been enhanced.

Very few differences were measured in most of the data collected in the on-farm study. There are several reports that a winter rye CC did not negatively affect corn yield (Andraski and Bundy 2005; Ball et al. 2005; Doran and Smith 1991; and Snapp and Surapur 2018). Thus, the negative effect of a winter rye CC on corn does not occur in all fields or years. The field used for our experimental plot study has a history of seedling disease (Acharya et al. 2017, 2018), but it is unknown whether there was a history of seedling disease in the fields used for the on-farm study. Additional research at more locations over more growing seasons is needed to fully understand the factors that play a role in reducing corn yield after a winter rye CC.

There are likely many factors that contribute to corn yield reduction after a winter rye CC. This research demonstrated that spatially separating a winter rye CC from corn reduced seedling disease and benefited corn growth, development, and yield. Consequently, an additional recommendation to field crop farmers incorporating a winter rye CC into their production system may include planting a winter rye CC in either 19 cm drilled winter rye rows or 76 cm drilled winter rye rows rather than broadcasting winter rye to reduce the negative effects of a winter rye CC on corn. Additionally, some farmers in central Iowa strip-till their cover crops a few weeks prior to planting. The corn is planted into the tilled strips. Preliminary data suggest strip-tilling reduces the negative effect of winter rye on corn similarly to spatially separating the CC and corn by planting arrangements (Licht et al. 2021).

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Figures

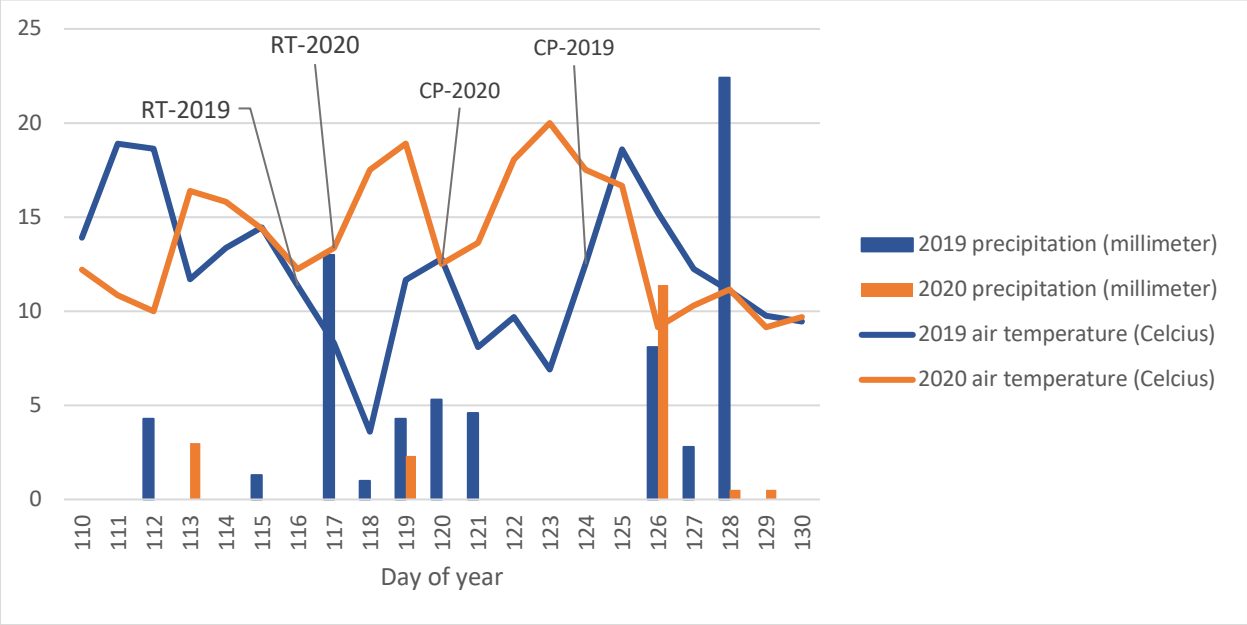

Figure 1. Average daily air temperature and precipitation from 20 April to 10 May 2019 and 19 April to 9 May 2020. Abbreviations: RT = rye termination, CP = corn planting.

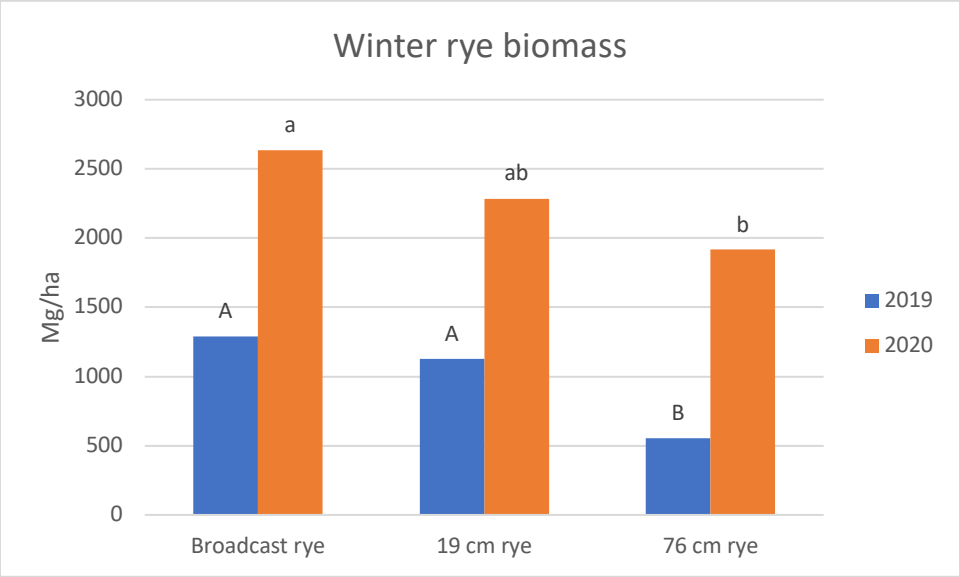

Figure 2. Biomass of winter rye cover crop treatments in 2019 and 2020. Bars topped with the same letter are not significantly different at *P* value = 0.10.

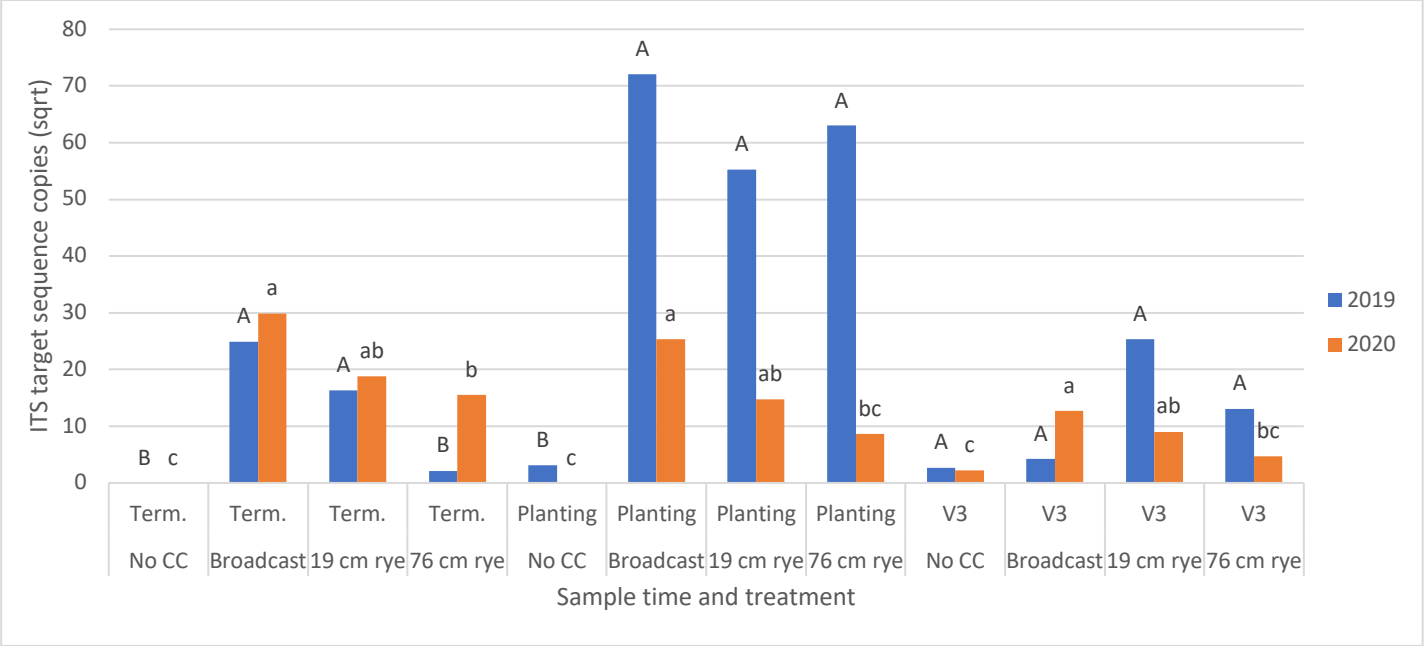

Figure 3. Density of *Pythium* spp. belonging to clade B in soil in the 2019 and 2020 experiment field study, in relationship to soil sampling time (termination, planting, and V3) and winter rye spacing arrangement treatments (no CC, broadcast rye, 19 cm rye, and 76 cm rye). Density is expressed as target pathogen ITS sequence copies per synthesized DNA, and is reported as copy number means, square root transformed. Means followed by the same letter within a sample time and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Tables

Table 1. Average monthly temperature and total monthly precipitation in the spring and summer of 2019 and 2020 in the experimental plot study.

	Temperature (°C)		Precipitation (mm)	
Month	**2019**	**2020**	**2019**	**2020**
March	0.11	5.7	38.1	67.3
April	11.2	9.1	49.3	37.8
May	14.9	15.3	211.3	134.1
June	21.7	23.9	100.8	39.9
July	24.3	24.4	117.1	70.9
August	21.5	22.7	33.0	25.9

[z] Months were selected based on timing of spring rye growth and corn growing season.

Table 2. Average biomass, carbon, and nitrogen content of winter rye spacing treatments in the experimental plot study.

Year	Treatments	Rye biomass (kg/ha)[x]	Rye shoot N accumulation	Rye shoot C:N
2019				
	No rye	NA[y]	NA	NA
	Broadcast rye	1291.6 a[z]	30.0 a	17.8 a
	19 cm rye	1125.6 a	25.4 a	18.6 a
	76 cm rye	551.9 b	16.7 b	13.6 b
	P > *F*	0.0026	0.006	0.0005
2020				
	No rye	NA	-	-
	Broadcast rye	2634.7 a	-	-
	19 cm rye	2285.4 b	-	-
	76 cm rye	1917.8 c	-	-
	P > *F*	0.0098		

[x] Rye biomass was collected by cutting the shoots at ground level approximately a day before glyphosate was applied.

[y] NA = Not applicable.

[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 3. Average effects of winter rye treatments on density of *Pythium* spp. belonging to clade B in the soil at different time points in the experimental plot study.

		Pythium clade B densities[v]		
Year	Treatments	Rye termination[w]	Corn planting[x]	V3[y]
2019				
	No CC	0.0 b[z]	3.1 b	2.7
	Broadcast rye	24.9 a	72.1 a	4.2
	19 cm rye	16.3 a	55.3 a	25.3
	76 cm rye	2.1 b	63.0 a	13.1
	P > *F*	0.0008	0.042	0.3702
2020				
	No CC	0.0 c	0.0 c	2.2 c
	Broadcast rye	29.9 a	25.4 a	12.7 a
	19 cm rye	18.8 ab	14.7 ab	9.0 ab
	76 cm rye	15.5 b	8.6 bc	4.7 bc
	P > *F*	0.0086	0.0116	0.0215

[v] Density is expressed as target pathogen ITS sequence copies per synthesized DNA, and is reported as copy number means, square root transformed.

[w] Soil samples at rye termination were collected one day before rye was sprayed with glyphosate in both years.

[x] Soil samples at corn planting were collected the day corn was planted (approximately eight days after rye was sprayed in 2019 and three days after rye was sprayed in 2020).

[y] Soil samples at growth stage V3 were collected 30 DAP in 2019 and 33 DAP in 2020.

[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 4. Average effects of winter rye treatments on corn seedling growth parameters, root rot, density of *Pythium* spp. belonging to clade B in radicles of corn at growth stage V2 to V4 in 2019 and 2020 in the experimental plot study.[s]

Year	Treatments	Shoot height (cm)[t]	Growth stage	Shoot dry weight (g)[u]	Radicle rot incidence (%)[v]	Radicle rot severity (%)[w]	Seminal rot incidence (%)[x]	Seminal rot severity (%)[y]	Radicle clade B (copies)
2019									
	No CC	30.4 b	3.7	5.6 b	70.0	3.1	92	5.4	3.5 b[z]
	Broadcast rye	29.6 b	3.5	4.5 c	86.0	3.3	100	4.4	7.2 b
	19 cm rye	30.8 b	3.6	5.0 bc	86.0	5.3	96	4.8	29.2 a
	76 cm rye	32.6 a	3.7	6.5 a	78.0	3.6	100	5.4	14.1 b
	P > *F*	0.0458	0.2186	0.0092	0.4545	0.49	0.228	0.8079	0.0455
2020									
	No CC	33.9	3.0 a	5.9 a	74.0	13.6 bc	30	1.2 b	7.8 c
	Broadcast rye	34.5	2.7 b	3.1 b	83.8	28.12 a	52	2.8 a	32.2 ab
	19 cm rye	34.2	2.4 c	3.2 b	80.0	27.3 ab	38	1.8 ab	47.4 a
	76 cm rye	35.6	3.0 a	5.3 a	66.0	9.8 c	20	0.6 b	28.3 b
	P > *F*	0.2852	0.0003	< 0.0001	0.4964	0.0898	0.1184	0.0565	0.0209

[s] Density is expressed as target pathogen ITS sequence copies per synthesized DNA, and is reported as copy number means of corn seedlings (N = 5 samples), square root transformed.
[t] Corn shoot height was measured from the ground to the extended leaf.
[u] Corn shoots were dried in an oven at 60°C for one week and weighed.
[v] Radicle rot incidence was calculated as the percentage of germinated seedlings with lesions on the radicle.
[w] Radicle rot severity was visually assessed by estimating the percent area of rot on the radicle root.
[x] Seminal rot incidence was calculated as the percentage of germinated seedlings with lesions on the seminal roots.
[y] Seminal rot severity was visually assessed by estimating the percent area of rot on the seminal roots.
[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 5. Average effects of winter rye cover crops on agronomic parameters of corn in the experimental plot study.

Year	Treatments	Corn stand/ha at V1[v]	Corn stand/ha at R6[w]	Chlorophyll reading at VT[x]	Corn height at VT (cm)[y]	Stalk rot index (%)
2019						
	No rye	83183	83700	47.0 a[z]	233.6 a	20
	Broadcast rye	77500	78189	39.3 c	203.4 b	20
	19 cm rye	80944	80600	39.4 c	203.8 b	17
	76 cm rye	82322	80944	43.4 b	229.2 a	22
	P > *F*	0.1215	0.1636	0.0008	< 0.0001	0.8643
2020						
	No rye	85078 a	84044	59.8 a	264.7 a	33 ab
	Broadcast rye	77845 b	83872	34.5 d	161.4 d	23 b
	19 cm rye	79394 b	84217	42.1 c	190.1 c	44 a
	76 cm rye	76639 b	83356	54.0 b	224.3 b	41 a
	P > *F*	0.0464	0.9719	< 0.0001	< 0.0001	0.078

[v] Corn stand counts were recorded within 15 m of the third row of each plot at growth stage V1.
[w] Corn stand counts were recorded within 15 m of the third row of each plot at growth stage R6.
[x] Chlorophyll readings were recorded in rows eight and nine from each plot at growth stage VT.
[y] Corn height was recorded in row eight from each plot at growth stage V18 in 2019 and VT in 2020.
[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 6. Average effects of winter rye cover crops on ear data and yield of corn in the experimental plot study.

Year	Treatments	Barren plants/ha[v]	Ear length (cm)[w]	Kernel number[x]	Kernel weight per 100 seeds (g)	Yield (Mt/ha)[y]
2019						
	No rye	1894	15.9	2728.4	31.3 a[z]	14.6 a
	Broadcast rye	3789	16.0	2803.8	30.1 ab	12.9 b
	19 cm rye	2411	15.8	2772.8	28.4 b	12.8 b
	76 cm rye	2411	16.1	2791.0	31.2 a	15.2 a
	P > *F*	0.1313	0.9375	0.8761	0.0424	0.0001
2020						
	No rye	1550 b	17.2	2922.6 a	29.5 a	12.4 a
	Broadcast rye	8267 a	16.6	2470.6 c	24.9 c	6.6 d
	19 cm rye	3100 b	16.7	2691.6 b	25.7 bc	9.0 c
	76 cm rye	1378 b	16.1	2641.4 bc	26.8 b	11.4 b
	P > *F*	0.0037	0.2181	0.0226	0.0035	< 0.0001

[v] Barren plants were counted within the same 15 m that stand counts were recorded in the third row at growth stage R6.

[w] Ear length is the average length of five ears from each plot.

[x] Kernel number is the total number of kernels from five ears from each plot.

[y] Corn yield data were collected from the last five rows of each plot.

[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 7. Average effects of winter rye treatments on density of *Pythium* spp. belonging to clade B in the soil collected from in the corn row and in the interrow in the On-farm study.

		Pythium clade B densities[x]	
Year	Treatments	Corn row[y]	Interrow[z]
2019			
	No CC	0.0	4.0
	38 cm rye	2.6	12.5
	76 cm rye	0.7	5.1
	P > *F*	0.6145	
2020			
	No CC	30.9	20.4
	38 cm rye	3.6	6.6
	76 cm rye	86.7	4.8
	P > *F*	0.4189	

[x] Density is expressed as target pathogen ITS sequence copies per synthesized DNA, and is reported as copy number means, square root transformed.

[y] Soil was sampled directly in the corn row.

[z] Soil was sampled in the interrow approximately 38 cm away from the corn row

Table 8. Average effects of winter rye cover crop treatments on corn seedling root rot, density of *Pythium* spp. belonging to clade B in radicles, and growth parameters of corn at growth stage V2 to V4 in the On-farm study.[u]

Year	Treatments	Radicle rot severity %[v]	Seminal rot severity %[w]	Corn radicle clade B (copies)	Corn shoot height (cm)[x]	Corn shoot dry weight (g)[y]	Growth stage
2019							
	No CC	7.1 ab[z]	6.1	25.8	27.9	3.4	3.4
	38 cm rye	9.7 a	5.6	21.1	27.8	3.4	3.3
	76 cm rye	3.1 b	5.1	24.8	28.8	3.7	3.3
	P > *F*	0.0514	0.7145	0.8594	0.4189	0.6838	0.8376
2020							
	No CC	11.8 b	2.9 b	40.8	31.3	5.1	2.8
	38 cm rye	25.3 a	5.5 a	99.8	31.1	5.1	2.9
	76 cm rye	11.7 b	2.2 b	68.1	32.4	5.3	3.0
	P > *F*	0.0016	0.0758	0.2128	0.28	0.7543	0.1453

[u] Density is expressed as target pathogen ITS gene copies per synthesized DNA, and is reported as copy number means of corn seedlings (N = 5 samples), square root transformed.

[v] Radicle rot severity was visually assessed by estimating the percent area of rot on the radicle root.

[w] Seminal rot severity was visually assessed by estimating the percent area of rot on the seminal roots.

[x] Corn shoot height was measured from the ground to the extended leaf.

[y] Corn shoots were dried in an oven at 60°C for one week and weighed.

[z] Means followed by the same letter within a column and year are not significantly different at *P* value 0.10 using Fisher's protected least significant difference.

Table 9. Effects of winter rye cover crops on initial and final stand, barren plants, and yield of corn in the on-farm study.

Year	Treatments	Corn stand/ha at V3[w]	Corn stand/ha at R6[x]	Barren plants/ha[y]	Yield (Mt/ha)[z]
2019					
	No CC	79328	79113	5381.9	14.6
	38 cm rye	77606	82019	3121.4	14.7
	76 cm rye	76960	80621	2906.1	14.4
	P > *F*	0.3814	0.3481	0.3633	0.5383
2020					
	No CC	89021	87647	3382.9	10.7
	38 cm rye	87514	88262	3690.4	10.8
	76 cm rye	89128	89185	2767.9	10.9
	P > *F*	0.2838	0.8591	0.8236	0.4768

[w] Corn stand counts were recorded within the first row of each 15 m small plot at growth stage V3.
[x] Corn stand counts were recorded within the first row of each 15 m small plot at growth stage R6.
[y] Barren plants were counted within the same 15 m that stand counts were recorded at growth stage R6.
[z] Corn yield data were collected from all rows in each strip.

CHAPTER 4. CONCLUSION

A winter rye CC has been shown to reduce yield in corn. There are many potential reasons for this yield decline including seedling disease in corn caused by pathogens harbored in winter rye roots. The goal of this research was to understand how planting corn at a distance from winter rye CC residue would affect corn seedling disease, growth and development. A growth chamber study, and two years of experimental field plot and on-farm trials in Iowa were conducted.

All studies from this research demonstrated that increasing the distance between a winter rye CC and corn reduced seedling disease and benefits corn growth, development. In the growth chamber study (Ch. 2), corn was planted 8-10 cm from winter rye residue and less seedling disease was observed, and corn seedling dry weight was greater indicating more vigorous seedlings.

The results of the growth chamber study were verified in both years of the experimental field plot study (Ch. 3) where the incidence and severity of seedling disease in corn planted approximately 38 cm from the winter rye residue was reduced compared to corn planted 19 cm from and within the winter rye residue. Furthermore, the experimental field plot study (Ch. 3) demonstrated that increasing the distance between winter rye and corn increased yield in corn. However, corn yield from the on-farm study showed no difference among treatments (Ch. 3). This shows that the negative effect of a winter rye CC on corn does not occur in all fields or years and further research is needed to fully understand the factors that play a role in reducing corn yield after a winter rye CC.

Different trends in *Pythium* clade B populations in the soil were observed in the field trials. Generally, *Pythium* clade B populations in the soil declined within four weeks after terminating winter rye. More frequent soil sampling is needed to understand the *Pythium* clade B population dynamics in the soil after winter rye is terminated.

This research provides an additional recommendation to field crop farmers incorporating a winter rye CC into their production system to reduce the negative effects of a winter rye CC on corn: planting a winter rye CC in rows at a distance away from the future corn row rather than broadcasting winter rye.